Systematic, phylogenetic and pollination studies of *Specklinia* (Orchidaceae)

Adam P. Karremans

... this work is dedicated to my mother who has introduced me to the wonderful world of orchids and kept pushing me further, look what you have made me do....

Systematic, phylogenetic and pollination studies of *Specklinia* (Orchidaceae)
Adam P. Karremans
October, 2015

Cover: *Specklinia remotiflora* Pupulin & Karremans illustrated by Sylvia Strigari, and reproduced with her kind permission.

It is not the intention of the author to publish any taxon names in this thesis. Names featured are thus not to be considered effectively published under article 30.8 of the International Code of Nomenclature for algae, fungi, and plants (Melbourne Code).

Systematic, phylogenetic and pollination studies of *Specklinia* (Orchidaceae)

Proefschrift
ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van de Rector Magnificus prof.mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op 25 november 2015
13:45 uur

door

Adam Philip Karremans

geboren te Leiden
in 1986

Hombre

Soy hombre, he nacido,
tengo piel y esperanza.
Yo exijo, por lo tanto,
que me dejen usarlas.
No soy dios: soy un hombre
(como decir un alga).
Pero exijo calor en mis raíces,
almuerzo en mis entrañas.
No pido eternidades
llenas de estrellas blancas.
Pido ternura, cena,
silencio, pan, casa...

Soy hombre, es decir,
animal con palabras.
Y exijo, por lo tanto,
que me dejen usarlas.

Jorge Debravo (1966)

Man

I am a man, I have been born,
I have skin and hope.
I demand, therefore,
to be allowed to use them.
I am not a god: I am a man
(as if to say seaweed).
But I demand warmth in my roots,
a meal in my guts.
I don’t ask for eternities
full of white stars.
I ask for tenderness, dinner,
silence, bread and home...

I am a man, as if to say,
an animal with words.
And I demand therefore,
to be allowed to use them.

Loose translation by the author

Thesis committee

Promotor
Prof. dr. E.F. Smets, Leiden University, Naturalis Biodiversity Center and KU Leuven

Co-promotors
Dr. B. Gravendeel, Leiden University and Naturalis Biodiversity Center
Dr. F. Pupulin, Lankester Botanical Garden, University of Costa Rica

Other members
Prof. dr. H.P. Spaink, Institute of Biology, Leiden University
Prof. dr. M. Schilthuizen, Institute of Biology, Leiden University and Naturalis Biodiversity Center
Prof. dr. P.C. van Welzen, Institute of Biology, Leiden University and Naturalis Biodiversity Center
Prof. dr. M.S.M. Sosef, Wageningen University and Botanic Garden Meise, Belgium
Dr. A. Pridgeon, Royal Botanic Gardens Kew, UK

Table of Contents

Systematic, phylogenetic and pollination studies of *Specklinia* (Orchidaceae)

Adam Philip Karremans

General Introduction

General Introduction

The rich biodiversity of Costa Rica

Uniting the large territories of North and South America there is a thin strip of land known as Central America. Central America serves both as a bridge for the northern and southern flora and fauna as well as a barrier for marine life trying to cross from east to west, and vice-versa (Fig. 1). However this bridge and barrier has not always been there. In the Miocene, Central America was an archipelago without continental connection, and it is estimated that the land finally closed between 3 and 15 million years ago (Montes *et al.* 2015).

The Costa Rican landscape (Fig. 2) is quite mountainous, with elevations that go from sea-level to above 3800 m on Cerro Chirripó, and with several peaks over 3000 m. The climate in the area is quite variable, but there are two basic water regimes in Central America, the Atlantic (Caribbean) is more rainy in November to January, and the Pacific one in which rain is almost absent from December to April. In between, a mix of both regimes is found, with the highest rainfall found at mid-elevations. Rainfall increases with elevation until a certain point and then it decreases again. In Costa Rica this turning point was calculated at about 1000 m. The large valleys have the lowest rainfall, while the highest rainfall can be found at some mountain bases. The areas around Tapantí National Park can have more than 315 days of rain. Costa Rica, being close to the Equator, has a very stable temperature. The average temperature of the warmest month does not exceed the average temperature of the coldest month by 5 degrees. With higher elevations the temperatures become lower, and the difference of day vs. night temperature also decreases. Northern winds are frequent from January to March, and can be up to 90 km/hr in some areas. On the Caribbean coast, winds are more constant and have an average speed of 7 km/hr (Janzen 1991).

The effects of climate on the biology of plants and animals in Costa Rica is poorly known, Janzen (1976) suggests that in tropical conditions, where the climate fluctuates very little, the high mountain peaks are probably a much greater barrier than in temperate regions, where the organisms are more used to seasonal changes. There are about 20 vegetation types that can be recognized in Costa Rica using the Life Zone system of Holdridge (1987), ranging from tropical dry forest, to sub-alpine rain paramo, with many transitional zones (Dressler 1993a). This complex landscape combined with different environmental conditions allow for a plethora of micro-climates to which epiphytic orchids are especially sensitive, and explain the high variety of species found in such a small country.

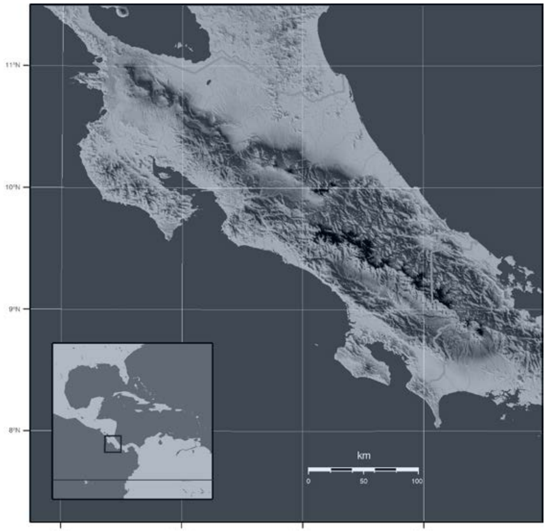


FIGURE 1. Map of Costa Rica and its position in Central America. Courtesy of Franco Pupulin.

FIGURE 2. View of the Talamanca mountain range in Costa Rica. Photograph by A.P. Karremans.

Orchidology in Costa Rica

With more than 1,600 reported species, Costa Rica has one of the richest orchid floras in tropical America, currently surpassed in total species number only by Brazil, Colombia, Ecuador and Peru. However, with an area of only 51,000 km^2, Costa Rica is five to 150 times smaller than each of those countries. As such, with 1 species per each 32 km^2, Costa Rica has the highest orchid species/area ratio in the world. The large number of reported orchid species is clearly a direct result of the combination of high diversity, intense botanical exploration and the presence of active research groups and institutions (Karremans & Bogarín 2013).

During the colonial period botanical exploration in Central America was almost non-existent, and it is not until their independence that interest in the local flora begins. The collections of George W. **Barclay** are likely to be the first documented orchid specimens from Costa Rica. He travelled under the command of the British Royal Navy admiral Sir Edward Belcher (Fig. 3) in the voyage of the HMS Sulphur off the Pacific Coast of Central America. *Rossioglossum ampliatum* (Lindl.) M.W.Chase & N.H.Williams, collected in 1839, was among the first species recorded from the country. The visit of Anders Sandoe **Oersted** to Costa Rica in 1846 is the first of a long list of naturalists who would visit the country in the following decades. He was followed by Józef **Warszewicz** Ritter von Rawicz and Hermann A. **Wendland**, among others. Their orchid collections in Costa Rica were studied and described by Heinrich Gustav **Reichenbach**, the most prominent authority on orchidology in the nineteenth century after the death of John **Lindley** (Ossenbach 2002; Bogarín *et al.* 2013a). Reichenbach *filius* also worked extensively with the collections of Auguste (Augustus) **Endrés**. Endrés' specimens, plants descriptions and drawings were extremely carefully and accurately prepared, very unlike his time. Endrés, an Alsace native of German origin, would initially start collecting orchids in Costa Rica employed by George Ure **Skinner** and James **Bateman** in 1866. Unfortunately his very early and impressive orchid flora of Costa Rica remained unpublished after his untimely death in Colombia, and it would be kept mostly "hidden" in Reichenbach's herbarium at the Natural History Museum in Vienna (Pupulin *et al.* 2014). On his drawing of «Pleurothallis mellifera» (which was later named *Specklinia endotrachys*) Endrés wrote "inner surface of sepals slightly viscous? / much visited by a small fly" and in his description again "sepals scabrous in inner surface (exuding honey)" in what is likely to be the first ever published observation on pollination in Pleurothallidinae [cited by Pupulin *et al.* 2012 (Chapter 1)].

Anastasio **Alfaro**, Paul **Biolley**, Alexander Curt and Alfred **Brade**, Alberto Manuel **Brenes**, Charles Herbert **Lankester**, Richard **Pfau**, Henry François **Pittier**, Paul C. **Standley**, Jean François Adolphe **Tonduz** and Karl **Wercklé** contributed extensively to collecting Costa Rican orchids by the end of the nineteenth and beginning of the twentieth centuries. Their collections were the basis for the creation of the Herbario Nacional, and allowed for the study of Costa Rican orchids by some of the worlds most renowned scientists of the time. Oakes **Ames**, Robert Allen **Rolfe**, Rudolf **Schlechter** and Charles **Schwienfurth** described hundreds of new orchid species on the basis of Costa Rican material collected by the first, and contributed significantly to our knowledge of the country's flora (Ossenbach 2002; Bogarín *et al.* 2013a).

In the second half of the twentieth century, a new cohort of students of Costa Rican orchids would appear. In contrast with their predecessors these would do both the "field" and "desk" work of more specific groups of orchids, and therefore contribute immensely to our complete understanding of the whole orchid flora. Including, Paul H. **Allen**, John **Atwood**, Calaway H. **Dodson**, Robert L. **Dressler**, James A. **Fowlie**, Leslie A. **Garay**, Eric **Hágsater**, Clarence K. **Horich**, Alex D. **Hawkes**, Carlyle **Luer**, Dora Emilia **Mora**, Rafael Lucas **Rodríguez Caballero** and Carl **Withner** (Ossenbach 2002; Bogarín *et al.* 2013a).

Figure 3. Portrait of British Royal Navy admiral Sir Edward Belcher by Stephen Pearce.

The turn of the century was marked by the appearance of the "Catálogo anotado de orquídeas de Costa Rica" in 2002, and the "Manual de Plantas de Costa Rica", in 2003. Both included more than 1,400 species of the Orchidaceae family, neatly summarizing the activities of the mentioned naturalists and scientists in almost two centuries of botanical exploration in the country. It also coincided with the creation of a research department at Lankester Botanical Garden (JBL), of the University of Costa Rica. JBL is dedicated exclusively to the study of orchids at the hand especially of Franco **Pupulin** and Jorge **Warner**. A healthy and young group of researchers, including Mario A. **Blanco**, Diego **Bogarín**, Melania **Fernández** and myself, and an upcoming group of students are now part of the orchidology team at JBL. In an effort to produce a comprehensive flora of the Orchidaceae for Costa Rica, dozens of scientific publications in the last decade have added more than 200 species of orchids to the Costa Rican flora (Karremans *et al.* 2012; Karremans & Bogarín 2013; Fernández *et al.* 2014).

The Pleurothallidinae, a major challenge for systematic research

Pleurothallis R.Br., type genus of subtribe Pleurothallidinae Lindl., was described already more than two centuries ago. Historically, *Pleurothallis* and Pleurothallidinae have been treated almost as synonyms, with the exclusion from *Pleurothallis* of only a few morphologically well-recognizable genera throughout the years. The first systematic classification of the members of Pleurothallidinae is possibly that of Lindley (1836; 1859). Several authors followed with additional proposals to tackle *Pleurothallis* and its segregate genera (i.e. Reichenbach f., Barbosa Rodrigues, Cogniaux, Schlechter, Garay, Dressler, and others). Members of the genus had however not suffered as many changes as they have in the last 30 years. Luer's first monograph of the group in 1986 triggered a proliferation of systematic studies that would have been impossible before. He published dozens of monographs thereafter. The first molecular phylogeny of the subtribe was published by Pridgeon *et al.* (2001), and was followed by a proposal to redefine it completely (Pridgeon & Chase 2001; Pridgeon 2005). However, the significant systematic and taxonomic changes proposed after that, in addition to the rapid increase in species numbers within Pleurothallidinae, has more than ever fueled the need to have a comprehensive picture of phylogenetic relationships within the subtribe.

The issue with the classification of the megadiverse *Pleurothallis* (in a traditional sense) has historically been the same one: the realization that it is not monophyletic, but the impossibility of resolving its systematics with the available data. In 1859, Lindley said about *Pleurothallis* that "I think it necessary to preserve this great and difficult genus without dismemberment. Not that I regard it as a really single aggregation of species...". A century later Luer would say that "*Pleurothallis* is indeed capable of being divided, but because of the various interrelationships, most divisions at the subgeneric and sectional levels seem more practical" and added "A *Pleurothallis* might be described as any pleurothallid that does not fit into any of the other genera" (Luer 1986). After their morphologically based cladistic study of the group, Neyland *et al.* (1995) wrote "the large genus *Pleurothallis* is polyphyletic and, therefore, may be divided into several genera", something that Garay had already noticed two decades before, "It is quite possible that *Pleurothallis* may be drastically segmented in the future; the most likely candidate is the former genus *Specklinia*" (Garay 1974). But, it was not until Pridgeon and Chase (2001), relying on the molecular-based studies by Pridgeon *et al.* (2001), that *Pleurothallis* was finally dismembered. They pleaded that it "has been nothing but a polymorphic assemblage for almost two centuries" and that "many taxa with conspicuous autapomorphies were segregated from it, gradually leaving the genus itself with no defining synapomorphies".

In their phylogenetic study of the group, Pridgeon and Chase (2001) found that "many characters are difficult to score in cladistic analyses because they are either continuous or probably not homologous. These same characters show up repeatedly in his [Luer's] artificial key to the subgenera". Nevertheless, the complexity of the group and their limited sampling size forced them to admit that "for nomenclatural transfers we extrapolated from the study taxa to morphologically similar taxa as recognized by Luer". Not surprisingly, subsequent phylogenetic studies within the Pleurothallidinae have been clear evidence that the generic, subgeneric and sectional systematics of the subtribe were far from fully resolved. Re-circumscriptions and emends were either made or at least suggested by several authors that used novel analytical methods and/or included a broader sampling of species (Stenzel 2004; Abele 2008; Karremans

2010; Chiron *et al.* 2012; Karremans *et al.* 2013a; Karremans 2014, Chapter 7; Karremans *et al.* unp., Chapter 6). Meanwhile, hundreds of species' names, be it new species or combinations, and dozens of new genera have since then been proposed by Luer (2002; 2004; 2005; 2006; 2007; 2009) and others, mostly on the basis of morphology. There is a pressing necessity of reviewing the phylogenetic relationships of many groups within the pleurothallids.

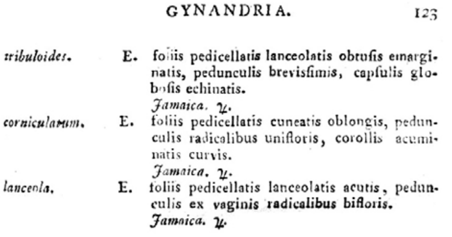
The genus Specklinia

The first species attributable to *Specklinia* ever to be described were *Epidendrum corniculatum* Sw., *E. lanceola* Sw. and *E. tribuloides* Sw. (Fig. 4) from Jamaica. They were described simultaneously by Olof Swartz in his *Nova genera & species plantarum; seu, Prodromus descriptionum vegetabilium, maximam partem incognitorum quœ sub itinere in Indiam Occidentalem annis 1783-87 digessit* in 1788. The original description included not more than a dozen words (Fig. 5), typical of the epoch and which has made their interpretation quite hazardous.

The generic name *Specklinia* appeared for the first time in 1830 in John Lindley's The Genera and Species of Orchidaceous Plants. Lindley honored Rudolph Specklin with the generic name, pleading that he had been an "outstanding sculptor in wood, whose grandfather's exceptional illustrations of plants well-deserved to be included in Fuchs' *Historia stirpium*". Little is known about Rudolph Specklin, Luer (2006) writes that he was an early nineteenth century English engraver, nevertheless this was quite unlikely. Based on Lindley's original statement in Latin, Veit Rudolph Specklin (Fig. 6) a renowned woodcutter, who famously illustrated the plants presented in *De Historia Stirpium Commentarii Insignes* of Leonhart Fuchs (Fig. 7), was the grandfather of his honoree Rudolph Specklin. Veit Rudolph Specklin, who lived in Straßburg and was of Alsatian decent, passed away in 1550. He left five children, of which the last would pass away in 1600. His grandson, Rudolph Specklin therefore likely lived from around the end of the sixteenth century to the beginning of the seventeenth century. Lindley was born two hundred years after, and surely did not know any of the Specklins personally.

In the original publication, Lindley included only five names in *Specklinia*. Swartz's *Epidendrum corniculatum* and *E. lanceola*, and three other species that are now generally placed in the genera *Anathallis* and *Acianthera*. He did not designate a type species for the genus. Garay and Sweet (1972) lectotypified the genus using *Specklinia lanceola* (Sw.) Lindl. It was chosen as type species by the authors because it "is the one which most approximates the generic characters given by Lindley". Lindley's generic description

Figure 4. *Epidendrum corniculatum* Sw., *E. lanceola* Sw. and *E. tribuloides* Sw., now better known as *Specklinia corniculata, S. lanceola and S. tribuloides* respectively. Photographs by A.P. Karremans.



GYNANDRIA. 123

tribuloides. E. foliis pedicellatis lanceolatis obtuſis emarginatis, pedunculis breviſſimis, capſulis globoſis echinatis.
Jamaica. ♃.
corniculatum. E. foliis pedicellatis cuneatis oblongis, pedunculis radicalibus unifloris, corollis acuminatis curvis.
Jamaica. ♃.
lanceola. E. foliis pedicellatis lanceolatis acutis, pedunculis ex vaginis radicalibus bifloris.
Jamaica. ♃.

Figure 5. Original publication of the first three species of *Specklinia* to be described. Taken from *Nova genera & species plantarum* by Olof Swartz.

FIGURE 6. Self-portraits of the illustrators of *De Historia Stirpium Commentarii Insignes* by Leonhart Fuchs, as found within the book itself.

FIGURE 7. Woodcutting of Leonhart Fuchs by Veit Rudolph Specklin as found in *De Historia Stirpium Commentarii Insignes* itself.

seems to be easily applicable to all of the other cited species, but in the sake of nomenclatural stability it is best to adhere to the proposed lectotype.

Traditionally, *Specklinia* Lindl. (Orchidaceae: Pleurothallidinae) had been considered a synonym of *Pleurothallis* R.Br. (Luer 1986). However, the generic limits of the large genus *Pleurothallis* were recircumscribed by Pridgeon and Chase (2001) on the basis of molecular studies by Pridgeon *et al*. (2001). The authors presented new evidence to re-establish *Specklinia*, recognizing 86 species. The recircumscribed *Specklinia* included species of *Pleurothallis* subgen. *Specklinia* (Lindl.) Garay [*P*. sects. *Hymenodanthae* Barb.Rodr., *Tribuloides* Luer, Muscariae Luer], subgen. *Empusella*, subgen. *Pseudoctomeria* and *Acostaea* Schltr., showing low levels of sequence divergence (Pridgeon & Chase 2001). *Specklinia* was difficult to characterize on the basis of a particular set of distinguishing morphological features (Karremans 2014, Chapter 7; Karremans *et al.* unp., Chapter 6), promoting the creation of several new genera, expressly designed to fit one or more morphologically aberrant species of the genus (Luer 2004; 2006). Due to the different interpretations of the circumscription of *Specklinia*, it had been difficult to estimate the actual number of species belonging to the genus. Pridgeon (2005) accounted for 200 species, but one year later Luer (2006) reduced the genus to no more than 40 species. Most recently Barros & Trettel Rodrigues (2009) accounted for 420 binomials, about five times the original number transferred by Pridgeon & Chase (2001). Finally the broadly sampled molecular phylogeny combined with morphological and geographical data presented here (Chapter 6) shows that 95 specific epithets are attributable to *Specklinia* at this time.

Specklinia has not been accepted by everyone yet. Some authors continue to place it under the synonymy of *Pleurothallis* (Ackerman 2014), this is especially true among orchids growers. Nevertheless, it has been amply proven that *Specklinia* species are not closely related to *Pleurothallis*, in fact they are closely related to other, generally accepted and traditionally used genera such as *Dryadella* Luer, *Platystele* Schltr. and *Scaphosepalum* Pfitzer (Pridgeon *et al*. 2001; Chapter 6).

Outline of this PhD thesis

The present work brings together the results of systematic, phylogenetic and pollination studies of species belonging to the genus *Specklinia*, with special emphasis on Costa Rican species. It is organized in three distinct sections that contain manuscripts of similar topics for ease of the reader.

Contributions towards our systematic knowledge of *Specklinia*:—Almost without exception any biological study on a particular species or group of species would be seriously flawed without the definition, clarification and understanding of the subject itself and the taxonomic name that should be applied to it. If, like in the case of the species studied here, our initial concept of a specific taxon is mistaken then we risk either not being able to answer our biological questions or doing it wrongly. In molecular phylogenies, the initial determination of a sampled taxon is key in the assessment of the resulting trees. Having misidentified terminals can lead to (1) the allocation of species to wrong genera, (2) the assumption that species or genera are non-monophyletic, (3) and the grouping of unrelated species while displacing close relatives. In ecological studies, the use of broad species' concepts, which actually include more than one species can have a detrimental effect. Mixing ecological preferences and interactions of different species will lead to missing the actual patterns of each species. Similarly, but possibly not as grim, over-splitting a species will lead to having several species with the same ecological preferences and interactions.

Applying the correct taxonomical name is essential. It is important to remember at this point that for any species only type specimens can be determined with complete certainty, everything else is our own interpretation. As such our conclusions based on non-type material should be handled with care. The chapters in this section attempt to clarify the taxonomic status of a series of *Specklinia* species. The species of the *Specklinia endotrachys* complex are disentangled in chapters 1 and 2, while the species of the *Specklinia glandulosa* complex are treated in chapter 3. Additional taxonomic novelties encountered during the different stages of this study are presented in chapters 4 and 5. In total thirteen *Specklinia* species are being characterized and illustrated, of which six are new to science.

Phylogenetic reassessment of *Specklinia* and its allied genera:—One of the initial challenges of proposing a study on *Specklinia* was the difficulty in trying to circumscribe the genus. The lack of consistency among authors as to how many and which species belonged to the genus in recent literature was a mayor issue. A broadly sampled phylogenetic analysis of *Specklinia* and its closest relatives was necessary to establish not only the below genus-level relationships, but also how the genus relates to other genera.

One of our main goals was to understand the relationships among species from all the proposed genera within this species' group, which includes the generic concepts of *Acostaea* Schltr., *Areldia* Luer, *Cucumeria* Luer, *Dryadella* Luer, *Gerardoa* Luer, *Incaea* Luer, *Muscarella* Luer, *Platystele* Schltr., *Pseudoctomeria* Kranezl., *Rubellia* (Luer) Luer, *Sarcinula* Luer, *Scaphosepalum* Pfitzer, *Specklinia*, *Sylphia* Luer, *Teagueia* (Luer) Luer, *Tribulago* Luer, *Tridelta* Luer, *Trigonanthe* (Schltr.) Brieger and *Verapazia* Archila.

Assessing the pollination mechanisms of *Specklinia*:—Chapter 8 is dedicated to a multi-disciplinary study of the pollination syndrome of *Specklinia* species. The study focuses on the *Specklinia endotrachys* complex, a group of orange-flowered *Specklinia* species that are visited and pollinated by *Drosophila* species. Our main questions were: 1) how are the *Specklinia* attracting their pollinators?, 2) are the *Specklinia* deceitful or rewarding?, 3) which and how many species of *Drosophila* are pollinating the *Specklinia*?, 4) what behaviors do the *Drosophila* show whilst visiting the flowers? 5) how do these species of *Specklinia* prevent hybridization?

General discussion:—The final chapter of the thesis brings together the most relevant results of all the previous chapters. An integrative reflection is given with thoughts about the findings and possible complementary future studies.

Contributions towards our systematic knowledge of *Specklinia*

Chapter 1

A reconsideration of the empusellous species of *Specklinia* (Orchidaceae: Pleurothallidinae) in Costa Rica

Franco Pupulin
Adam P. Karremans
Barbara Gravendeel

This paper focuses on the systematics of the *Specklinia endotrachys* species complex in Costa Rica. Traditionally considered a variable species, *S. endotrachys* is here treated as one of at least four, albeit closely related, taxa. Of these species, *S. endotrachys*, *S. pfavii*, and *S. spectabilis* are described and illustrated from living material, and *S. remotiflora* is described and illustrated as new to science. *Specklinia remotiflora* is compared with *S. endotrachys* and *S. spectabilis*, from which it differs in the repent habit, lax inflorescence and campanulate flowers provided with convergent sepals and non-apiculate petals. New combinations are proposed for *Pleurothallis pfavii* and *P. spectabilis*. A lectotype is selected for *Pleurothallis endotrachys*. Observations on the pollination of *S. remotiflora* and *S. spectabilis* in cultivation are given.

Keywords: Neotropical orchids, pollination, *Specklinia endotrachys* complex, *Specklinia remotiflora*

Introduction

Recircumscription of the generic limits of the mammoth genus *Pleurothallis* R.Br. (Orchidaceae: Pleurothallidinae) as a result of molecular studies (Pridgeon *et al.* 2001; Pridgeon & Chase 2001) and the consequent creation of several more segregate genera (Pridgeon & Chase 2001; 2002; Luer 2004; 2005; 2006; 2007; 2010; 2011) has made the taxonomy of some concepts fluid. In particular, the paper by Pridgeon and Chase (2001) presented new evidence to re-establish *Specklinia* Lindl., recognizing 86 species, most of which were transferred by the authors. Both in the bootstrap consensus trees of the *matK*/*trnL-F* dataset and the most parsimonious tree from the combined *matK*/*trnL-F*/ITS nrDNA dataset their "clade F" unites a morphologically highly heterogeneous set of taxa, including *Dryadella simula* (Rchb.f.) Luer, *Pleurothallis costaricensis* Rolfe, *P. lentiginosa* F.Lehm. & Kraenzl., *P. endotrachys* Rchb.f., *Acostaea costaricensis* Schltr., and species of the genera *Platystele* Schltr. and *Scaphosepalum* Pfitzer. In one of the most parsimonious trees of the complete ITS nrDNA matrix, based on a larger sampling, clade F also includes other species of *Pleurothallis*, among which *P. lanceola* (Sw.) Spreng.—the type species of the genus *Specklinia*—together with *P. endotrachys*, *P. fulgens* Rchb.f., *P. lateritia* Endrés ex Rchb.f., *P. lentiginosa*, and *P. tribuloides* (Sw.) Lindl., form a distinct subclade treated by the authors as the "core" *Specklinia*. Even with the removal of the basal *Dryadella* Luer and the derived *Platystele* and *Scaphosepalum* from clade F, the resulting circumscription of *Specklinia* is variable both in terms of vegetative and floral morphology.

Specklinia sensu Pridgeon & Chase (2001) is difficult to characterize on the basis of a particular set of distinguishing morphological features, which has promoted the creation of several new genera, expressly designed to fit one or more morphologically aberrant species of *Specklinia* (Luer 2004; 2006). Due to the different interpretations of the circumscription of *Specklinia*, it is difficult to estimate the actual number of species of this genus, but Barros and Trettel Rodrigues (2009) recently accounted for 420 binomials (Barros 2004; 2006; Barros & Trettel Rodrigues 2009; Luer 2004; 2007), more than twice the number originally included by Pridgeon (2005).

Among segregates from *Specklinia*, we focus here on the species of the supposedly monotypic genus *Empusella* (Luer) Luer, elevated to generic rank and based on *Pleurothallis* subgen. *Empusella* Luer (1986) to accommodate

Pleurothallis endotrachys [syn. *Specklinia endotrachys* (Rchb.f.) Pridgeon & M.W.Chase, 2001] (Luer 2004). According to Luer (1986), *Pleurothallis* subgen. *Empusella* may be recognized by the caespitose habit with short stems ("ramicauls"), an inflorescence born from an annulus near the base of the ramicaul, and a laterally compressed peduncle. The name is derived from the Latin *empusella*, a little hobgoblin, in allusion to the fancied appearance of the flower (Luer 2007). In his diagrammatic representation of possible relationships between groups of *Pleurothallis*, Luer (1986) placed subgen. *Empusella* as a derived member of the "affinity *Specklinia*", close to *Masdevallia* Ruiz & Pavón and *Scaphosepalum*. He considered the "bizarre" *Pleurothallis endotrachys* as the only member of the subgenus, "without close relatives", noting how other names for the species (which he considered synonyms) had been placed in four genera in the past. Luer formally transferred *P. endotrachys* to *Empusella* Luer, without rationale either for creation of the new genus or its phylogenetic relationships. In his later treatment of *Specklinia* and other vegetatively similar genera, Luer (2006) did not include *Empusella* in the discussion, possibly considering it only distantly related to his concept of *Specklinia*.

The results of nuclear (ITS) and plastid DNA (*trnL-F, matK*) sequencing for 185 taxa of the Pleurothallidinae (Pridgeon *et al.* 2001) showed, however, that *Pleurothallis* subgen. *Empusella* is placed in a clade sister to the *Scaphosepalum-Platystele* clade, which comprises several sections of *Pleurothallis* subgen. *Specklinia* (Lindl.) Garay (including the type of the genus, *S. lanceola*) and *Pleurothallis lentiginosa* Lehm. & Kraenzl., the last previously treated as the monotypic genus *Pseudoctomeria* Kraenzl. on the basis of its highly divergent floral morphology. Due to the absence of reliable morphological characters to interpret as synapomorphies and the strong support provided by the genetic evidence, Pridgeon and Chase (2001) resurrected and redefined *Specklinia* as sister to the *Scaphosepalum-Platystele* clade. The recircumscribed *Specklinia* included species of *Pleurothallis* subgenera *Specklinia* [*P.* sects. *Hymenodanthae* Barb.Rodr., *Tribuloides* Luer, *Muscariae* Luer], *Empusella* and *Pseudoctomeria*, and *Acostaea* Schltr., showing low levels of sequence divergence. Among the morphological features useful to define *Specklinia*, the authors indicated the usually small plants provided with an abbreviated stem with an annulus, the variously connate sepals, and the hinged lip; the sepals and petals of *Specklinia* are mostly membranous, and the column is provided with a toothed apex, and ventral anther and stigma.

Our independent genetic analyses (Karremans *et al.* unp., Chapter 6) confirm the phylogenetic placement of the empusellous species close to the core of *Specklinia*. Therefore, recognizing this small group of species as a separate genus would result in recognition of most of the segregate genera from *Specklinia*, including *Acostaea*, *Gerardoa* Luer, *Muscarella* Luer, *Pseudoctomeria*, *Sarcinula* Luer, and *Tribulago* Luer, but would also require creation of at least two additional genera, reducing *Specklinia* sensu stricto to just a few species.

The empusellous species of *Specklinia* belong to a clade that includes *Specklinia barbae* (Schltr.) Luer, *S. chontalensis* (A.H.Heller & A.D.Hawkes) Luer, *S. corniculata* (Sw.) Steud., *S. fulgens* (Rchb.f.) Pridgeon & M.W.Chase, *S. guanacastensis* (Ames & C.Schweinf.) Pridgeon & M.W.Chase, *S. glandulosa* (Ames) Pridgeon & M.W.Chase, *S. lanceola, S. lentiginosa, S. psichion* (Luer) Luer, and *S. tribuloides* (Sw.) Pridgeon & M.W.Chase. This singular group is comprised of morphologically heterogeneous species that have never before been presumed to be closely related, the only constant morphological character they seem to share is the color of the flowers. All species mentioned, except for *S. lentiginosa*, have intense bright orange flowers with a fruity odor. What makes this clade unusual is not only the fact that more than a dozen species share a common color, but that the different bright orange shades are unique within *Specklinia*, uncommon in Pleurothallidinae, and not at all common among Orchidaceae in general. Whereas most orange- flowered orchid species appear to be members of a clade of hummingbird-pollinated within mainly non-orange, bee-pollinated group (i.e. *Elleanthus* Presl, *Ornithidium* Salisb. ex R.Br., *Scaphyglottis* Poepp. & Endl.), in this case the species of the *S. endotrachys* complex are pollinated by fruit flies, *Drosophila*, as it was early noted out by Endrés (in Reichenbach 1878) in the nineteenth century and confirmed by Chase (1985) with cultivated plants.

Therefore, we will treat here the species close to *Pleurothallis endotrachys* as members of *Specklinia*, making the necessary combinations.

Materials and Methods

This study was conducted at the Lankester Botanical Garden (JBL) of the University of Costa Rica and the Hortus Botanicus of Leiden University in The Netherlands between 2010 and 2012. Cited specimens belong to the Specklinia endotrachys complex and include vouchers kept mainly at CR, JBL, INB, L, MO and USJ. Phenological data were recorded in the field and from cultivated specimens or herbarium labels. Herbarium specimens were deposited at CR, JBL and L. Sketches of specimens were drawn with a Leica MZ 9.5 stereomicroscope with a drawing tube and conserved in the reference collections of JBL and L. The species were illustrated by composite line drawings from living specimens by Pupulin and Winkel. Descriptions were prepared from living specimens and herbarium material from JBL and L.

Key to the Costa Rican species of Specklinia close to S. endotrachys

1. Flowers yellow with red petals; lip acute, yellow, with a central red line; column wings broad, entire; clinandrium entire .... *S. pfavii*
- Flowers entirely orange; lip rounded to truncate, concolorous orange; column wings narrow, denticulate; clinandrium erose-dentate 2

2. Plant repent; inflorescence lax; flowers not spreading; petals not apiculate .... *S. remotiflora*
- Plant caespitose; inflorescence congested; flowers spreading; petals distinctly apiculate 3

3. Floral bracts subequal to the length of the pedicel; dorsal sepal lanceolate; petals retuse, densely and coarsely papillose, provided with a long mucron abruptly inserted within the sinus .... *S. endotrachys*
- Floral bracts much shorter than the length of the pedicel; dorsal sepal linear-triangular; petals acute, tapering, microscopically papillose, the mucron continuous with the apex .... *S. spectabilis*

Taxonomic treatment

1. *Specklinia endotrachys* (Rchb.f.) Pridgeon & M.W.Chase.

Basionym: *Pleurothallis endotrachys* Rchb.f. Type: COSTA RICA. Alajuela: San Ramón, along the Barranca River, *Endres 92* (lectotype, selected by Pupulin *et al.* (2012), W 0021581; isolectotypes, W 1889-003365, W 0020331, W 0020150, AMES 118500). Additional material associated with the type is recorded by Pupulin *et al.* (2011).

Homotypic synonyms: *Humboltia endotrachys* (Rchb.f.) Kuntze. *Empusella endotrachys* (Rchb.f.) Luer. Non *Pleurothallis endotrachys* Lehm. & Kraenzl., *nom. illeg.* [= *Pleurothallis lehmanniana* Schltr].

Epiphytic, caespitose, erect herbs to 16 cm tall. Roots fibrous, flexuous, glabrous, to 0.7 mm in diameter. Stem abbreviated, terete, slender, to 1.5 cm long, monophyllous, completely covered by a glumaceous, imbricating, acute sheath to 2 cm long, becoming dry-papyraceous with age. Leaf oblong-elliptic, obtuse, minutely retuse, the midvein protruding abaxially into a small apicule, 11.0–16.0×1.7–2.2 cm, gradually tapering toward the base into a deeply conduplicate-rounded petiole provided with ancipitous margins, subcoriaceous. Inflorescence produced laterally from the apex of the stem, without an annulus, and emerging from a short, spathaceous, acute, carinate bract ca. 2 mm long, a successively, many-flowered (to 19+), distichous congested raceme to 27 cm long; peduncle flattened, ancipitous, to 20 cm long, with 3 distant, imbricating- tubular, ancipitous, acute bracts, 9–10 mm long; rachis fractiflex, almost completely covered by the floral bracts. Floral bracts tubular-amplectent, strongly conduplicate-ancipitous, broadly ovate, acute, uncinate in lateral view, 8–10×8–9 mm. Pedicel cylindric, subclavate, glabrous, 8–10 mm long, persistent. Ovary linear, subtrigonous, 6 mm long, orange. Flowers with pale orange dorsal sepal, striped with darker orange, the lateral sepals, petals and lip bright orange, the column pale orange. Sepals densely papillose in the inner surface except at the base, the lateral ones fleshy and thickened along the external margin, the dorsal one thinner; dorsal sepal erect, elliptic-lanceolate, 5-veined, acuminate, geniculate at the middle, semi-hyaline, flushed with orange along the veins, 27×8 mm, the distal two thirds densely papillose, the papillae extending along the margins almost to the base; lateral sepals lanceolate, acuminate, ending into a filiform mucro, 3- veined, 24×5 mm, connate at the base ca. 4 mm to from a deeply saccate mentum in front to the column foot, membranous-hyaline at the base, then thick, papillose, with a prominent keel abaxially along the midvein, the margins slightly revolute-thickened. Petals small, linear-ligulate, falcate, subspathulate, retuse, provided with a filiform mucro in the sinus, 4.5×1.0 mm, univeined, the apex and the distal labellar margin papillose. Lip small, arched-convex in natural position, thinly articulate with the column foot by a hyaline claw, ligulate- spathulate when expanded, rounded-subretuse, 5.5×1.8 mm, the central portion provided with a pair of thin, elevated keels converging and running to the base of the apical lobe. Column arched, semi-terete, 5–6 mm long without the foot, margins of the stigma dilated into semi-rhombic, crenulate, obtuse, membranous wings, the clinandrium apically tridentate, flanked by a small tooth laterally; column foot forward-projecting, fleshy, incurved, ca. 2.0 mm long. Anther cap cucullate, ovate, strongly keeled above, the keel protruding into a small mucro, 2-celled. Pollinia 2, ligulate-obovate, semi-convex, the sub hyaline base flattened, minutely uncinate. Fruit not seen.

Other material examined:—COSTA RICA. Puntarenas: Monteverde: San Gerardo de Santa Elena, vertiente Altántica de la Cordillera de Tilarán, ca. 1000 m, 23 Enero 1999, floreció en cultivo en el JBL el 11 Abril 2000, *Blanco 959 & Arias* (USJ!); Same locality and collection date, *Blanco 961* (JBL-Spirit!, CR!; Fig. 8, 12). Alajuela: Upala, Aguas Claras de Buenos Aires, Hotel Termales Azules, laderas del Volcán Rincón de La Vieja, 10°49'09" N - 85°16'04" W. 700–1000 m, 05 de Abril del 2004, *Karremans 218* (JBL-Spirit!). Without specific locality, *Endrés s.n.* (W!).

Distribution:—Endemic to Costa Rica, where it has been recorded from the Pacific watershed of the northern volcanic and Tilarán mountain chains, at 700–1100 m elevation (Fig. 9).

Notes:—*Pleurothallis endotrachys* was described by Reichenbach (1876) as a member of his *Pleurothallis* series *Sicariae* on the basis of a plant collected by Endrés (no. 92) in Costa Rica at San Ramón, along the Barranca River on the Pacific side of the Tilarán range. The sheet at W with the handwritten description by Reichenbach (W 0020331), which Luer annotated as lectotype (correction label, 1992), is sterile, as well as W 0020150, whereas flowers are present on W 0021581 (with a detailed description by Endrés), selected here as the lectotype, and W 1889-0033651 (all part of the same collection under *Endrés 92*). Another sheet with two fertile specimens from the same collection is conserved at AMES (118500). Two sheets at W include drawings prepared by Endrés of his collection number 92 (W 0020151, 0020152). On one of the drawings (W 0020152) Endrés wrote: "Inner surface of sepals slightly viscous? / much visited by a small fly!"; and in the description he stated: "Sepals scabrous in inner surface (exuding honey)" (W 0021581). This explains the intended name of "Pleurothallis mellifera", proposed by Endrés in the description he sent to Reichenbach. Endrés' note, translated in Latin by Reichenbach in 1878 ("*Flos intus viscidus a parva mosca quadam diligentissime visitatus*"), is probably one of the earliest (if not the first) published observations about pollination in Pleurothallidinae.

Chase (1985) reported on the pollination of Costa Rican populations of *S. spectabilis* (as *Pleurothallis endotrachys*) cultivated in Michigan (USA), observing that early in the day the flowers emit a faint rotten-fruit odor and that only fragrant flowers were of interest to the flies. The species of *Drosophila* observed, *D. immigrans*, has also been noted from Costa Rica and hence could be the natural pollinator (Chase 1985).

In the research greenhouses of Lankester Botanical Garden, species of *S. endotrachys* group are frequently visited by small drosophiloid flies (Fig. 10). The visits extend over the whole day, but they are apparently more frequent in the hottest hours of the afternoon. Flies spend a long time on the flowers, exploring both the abaxial and adaxial sides of the sepals with their mouthparts, but mostly they walk around the inner base of the sepals and the lip. It is not uncommon to observe the flies resting immobile on the sepals for up to one hour. When the fly goes up along the strongly convex lip, its weight causes a shift in balance, provoking the blade to shift and to trap the fly against the column (Fig. 11). The column

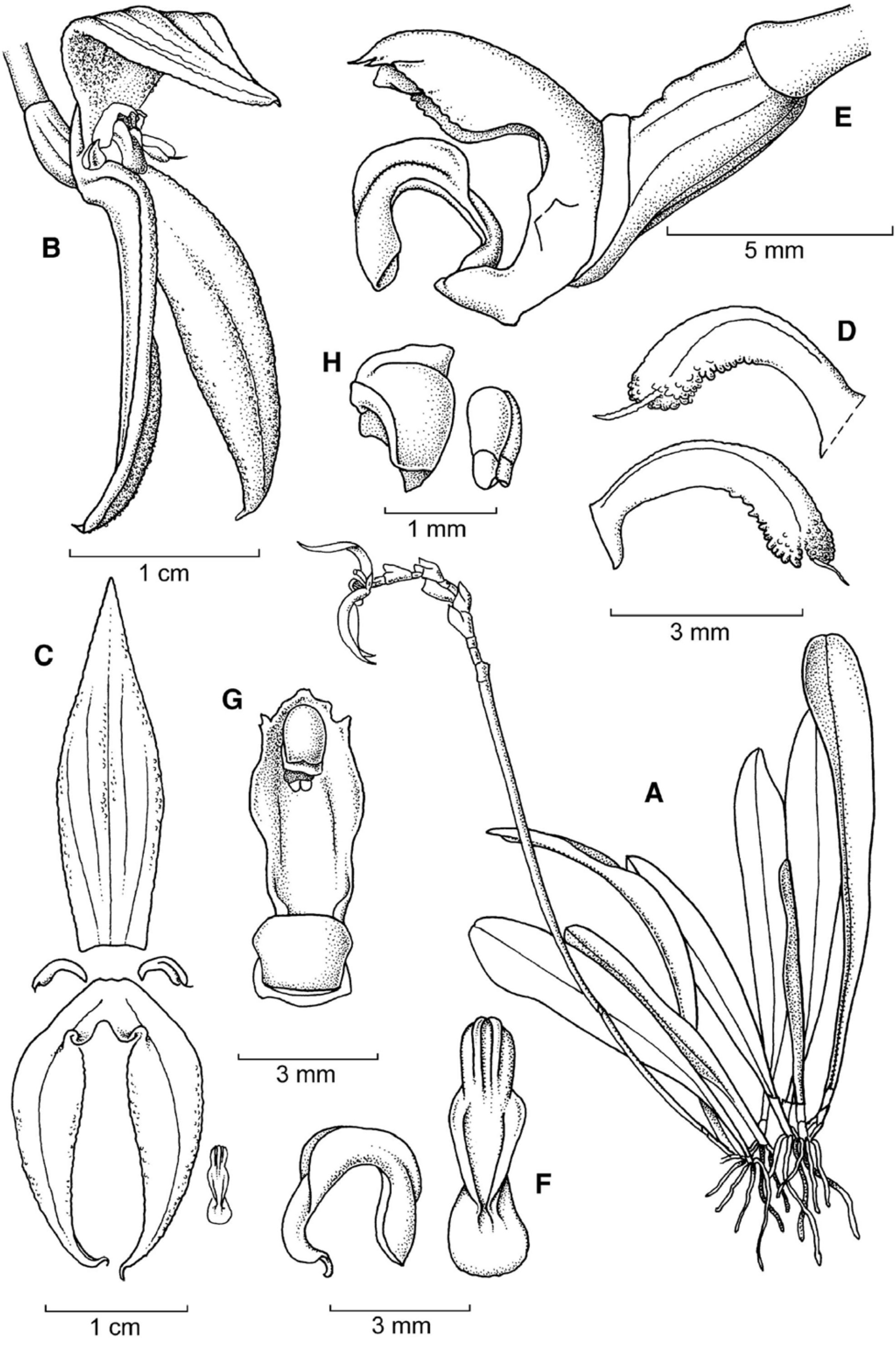


Figure 8. *Specklinia endotrachys* (Rchb.f.) Pridgeon & Chase. A. Habit. B. Flower. C. Dissected perianth. D. Petals. E. Column and lip, lateral view. F. Lip, front and side views. G. Column, ventral view. H. Anther and pollinia. Drawn by F. Pupulin & E. Winkel from *Blanco 861* (JBL-spirit).

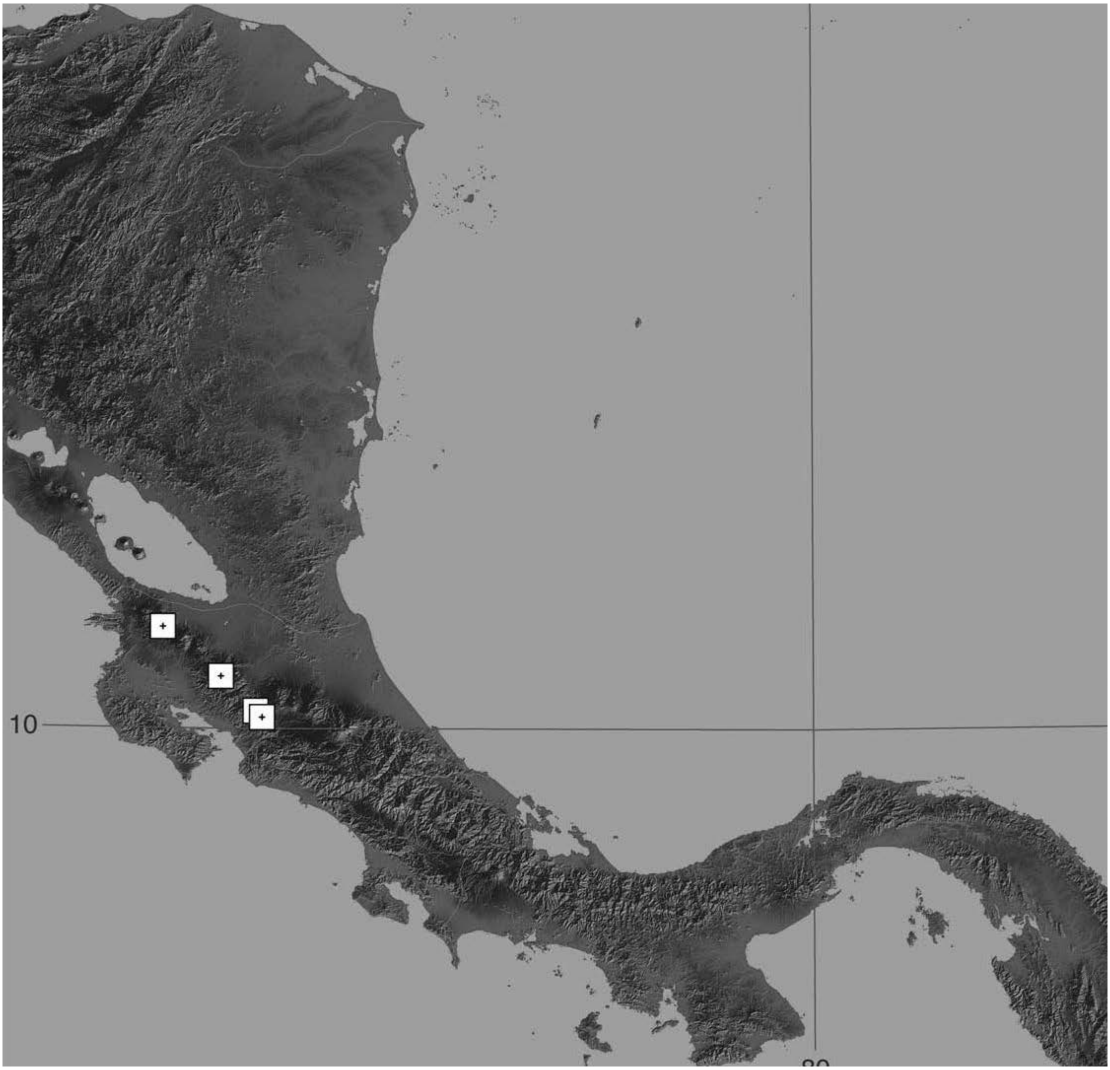


Figure 9. Distribution map of *Specklinia endotrachys.*

wings and porrect petals have a clear function in maintaining the body of the fly in the correct position to receive the pollinia on the underside of the scutellum, where they firmly adhere with their hooked apex (Fig. 10). We cannot confirm presence of any "meal" or nectar on the surface of the fresh flowers we examined, as suggested by Endrés. Also Chase (1985) observed no nectar on the flowers, nor did he observe the flies removing anything from the roughened areas of the sepals. Further ultrastructural and chemical studies aimed to understand the powerful attraction of these flowers on drosophilid flies are under way.

In the protologue of *Pleurothallis endotrachys*, Reichenbach (1876) mentioned the uncinate floral bracts, longer than the subtended pedicels ("*bracteis* [...] *introrsum curcatis, ovariis pedicellatis multo longioribus*"), the retuse apex of the petals ("*tepalis linearibus retusiusculis*"), the triangular wings of the column and the denticulate clinandrium that are diagnostic of the species.

Among the species of this group, *S. endotrachys* is easily distinguished by the combination of caespitose habit, long floral bracts hiding the pedicels, orange flowers, twisted lateral sepals and distinctly retuse petals provided with a long mucron abruptly inserted within the sinus.

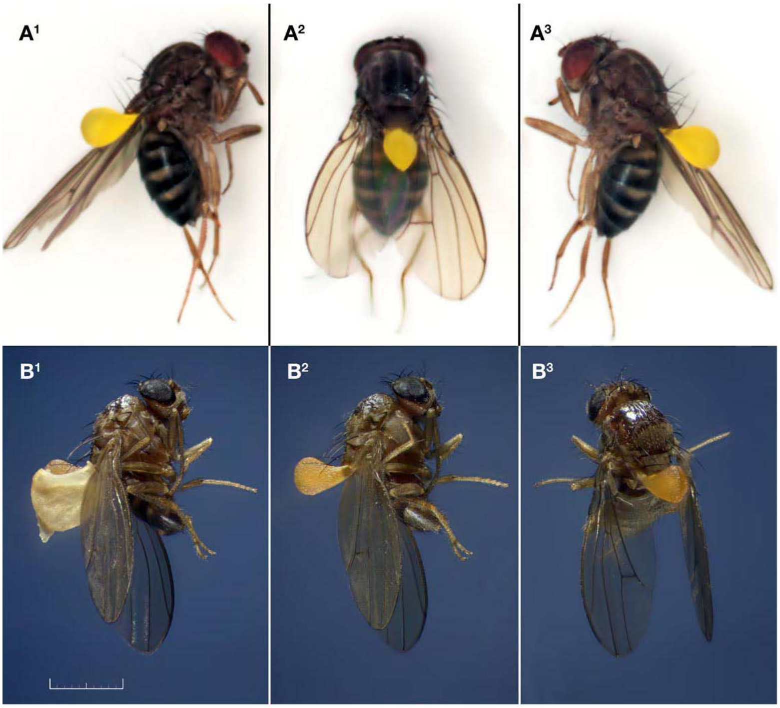


Figure 10. Pollinators of *Specklinia* species. A1–A3. Drosophilideae species with pollinarium of *S. remotiflora* (*Bogarín 8181*). B1–B3. Drosophilideae species with pollinarium of *S. spectabilis* (*JBL-02643*). In B1 the pollinarium is still enclosed within the anther cap. Scale bar=1 mm. Photographs by Franco Pupulin.

Figure 11. Pollinator of *Specklinia spectabilis* (*JBL-02643*), trapped against the column of the flower. Sepals are removed. Scale bar = 5 mm. Photograph by Franco Pupulin.

2. *Specklinia pfavii* (Rchb.f.) Pupulin & Karremans, Phytotaxa 63: 8. 2012.
Basionym: *Pleurothallis pfavii* Rchb.f. Type: [COSTA RICA or PANAMA]. Chiriquí, *Pfau. s.n.* (holotype, W).
Heterotypic synonyms: *Masdevallia platyrachis* Rolfe. *Pleurothallis platyrachis* (Rolfe) Rolfe, *comb. inval*.; (Rolfe) Rolfe ex Hook. *Kraenzlinella platyrachis* (Rolfe) Rolfe. TYPE: COSTA RICA: "The plant was imported from Costa Rica by Mr. Shuttleworth, and sent to Kew in 1884, in which year a scape was produced. It has now become fully established and is bearing several scapes, the first flower having recently expanded", *E. Shuttleworth s.n.* (holotype, K).

Epiphytic, caespitose, erect herb to 17 cm tall. Roots fibrous, flexuous, glabrous, to 1.5 mm in diameter. Stem abbreviated, terete, to 1.5 cm long, monophyllous, completely concealed by 2 glumaceous, slightly loose, ancipitous, acute sheaths to 1.8 cm long, becoming brown-papyraceous with age. Leaf elliptic, 10.7–14.2×2.4–3.0 cm, minutely retuse, the midvein protruding abaxially into a small apicule, gradually tapering toward the base into a conduplicate petiole, the margins ancipitous, subcoriaceous. Inflorescence born laterally from the apex of the stem, without an annulus, an erect-subarching, congested, successively- flowered, distichous raceme to 35 cm long; the rachis strongly fractiflex, producing up to 30+ flowers; peduncle flattened, ancipitous, to 28 cm long, with 3–4 distant, tubular-amplectent, ancipitous, apically subuncinate, acute bracts, 6–7 mm long. Floral bracts infundibuliform, subuncinate laterally, ovate, abaxially ancipitous, subacuminate, 7×6 mm. Pedicel cylindric, glabrous, to 12 mm long, persistent. Ovary subclavate, subtrigonous, to 5 mm long, green. Flowers with bright yellow sepals and lip, lip with longitudinal orange stripes, petals dark red, column yellow. Sepals fleshy, densely papillose on the inner surface with the exception of the hyaline basal third, margins revolute, strongly keeled abaxially along veins; dorsal sepal lanceolate, triveined, acute, the base hyaline, flushed with yellow along veins, the distal two-thirds densely papillose, 24×8 mm; lateral sepals narrowly elliptic-subfalcate, 3-veined, 23.0×3.5 mm, connate at the base for about 3 mm into a deeply concave mentum, membranaceous-hyaline at the base, then densely papillose, margins slightly revolute. Petals small, ligulate-subfalcate, rounded, porrect, univeined, 12×3 mm, with a rounded keel abaxially along the vein, the apex thickened, minutely papillose inside, the labellar margin provided with low papillae in the basal half. Lip lanceolate, articulate with the apex of column foot by a hyaline claw, strongly arched-convex in natural position, triveined, subtrullate when expanded, obtuse to subacute, with a small, rounded apicule, the distal half provided with 2 thin, high keels converging toward the apex but not reaching it, the clawed base thickened, subquadrate; entire lip 18×8 mm. Column arched, semiterete, provided with a foot, 6.5 mm long without the foot, with 2 broad, thin, membranaceous, rounded wings in the middle portion, the apex rounded, deeply cucullate, the clinandrium shallow, entire; column foot forward-projecting, stout, fleshy, 4 mm long. Anther cap ovate, deeply cucullate, strongly keeled in the middle, 2-celled. Pollinia 2, obovate-complanate, hooked at attenuate base. Fruit not seen.

Other material examined:—COSTA RICA. Pérez Zeledón: without any additional collection data, cultivated by Wubben in The Netherlands, flowered in cultivation at the Hortus Botanicus in Leiden, 1 Dec 2011, *Karremans 4825* (L-Spirit!; Fig. 13). Without collecting data, flowered in cultivation at Lankester Botanical Garden, *JBL-11086* (JBL-Spirit!, CR!; Fig. 12).

Distribution:—Endemic to the lowlands of southern Costa Rica and western Panama, on the Pacific watershed of the Talamanca-Chiriquí range, at around 500 m elevation (Fig. 14).

Reichenbach (1886) described *Pleurothallis pfavii* on the basis of a living plant he received from Pfau, who collected it in "Chiriquí". He precisely noted the colors of the perianth: "*Flores intense sulphurei pollicem longi. Tepalo pulcherrime cinnamomeo brunnea. Labellum fiavum linea mediana rufa. Columna viridula*". The exact locality of the original collection is unknown, and the region of Chiriquí (actually in Panama) was at the time shared by Panama and Costa Rica. The Swiss Rudolf Richard Pfau (?—1897) collected mostly in Costa Rica, where he eventually

Figure 12. Comparison of flowers of species of the *Specklinia endotrachys* group. A. *Specklinia endotrachys* (*Blanco 961*). B. *Specklinia pfavii* (*JBL-11086*). C. *Specklinia remotiflora* (*Bogarín 8181*). D. *Specklinia spectabilis* (*JBL-02641*). All flowers shown in front, three-quarters side, and side views. Scale bar = 1 cm. Photographs by Franco Pupulin.

owned a nursery in San José. According to the protologue, *Pleurothallis pfavii* has falcate, obtuse petals provided with a thickened external margin and acute (“*quasi sagittato*”) lip (Reichenbach 1886), a set of features that makes it unmistakable.

Originally described in the Gardeners’ Chronicle in August 1888, *Masdevallia platyrachis* was illustrated shortly after in the Botanical Magazine (Hooker 1890: sub pl. 7129) under the name of *Pleurothallis platyrachis*. The original description of *Masdevallia platyrachis* was based a plant cultivated at Kew and received from Shuttleworth of Charlesworth & Co., where it was supposedly introduced from Costa Rica. Edward Shuttleworth (1829-1909) collected orchids in Colombia, but he never went to Costa Rica, and no records remain of the collectors employed by the commercial nursery of Charlesworth in Central America during the last decades of nineteenth century. This leaves the exact type locality of *M. platyrachis* unresolved. Rolfe (1890) transferred it to *Pleurothallis* in his reconsideration of *Scaphosepalum*, but as he did not expressly associate the epithet with the genus *Pleurothallis*, the combination is invalid according to art 33.1 of the ICBN. A valid combination was published that same year, when *Pleurothallis platyrachis* was first illustrated (Hooker 1890). The precise illustration by Fitch, showing the plant habit and details of the petals, lip, and column, leaves no doubts about the identity of *Pleurothallis platyrachis* as conspecific with *P. pfavii*.

The combination of bright yellow flowers with red petals, the yellow with a central red line, acute lip, and the rounded, not apiculate petals, distinguish *S. pfavii* from other members of the *S. endotrachys* complex.

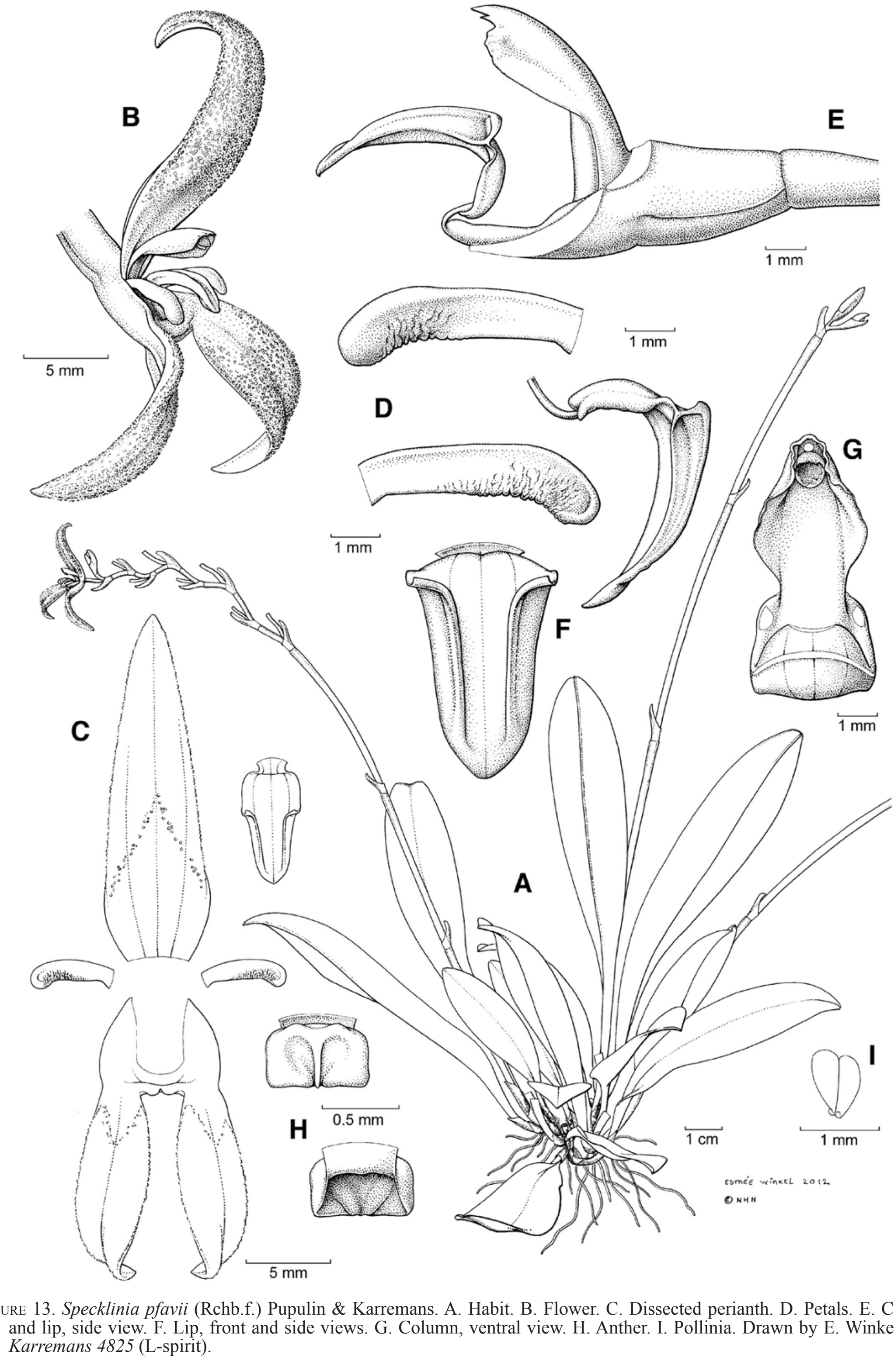


Figure 13. *Specklinia pfavii* (Rchb.f.) Pupulin & Karremans. A. Habit. B. Flower. C. Dissected perianth. D. Petals. E. Column and lip, side view. F. Lip, front and side views. G. Column, ventral view. H. Anther. I. Pollinia. Drawn by E. Winkel from *Karremans 4825* (L-spirit).

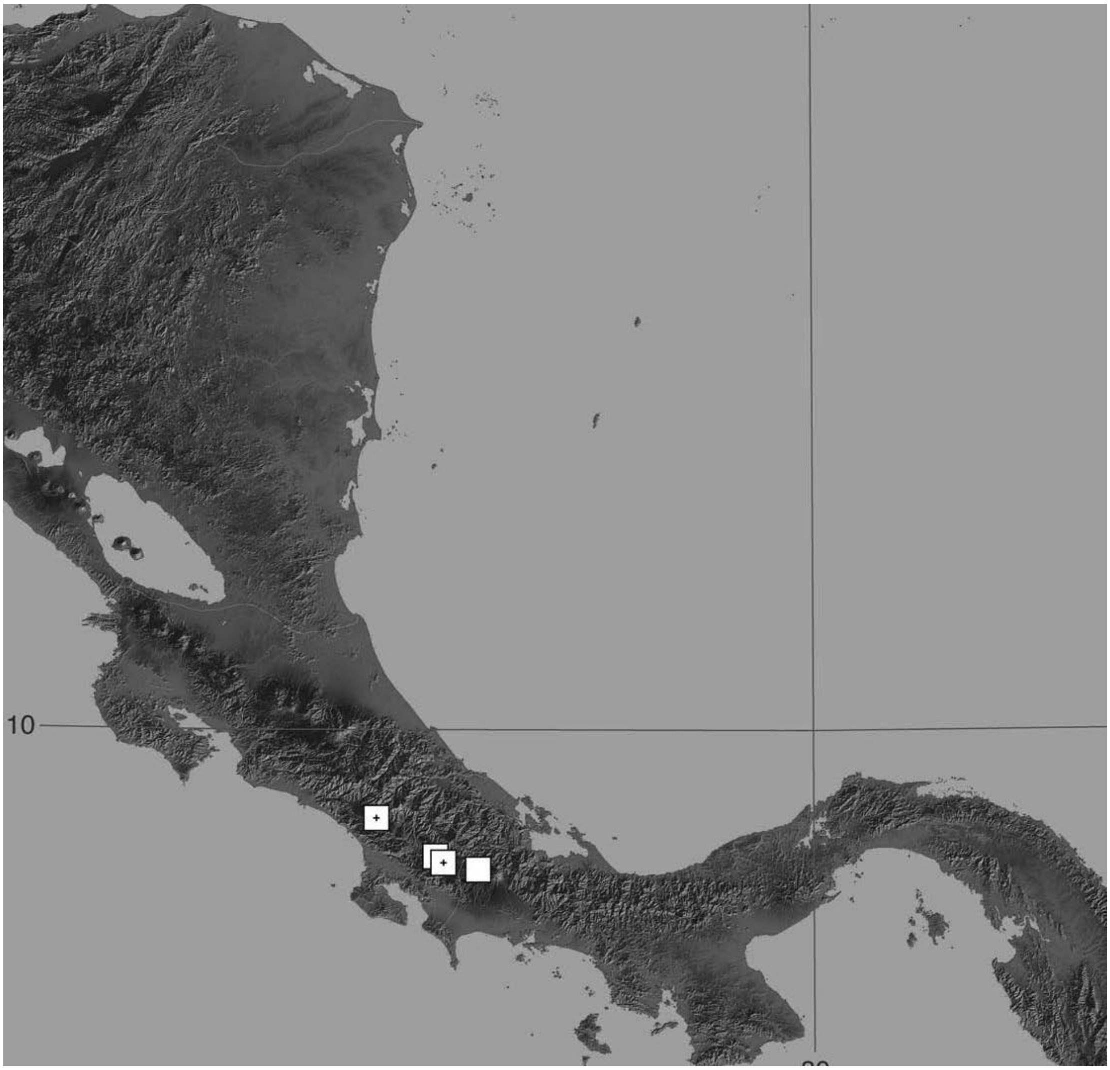


Figure 14. Distribution map of *Specklinia pfavii*.

3. ***Specklinia remotiflora*** Pupulin & Karremans, Phytotaxa 63: 11. 2012.

> *A Pleurothallide endotrachyde* Rchb.f. *similis, habitu repente, inflorescentia laxa, floribus subcampanulatis sepalibus convergentibus petalibusque truncatis nec apiculatis recedit*.

Type:—COSTA RICA. Coto Brus: Sabalito, Zona Protectora Las Tablas, 15 km al noreste de Lucha, Sitio Coto Brus, Finca de Miguel Sandí, bosque muy húmedo montano, ad ager Sandiorum “El Surá”, crescenti epiphytica in sylvis humidis versus pascues prope flumen Surá, 7 October 2010, *Bogarín 8181, Dressler, Fernández & Pupulin* (holotype, USJ; isotype, JBL-Spirit!). Fig. 12, 15.

Epiphytic, subrepent-ascending, erect herb to 30 cm tall. Roots fibrous, flexuous, glabrous, to 1.5 mm in diameter. Stem abbreviated, terete-cylindric, to 2.2 cm long, monophyllous, completely concealed by a papyraceous, subancipitous, acute sheath to 3.5 cm long, eventually disintegrating into fibrous remains. Leaf narrowly obovate-oblanceolate, 17.0–29.0×3.2–3.9 cm, minutely and irregularly emarginate at apex, the midvein protruding abaxially into a small apicule, gradually tapering toward the base into a deeply conduplicate petiole with strongly ancipitous margins, subcoriaceous. Inflorescence born laterally from the apex of the stem, without an annulus, a lax, distichous, successively 4–7-flowered raceme, up to 40 cm long; peduncle flattened, ancipitous, to 30 cm long, with 4–5 distant, amplectent, ancipitous, subacute bracts, 7–8 mm long. Floral bracts infundibuliform, broadly ovate, abaxially ancipitous, acute to subacuminate, 10×8 mm. Pedicel cylindric, glabrous, 13–15 mm long, persistent. Ovary subclavate, with low, irregularly crenulate crests, 4–5 mm long, green. Flowers with dark orange-red sepals and petals, lip red, column yellow. Sepals fleshy, densely papillose on the inner surface except at base, margins thickened-revolute; dorsal sepal lanceolate-elliptic, triveined, acute, the base semi-hyaline, flushed with orange along veins, the distal two- thirds densely papillose, the revolute margins glabrous, 20×7 mm; lateral sepals narrowly elliptic-oblanceolate, subfalcate, triveined, 19–20×5 mm, connate for about one-quarter to one-third of their length, the base saccate, membranaceous-hyaline, the apex acute, gently deflexed at the middle, the midvein strongly carinate abaxially. Petals small, ligulate-falcate, truncate, porrect, 6.0–7.0×1.0–1.5 mm, univeined, papillose-thickened toward the concave apex, the labellar margin provided with coarse papillae arranged in two rows, the outer margin thickened. Lip small, longitudinally arched-convex in natural position, thinly articulate with the column foot by a hyaline claw, narrowly elliptic-lanceolate when expanded, obtuse, the apex reflexed, appearing minutely retuse, the clawed base thickened, transversely minutely gibberose, 8.0–9.0×2.0–2.5 mm, provided with 2 slender keels, fringed-lacerate at the base, gently converging from the base of the lamina to near the apex, then spreading. Column arched, terete-slender at the base, 6.0–6.5 mm long without the foot, provided with broad membranous wings serrulate along the margins, at the apex forming a deeply cucullate, sharply lacerate clinandrium; column foot forward-projecting, stout, fleshy, incurved, 2.0–2.5 mm long. Anther cap deeply cucullate, ovate, 2-celled. Pollinia 2, obovate-complanate, minutely hooked at the base. Fruit not seen.

Other material examined:—COSTA RICA. Puntarenas: Coto Brus, Sabalito, Las Mellizas, siguiendo la línea divisoria entre Costa Rica y Panamá, entre Cerro Nubes y Cerro Pando, hito geográfico 340, camino de la Sierra, 08°55’18” N, 82°43’30” W, 2465 m, 15 Aug. 1989, *Herrera 3411* (USJ!; INB!); Buenos Aires: P.N. La Amistad, Cuenca Térraba-Sierpe, sendero a Valle del Silencio, colectado a orilla de bosque, 9°04’51.0781” N -82°58’47.5188” W 2300 m, 18 Apr. 2001, *Alfaro 3646* (INB!); Coto Brus: Sabalito, Las Mellizas, siguiendo linea divisoria entre Costa Rica y Panama, entre Cerro Nubes y Cerro Pando, hito geografico 340, camino de la Sierra, 8°55’ N, -82°43’ W, 2465 m, 21 Sept. 1996, *Navarro 476* (INB!); Coto Brus: Sabalito, Zona Protectora Las Tablas, 13 km al noreste de Lucha, Sitio Coto Brus, entre Río Surá y Quebrada Sutú, Finca de Miguel Sandí, 8°56’46.1” N 82°44’30.9” W, 1778 m, bosque pluvial montano bajo, epífitas en potreros arbolados, 6 Jun. 2010, *Bogarín 7773 & Karremans* (JBL-Spirit!). Same date and locality, *Karremans 2856 & Bogarin* (JBL-Spirit!); Coto Brus: Sabalito, Zona Protectora Las Tablas, 15 km al noreste de Lucha, Sitio Coto Brus, Finca de Miguel Sandí, bosque muy húmedo montano, ad ager Sandiorum “El Surá”, *crescenti epiphytica in sylvis humidis versus pascues prope flumen Surá*, 7 Oct. 2010, *Bogarín 8180, Dressler, Fernández & Pupulin* (JBL-Spirit!); same locality and date *Bogarín 8183, Dressler, Fernández & Pupulin* (JBL-Spirit!); Coto Brus: Sabalito, Zona Protectora Las Tablas, 15 km al noreste de Lucha, Sitio Coto Brus, 8°56’ N 82°44’ W, 2000 m, finca de Miguel Sandí, en bordes de bosque subiendo por el margen del río Surá en el cerro al noroeste de la finca, bosque muy húmedo montano, 7 Oct. 2010, *Fernández 402, Dressler, Bogarín & Pupulin* (JBL-Spirit!). Limón: Talamanca, Bratsi, P.N. La Amistad, Atlantic slope, south side of unnamed cordillera between the Rio Terbi and Rio Sini, 2-4 airline km W of the Costa Rican-Panamanian border, 09°11’ N, -82°58’ W, 2300–2500m, 11/ Sept. 1984, *Davidse 28921, Herrera & Grayum* (INB!); Talamanca: P.N. La Amistad, Tararia, Valle del Silencio, Sendero el Alto, colectado en bosque. 9°06’02.6103” N -82°58’03” W, 1714 W 2440 m, 20/jun/2003, *Alfaro 4597, Alfaro & Alfaro* (INB!); Talamanca: Bratsi, P.N. La Amistad, Valle del Silencio, sector de acampar a los jardines, 9°07’ N, -82°57’ W, 2500 m, bosque primario, 14 Apr.1996, *Quesada 1481* (INB!); Talamanca: Bratsi, P.N. La Amistad, Sendero Valle del Silencio al Jardin Natural, 9°07’ N, -82°57’ W, 2400 m, 01 Jun. 1996, *Quesada 1574* (INB!). COSTA RICA—PANAMA. Puntarenas-Chiriquí: Coto Brus-Renacimiento, línea fronteriza hacia el Cerro Pando, después del mojón, N.338, 8°55’11.22” N 82°43’18.18”

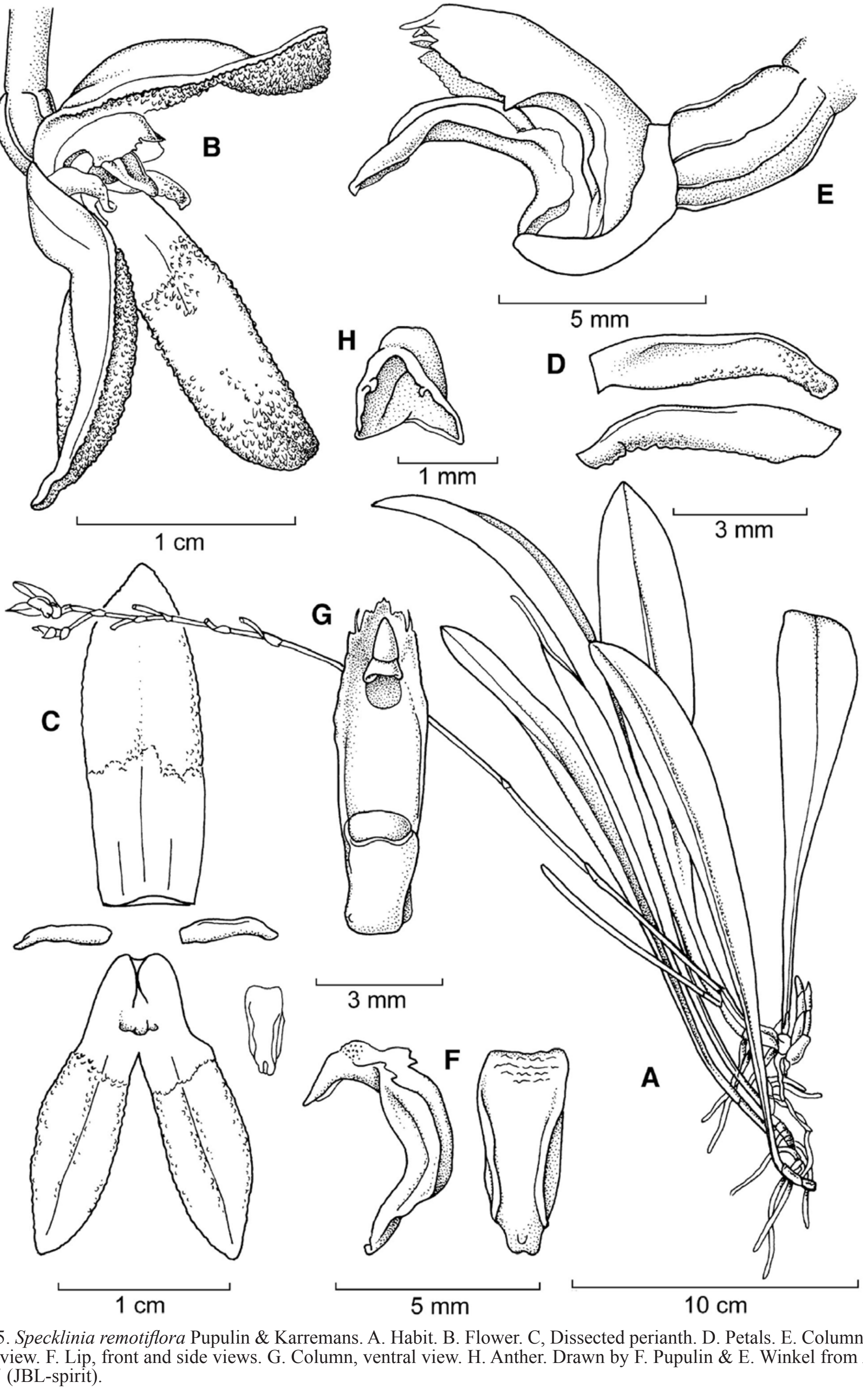


Figure 15. *Specklinia remotiflora* Pupulin & Karremans. A. Habit. B. Flower. C, Dissected perianth. D. Petals. E. Column and lip, side view. F. Lip, front and side views. G. Column, ventral view. H. Anther. Drawn by F. Pupulin & E. Winkel from *Bogarín 8181* (JBL-spirit).

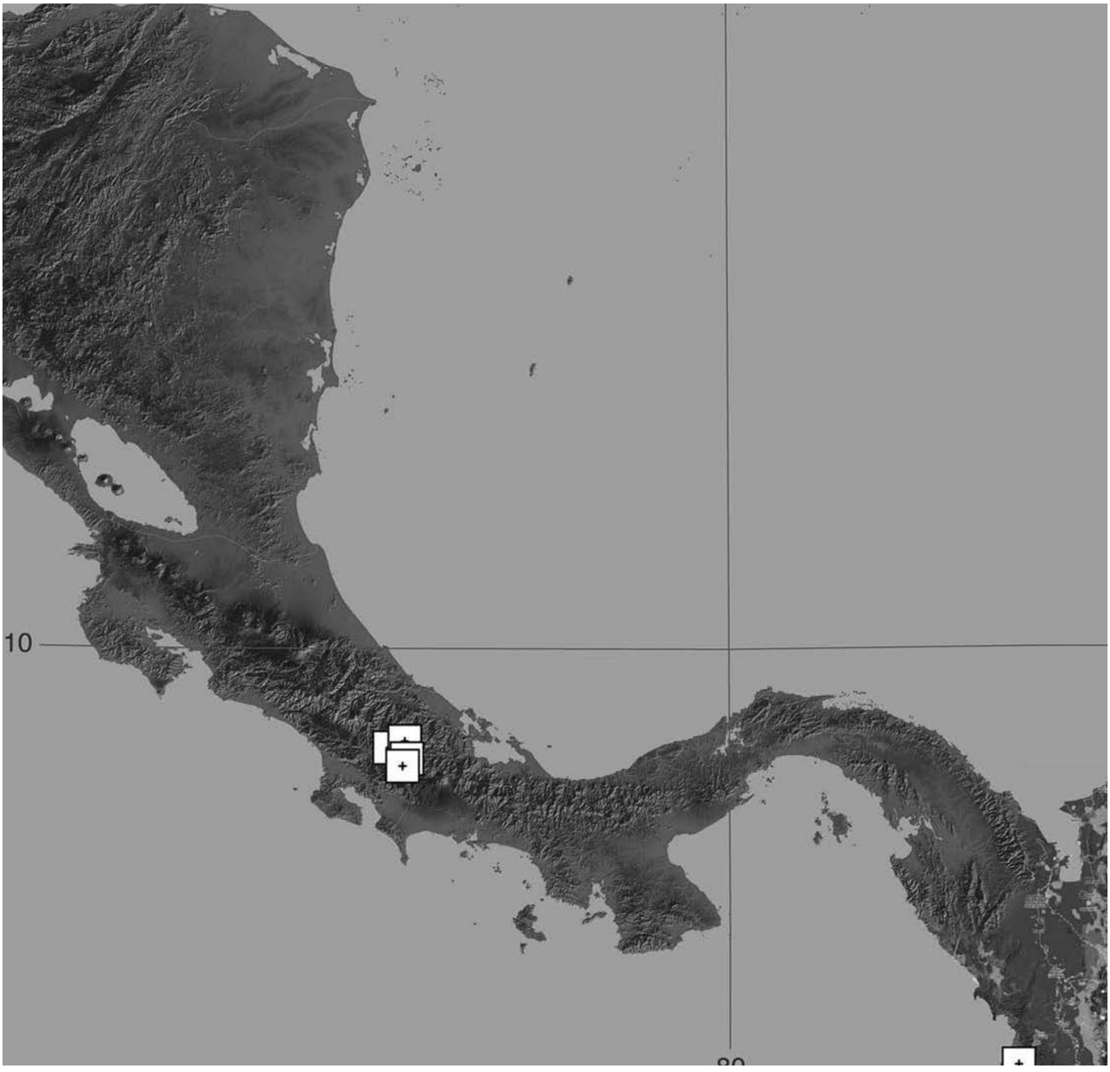


Figure 16. Distribution map of *Specklinia remotiflora.*

W, 2446 m, bosque muy húmedo montano bajo, epífitas en bosque primario, *in sylvis virginis versus montium Pando in itinere ad summum Costa Rica austro- orientalis in finibus utrimque Costa Rica et Panama*, 19 Apr. 2011, *Karremans 4023, Bogarín & Jiménez* (JBL-Spirit!); same date and locality, *Karremans 4024, Bogarín & Jiménez* (JBL-Spirit!); Same date and locality, *Bogarín 8656, Karremans & Jiménez* (JBL-Spirit!, CR!). PANAMA. Chiriquí: NW of Cerro Punta, at INRENARE station in Parque Amistad; trail below station; forested slopes; collected with 08°54'N 082°35'W, 2100 m, 20 Oct. 1992, *McPherson & Richardson 15941* (MO!). COLOMBIA. Chocó: south of Cabo Marzo, Bahía del Aguacate, sea level, *Misas Urreta 291* (HPUJ). Unknown country of origin, cultivated by Wubben in The Netherlands, flowered in cultivation at the Hortus Botanicus in Leiden 1 Dec. 2011, *Karremans 4798* (L-spirit!); same data, *Karremans 4846* (L-spirit!); cultivated by Sijm in The Netherlands, flowered in cultivation, 9 Jan. 2012, *Karremans 4854* (L-spirit!).

Distribution:—Relatively frequent in southern Costa Rica and western Panama, where it grows on the Talamanca-Chiriqui range, at 1750–2500 m. Also reported from the Colombian northern Pacific coast at sea level, perhaps ranging to Ecuador (Fig. 16).

Notes:—*Specklinia remotiflora* is apparently common along the Pacific watershed of southern Talamanca Mountains in Costa Rica and adjacent Panama. Even though we are not aware of any record in central and western Panama, the distribution of the species is likely continuous southward at least to the Pacific coastal range of Baudó in northern Colombia, where populations morphologically similar to *S. remotiflora* were documented by Misas Urreta (2006). It is noteworthy, however, that Colombian plants were found growing at sea level, whereas in Costa Rica and Panama *S. remotiflora* is exclusively known from submontane and montane wet forests at 1750-2500 m. Plants in cultivation at Ecuagenera (Pupulin, pers. obs. 2009), supposedly collected in Ecuador but without specific locality data, also correspond to this species.

The repent habit, lax inflorescence, and subcampanulate, orange flowers provided with obtuse, non- apiculate petals easily distinguish *S. remotiflora* from *S. endotrachys* and *S. spectabilis*, to which it is most similar. It has non-mucronate petals like *S. pfavii*, but the latter has a caespitose habit (vs. repent in *S. remotiflora*), congested inflorescence (vs. lax), yellow flowers with red petals (vs. orange), and oblong, entire column wings (vs. triangular, denticulate).

4. *Specklinia spectabilis* (Ames & C.Schweinf.) Pupulin & Karremans, Phytotaxa 63: 15. 2012.
Basionym: *Pleurothallis spectabilis* Ames & C.Schweinf.. Type:—PANAMA. Veraguas: Santa Fé, Feb. 1924, 1500 ft, *Powell 382* (holotype, AMES!; isotype, MO; photo of type, AMES!).

Epiphytic, caespitose, erect herb to 18 cm tall. Roots fibrous, flexuous, glabrous, to 1 mm in diameter. Stem abbreviated, terete-subcomplanate, slender, monophyllous, 1.2–1.5 cm long, covered by a glumaceous, adpressed, obtuse sheath, becoming dry-papyraceous with age and eventually dissolving. Leaf narrowly oblanceolate, minutely retuse, subcoriaceous, 11.5–16.5×1.2–1.7 cm, the adaxial midvein protruding at apex into a small apicule, gradually tapering toward the base into a strongly conduplicate-channeled, ancipitous petiole to 3.5 cm long. Inflorescence produced laterally from the apex of the stem, with an annulus, born from a small, papyraceous, spathaceous bract to 4 mm long, erect to arching, distichous, congested, successively many-flowered (to 23+) raceme, to 36 cm long; peduncle flattened, ancipitous, to 28 cm long, provided with 3 distant, tubular-amplectent, ancipitous, acute bracts to 9 mm long; rachis complanate, gently fractiflex. Floral bracts broadly ovate, acute, strongly flattened, abaxially ancipitous, apically shortly recurved-subuncinate in lateral view, 10×6 mm. Pedicel cylindric, glabrous, to 11 mm long, persistent. Ovary cylindric-subclavate, 4 mm long, green. Flowers orange, sepals semi-hyaline at the base, tinged orange along the veins, column yellow. Sepals densely papillose adaxially except at the base, where they become semi-hyaline; dorsal sepal erect, triangular-lanceolate, acute, 5-veined, slightly concave at the base, inner surface densely papillose on the distal two-thirds, papillae extending almost to the base along the thickened margins, base semi-hyaline, the veins flushed with orange, 18.4–20.2×4.8–5.2 mm; lateral sepals semigeniculate, born subparallele and then twisted outwards, lanceolate-subfalcate, triveined, subacuminate, ending into a short mucro, margins slightly revolute, 18.0–18.4×3.6–4.1 mm, connate at the base for ca. 5 mm to form a deeply saccate mentum around the column foot, base hyaline, ribbed abaxially along veins, then densely papillose, the midvein strongly carinate externally. Petals ligulate-falcate, acute, porrect, papillose at the concave apex, abruptly contracting into a mucro, univeined, 4.1–4.5×0.8–1.0 mm. Lip longitudinally arched-convex in natural position, thinly articulate with the column foot by a hyaline claw, rectangular-subpandurate when expanded, truncate, with a small apicule, 4.9–5.2×1.5–1.6 mm, provided with a pair of thin, erect keels arising from the middle margin and gently converging close to the apex, central portion with a shallow groove between the keels. Column semiterete, arched, 4.5 mm long without the foot, central portion expanded into broad, membranaceous, semihyaline wings, upper margin denticulate, clinandrium deeply lacerate-dentate; column foot stout, forward-projecting, incurved, slightly grooved at the base, ca. 2.0 mm long. Anther cap ovate- subquadrate, deeply cucullate, 2-celled. Pollinia 2, obovate-complanate, the subhyaline base contracted into a small hook. Immature fruit a green capsule, narrowly obovate, glabrous, with six crests, three taller, thus appearing triangular, 18 mm long, 10 mm wide at its widest point, just below the apex.

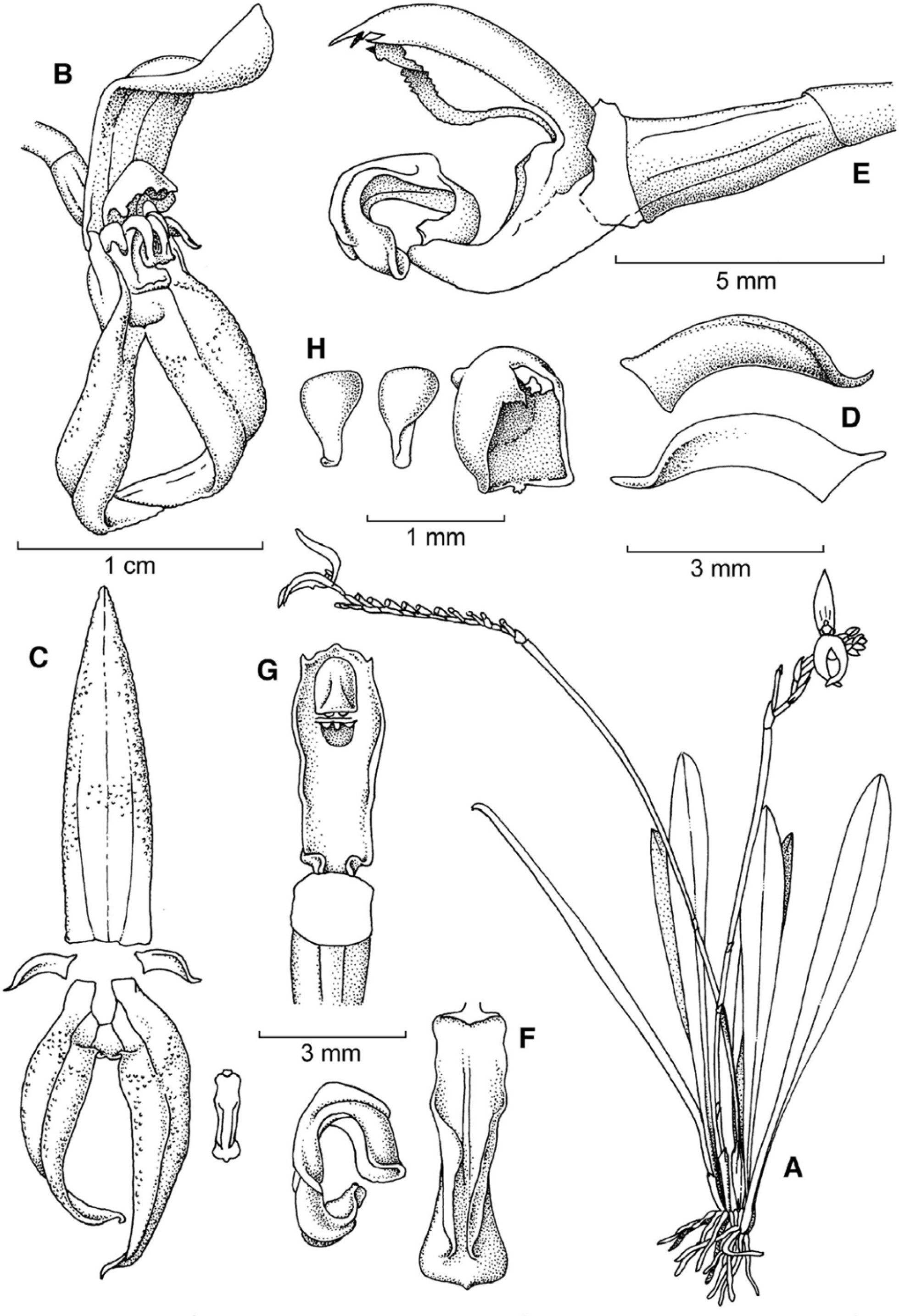


Figure 17. *Specklinia spectabilis* (Ames) Pupulin & Karremans. A. Habit. B. Flower. C. Dissected perianth. D. Petals. E. Column and lip, side view. F. Lip, front and side views. G. Column, ventral view. H. pollinaria and anther. Drawn by F. Pupulin & E. Winkel from *JBL-02641* (JBL-spirit).

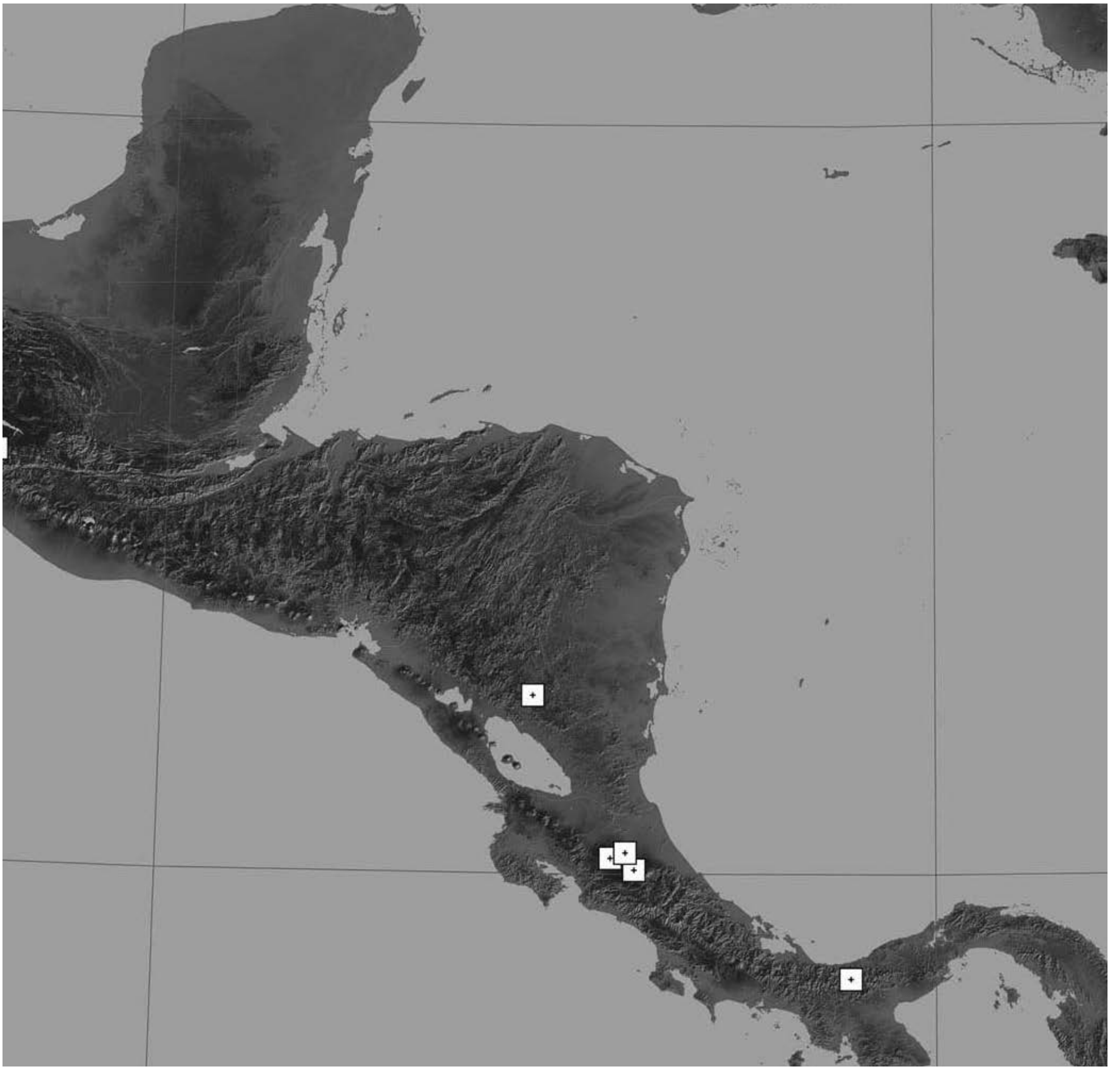


Figure 18. Distribution map of *Specklinia spectabilis*.

Other material examined:—MEXICO. Chiapas: *Soto 9484* (AMO). NICARAGUA. Chontales: Cerro Oluma, lower to middle E slopes; moist forest in quebradas, 12°18’06”N 085°23’22”W, 500–700 m, 30 Jan. 2008, *Stevens, Coronado, Montiel, Duarte 26820* (HULE; MO; photograph of flower, MO!). COSTA RICA. San José: trail up to water source for guard station in Parque Nacional Braulio Carrillo; humid forest, 700–750 m, 83°57’ N - 10°06’ W. 16/II/1984, *Chase 84218* (CR-98483!; CR-98381!; K-spirit); Bajo de la Hondura: Parque Nacional Braulio Carrillo, floreció en el JBL 16-XII-1993, *Mora s.n.* (USJ!); Vasquez de Coronado: Parque Nacional Braulio Carrillo, Sendero la Botella, 10°10’00” N 83°57’20” W, 750 m, 21 Sept. 1990, *Ingram 559 & Ferrell* (USJ!; INB!); Limón: Pococí, Guapiles, Reserva Teleférico del bosque lluvioso, parque atlántico, 10°10’24.5” N–83°54’48.3” W, 546 m, 14 Oct. 2008, *Quesada 2729, Serrano & Volio* (CR!); Heredia: Estación Carillo de 700 a 450 m. de la Fila Cañón del R. Sucio, bosque muy húmedo tropical-transición a premontano, 12/11/1983, *Chacón 1716 & Herrera* (CR-108241!); Parque Nacional Braulio Carrillo, Bajo de La Hondura, recolector desconocido, sin fecha, floreció en el JBL el 26 Oct. 2000, *Blanco 1653* (USJ!); Parque Nacional Braulio Carillo, Río Sucio, 1350 m, 10 Oct. 2001, *Bosch s.n.* (USJ!); Pococí: Parismina, recolectada por Gerson Villalobos, floreció en cultivo en el Jardín Botánico Lankester, 30 Octubre 2009, *Bogarín 7401* (JBL-Spirit!); same locality and data, *Bogarín 7403* (JBL-spirit!); Alajuela: Potrerillos, Piedades de San Ramón, 1150m, 6/XII/1922, *Brenes 495* (CR!); Piedades de San Ramón, 1100 m, 26/X/1925, *Brenes 273(1458)* (CR!); without collection data, flowered in cultivation at Lankester Botanical Garden, *JBL- 02643* (JBL-spirit!, CR!); flowered in cultivation at Lankester Botanical Garden, *JBL-02641* (JBL-spirit!) (Fig. 12, 17); flowered in cultivation at Lankester Botanical Garden, *JBL-02535* (JBL-spirit!); flowered in cultivation at Lankester Botanical Garden, *JBL-02532* (JBL-spirit!).

Distribution:—Southern Mexico (Chiapas) to central Panama, mostly along the Caribbean watershed, at 450-1350 meters (Fig. 18).

Ames described *Pleurothallis spectabilis* from Central Panama, comparing it with *P. pfavii* and its synonym, *P. platyrachis*, and distinguishing it by the truncate-retuse lip, and dentate wing of the column (Ames & Schweinfurth 1925). The short rhizome, congested inflorescence with floral bracts shorter than pedicels, and apiculate-mucronate petals are diagnostic of the species.

We accept here populations from Nicaragua as belonging to *S. spectabilis*, even though the available material is scanty and illustrations somewhat confused. The plant illustrated by Hamer (1984) from Nicaragua and supposedly based on *Stevens & Hahn 18980* (MO) is actually a mix of that collection and the flower from a specimen from El Salvador, previously illustrated under *Hamer 482* in his series on orchids of El Salvador (Hamer 1981). Whereas the size of the cespitose plant, floral bracts shorter than pedicels, truncate lip and serrulate column-wings are apparently consistent with the concept treated here as *S. spectabilis*, the petals of both specimens illustrated from El Salvador and Nicaragua are not mucronate, but instead acute and rounded- involute, respectively. It may well be that populations from El Salvador on the Pacific watershed of Central America continental division represent a still undescribed taxon. On the other hand, photographs of flowers of *Stevens 26820* (MO), also from Nicaragua, are consistent with *S. spectabilis*. Finally, the presence in Mexico of *S. spectabilis* (Solano & Soto 2008) confirms that this taxon reaches the northernmost distribution for the group and strengthens our interpretation of intermediate populations as belonging to this species.

PLoS ONE 10(7): e0131971

Chapter 2

Specklinia dunstervillei, a new species long confused with *Specklinia endotrachys* (Orchidaceae: Pleurothallidinae)

Adam P. Karremans
Franco Pupulin
Barbara Gravendeel

Specklinia dunstervillei is described as a new species in recognition of the distinctness of a Venezuelan species related to and confused with *Specklinia endotrachys*. It was illustrated for the first time by G. C. K. Dunsterville in 1963 from a plant collected in Trujillo on the Cordillera de Merida. The newly named species can be easily recognized by its small habit, short leaves and small reddish-orange flowers, the non-ascending dorsal sepal and the obtuse petals that are shortly apiculate. *Specklinia dunstervillei* is formally described and illustrated once again and compared morphologically and genetically with its closest relatives.

Keywords: Colombia, Costa Rica, G. C. K. Dunsterville, *Empusella*, *Specklinia*, Venezuela

Introduction

In the systematic study of the *Specklinia endotrachys* species complex, Pupulin *et al.* (2012) found that, aside from the latter, *Specklinia pfavii* (Rchb.f.) Pupulin & Karremans, *S. remotiflora* Pupulin & Karremans and *S. spectabilis* (Ames) Pupulin & Karremans could all be recognized as distinct species in the complex. *Specklinia endotrachys* (Rchb.f.) Pridgeon & M.W.Chase had traditionally been considered a widely distributed and highly variable albeit morphologically quite unique species, nevertheless, the four proposed taxa could be easily distinguished morphologically and ecologically from each other. At the time, the authors recognized that not all the available material labeled *S. endotrachys* could be adequately placed into one of those four species concepts, and suspected more taxa would be recognized in the complex in the future (Pupulin *et al.* 2012; 2013a; 2013b).

The fifth species to be added to the complex was the Guatemalan *Specklinia juddii* (Archila) Pupulin & Karremans, unknown at the time of the first publication and later placed amongst its relatives (Pupulin *et al*. 2013a; 2013b). It had been published under the generic name *Empusella* (Luer) Luer, a monotypic genus typified by *S. endotrachys* and here regarded as a synonym of *Specklinia* Lindl. (Pridgeon *et al.* 2001; Bogarín *et al.* 2013b; Karremans *et al.* 2013a). Similarly, the Venezuelan material labeled *S. endotrachys* and illustrated for the first time in Dunsterville and Garay (1965), morphologically did not resemble the other five species in the complex and had remained a mystery to the authors (Pupulin *et al.* 2012; 2013a; 2013b). Now with additional material at hand we are able to compare the Venezuelan material with the other species of this complex, both morphologically (for all species currently recognized) and genetically (for all except *S. juddii*).

Materials and Methods

This study was conducted at Jardín Botánico Lankester (JBL) of the Universidad de Costa Rica and Naturalis Biodiversity Center - Leiden University, from 2012 to 2014. Plants were collected under the scientific permits handed by the Costa Rican Ministry of Environment (MINAE) to researchers at JBL. Individual plants were photographed, illustrated and preserved as DNA samples, herbarium specimens and spirit specimens in formaldehyde: acetic acid: ethanol [FAA (53% ethanol, 37% water, 5% formaldehyde and 5% glycerol)] (only including flowers) for future reference, deposited at JBL-spirit and L-spirit and the DNA bank of Naturalis Biodiversity Center. Taxon names mostly follow Pridgeon (2005).

Photography

The Lankester Composite Digital Plate (LCDP) and color illustrations of the flowers were made using a Nikon D5100 digital camera with a AF-S VR Micro-NIKKOR 105mm f/2.8G IF-ED lens and an Epson Perfection Photo Scanner V600, and a Leica MZ9.5 stereomicroscope fitted with a Leica DFC295 digital microscope color camera with Leica FireCam 3.4.1 software.

Phylogenetic analysis

The data matrix included DNA sequences of 50 individuals (Table 1), 27 of which were produced in this study. The remaining data were obtained from GenBank (Pridgeon *et al.* 2001; Bogarín *et al.* 2013b; Chiron *et al.* 2012). Fresh leaf and flower cuttings of approximately 1 cm2 were dried with silica gel. Samples (20 mg) were pulverized and extraction performed following the DNEasy procedure (Qiagen). The nuclear ribosomal internal transcribed spacer (nrITS) region was amplified using the methods and primers for sequencing and amplification described by Sun *et al.* (1994), and Sanger sequencing was done commercially by Macrogen on a 96-capillary 3730xl DNA Analyzer automated sequencer (Applied Biosystems, Inc.) using standard dye-terminator chemistry (Macrogen, Inc.).

The Staden *et al.* (2003) package was used for editing of the sequences. Contigs were exported as .fas files and opened in Mesquite v2.72 (Maddison & Maddison 2007), where they were checked for base calling errors, the matrix was aligned manually (S1 Sequence Matrix). The ends of each data set were trimmed to eliminate possible erroneous data, and gaps were regarded as missing data (filled with Ns). *Phloeophila peperomioides* was used as the outgroup, as it was found to be one of the most distantly related of all included species in this phylogenetic analysis (Pridgeon *et al.* 2001). The trees were produced with an analysis of the nrITS dataset of 43 sequences using BEAST v1.6.0. (Drummond & Rambaut 2007). Parameters were set to preset, except for substitution model GTR with 10 categories, clock model uncorrelated lognormal, tree prior Yule process, and number of generations 20,000,000. The resulting trees were combined using TreeAnnotator v1.6.0., where the first 3000 trees were used as burn-in. FigTree v1.3.1. (Rambaut 2009) was used to edit the resulting tree. Posterior probabilities are given for each node in decimal form.

A pairwise comparison of the ITS sequence of the accessions of *S. dunstervillei*, *S. endotrachys*, *S. montezumae* (as an outgroup), *S. pfavii*, *S. spectabilis* and *S. remotiflora* is presented in Table 2. Each different base was counted as an individual change, even when concurrent; insertions and deletions were counted as a single change regardless of length. All the mentioned accession of each species in Table 1 were combined and used for the comparison except for AF262859, a sequence labeled *S. endotrachys* by Pridgeon *et al.* (2001) but which we suspect (based on DNA data) should be *S. remotiflora* or a closely related species.

Nomenclature

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS ONE article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Results

Photography:— The color illustrations of species of the *Specklinia endotrachys* complex (Fig. 19) shows a morphologically distinct entity, *Specklinia dunstervillei* (Figs. 19A and 19B), recognized amongst others by the smaller flowers and shortly apiculate petals.

Table 1. List of the 50 accessions used in the phylogenetic analysis. The vouchers, NCBI GenBank accession number and source are given. Scientific names follow Pridgeon 2005.

Taxon	Sequence Voucher	GenBank Accession Number	Sequence Source
Dryadella simula (Rchb. f.) Luer	*Chase 1095*	AF262825	Pridgeon *et al.* (2001)
Dryadella susanae (Pabst) Luer	*Chiron 11240*	JQ306486	Chiron *et al.* (2012)
Phloeophila peperomioides (Ames) Garay	None	AF275690	Pridgeon *et al.* (2001)
Platystele compacta (Ames) Ames	*Chase 5637*	AF262822	Pridgeon *et al.* (2001)
Platystele misera (Lindl.) Garay	*Chase 5625*	AF262823	Pridgeon *et al.* (2001)
Platystele stenostachya (Rchb. f.) Garay	*Chase 5618*	AF262821	Pridgeon *et al.* (2001)
Scaphosepalum grande Kraenzl.	*Chase 1107*	AF262819	Pridgeon *et al.* (2001)
Scaphosepalum swertiifolium (Rchb. f.) Rolfe	*Chase 1383*	AF262818	Pridgeon *et al.* (2001)
Scaphosepalum verrucosum (Rchb. f.) Pfitzer	*Chase 1331*	AF262820	Pridgeon *et al.* (2001)
Specklinia absurda Bogarín, Karremans & Rincón	*Bogarín 8711* (JBL-Spirit)	KC425827	Bogarín *et al.* (2013b)
Specklinia barbae (Schltr.) Luer	*Karremans 4853*	KC425771	This Study
Specklinia barbae (Schltr.) Luer	*Karremans 3928*	KC425769	This Study
Specklinia chontalensis (A.H.Heller & A.D.Hawkes) Luer (1)	*Pupulin 6543*	KC425776	This Study
Specklinia chontalensis (A.H.Heller & A.D.Hawkes) Luer (2)	*Pupulin 6543*	KF747799	This Study
Specklinia costaricensis (Rolfe) Pridgeon & M.W.Chase	*Chase 5612*	AF262862	Pridgeon *et al.* (2001)
Specklinia digitalis (Luer) Pridgeon & M.W.Chase	*Karremans 5737*	KF747806	This Study
Specklinia dunstervillei	*Karremans 5966*	KP012456	This Study
Specklinia endotrachys (Rchb.f.) Pridgeon & M.W.Chase (1)	*Blanco 961*	KC425784	This Study
Specklinia endotrachys (Rchb.f.) Pridgeon & M.W.Chase (2)	*Blanco 961*	KF747810	This Study
Specklinia fuegi (Rchb.f.) Solano & Soto Arenas	*Karremans 5600* (JBL-Spirit)	KC425786	Bogarín *et al.* (2013b)
Specklinia fulgens (Rchb.f.) Pridgeon & M.W.Chase	*Chase 5630*	AF262872	Pridgeon *et al.* (2001)
Specklinia glandulosa (Ames) Pridgeon & M.W.Chase	*Karremans 5501*	KC425792	This Study
Specklinia glandulosa (Ames) Pridgeon & M.W.Chase	*Karremans 3265*	KC425791	This Study
Specklinia glandulosa (Ames) Pridgeon & M.W.Chase	*Karremans 2945*	KP012452	This Study
Specklinia glandulosa (Ames) Pridgeon & M.W.Chase	*Karremans 5944*	KP012453	This Study
Specklinia glandulosa (Ames) Pridgeon & M.W.Chase	*Bogarín 2895*	KP012454	This Study
Specklinia glandulosa (Ames) Pridgeon & M.W.Chase	*Karremans 3268*	KP012455	This Study
Specklinia grobyi (Bateman ex Lindl.) F.Barros	*Chiron 04524*	JQ306485	Chiron *et al.* (2012)
Specklinia lanceola (Sw.) Lindl. (2)	*Pridgeon s.n.*	KC425838	Bogarín *et al.* (2013)
Specklinia lanceola (Sw.) Lindl. (3)	*Chase 1433*	AF262861	Pridgeon *et al.* (2001)
Specklinia lentiginosa (F.Lehm. & Kraenzl.) Pridgeon & M.W.Chase	None	AF275692	Pridgeon *et al.* (2001)
Specklinia montezumae (Luer) Luer	*Karremans 229* (JBL-Spirit)	KC425811	Bogarín *et al.* (2013b)
Specklinia montezumae (Luer) Luer	*Karremans 5751*	KF747816	This Study
Specklinia picta (Lindl.) Pridgeon & M.W.Chase	*Van Den Berg 2146*	JQ306384	Chiron *et al.* (2012)
Specklinia pissina	*Karremans 4797*	KC425795	This Study
Specklinia pissina	*Karremans 4839*	KC425797	This Study
Specklinia pfavii (Rchb.f.) Pupulin & Karremans	*Karremans 4825*	KC425814	This Study
Specklinia pfavii (Rchb.f.) Pupulin & Karremans	*Karremans 3656*	KF747819	This Study
Specklinia pfavii (Rchb.f.) Pupulin & Karremans	*JBL-11086*	KF747820	This Study
Specklinia remotiflora Pupulin & Karremans (4)	*Chase 1303*	AF262859	Pridgeon *et al.* (2001)
Specklinia remotiflora Pupulin & Karremans (1)	*Karremans 4798a*	KC425818	This Study
Specklinia remotiflora Pupulin & Karremans (2)	*Karremans 4798b*	KC425819	This Study
Specklinia remotiflora Pupulin & Karremans (3)	*Karremans 4854*	KC425820	This Study
Specklinia sp.	*Karremans 6025*	KP012457	This Study
Specklinia sp.	*Pupulin 7709*	KC425824	This Study
Specklinia spectabilis (Ames & C.Schweinf.) Pupulin & Karremans	*Bogarín 7401*	KC425830	This Study
Specklinia spectabilis (Ames & C.Schweinf.) Pupulin & Karremans	*Karremans 5699*	KC425828	This Study
Specklinia subpicta (Schltr.) F.Barros	*Chiron 11046*	JQ306389	Chiron *et al.* (2012)
Specklinia succulenta Bellone & Archila	*Bellone 680*	JQ306383	Chiron *et al.* (2012)
Specklinia tribuloides (Sw.) Pridgeon & M.W.Chase (1)	*Chase 5615*	AF262867	Pridgeon *et al.* (2001)

Phylogenetic Analyses:—The consensus gene tree (Fig. 20) was obtained from a BEAST analysis of a matrix of 45 ITS sequences (Table 1), including 12 accessions belonging to 5 different species of the *Specklinia endotrachys* complex. The accessions of *S. dunstervillei*, *S. endotrachys*, *S. pfavii*, *S. spectabilis* and *S. remotiflora* are found in a highly supported monophyletic clade (P.P. = 0.99), sister to the accessions of *S. montezumae*.

The pairwise comparison of the ITS sequence of the accessions of the S. endotrachys species complex (Table 2) shows that the sequences of *Specklinia endotrachys* and *S. spectabilis* do not differ from each other, while *S. dunstervillei* differs in 2 bases from those species. *Specklinia pfavii* and *S. remotiflora* differ in 4 and 10 bases respectively, from the three before mentioned species. *Specklinia montezumae* differs in 9 to 11 bases from each of the members of the *S. endotrachys* species complex.

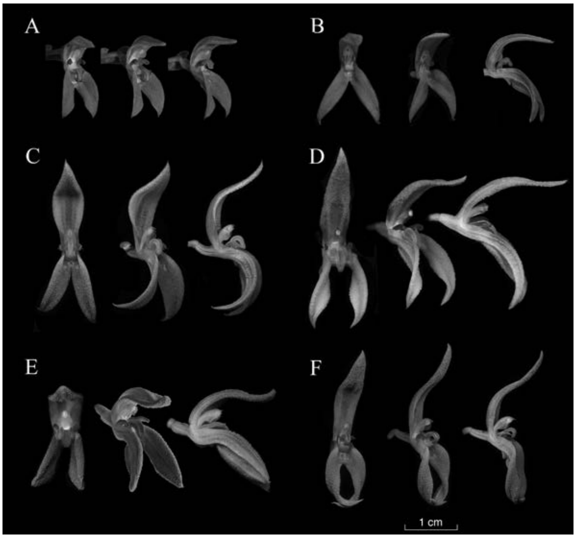


FIGURE 19. Comparison of flowers of species of the *Specklinia endotrachys* group. A. *S. dunstervillei* (*Karremans 5966*). B. *S. dunstervillei* (*Karremans 5899*). C. *S. endotrachys* (*Blanco 961*). D. *S. pfavii* (*JBL-11086*). E. *S. remotiflora* (*Bogarín 8181*). E. *S. spectabilis* (*JBL-02641*). All flowers shown in front, three-quarters side, and side views. Photographs by F. Pupulin (B-F) and R. van Vugt (A).

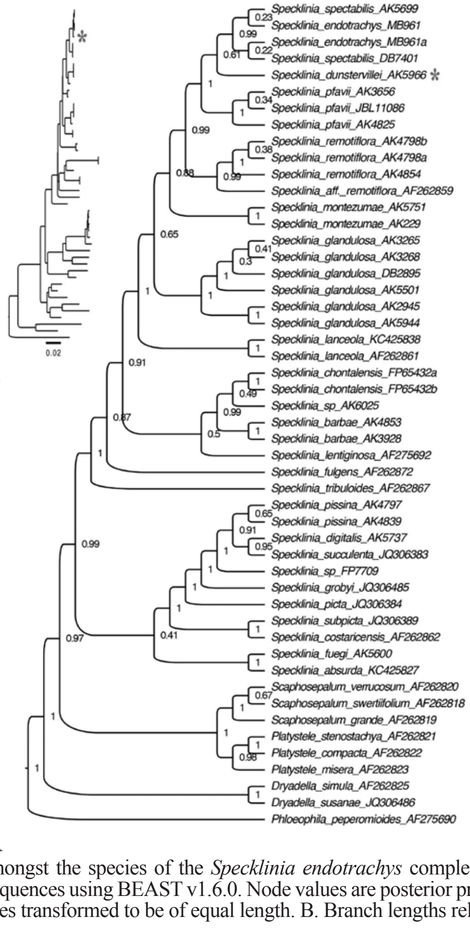


FIGURE 20. Phylogenetic relationship amongst the species of the *Specklinia endotrachys* complex. The trees were produced with an analysis of the nrITS dataset of 50 sequences using BEAST v1.6.0. Node values are posterior probabilities. The tree was edited using FigTree v.1.3.1. A. Tree with branches transformed to be of equal length. B. Branch lengths relative to relative number of changes.

Table 2. Pairwise comparison of the number of single base differences amongst the nrITS sequences of *S. dunstervillei*, *S. endotrachys*, *S. montezumae*, *S. pfavii*, *S. spectabilis* and *S. remotiflora*.

	S. dunstervillei	***S. endotrachys***	***S. pfavii***	***S. remotiflora***	***S. spectabilis***	***S. montezumae***
Specklinia dunstervillei	-	2	4	10	2	10
Specklinia endotrachys	2	-	4	10	0	10
Specklinia pfavii	4	4	-	12	4	11
Specklinia remotiflora	10	10	12	-	10	9
Specklinia spectabilis	2	0	4	10	-	10
Specklinia montezumae	10	10	11	9	10	-

Discussion

Considering all the available evidence, including morphology, genetics, distribution and ecology, we find that the Venezuelan material labeled *Specklinia endotrachys*, actually belongs to an unnamed species, described here forth:

5. *Specklinia dunstervillei* Karremans, Pupulin & Gravend., PLoS ONE 10(7): e131971 (5). 2015.

> *The species is similar to Specklinia endotrachys but can be distinguished by the small habit, shorter leaves, smaller flowers, the flat, non-ascending dorsal sepal (vs. ascending), and the obtuse, shortly apiculate (vs. emarginate and long mucronate) petals.*

Type:—VENEZUELA. Without collecting data, cultivated by Jacobus Wubben in the Netherlands. Flowered in cultivation on March 29th 2013, *A.P. Karremans 5966 & B. Gravendeel* (holotype, JBL-spirit!; isotype, L-spirit!; Figs. 19 and 21).

Epiphytic, caespitose, erect herb to 10 cm tall, excluding the inflorescence. Roots fibrous, flexuous, glabrous. Stem abbreviated, terete-cylindric, to 1 cm long, monophyllous, completely concealed by papyraceous, subancipitous, acute sheaths to 1 cm long. Leaf narrowly obovate-oblanceolate, 6–10 x 1.2–1.8 cm, minutely emarginate at apex, the midvein protruding abaxially into a small apicule, gradually tapering toward the base into a deeply conduplicate petiole with strongly ancipitous margins, subcoriaceous. Inflorescence borne laterally from the apex of the stem, without an annulus, a distichous, successively flowered raceme, with only one flower open at once, up to 12–13 cm long; peduncle flattened, ancipitous, to 11–12 cm long, with 2–3 amplectent, ancipitous, subacute bracts, 5–6 mm long. Floral bracts infundibuliform, broadly ovate, acute to subacuminate, 2–3 mm long. Pedicel cylindric, glabrous, 4 mm long, persistent. Ovary subclavate, 2 mm long, green tinted with orange. Flowers with reddish-orange sepals, petals and lip, the column greenish-yellow, lightly tinted orange. Sepals fleshy, densely papillose in the inner surface except at the base; dorsal sepal elliptic, 3-veined, acute, the base whitish semi-hyaline, flushed with orange along the veins, the distal two thirds densely papillose, 14–16 × 5-6 mm; lateral sepals narrowly elliptic-oblanceolate, subfalcate, 3–veined, 13–15 × 4 mm, the base saccate, membranaceous-hyaline, the apex acute, gently twisting above the middle, the midvein strongly carinate abaxially. Petals small, ligulate-falcate, truncate, shortly apiculate, porrect, 3–4 × 1 mm, 1-veined, papillose-thickened toward the concave apex. Lip small, longitudinally arched-convex in natural position, thinly articulate with the column foot by a hyaline claw, narrowly elliptic-lanceolate when expanded, obtuse, the apex reflexed, appearing minutely retuse, the clawed base thickened, transversely minutely gibberose, 5–6 × 2 mm, provided with 2 slender keels, fringed-lacerate at the base, gently converging from the base of the lamina to near the apex. Column arched, terete-slender at the base, 4.5–5.0 mm long without the foot, provided with broad membranous wings serrulate along the margins, at the apex forming a deeply cucullate, serrulate clinandrium; column foot forward-projecting, stout, fleshy, incurved, 1.5 mm long. Anther cap deeply cucullate, ovate, 2-celled. Pollinia 2, obovate-complanate, minutely hooked at the base, lacking caudicles. Note: Only the specimens from Venezuela were used for the description (*Dunsterville 757* and *Karremans 5966*).

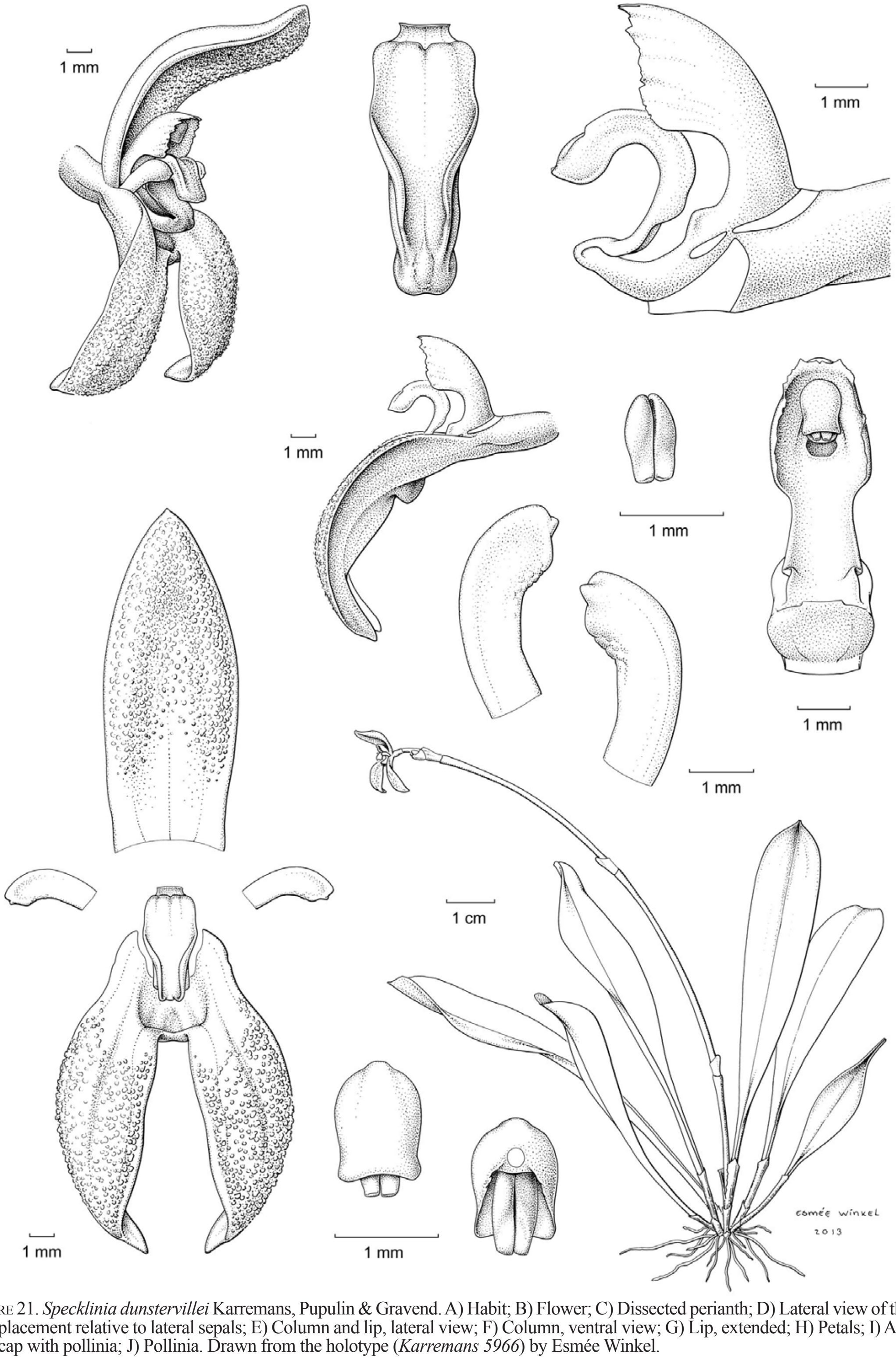


Figure 21. *Specklinia dunstervillei* Karremans, Pupulin & Gravend. A) Habit; B) Flower; C) Dissected perianth; D) Lateral view of the lip placement relative to lateral sepals; E) Column and lip, lateral view; F) Column, ventral view; G) Lip, extended; H) Petals; I) Anther cap with pollinia; J) Pollinia. Drawn from the holotype (*Karremans 5966*) by Esmée Winkel.

Etymology:—The name honors G. C. K. Dunsterville, who prepared the first known illustration of the species.

Other material examined:—COSTA RICA. Without collecting data, cultivated by Gerson Villalobos in Moravia, San José. Flowered in cultivation on September 1st 2013, *A.P. Karremans 5899* (JBL-spirit!; Figs. 22 and 23). Venezuela. Boconó-Guaramacal penetration road. About 8000 ft. in rain forest, *G. C. K. Dunsterville 757* [illustration of voucher in Dunsterville and Garay (1965)!; Figs. 24 and 25].

Other records:—COLOMBIA. Without collecting data, illustration of voucher in Ortiz and Uribe (2007).

Distribution:—The material collected by Dunsterville in Venezuela comes from the road between Boconó and Guaramacal in Trujillo, on the Cordillera de Merida, a branch of the Colombian Andes, at an elevation of about 2400 m. A few specimens that have been found in private collections in Costa Rica and Colombia lack precise location data.

Figure 22. Acuarela of *Specklinia dunstervillei* Karremans, Pupulin & Gravend. By Sylvia Strigari, based on *Karremans 5899* (JBL).

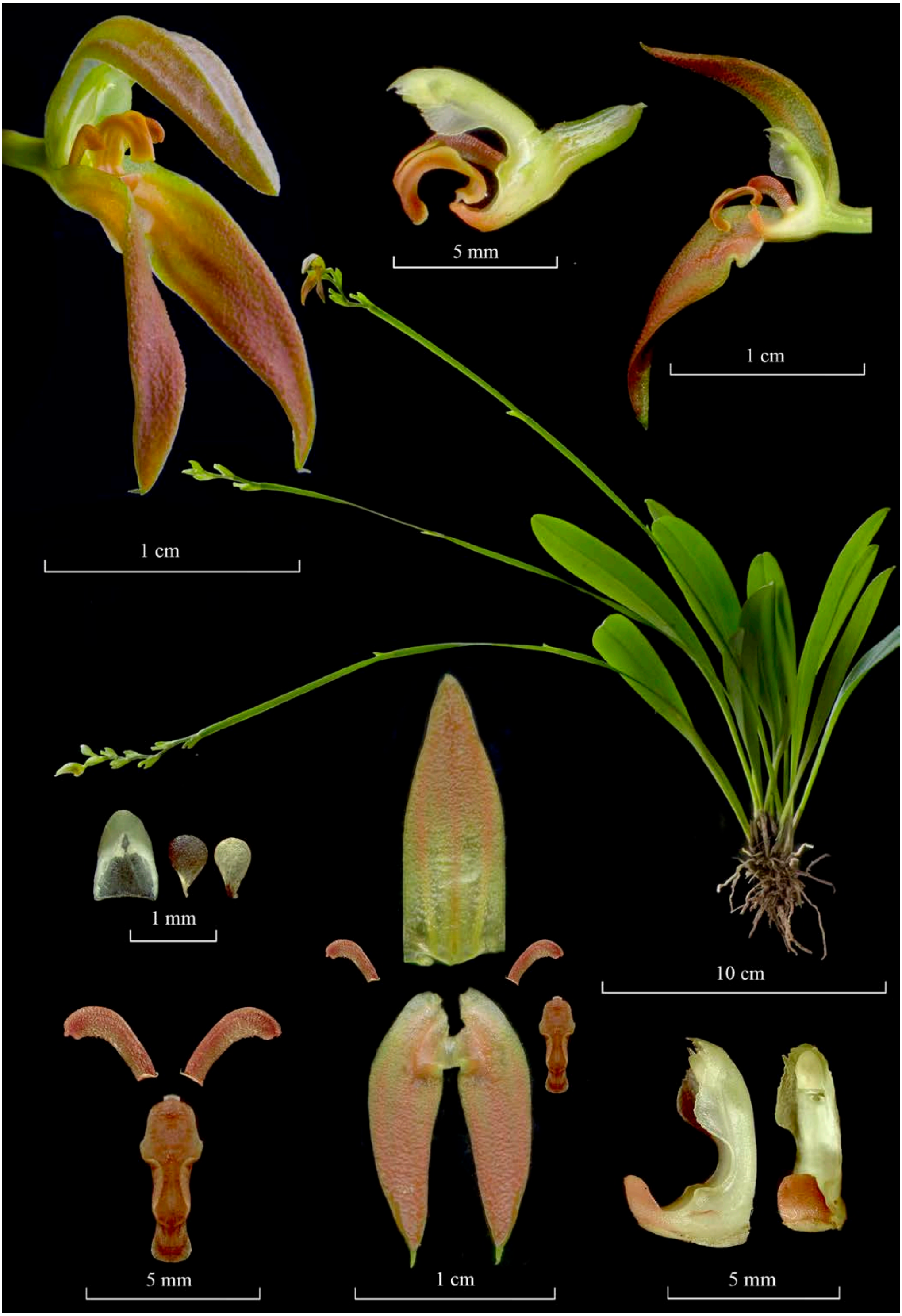


Figure 23. Lankester Composite Dissection Plate (LCDP) of *Specklinia dunstervillei* Karremans, Pupulin & Gravend. A. Habit. B. Flower. C. Transverse section of a whole flower. D. Dissected perianth. E. Column and lip, lateral view. F. Petals and lip. G. Column in ventral and lateral view. H. Pollinia and anther cap. Based on photographs of *Karremans 5899* (JBL) by A.P. Karremans.

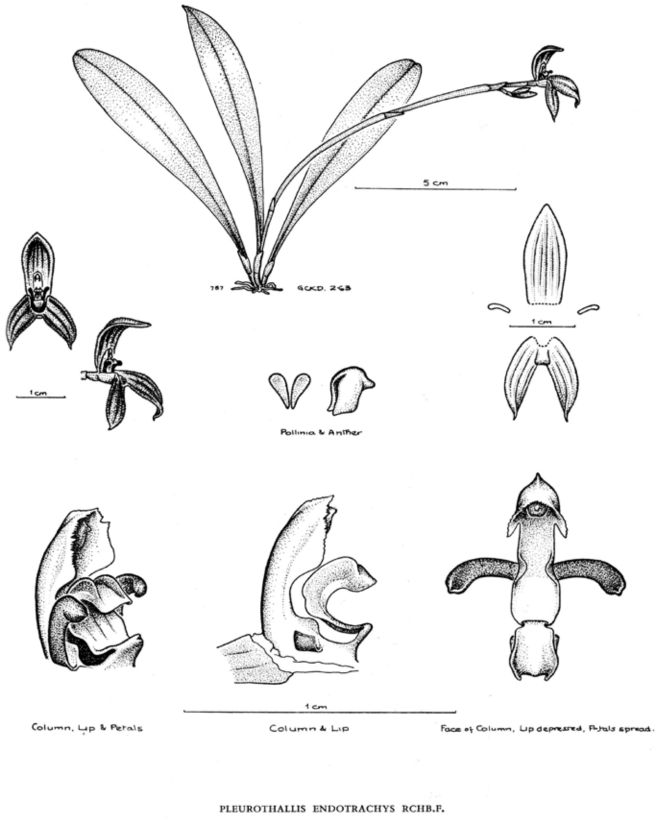


Figure 24. *Specklinia dunstervillei* Karremans, Pupulin & Gravend. Illustrated by G. C. K. Dunsterville in Dunsterville and Garay (1965) from a plant found in Trujillo, Venezuela (*Dunsterville 757*). Reproduced with the kind permission of the Orchid Herbarium of Oakes Ames, the Harvard University Herbaria.

Figure 25. *Specklinia dunstervillei* Karremans, Pupulin & Gravend. Unpublished photographs by G. C. K. Dunsterville from the plant found in Trujillo, Venezuela (*Dunsterville 757*). A. The plant habit. B. Detail of the flower. Reproduced with the kind permission of the Orchid Herbarium of Oakes Ames, the Harvard University Herbaria.

Key to the empsellous species of Specklinia

1. Flowers yellow with red petals; lip acute, yellow, with a central red line; column wings broad, entire; clinandrium entire .... *S. pfavii*
- Flowers reddish-orange; lip rounded to truncate, concolorous orange; column wings narrow or broad, erose-denticulate; clinandrium erose-dentate 2

2. Flowers campanulate, with lateral sepals straight, not spreading, petals obtuse 3
- Flowers spreading, lateral sepals twisted and/or bent, petals apiculate to mucronate 4

3. Plant repent, inflorescence lax; flowers bright orange in both outer and inner surfaces; column with rectangular wings and erose-dentate clinandrium .... *S. remotiflora*
- Plant caespitose, inflorescence congested; flowers green outside, orange brown within; column with broadly elliptic wings and entire clinandrium .... *S. juddii*

4. Plant and flowers small (leaf up to 10 cm long, dorsal sepal about 1.5 cm long), dorsal sepal bent forward, but not twisted, petals shortly apiculate .... *S. dunstervillei*
- Plant and flowers larger (leaf longer than 10 cm long and sepals at least 2 cm long), dorsal sepal erect, ascending, petals long-mucronate 5

5. Floral bracts subequal to the length of the pedicel; dorsal sepal lanceolate, lateral sepals bent but not twisted; petals retuse, with a long mucron abruptly inserted within the sinus .... *S. endotrachys*
- Floral bracts much shorter than the length of the pedicel; dorsal sepal linear-triangular, lateral sepals twisted; petals acute, tapering, the mucron continuous with the apex .... *S. spectabilis*

Ecology:—The typical form of *Specklinia dunstervillei* is that found in Colombia and Venezuela (Figs. 19a, 21 and 24). The material illustrated by Dunsterville, which lacks an herbarium voucher (Gustavo Romero pers. comm.), was collected on the Cordillera de Merida at about 2400 m in elevation. That makes the new species the one with the most Easternly distribution in the complex and, together with *S. remotiflora*, the only one to grow in cloud forests at high elevations above 1800 m. A photograph of this species was published in Ortiz and Uribe (2007) without precise locality, nonetheless it was definitively taken from a Colombian plant (Carlos Uribe V. pers. comm.). Considering that the Cordillera de Merida extends into Colombia, it is indeed not surprising that the species is also found there. Finally, we have chosen to regard the material found in Costa Rican private collections as *S. dunstervillei* as they are morphologically most similar to that species concept, nevertheless they do show quite some variation (Figs. 19b, 22 and 23). The studied Costa Rican specimens lack specific collection data.

Like the other species of the *S. endotrachys* complex, *S. dunstervillei* has nectar secreting stomata placed on the apex of the warts that cover the surface of the sepals (Pupulin *et al.* 2012, Chapter 1; 2013a; 2013b; Karremans *et al.* 2013a). The released nectar gives the sepals a wet-glossy appearance, as noted by Dunsterville and Garay (1965), and is given special attention by visiting fruit flies. At Lankester Botanical Garden the flowers were frequently visited by up to 6 individuals of *Drosophila* spp. The flies wander about on the sepals, sucking on the papillae rich surfaces, occasionally removing the pollinia, after stepping on the movable lip and being adpressed against the column (Fig. 26).

Morphological recognition:—*Specklinia dunstervillei* is morphologically similar to other members of the *S. endotrachys* complex. Within *Specklinia* they are recognized by the relatively large habit and flowers, the long, successive, flat, ancipitous inflorescence, the bright yellow to orange-red flowers, the verrucose sepals, the relatively minuscule petals and the highly sensitive strongly concave lip. Amongst its closest relatives, *S. dunstervillei* is recognized by the tiny habit, caespitose plants, short leaves (up to about 10 cm), the small flowers (dorsal sepal 14–16 × 5-6 mm), the flattening, non-ascending dorsal sepal, the lateral sepals that spread, slightly twisting downwards and the obtuse petals, which are shortly apiculate.

Figure 26. *Drosophila* sp. with the pollinium, still within the anther cap, of *Specklinia dunstervillei* Karremans, Pupulin & Gravend. (*Karremans 5899*). Photographed by A.P. Karremans.

Genetic recognition:—The nrITS sequence of the accession of *Specklinia dunstervillei* differs in two base pairs (out of just under 800bp) from the accessions of both *S. endotrachys* and *S. spectabilis*, which are identical to each other (Table 2). Considering that the species of the *S. endotrachys* complex differ in only 9 to 11 bases (between 1.1 and 1.4%) from an outgroup as morphologically distinct as *S. montezumae*, it becomes highly plausible that species with significant morphological and ecological differences within the complex itself, such as *S. endotrachys* and *S. pfavii*, can differ in just about 0.5% bases. Therefore it is also not unusual that more closely related species such as *S. endotrachys* and *S. dunstervillei* differ in only 2 bases, or that the sisters *S. endotrachys* and *S. spectabilis* do not differ at all in this particular DNA barcode.

The internal transcribed spacer (ITS) of nuclear ribosomal DNA has consistently shown high levels of discrimination among many species of plants (Kress *et al.* 2005) including Pleurothallidinae species, and are well suited for a broad range of phylogenetic studies (Sun *et al.* 1994; Pridgeon *et al.* 2001; Karremans 2010; Karremans *et al.* 2013a). However, in current literature DNA data are mostly used to support so called "cryptic" novel taxa, when the accession of a said taxon does not cluster with other accessions of the a priori believed same taxon (Bogarín 2007; Lahaye *et al.* 2008; Chase *et al.* 2009; Leopardi *et al.* 2012). When different accessions of a species complex cluster together authors seem to believe that there is evidence that they are a single species, however that is not only an illogical conclusion but also frequently untrue. Be it accessions of the same species, or sister species, or species of a single genus or the same family, any two accessions will cluster together with high support if they are relatively much more similar to each other than to the other sequences analyzed. The only measurable difference between the mentioned sister pairs would be the branch length (as a measure of number of base pair changes).

However, as there is no established similarity threshold above which one should consider two sequences to belong to two different species, or below which they should be considered a single species, it is at this time also not possible to infer from branch lengths alone if we are dealing with a single or more than one species. Meyer and Paulay (2005) found that this especially counts for taxonomically understudied groups that are not yet thoroughly sampled.

In Pleurothallidinae, DNA data have been seldomly used to support novel species descriptions. Ramos-Castro *et al.* (2012) used an nrITS based phylogeny to prove that their novel taxon *Stelis zootrophionoides* Castañeda-Zárate & Ramos-Castro belonged in that genus, however they did not venture into using DNA data to set their novelty apart from other taxa in the genus. In the phylogeny presented, the single accession of *S. zootrophionoides* clusters, as would be expected, with that of the morphologically similar *S. nigriflora* (L.O.Williams) Pridgeon & M.W.Chase. Therefore, their phylogeny by itself could not prove that the accessions belonged to two different species, and the authors relied exclusively on morphological differences to establish their novelty. A species labeled *Specklinia* sp. in Chiron *et al.* (2012), of which the nrITS sequence clustered with that of *Specklinia marginata* (Lindl.) Pridgeon & M.W.Chase, was later published as a distinct species using mainly morphological characters. In both cases the authors could also have argued that the nrITS sequence of the accession of their novelty differed in about 1% from the sequence of the accession of its closest relative with which it clustered. A different approach was that of Meyer *et al.* (2012), who used the presence of insertions/deletions in the *trnH-psbA* and *ycf1* sequences of accessions of *Dracula radiosa* (Rchb.f.) Luer and close relatives to support the establishment of their novel species *Dracula trigonopetala* Gary Mey. & L. Baquero. In that case their novelty differed in less than 0.3% from its closest relatives (as an insertion/deletion counts for a single change), however, the 26 bp unique insertion in the *trnH-psbA* sequence of their novelty stands out in an otherwise quite conserved region and therefore easily sets it apart from its close relatives.

Chapter 3

The glandulous *Specklinia*: morphological convergence versus phylogenetic divergence

Adam P. Karremans
Diego Bogarín
Franco Pupulin
Carlyle A. Luer
Barbara Gravendeel

The present paper focuses on the systematics of the *Specklinia glandulosa* species complex. Traditionally, *S. glandulosa* has been considered a widely distributed and variable species, ranging from Mexico to the Guiana Shield. Here it is treated as one of at least six different, albeit closely related, species. Of these species, *S. pertenuis* and *S. vittariifolia*, are recognized as distinct species and removed from the synonymy of *S. glandulosa*, and *S. alajuelensis* and *S. gersonii* are described and illustrated as new to science. *Specklinia chontalensis* is described and illustrated from living, Costa Rican material. *Specklinia alajuelensis* is compared with *S. glandulosa* and *S. vittariifolia*, from which it differs in its broader leaves, multi-flowered, lax inflorescence that surpasses the leaves, and smaller petals and sepals. *Specklinia gersonii* is compared with S. glandulosa, from which it differs in the smaller leaves, and the smaller, orange flowers.

Keywords: Orchidaceae, Pleurothallidinae, *Specklinia*, *S. alajuelensis*, *S. chontalensis*, *S. gersonii*, *S. glandulosa*, *S. vittariifolia*

Introduction

Frequently, when a few outstanding morphological features are shared by a number of similar specimens authors tend to accept them as variations of a single species. In such cases the similarities amongst the group of specimens appear much larger than their individual differences. However, this can be misleading and such variations may not always represent the variation of a single taxon. A larger sampling of the alleged variable species might show that those supposedly unique morphological features are actually diagnostic to a whole lineage of well established species. Long, flattened inflorescences bearing large, bright reddish-orange flowers, led authors to believe that *Specklinia endotrachys* (Rchb.f.) Pridgeon & Chase, *S. pfavii* (Rchb.f.) Pupulin & Karremans, *S. remotiflora* Pupulin & Karremans and *S. spectabilis* (Ames & C.Schweinf.) Pupulin & Karremans, were all a single variable species despite their obvious morphological differences and completely different ecological preferences (Pupulin *et al.* 2012). The *Specklinia condylata* complex was another such example (Bogarín *et al.* 2014). Similarly a high morphological variation has been traditionally accepted in *Specklinia glandulosa* (Ames) Pridgeon & Chase, a name used for classifying any *Specklinia* specimen with orange flowers and fully glandular, single-flowered inflorescences (Luer 2006). However, when considering additional evidence it becomes evident that *S. glandulosa* is actually a species complex in need of disentanglement.

August Endrés was the first to collect and illustrate a member of the glandulous *Specklinia* species around 1867. The origin of his material was Costa Rica without any precise locality, but the illustrations and descriptions are still kept at Reichenbach's herbarium in Vienna. Nevertheless, the first name applicable to this group of species appeared much later. *Pleurothallis glandulosa* was described by Oakes Ames from a plant collected by Powell in central Panama in 1923. The fully glandular pedicel, rachis, peduncle, ovary and external surface of sepals, which prompted its name, were a unique combination of features amongst its known relatives at the time. The same year, Rudolf Schlechter described *Pleurothallis vittariifolia* Schltr. from a plant collected by Wercklé in San Jerónimo, Costa Rica. A third name appeared a bit more than a decade later when *Pleurothallis pertenuis* C.Schweinf.) was published based on material from Guyana. Due to their very particular bright yellow-orange flowers, glandular inflorescences and thin leaves, the three were regarded as synonymous (Luer 2006).

Nevertheless, besides many conspicuous similarities we encounter several unique differences among specimens identified as *S. glandulosa* along its unusually broad distribution (Fig. 27). Therefore we conducted a morphological and genetic study of those specimens from their entire geographical range and present our findings here.

Materials and methods

This study was conducted at Jardín Botánico Lankester (JBL) of the Universidad de Costa Rica and Naturalis Biodiversity Center-Leiden University, from 2012 to 2015. Plants were collected under the scientific permits handed by the Costa Rican Ministry of Environment (MINAE) to researchers at JBL. Individual plants were photographed, illustrated and preserved as DNA samples, herbarium specimens and spirit specimens in FAA (53% ethanol, 37% water, 5% formaldehyde and 5% glycerol). Specimens were deposited at JBL-spirit and L-spirit and in the DNA bank of Naturalis Biodiversity Center.

Scanning Electron Microscopy (SEM): Tissue samples of floral parts were prepared for SEM observation by harvesting tissue from the flowers up to 48 h after the beginning of anthesis, fixing in FAA (ethanol 50%, acetic acid, formalin at a proportion of 18:1:1 v/v), and dehydration through a series of ethanol steps and critical-point drying using liquid CO2. Dried samples were mounted and sputter-coated with gold and observed with a JEOL JSM-5300 scanning electron microscope, at an accelerating voltage of 5 to 10 kV. All images were processed digitally.

Macrophotography: Color illustrations of plants and flowers were made using a Nikon® D5100, D5300 or D7100 digital camera, a DFC295 Leica® digital microscope color camera with Leica FireCam version 3.4.1 software, and an Epson® V370 Photo Scanner. Adobe Photoshop® was used for editing images and stacking whenever necessary.

Phylogenetic analysis: The data matrix included DNA sequences of 50 individuals (Table 3). Fresh leaf and flower cuttings of approximately 1 cm2 were dried with silica gel. Samples (20 mg) were pulverized and extraction performed following the DNEasy procedure (Qiagen). The nuclear ribosomal internal transcribed spacer (nrITS) region was amplified using the methods and primers for sequencing and amplification described by Sun *et al.* (1994), and Sanger sequencing was done commercially by Macrogen on a 96-capillary 3730xl DNA Analyzer automated sequencer (Applied Biosystems, Inc.) using standard dye-terminator chemistry (Macrogen, Inc.).

The Staden *et al.* (2003) package was used for editing of the sequences. Contigs were exported as .fas files and opened in Mesquite v2.72 (Maddison & Maddison 2007), where they were visually checked for base calling errors. Edited contigged sequences were aligned manually. The ends of each data set were trimmed to eliminate possible erroneous data, and gaps were regarded as missing data (filled with Ns). *Phloeophila peperomioides* (Ames) Garay was used as outgroup, as it was found to be one of the most distantly related of all included species in this phylogenetic analysis (Pridgeon *et al.* 2001). The trees were produced with an analysis of the nrITS dataset of 43 sequences using BEAST v1.6.0. (Drummond & Rambaut 2007). Parameters were set to preset, except for substitution model GTR with 10 categories, clock model uncorrelated lognormal, tree prior Yule process, and number of generations 20,000,000. The resulting trees were combined using TreeAnnotator v1.6.0., where the first 3000 trees were used as burn-in. FigTree v1.3.1. (Rambaut 2009) was used to edit the resulting tree. Posterior probabilities are given for each node in decimal form.

A pairwise comparison of the ITS sequence of analyzed glandulous *Specklinia* is presented in Table 4. Each different base was counted as an individual change, even when concurrent; insertions and deletions were counted as a single change regardless of length.

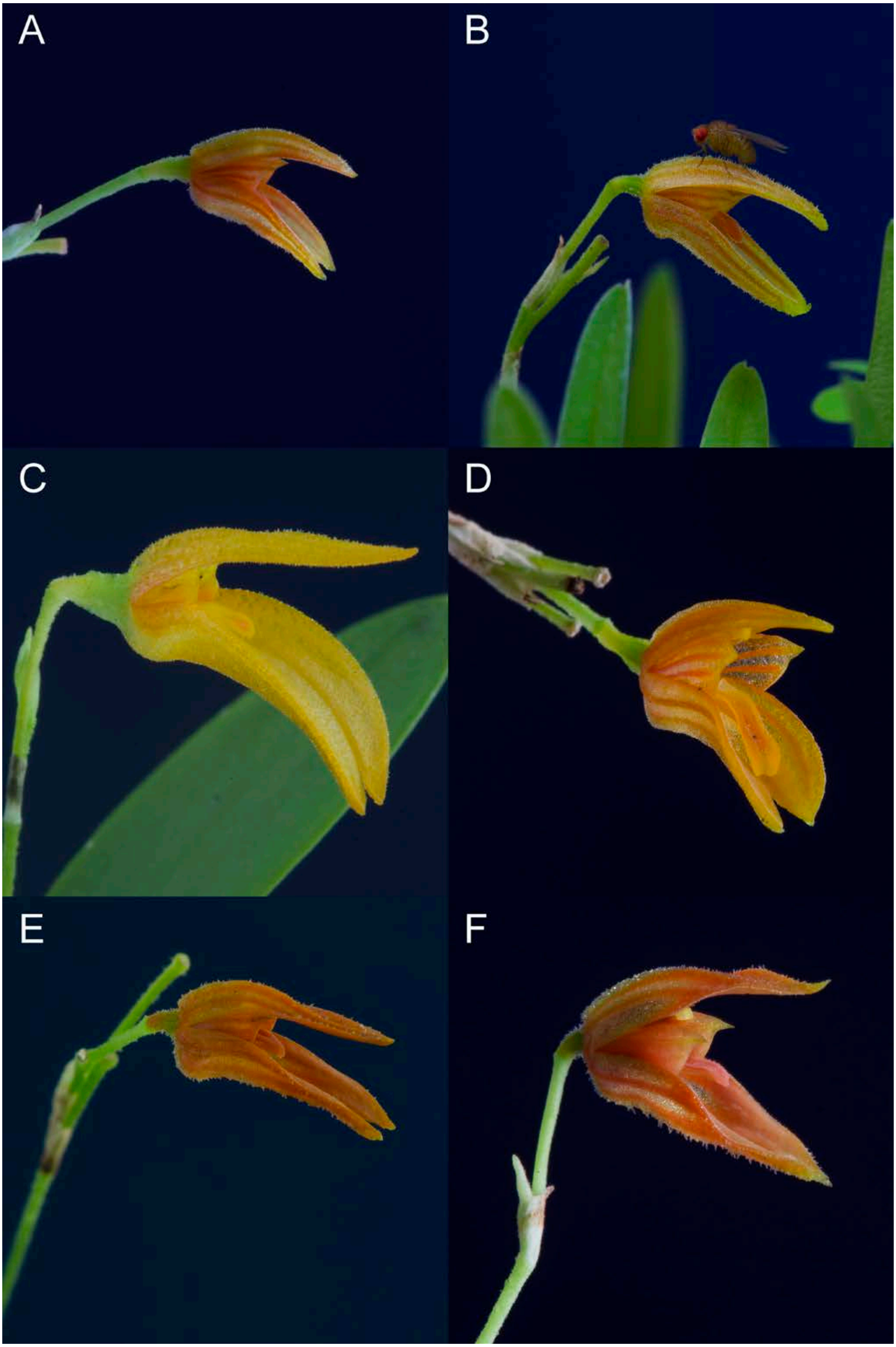


Figure 27. The glandulous *Specklinia* species. A. *S. alajuelensis* 1 (*Karremans 3265*). B. *S. alajuelensis* 2 (*Bogarín 2895*). C. *S. chontalensis* (*Pupulin 6543*). D. *S. gersonii* (*Karremans 6025*). E. *S. glandulosa* (*Karremans 6306*). F. *S. vittariifolia* (*Karremans 2945*). Photographs by A.P. Karremans.

TABLE 3. List of the 50 accessions used in the phylogenetic analysis. The vouchers, NCBI GenBank accession number and source are given.

Taxon	Sequence Voucher	GenBank Accession Number	Sequence Source
Dryadella simula (Rchb. f.) Luer	*Chase 1095*	AF262825	Pridgeon *et al.* (2001)
Dryadella susanae (Pabst) Luer	*Chiron 11240*	JQ306486	Chiron *et al.* (2012)
Phloeophila peperomioides (Ames) Garay	None	AF275690	Pridgeon *et al.* (2001)
Platystele compacta (Ames) Ames	*Chase 5637*	AF262822	Pridgeon *et al.* (2001)
Platystele misera (Lindl.) Garay	*Chase 5625*	AF262823	Pridgeon *et al.* (2001)
Platystele stenostachya (Rchb. f.) Garay	*Chase 5618*	AF262821	Pridgeon *et al.* (2001)
Scaphosepalum grande Kraenzl.	*Chase 1107*	AF262819	Pridgeon *et al.* (2001)
Scaphosepalum swertiifolium (Rchb. f.) Rolfe	*Chase 1383*	AF262818	Pridgeon *et al.* (2001)
Scaphosepalum verrucosum (Rchb. f.) Pfitzer	*Chase 1331*	AF262820	Pridgeon *et al.* (2001)
Specklinia absurda Bogarín, Karremans & Rincón	*Bogarín 8711*	KC425827	Bogarín *et al.* (2013b)
Specklinia alajuelensis Karremans & Pupulin 1 (a)	*Karremans 3268*	KP012455	Karremans *et al.* (unp.)
Specklinia alajuelensis Karremans & Pupulin 1 (b)	*Karremans 3265*	KC425791	Karremans *et al.* (unp.)
Specklinia alajuelensis Karremans & Pupulin 2 (a)	*Karremans 5501*	KC425792	Karremans *et al.* (unp.)
Specklinia alajuelensis Karremans & Pupulin 2 (b)	*Bogarín 2895*	KP012454	Karremans *et al.* (unp.)
Specklinia barbae (Schltr.) Luer (a)	*Karremans 4853*	KC425771	Karremans *et al.* (unp.)
Specklinia barbae (Schltr.) Luer (b)	*Karremans 3928*	KC425769	Karremans *et al.* (unp.)
Specklinia chontalensis (A.H.Heller & A.D.Hawkes) Luer (b)	*Pupulin 6543*	KC425776	Karremans *et al.* (unp.)
Specklinia chontalensis (A.H.Heller & A.D.Hawkes) Luer (b)	*Pupulin 6543*	KF747799	Karremans *et al.* (unp.)
Specklinia costaricensis (Rolfe) Pridgeon & M.W.Chase	*Chase 5612*	AF262862	Pridgeon *et al.* (2001)
Specklinia digitalis (Luer) Pridgeon & M.W.Chase	*Karremans 5737*	KF747806	Karremans *et al.* (2015a)
Specklinia endotrachys (Rchb.f.) Pridgeon & M.W.Chase (a)	*Blanco 961*	KC425784	Karremans *et al.* (unp.)
Specklinia endotrachys (Rchb.f.) Pridgeon & M.W.Chase (b)	*Blanco 961*	KF747810	Karremans *et al.* (unp.)
Specklinia fuegi (Rchb.f.) Solano & Soto Arenas	*Karremans 5600*	KC425786	Bogarín *et al.* (2013b)
Specklinia fulgens (Rchb.f.) Pridgeon & M.W.Chase	*Chase 5630*	AF262872	Pridgeon et al. (2001)
Specklinia gersonii Bogarín & Karremans	*Karremans 6025*	KP012457	Karremans *et al.* (unp.)
Specklinia grobyi (Bateman ex Lindl.) F.Barros	*Chiron 04524*	JQ306485	Chiron *et al.* (2012)
Specklinia lanceola (Sw.) Lindl. (a)	*Pridgeon s.n.*	KC425838	Bogarín et al. (2013)
Specklinia lanceola (Sw.) Lindl. (b)	*Chase 1433*	AF262861	Pridgeon *et al.* (2001)
Specklinia lentiginosa (F.Lehm. & Kraenzl.) Pridgeon & M.W.Chase		AF275692	Pridgeon *et al.* (2001)
Specklinia lugduno-batavae Karremans, Bogarín & Gravend.	*Pupulin 7709*	KC425824	Karremans *et al.* (2015a)
Specklinia montezumae (Luer) Luer (a)	Karremans 229	KC425811	Bogarín *et al.* (2013b)
Specklinia montezumae (Luer) Luer (b)	*Karremans 5751*	KF747816	Karremans *et al.* (unp.)
Specklinia picta (Lindl.) Pridgeon & M.W.Chase	*Van Den Berg 2146*	JQ306384	Chiron *et al.* (2012)
Specklinia pissina (Luer) Solano & Soto Arenas (a)	*Karremans 4797*	KC425795	Karremans et al. (2015a)
Specklinia pissina (Luer) Solano & Soto Arenas (b)	*Karremans 4839*	KC425797	Karremans et al. (2015a)
Specklinia pfavii (Rchb.f.) Pupulin & Karremans (a)	*Karremans 4825*	KC425814	Karremans *et al.* (unp.)
Specklinia pfavii (Rchb.f.) Pupulin & Karremans (b)	*Karremans 3656*	KF747819	Karremans et al. (unp.)
Specklinia pfavii (Rchb.f.) Pupulin & Karremans (c)	*JBL-11086*	KF747820	Karremans et al. (unp.)
Specklinia aff. remotiflora Pupulin & Karremans	*Chase 1303*	AF262859	Pridgeon *et al.* (2001)
Specklinia remotiflora Pupulin & Karremans (a)	*Karremans 4798a*	KC425818	Karremans *et al.* (unp.)
Specklinia remotiflora Pupulin & Karremans (b)	*Karremans 4798b*	KC425819	Karremans *et al.* (unp.)
Specklinia remotiflora Pupulin & Karremans (c)	*Karremans 4854*	KC425820	Karremans *et al.* (unp.)
Specklinia sp.	*Karremans 5966*	KP012456	Karremans *et al.* (unp.)
Specklinia spectabilis (Ames & C.Schweinf.) Pupulin & Karremans (a)	*Bogarín 7401*	KP012457	This Study
Specklinia spectabilis (Ames & C.Schweinf.) Pupulin & Karremans (b)	*Karremans 5699*	KC425824	This Study
Specklinia subpicta (Schltr.) F.Barros	*Chiron 11046*	KC425830	This Study
Specklinia succulenta Bellone & Archila	*Bellone 680*	KC425828	This Study
Specklinia tribuloides (Sw.) Pridgeon & M.W.Chase	*Chase 5615*	JQ306389	Chiron *et al.* (2012)
Specklinia vittariifolia (Schltr.) Pridgeon & M.W.Chase (a)	*Karremans 2945*	JQ306383	Chiron *et al.* (2012)
Specklinia vittariifolia (Schltr.) Pridgeon & M.W.Chase (b)	*Karremans 5944*	AF262867	Pridgeon *et al.* (2001)

TABLE 4. Pairwise comparison of the number of single base differences amongst the nrITS sequences of *Specklinia alajuelensis*, *S. chontalensis*, *S. gersonii* and *S. vittariifolia*.

	S. alajuelensis	***S. chontalensis***	***S. gersonii***	***S. vittariifolia***
Specklinia alajuelensis	-	49–50	31–32	17–18
Specklinia chontalensis	49–50	-	44	45
Specklinia gersonii	31–32	44	-	26
Specklinia vittariifolia	17–18	45	26	-

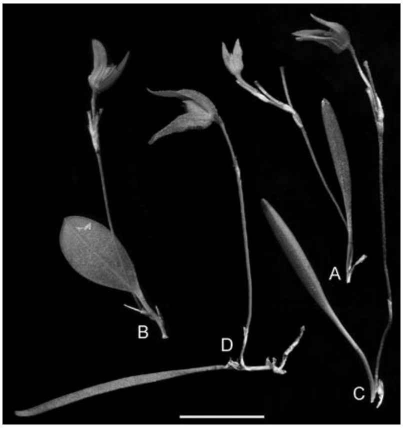


Figure 28. Comparison of the habits and flowers of species of the glandulous species of *Specklinia*. A. *S. alajuelensis* 1 (*Karremans 3265*). B. *S. gersonii* (*Karremans 6025*). C. *S. glandulosa* (*Karremans 6306*). D. *S. vittariifolia* (*Karremans 2945*). Scale bar = 1 cm. Photographs by A.P. Karremans.

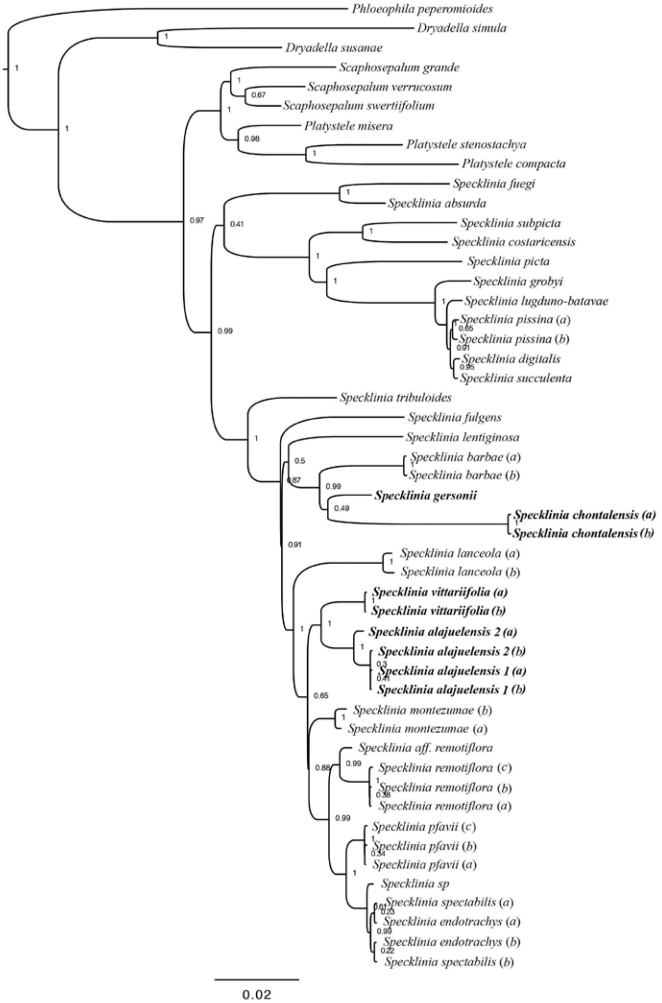


Figure 29. Phylogenetic relationship amongst the species of the glandulous *Specklinia*. The trees were produced with an analysis of a nrITS dataset of 50 sequences using BEAST v1.6.0. The resulting trees were combined using TreeAnnotator v1.6.0., where the first 3,000 trees were used as burn-in. Node values are posterior probabilities. The tree was edited using FigTree v.1.3.1. Branch lengths are relative to the relative number of changes. Names in bold correspond to species studied here.

Taxonomic Treatment

Phylogenetically, the glandulous *Specklinia* species are found in a clade, which includes *Specklinia lanceola* (Sw.) Lindl., the type species of the genus *Specklinia*. We recognize that at least 6 different *Specklinia* species share the small habit with a relatively long inflorescence, the single, bright yellow to orange flowers, flowering in succession, and a fully glandular pedicel, rachis, peduncle, ovary and external surface of the sepals (Fig. 28). These particular morphological features have evolved at least twice within the genus *Specklinia*, as the species treated here belong to two unrelated clades (Fig. 29). One clade includes *Specklinia chontalensis* (Heller & Hawkes) Luer, which is sister to *Specklinia barbae* (Schltr.) Luer. The second includes *Specklinia glandulosa*, which is sister to a clade that includes the species of the *Specklinia endotrachys* complex and *S. montezumae* (Luer) Luer.

Key to the glandulous species of Specklinia (as treated here)

1. Leaves elliptic to broadly elliptic, > 1 cm wide, peduncle lacking bracts, ovary trialate 2
\- Leaves narrowly linear to ligulate, < 4 mm wide, peduncle with a single bract, ovary terete 3

2. Flowers orange, sepals < 8 mm long, dorsal sepal with no wart-like transparencies, petals 3-veined, lip apically bifid-emarginate .... *S. gersonii*
\- Flowers yellow, sepals > 10 mm long, dorsal sepal with conspicuous wart-like transparencies, petals 2-veined, lip apically obtuse .... *S. chontalensis*

3. Inflorescence subequal to the leaf, arched to semi pendulous, flowers large, sepals exceeding 7.5 mm long and lip 4.5 mm long, lip with a pair of conspicuous sub-trapezoid lateral lobes .... *S. vittariifolia*
\- Inflorescence longer than the leaf, erect, flowers small, sepals between 4-6 mm long and lip 2.5-3.5 mm long, lip with a pair of relatively small triangular lateral lobes 4

4. Inflorescence distichous, frequently multi-flowered (up to 6 flowers) .... *S. alajuelensis*
\- Inflorescence sub-fascicled, few-flowered (frequently 1 or 2 flowers) 5

5. Inflorescence barely exceeding the leaf, flowers frequently cleistogamous, sepal ornamentation inconspicuous, petals narrow, oblong .... *S. pertenuis*
\- Inflorescence conspicuously exceeding the leaf, flowers not cleistogamous, sepal ornamentation conspicuous, petals falcate .... *S. glandulosa*

6. *Specklinia alajuelensis* Karremans & Pupulin, Phytotaxa 218(2): 108. 2015.

> *The species is similar to Specklinia endotrachys but can be distinguished by the small habit, shorter leaves, smaller flowers, the flat, non-ascending dorsal sepal (vs. ascending), and the obtuse, shortly apiculate (vs. emarginate and long mucronate) petals.*

Type:—VENEZUELA. Alajuela: San Ramón, Piedades Sur, San Miguel (La Palma). Camino a San Bosco, a orillas y dentro de un pequeño bosque secundario, 10º07’18.8”N 84º31’13.1”W, 1,062 m, 21 december 2010, *A.P. Karremans, J.A.J. Karremans & M. Contreras Fernández 3265* (holotype, JBL-spirit, D4704!; Figs. 27a, 28a & 30).

Epiphytic, caespitose, ascending, erect herb to 2.0–3.0 cm tall (excluding the inflorescence). Roots fibrous, flexuous, glabrous. Stem abbreviated, terete, to 0.2–0.9 cm long, completely concealed by papyraceous, subancipitous, acute sheaths to 0.5 cm long. Leaf narrowly obovate to linear, up to 18–27 × 2.5–3.5 mm, minutely and irregularly emarginate at apex, the mid-vein protruding abaxially into a small apicule, gradually tapering toward the base into a deeply conduplicate petiole, subcoriaceous. Inflorescence borne laterally from the apex of the stem, without an annulus, successively single flowered, up to 3.0–4.5 cm long, glandular; peduncle terete, to 4 cm long, with 1 distant, glandular, terete bract, 2–3 mm long. Floral bracts infundibuliform, glandular, broadly ovate, acute to subacuminate, 2 mm long. Pedicel terete, glandular, 13–15 mm long, persistent. Ovary subclavate, with low, irregularly crenulate crests, 1 mm long, green. Flowers up to 6 per inflorescence. Sepals fleshy, densely glandulose in the outer surface; dorsal sepal lanceolate-elliptic, 3-veined, acute, the base semi-hyaline, flushed with orange along the veins, the distal two thirds greenish, with reddish-orange veins, 5.0–6.0 × 1.5–2.0 mm; lateral sepals narrowly elliptic-oblanceolate, subfalcate, 3-veined, 5.0–6.0 × 2.5–3.5 mm, connate for about half their length, the base saccate, membranaceous-hyaline, the apex acute, the veins strongly carinate abaxially. Petals small, ligulate-falcate, acute, 2.0–2.5 × 1.0 mm, 2-veined. Lip reddish-orange, small, longitudinally arched-convex in natural position, thinly articulate with the column foot by a hyaline claw, the blade sagittate to sub-triangular when expanded, obtuse, 2.5 × 0.8 mm, provided with a pair of acute, triangular lateral lobes at about the middle of the blade, margin dentate-erose, especially apically. Column dark-red, arched, terete and slender at the base, 1.5 mm long without the foot, provided with membranous wings, serrulate along the margins, the apex prolonged into a deeply cucullate, lacerate clinandrium; column foot, stout, fleshy, 0.7 mm long. Anther cap deeply cucullate, ovate, 2-celled. Pollinia 2, obovate-complanate, minutely hooked at the base. *NOTE: Description based on *AK3265*, *AK3266* & *FP8470*.

Other material examined (morph 1):—COSTA RICA. Alajuela: San Ramón, Piedades Sur, San Miguel (La Palma). Camino a San Bosco, a orillas y dentro de un pequeño bosque secundario, 10º07’18.8”N 84º31’13.1”W, 1,062 m, 21 December 2010, *Karremans, Karremans & Contreras Fernández 3265* (JBL-spirit, D4704!); Idem, *Karremans et al. 3266* (JBL-spirit; D6078!, D5956!, D4721!); Idem, *Karremans et al. 3268* (JBL-spirit; D6074!). San Ramón, Santiago, road to Berlín, Balboa, 10º02’30”N 84º29’30”W, 1,230 m, premontane moist forest, epiphytic on trees along the roadside, 30 May 2013, *Pupulin, Bogarín, Díaz & Fernández 8469* (JBL-spirit); Idem, *Pupulin et al. 8470* (JBL-spirit; D6126!). Idem, *Pupulin et al. 8471* (JBL-spirit; D5873!). San Ramón, Santiago, camino a Balboa, 10°02’24.76”N 84°29’29.88”W, 1,222 m, epífitas en árboles aislados, bosque pluvial premontano, 30 May 2013, *Bogarín, Díaz, Fernández & Pupulin 10193* (JBL-spirit, D5867!). San Ramón, Piedades Sur, Potrerillos, 4 km E. of Piedades Sur, 1,235 m, 22 June 1969, *Lent 1762a* (CR!). San Ramón, Piedades, alt. 1,100 m, 21 June 1925, Brenes 1285 (78) (CR!). San Ramón, Piedades, alt. 1,100 m, 29 november 1925, *Brenes 1431 (244)* (CR!). San Ramón, camino de Piedades, alt. 1,025 m, 5 July 1924, *Brenes 2213 (32)* (CR!). San Ramón, el Socorro, alt. 1,050 m, 25 July 1924, *Brenes 2237 (84)* (CR!). Sine loc., ca. 1867, *Endrés 52* (W!).

Other material examined (morph 2):—COSTA RICA. Alajuela: Upala, Parque Nacional Rincón de la Vieja, road to Colonia Blanca by Quebrada Rancho Grande, 700 m, 7 July 1978, *Todzia 354* (CR!). San José: Vazquez de Coronado, Braulio Carrillo Nat. Park. Along sendero La Botella, 10º10’00”N 83º57’20”W, 750 m, 21 September 1990, *Ingram & Ferrell 558* (MO; CR!; SEL!). Limón: Pococí, Guápiles, 1 km después del puente sobre el Río Corinto en dirección a Guápiles, 10°12’40.9”N 83°52’38.5” W, 300 m, bosque muy húmedo tropical, epífitas en bosque secundario, 15 June 2006, *Bogarín, Dressler, Gómez-Laurito & Pupulin 2895* (JBL-spirit!; Fig. 27b & 31). Grecia, San Isidro, Coope Victoria, rio Rosales, del Puente 1 km al Sur, *Alfaro & Rodríguez 8* (Epidendra!). Guanacaste: Tilarán, Hno Jorge de la Cruz legit, *Karremans 5501* (JBL-spirit, D5148!). NICARAGUA. Rio San Juan: between Río Santa Cruz and Caño Santa Crucita, La Palma 11°02–04’N 84°24–26’W, elevation 40–60 m; tall evergreen forest, 30 November–2 December 1984, *Stevens, Montiel & Robleto 23460* (SEL!; MO!). Zelaya [Región Autónoma del Atlántico Sur]: along road to Colinia Yolaina, Colonia La Esperanza, etc., ca. 1.3 km SE of intersection with road between Nueva Guinea and Colonia Verdun, immediately upriver from bridge over Caño Sardina; ca. 11°40’N 84°26’W, elev. ca. 180–200 m; disturbed evergreen forest and river banks, 11–12 February 1978, *Stevens & Krukoff 6294* (MO!). Zelaya [Región Autónoma del Atlántico Norte]: 0.5–1.5 km from Plantel El Salto along road to Bonanza, slipe above Río Pis Pis; ca. 14°03’N 84°37’W, elevation ca. 140 m, tall evergreen forest on steep slopes and pastures, 16 December 1980, *Stevens & Krukoff 18814* (MO!).

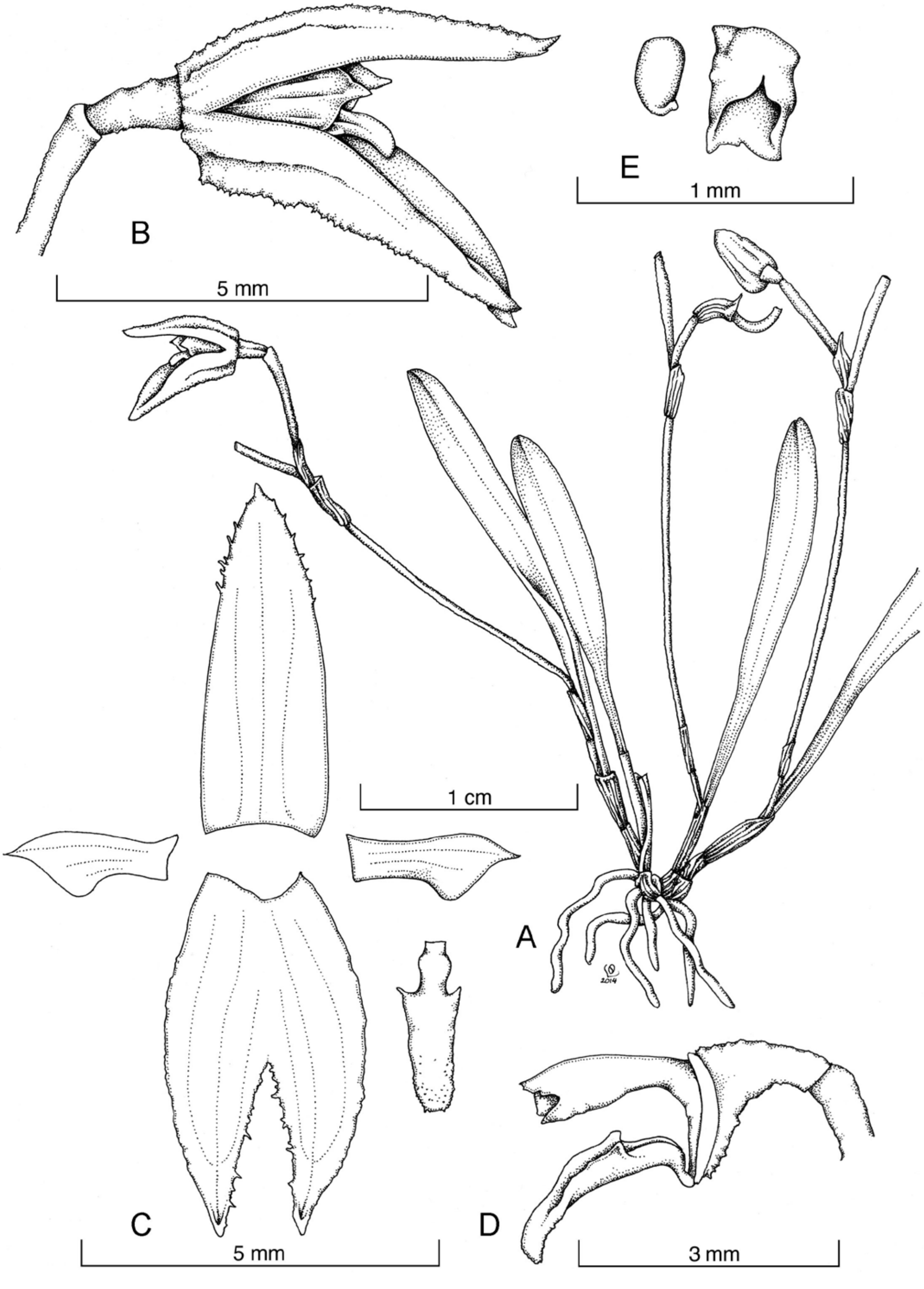


Figure 30. *Specklinia alajuelensis* Karremans & Pupulin. A: habit; B: flower; C: dissected perianth; D: column and lip, lateral view; E: anther and pollinaria. Drawn by A.P. Karremans & L. Oses from *A.P. Karremans 3265* (JBL-spirit).

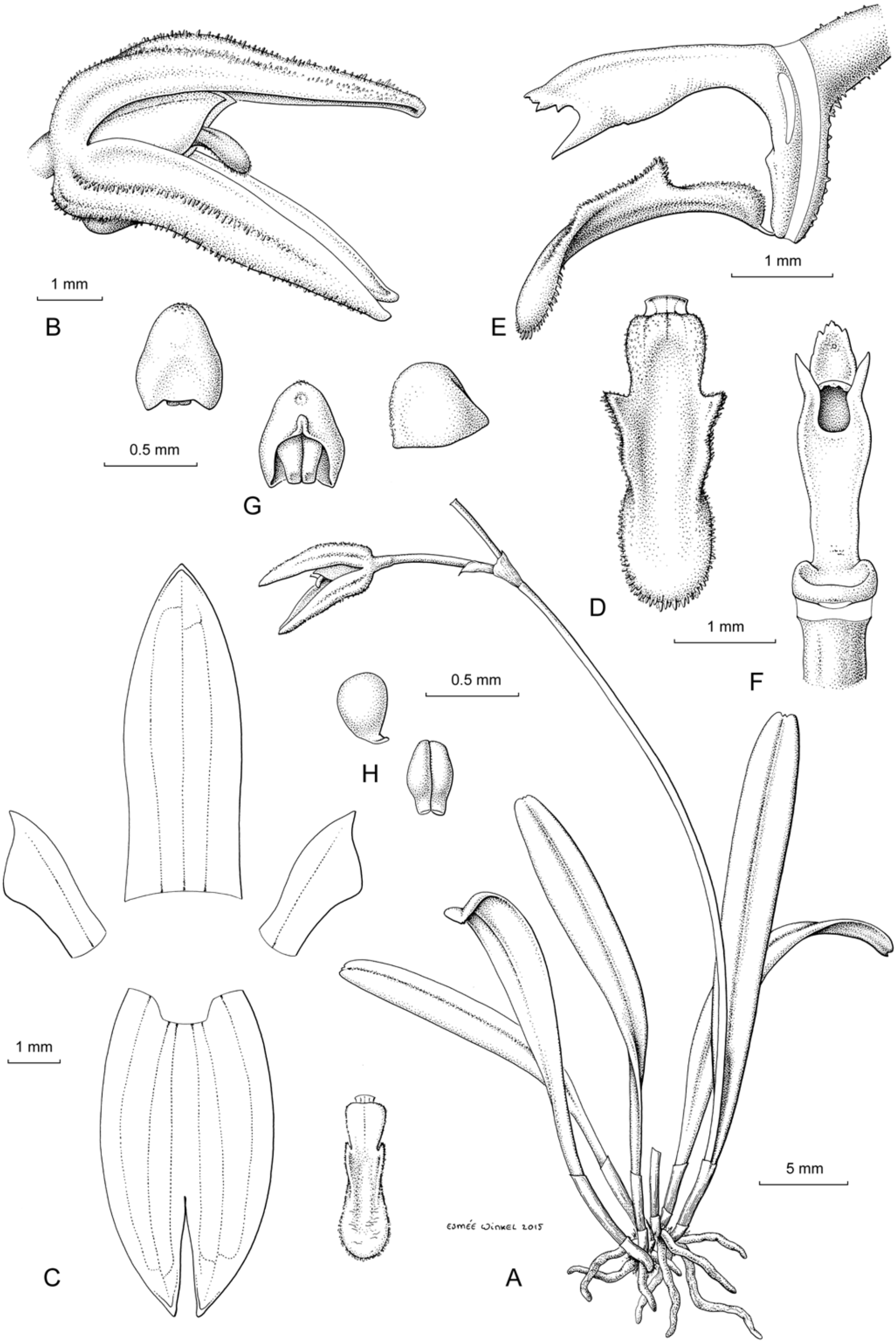


Figure 31. *Specklinia alajuelensis* Karremans & Pupulin. A: habit; B: flower; C: dissected perianth; D: lip; E: column and lip, lateral view; F: column, ventral view; G: anther with pollinaria; H: pollinaria. Drawn by E. Winkel from *D. Bogarín 2895* (JBL-spirit; L-spirit).

Etymology:—The name refers to the province of Alajuela in Costa Rica, where the type and most other specimens were collected.

Distribution:—Known only from Nicaragua and Costa Rica (Fig. 32), where it is found growing between 300–1,235 m in elevation.

Notes:—The first to collect this species was probably A. Endrés. *Endrés 52* was collected in Costa Rica, without a more specific locality. Nevertheless, a vast majority of his collections come from San Ramón, Alajuela, where this species is common. Luer also listed Endrés 46 under *Specklinia glandulosa*, however we believe that number 46 is actually *Specklinia acicularis* (Ames & C.Schweinf.) Pridgeon & M.W.Chase. The latter has similarly thin leaves but lacks the glandular ornamentation on the inflorescence and exterior of the sepals, and has a dark purplish to brownish coloration of the flower.

The typical form of this species (morph 1) has relatively short leaves, which are shorter than the multi-flowered inflorescence. The floral segments are typically not spreading. The species is common around San Ramón in Alajuela, at elevations between 1,025–1,235 m. Plants with similar characteristics (morph 2) are found in the Caribbean lowlands, at elevations between 300–750 m. Aside from the obvious ecological differences they can also be set aside morphologically, and could represent a different species. At this time we prefer to include them here until more evidence can be presented.

In Costa Rica, *S. alajuelensis* and *S. vittariifolia* have been confused with each other in herbaria. The first can be easily recognized from the second by the leaves that are less than 10 times as long as wide (while the second have extremely narrow leaves that can be more than 15 times longer than wide). The inflorescence of *S. alajuelensis* produces a lax inflorescence of an extremely slow succession, and always becomes longer than the leaf. The inflorescence of *S. vittariifolia* does not exceed the leaf length even though it can also produce several flowers over time; the flowers are born closely together making the inflorescence sub-fascicled.

7. *Specklinia chontalensis* (A.H.Heller & A.D.Hawkes) Luer.

Basionym: *Pleurothallis chontalensis* A.H.Heller & A.D.Hawkes. Type:—NICARAGUA. Chontales: Río Mico, epiphytic, alt. 1500 ft, Aug 1960, *A.H. Heller 3735* (holotype, AMES!).

Epiphytic, caespitose, ascending, erect herb to 6 cm tall (excluding the inflorescence). Roots fibrous, flexuous, glabrous. Stem abbreviated, terete, to 0.5–0.7 cm long, completely concealed by papyraceous, subancipitous, acute sheaths to 0.5 cm long. Leaf obovate-elliptic, up to 30–45 × 5–10 mm, minutely and irregularly emarginate at apex, gradually tapering toward the base into a deeply conduplicate petiole, subcoriaceous. Inflorescence borne laterally from the apex of the stem, without an annulus, successively single flowered, up to 4.0–5.0 cm long, glandular; peduncle terete, to 3.0–4.0 cm long, without bracts. Floral bracts infundibuliform, glandular, broadly ovate, acute, 1.0–1.5 mm long. Pedicel terete, glandular, 4–6 mm long, persistent. Ovary trialate, subclavate, 2–3 mm long, green. Flowers at least up to 5 per inflorescence, normally yellowish, rarely orange. Sepals fleshy, carinate, microscopically glandulose on both surfaces, and especially the margin; dorsal sepal lanceolate-elliptic, 3-veined, acute, the base semi-hyaline, conspicuously covered with inflated, wart-like transparencies, 10.5–11.0 × 3.0–3.2 mm; lateral sepals elliptic, 3-veined, 9.5–10.0 × 4.5 mm, connate for about three fourth of their length, the base saccate, membranaceous-hyaline, the apex acute, the veins strongly carinate abaxially. Petals small, ligulate-falcate, oblique, acute, 2.6–2.7 × 1.0 mm, 1 or 2-veined. Lip yellowish-orange, small, longitudinally arched-convex in natural position, thinly articulate with the column foot by a hyaline claw, ligulate when expanded, obtuse, 3.0 × 1.0–1.1 mm, fully papillose provided with a pair of acute, erect, triangular lateral lobes from just below to just above the middle, margin dentate-erose, especially apically. Column, arched, terete and slender at the base, 2.5–3.0 mm long without the foot, provided with membranous wings, serrulate along the margins, the apex prolonged into a deeply cucullate, lacerate clinandrium; column foot, stout, fleshy, 1.2–1.5 mm long. Anther cap deeply cucullate, ovate, 2-celled. Pollinia 2, obovate-complanate, minutely hooked at the base. *NOTE: Description based on *FP6543* and the original protologue.

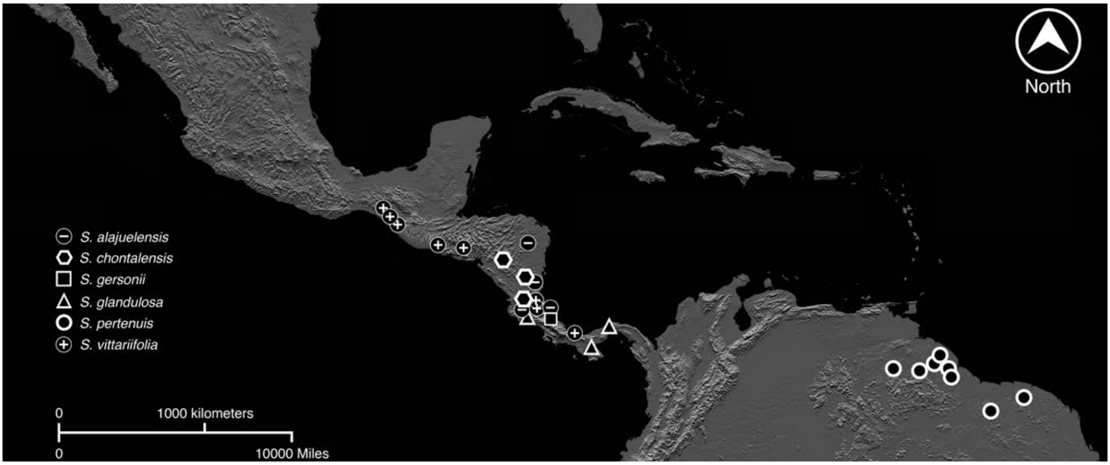


FIGURE 32. General distribution map of the glandulous species of *Specklinia*. Edited by D. Bogarín.

Other material examined:—COSTA RICA. Alajuela: Upala, road from Upala, ca. 3 km after Bijagua, turning toward Volcán Tenorio, northern slopes of Volcán Tenorio, Caribbean watershed, first bridge on the road, 10º45'52.2"N 85º01'04.4"W, 320 m, tropical moist forest, 28 March 2007, *F. Pupulin, D. Bogarín, S. Dalström, G. Gigot & M. Powell 6543* (JBL-spirit, D1926!, D2376!, D5150!; L-spirit!; Fig. 27c & 33). Upala, Aguas Claras, Colonia Blanca, camino entre Colonia Verde y Colonia Libertad hacia Buenos Aires, faldas al noreste del Volcán Rincón de La Vieja, 10°52'26.2"N 85°14'51.3"W, 550–600 m, bosque muy húmedo tropical, epífitas en potreros y árboles aislados, 4 febrero 2006, *D. Bogarín, J. Barrantes, R.L. Dressler, R. Gómez & A. Rojas 2557* (JBL-spirit, D0721!). Santa Maria National Park, primary forest, Caribbean slope, alt. 600 m, 8 Feb. 1978, *R. Liesner 5187* [MO; illustration by Luer (2006)!]. Guanacaste: Liberia, road from Potrerillos to Brasilia, proximity of Hacienda La Josefina, Pacific watershed of northern volcanic chain, 10º52'15.3"N 85º27'02.2"W, 620 m, tropical dry forest, 27 March 2007, *F. Pupulin, D. Bogarín, S. Dalström, G. Gigot & M. Powell 6521* (JBL-spirit, D3293!). NICARAGUA. Chontales: Río Mico, epiphytic, alt. 1400 ft, Aug 1960, *A.H. Heller 7827* (AMES). Cerro El Chamarro, La Liberta district, alt. 2,175 ft., *A.H. Heller 1036* (AMES).

Etymology:—The name refers to the department of Chontales, southern Nicaragua, where the type specimen was collected.

Distribution:—This species is only known from Nicaragua and Costa Rica (Fig. 32). It is found growing at elevations between 320–660 m.

Notes:—*Specklinia chontalensis* has been traditionally considered well distinguished from *S. glandulosa*. Of the species treated here this is probably the easiest to distinguish morphologically from the other members. The species does share some similarities in floral morphology, however it is mostly included here because of its glandular inflorescence and sepals, and it being sister to *S. gersonii.* The large yellow flowers with the dorsal sepal covered with inflated, wart-like transparencies set it aside immediately.

8. *Specklinia gersonii* Bogarín & Karremans, Phytotaxa 218(2): 112. 2015.

> *The species is similar to Specklinia glandulosa (Ames) Pridgeon & M.W.Chase, but can be easily distinguished by the wider and sub-orbicular (vs. linear) leaves, the lack of a bract on the peduncle, the trialate ovary (vs. terete), the wider dorsal sepal (2.5 vs 1.5 mm), and the widely rounded apical half of the sepals in natural position (vs. narrow and elongate).*

Type:—COSTA RICA. Limón: Guácimo, Pocora, La Argentina, camino a la catarata del río Dos Novillos, ca. 600 m, invenit Gerson Villalobos et Daniel Matamoros, floreció en cultivo en el Jardín Botánico Lankester, 12 abril 2012, *D. Bogarín 9565* (holotype, JBL-spirit, D5192!; isotype, JBL-spirit, D5197!; Fig. 34).

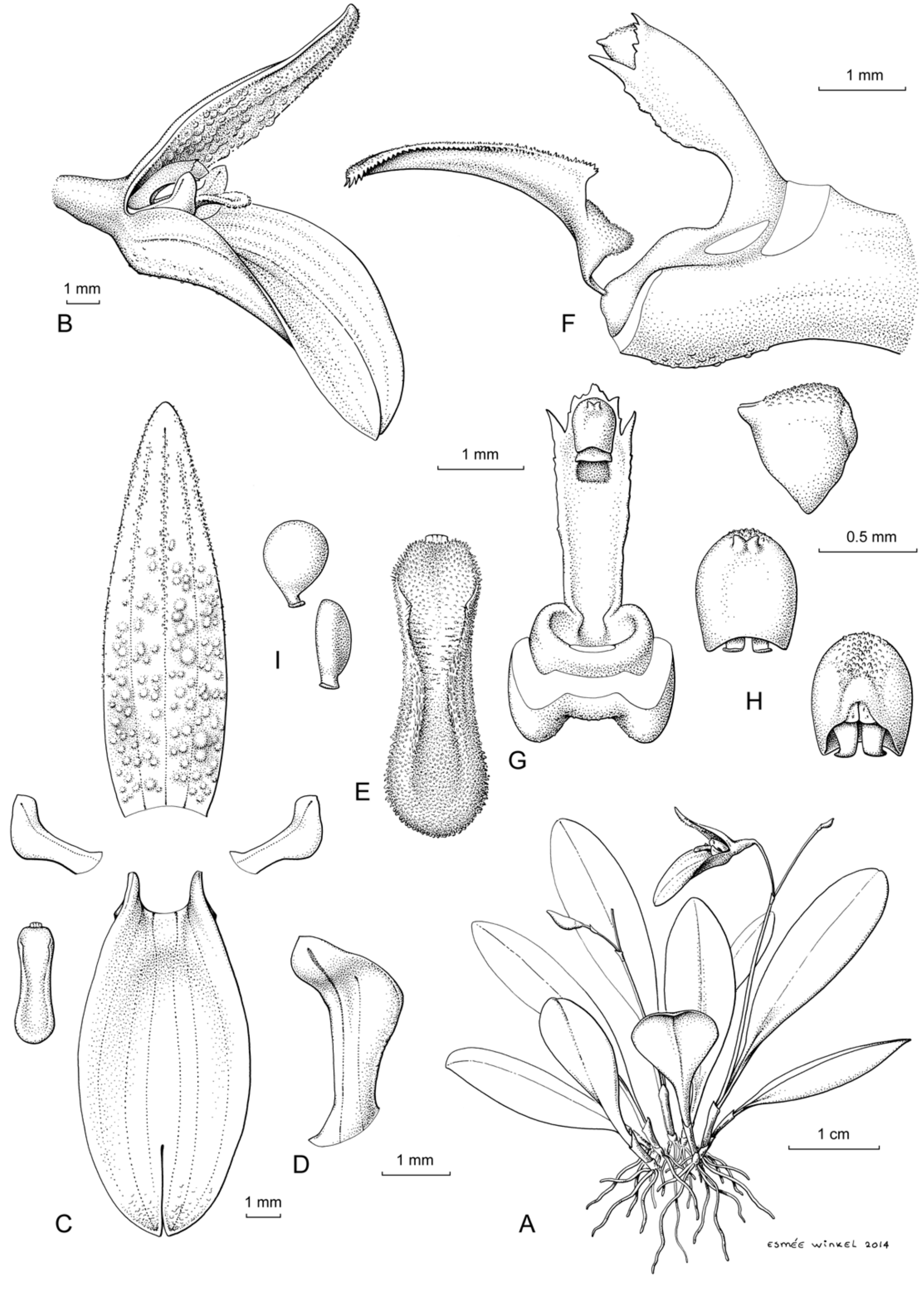


FIGURE 33. *Specklinia chontalensis* (A.H.Heller & A.D.Hawkes) Luer. A: habit; B: flower; C: dissected perianth; D: petal; E: lip; F: column and lip, lateral view; G: column, ventral view; H: anther with pollinaria; I: pollinaria. Drawn by E. Winkel from *F. Pupulin 6543* (JBL-spirit; L-spirit).

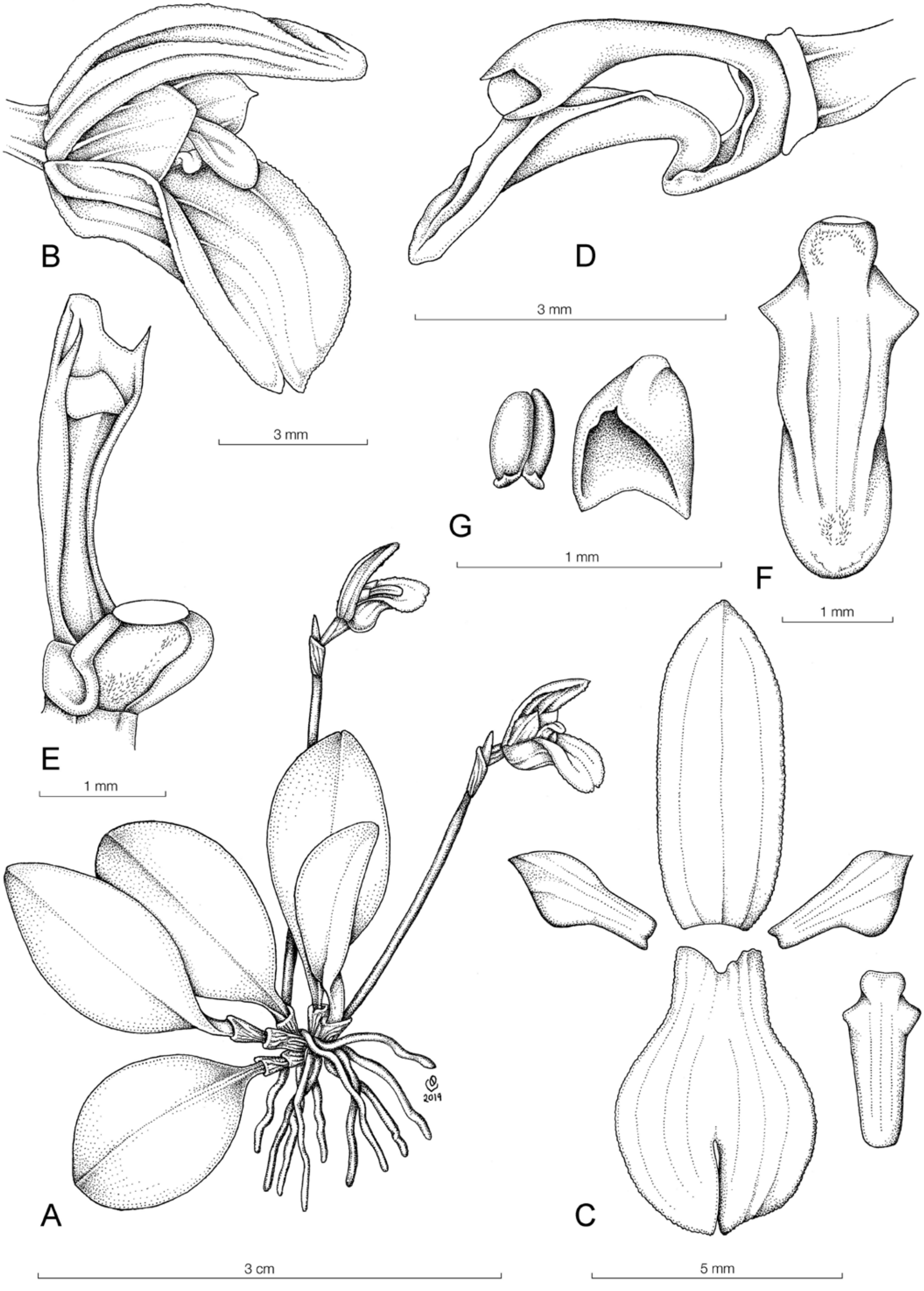


Figure 34. *Specklinia gersonii* Bogarín & Karremans. A: habit; B: flower; C: dissected perianth; D: column and lip, lateral view; E: column ventral view; F: lip; G: anther and pollinaria. Drawn by D. Bogarín & L. Oses from *Bogarín 9565* (JBL-spirit).

Epiphytic, caespitose, ascending, erect herb to 4.0 cm tall. Roots fibrous, flexuous, glabrous, to 1 mm in diameter. Stem abbreviated, terete, to 5 mm long, concealed by a papyraceous, subancipitous, acute sheath to 4 mm long. Leaf elliptic to broadly elliptic, up to 20 × 12 mm, minutely and irregularly emarginate at apex, the mid-vein protruding abaxially into a small apicule, gradually tapering toward the base into a deeply conduplicate petiole, subcoriaceous. Inflorescence borne laterally from the base of the leaf, without an annulus, successively single flowered, up to 3 cm long, glandular; peduncle terete, to 2 cm long, without bracts. Floral bracts infundibuliform, glandular, broadly ovate, acute to subacuminate, 4 mm. Pedicel terete, glandular, 3.5 mm long, persistent, appearing fascicled. Ovary trialate, subclavate, 2 mm long, greenish to orange. Flowers up to 4, only one developed at a time; with fruity fragrance around midday. Sepals fleshy, densely microscopically-glandulose on the outer surface; dorsal sepal narrowly-elliptic, 3-veined, acute, greenish orange, with bright orange-red, the margins microscopically glandulous, 7.0–8.0 × 2.5 mm; lateral sepals narrowly elliptic-oblanceolate, subfalcate, 3-veined, 6.5–7.5 × 4.0–5.0 mm, connate for about two thirds of their length, the midvein strongly carinate abaxially. Petals small, lanceolate-falcate, acute, 3.1–3.3 × 1.4–1.6 mm, 3-veined. Lip reddish-orange, small, longitudinally arched-convex in natural position, thinly articulate with the column foot by a hyaline claw, apically bifid-emarginate in natural position, sagittate to sub-triangular when expanded, obtuse, 3.5 × 1.4–1.5 mm, provided with a pair of sharp, triangular lateral lobes at the base. Column dark-red, arched, terete and slender at the base, 2.5 mm long without the foot, provided with membranous wings, the apex prolonged into a deeply cucullate, lacerate clinandrium; column foot, stout, fleshy, 1 mm long. Anther cap deeply cucullate, ovate, 2-celled. Pollinia 2, obovate-complanate, minutely hooked at the base. *NOTE: Description based on *DB9565* & *AK6025*.

Other material examined:—COSTA RICA. Limón: Guácimo, Pocora, La Argentina, camino a la catarata del río Dos Novillos, 10°06’07.71” N 83°39’28.74” W, 591 m, bosque muy húmedo tropical transición a premontano, 25 noviembre 2013, *A.P. Karremans, D. Bogarín & G. Villalobos 6025* (JBL-spirit!; Fig. 27d & 28b).

Etymology:—The name honors Gerson Villalobos, a Costa Rican orchid enthusiast who brought this species to our attention.

Distribution:—Known only from Costa Rica, growing at elevations around 600 m (Fig. 32).

Notes:—Despite its appealing bright orange flowers and easily recognizable broadly elliptic leaves it seems that *Specklinia gersonii* Bogarín & Karremans had eluded botanists so far. The species is as far as we know very rare; in fact we are aware only of a handful of plants, all from a single population on a trail along the Dos Novillos river in Limón.

9. *Specklinia glandulosa* (Ames) Pridgeon & M.W.Chase.
Basionym: *Pleurothallis glandulosa* Ames. Type:—PANAMA. Juan Grande range, sea level, *C.W. Powell 306* (holotype, AMES!; isotypes, AMES!, MO!; Fig. 35).

Epiphytic, caespitose, ascending, erect herb to 2.5 cm tall (excluding the inflorescence). Roots fibrous, flexuous, glabrous. Stem abbreviated, terete, to 5 mm long, completely concealed by papyraceous, subancipitous, acute sheaths. Leaf narrowly linear, up to 18–21 × 1.5–2.0 mm, gradually tapering toward the base into a deeply conduplicate petiole, subcoriaceous. Inflorescence borne laterally from the apex of the stem, without an annulus, successively single flowered, up to 3.5 cm long, glandular; peduncle terete, with 1 distant, glandular, terete bract. Floral bracts infundibuliform, glandular, broadly ovate, acute. Pedicel terete, glandular, 4 mm long, persistent. Ovary terete. Flowers 1 (probably up to a few with time) per inflorescence, Pompeian red. Sepals fleshy, densely glandulose in the outer surface; dorsal sepal narrowly lanceolate, 3-veined, acute, 8.0 × 1.5 mm; lateral sepals linear-lanceolate, subfalcate, 3-veined, 8.0 × 1.0 mm, connate to below the middle, the base saccate, the apex acute, the veins strongly carinate abaxially. Petals small, ligulate-falcate, acute, 3 × 1.5 mm, 2-veined. Lip small, longitudinally arched-convex in natural position, thinly articulate with the column foot by a hyaline claw, subpandurate from a cordate-sagittate base when expanded, obtuse, 3.5 × 1 mm, provided with a pair of acute, triangular lateral lobes from just below to just above the middle, margin dentate-erose, especially apically. Column arched, terete and slender at the

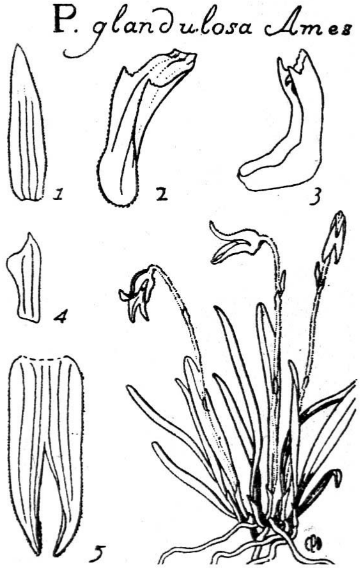


Figure 35. Type illustration of *Specklinia glandulosa* (Ames) Pridgeon & M.W.Chase, published in Ames (1923). Reproduced with the kind permission of the Orchid Herbarium of Oakes Ames, the Harvard University Herbaria.

base, 2 mm long without the foot, provided with membranous wings, margins irregular, the apex prolonged into a deeply cucullate, dentate clinandrium; column foot, stout, fleshy. *NOTE: Description adapted from the protologue.

Other material examined:—COSTA RICA. Puntarenas: Along N fork (known locally as "Quebrada Mona") of Quebrada Bonita, Carara reserve. 9º47'N 84º36'W, elev. 35–40 m. Epiphytic on branch of large fallen tree in primary forest. 31 August 1985, *Michael H. Grayum, R. Warner, P. Sleeper & S. Phelps 5939* (MO!). Without specific collection data, cultivated by Gerson Villalobos, flowered 29 August 2014, *A.P. Karremans 6306* (JBL-spirit!; Fig. 27e, 28c & 36). PANAMA. Herrera: Distrito de Las Minas, alrededor del primer Ciclo de Chepo, ca. 900 m, 7º43'N 80º50'O, bosque nuboso. 29 de septiembre de 1994, *C. Galdames, E. Montenegro, C. Chung & E. Valdez 1758* (PMA!). Chepo de las Minas, *K.S. Walter 78-1518*, flowered in cultivation at SEL, 29 Apr. 1980, C.A. Luer 5237 [SEL!; illustration by Luer (2006)!]. 18 km W of Las Minas, N slope of Cerro Alto Higo; 2400–3000 ft. (known locally as el Montoso); 6 Aug. 1978, *B. Hammel 4289* (MO!). Veraguas: Coiba. Parque Nacional Coiba, afluente del río Santa Clara, orilla del río, 24 julio 2005, *A. Ibáñez & C. Núñez 4342* (PMA!). Panama: Cerro Azul, near Goofy Lake, 24 Aug. 1960, *J.E. Ebinger 984* (MO!). Los Santos: Loma Prieta, Cerro Grande, alt. 2400–2800 ft. Cloud forest and disturbed margins, 8 June 1967, *W.H. Lewis, R.K. Baker, B. MacBryde & R.L. Oliver 2214* (MO!).

Etymology:—The name comes from the Latin *glandula* meaning "diminutive of gland", referring to the conspicuous presence of small glands covering most floral parts and inflorescence.

Distribution:—Known only from Costa Rica and Panama, from around sea level to up to 900 m (Fig. 13).

Notes:—The name *Specklinia glandulosa* has been applied to most of the species treated here at some point. Nevertheless, it can be recognized by the extremely long inflorescence in relation to the leaf (close to twice the length). The sepals are relatively long and narrow in comparison with *S. alajuelensis*, *S. pertenuis* and *S. vittariifolia*. Although it can produce more than a single flower per inflorescence with time, these are closely placed (sub-fascicled), and the species normally appear to have a single flowered inflorescence.

In Costa Rica this species seems to be restricted to the lowlands of the Central and South Pacific, and it is apparently seen very rarely. Most known specimens are from Central Panama.

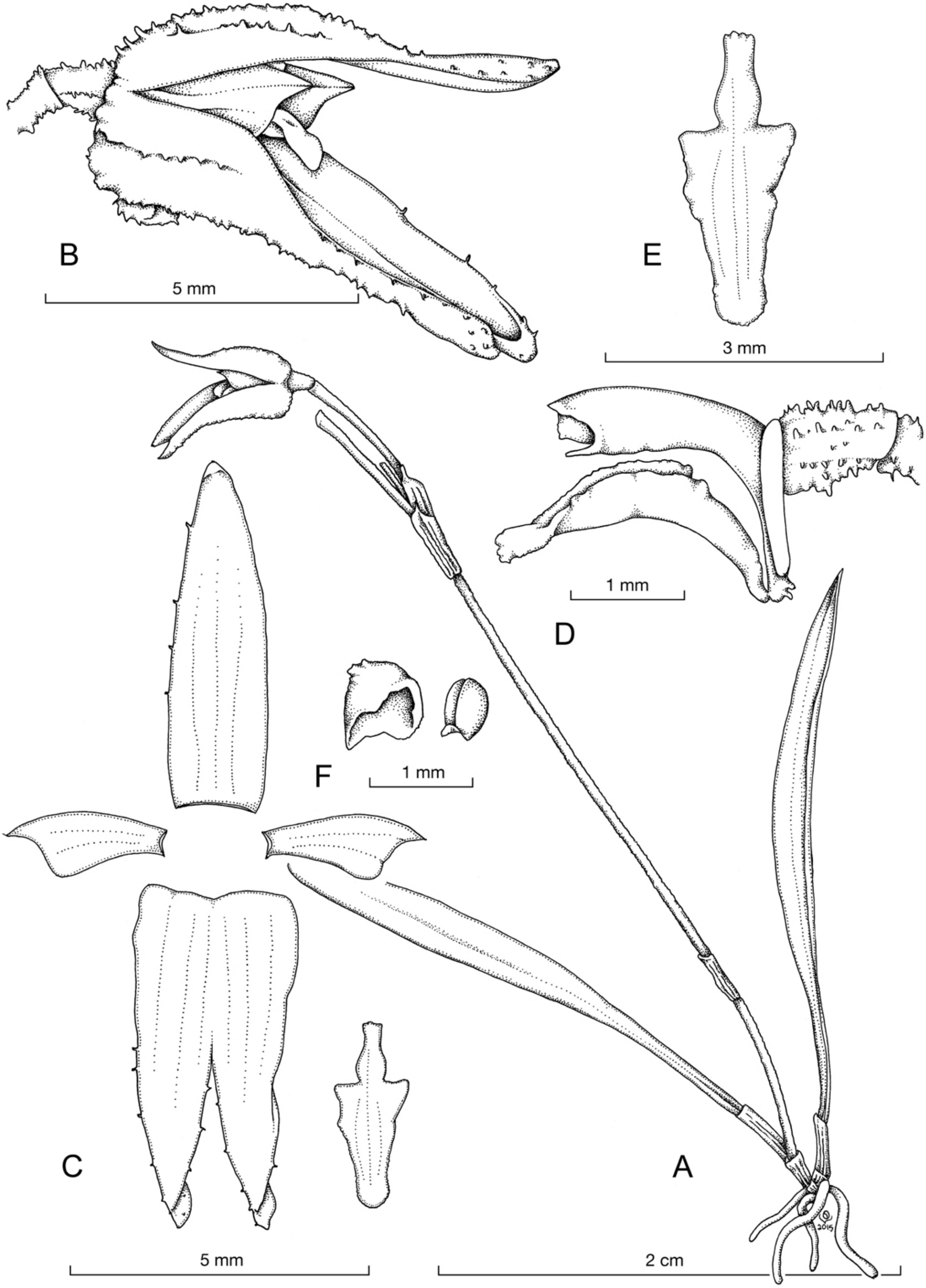


Figure 36. *Specklinia glandulosa* (Ames) Pridgeon & M.W.Chase. A: habit; B: flower; C: dissected perianth; D: column and lip, lateral view; E: lip; F: anther and pollinaria. Drawn by A.P. Karremans & L. Oses from *Karremans 6306* (JBL-spirit).

10. *Specklinia pertenuis* (C. Schweinf.) Karremans & Gravend., Phytotaxa 218(2): 116. 2015.
Basionym: *Pleurothallis pertenuis* C.Schweinf. Type:—GUYANA. Essequibo River, Moraballi Creek, near Bartica, nearly at sea-level. 15 Sept 1929, *N.Y. Sandwith 304* (holotype, K!; isotype, AMES; illustration of type, AMES!).

Epiphytic, caespitose, ascending, erect herb. Roots fibrous, flexuous, glabrous, to 1 mm in diameter. Stem abbreviated, terete, to 3.7 mm long, completely concealed by two papyraceous, subancipitous, acute sheaths. Leaf narrowly linear or oblanceolate-linear, coriaceous, fleshy, up to 22 × 1.7 mm, minutely and irregularly emarginate at apex, the mid-vein protruding abaxially into a small apicule, gradually tapering toward the base into a deeply conduplicate petiole, subcoriaceous. Inflorescence slender, successively single flowered, in anthesis shortly surpassing the leaves, minutely puberulent. Floral bracts infundibuliform, puberulent. Pedicel conspicuously protruding beyond the floral bract. Ovary slenderly terete, muricate. Flower buds brownish orange. Dorsal sepal lanceolate, 3-veined, acute, concave, 4 × 1.8 mm; lateral sepals elliptic-ovate, 3-veined, 4.1 × 2.2 mm, connate for about half of their length. Petals asymmetrical, oblong-spatulate, 2.7 × 1 mm, 1-veined. Lip sagittate-oblong, with acute, spreading angles just below the middle, 3 × 1.4 mm, rounded at the apex, finely papillose on the upper surface. Column stout, 2 mm long, narrowly winged on each side. *NOTE: Description is adapted from the original protologue.

Other material examined:—BRAZIL. Estado do Para, Municipio de Sao Felix do Xingu, vila Canaa, fazenda do Sr. Josue, 4 Jan. 1997, *J.B.F. da Silva 630* (MG!). GUYANA. Roriabo, 7 Nov. 1894, *E.F. im Thurn 84* (K; photograph of type, AMES!); Aruka, June 1897, *E.F. im Thurn 84* (K; photograph of type, AMES!); B.-B. [Barima-Barama] Road. Nov. 1896, *E.F. im Thurn 84* (K; photograph of type, AMES!) between Mazaruni Station and Labbakabra Creek, 27 Apr. 1937, *N.Y. Sandwith 1225* (K); Pomeroon River, Mar. 1884, *G.S. Jenman 1996* (K); Upper Mazaruni District, adjacent to Eboropu Mountain, alt. 470 m, 8 Apr. 1979, *P.J. Edwards 1188* (K). SURINAME. Brownsberg Mazaroni top, 16 April 1981, *R. Determann 148* (SEL!). Wilhelmina Mts., 15 July 1981, R. Determann 81-2168, flowered in cultivation 14 Feb. 1982, *C.A. Luer 6829* (illustration, SEL!). Lely Mountains, distr. Marowikne, 1 June 1976, *M. & P. Teunissen 1631* (SEL!). VENEZUELA. 113 km. south of El Dorado, in fairly dense forest at about 600 m, *G.C.K. Dunsterville 430* (illustration, AMES!; Fig. 37).

Etymology:—The name comes from the Latin *tenuis* meaning "thin or fine", referring to the "very slender" plants.

Distribution:—Known from Venezuela, Guyana, Suriname and Brazil, where it is typically found growing at low elevations, from around sea level to up to 600 m (Fig. 32).

Notes:—When describing *Specklinia pertenuis* (as *Pleurothallis pertenuis*), Schweinfurth recognized it closely resembled *P. vittariifolia* (= *Specklinia vittariifolia*). He distinguished the two species apart on the basis of the glabrous sepals of the first, which are characteristic of the second. Illustrations by Dunsterville and Luer of material from Venezuela and Suriname, respectively, show flowers with less conspicuous ornamentation than observed in *S. glandulosa* and *S. vittariifolia*, but nonetheless present. It is possible that the inconspicuous ornamentation of Schweinfurth's plants was even more difficult to observe in herbarium material. The disposition of the floral parts in the illustration and the statement in the protologue that "bud brownish orange", suggests that the type illustration was prepared from a cleistogamous flower. In fact, ovaries are swollen in many flowers of the herbarium specimens and illustrations.

Material from Brazil, Guyana, Surinam and Venezuela is therefore recognized a distinct species under the name Specklinia pertenuis. It can be recognized by the inflorescence that barely exceeds the leaf, and is rarely multi-flowered, the frequently bears cleistogamous flowers, with inconspicuous sepal ornamentation, very narrow sepals and petals, and a lip with sharp-triangular spreading angles just below the middle.

The plant illustrated by Silva & Silva (1997) of Brazilian origin (*Silva 369*; MG!) shows perianth parts typical of the *S. glandulosa* group, and comparable to those of *S. pertenuis* particularly. Nevertheless the leaves are much broader and have a different shape. Another Brazilian collection (*Silva 630*; MG!) does overlap well with *S. pertenuis*. Both specimens come from Sao Felix do Xingu, and at first glance do not seem to be the same species. Without more material it is difficult to determine them with certainty.

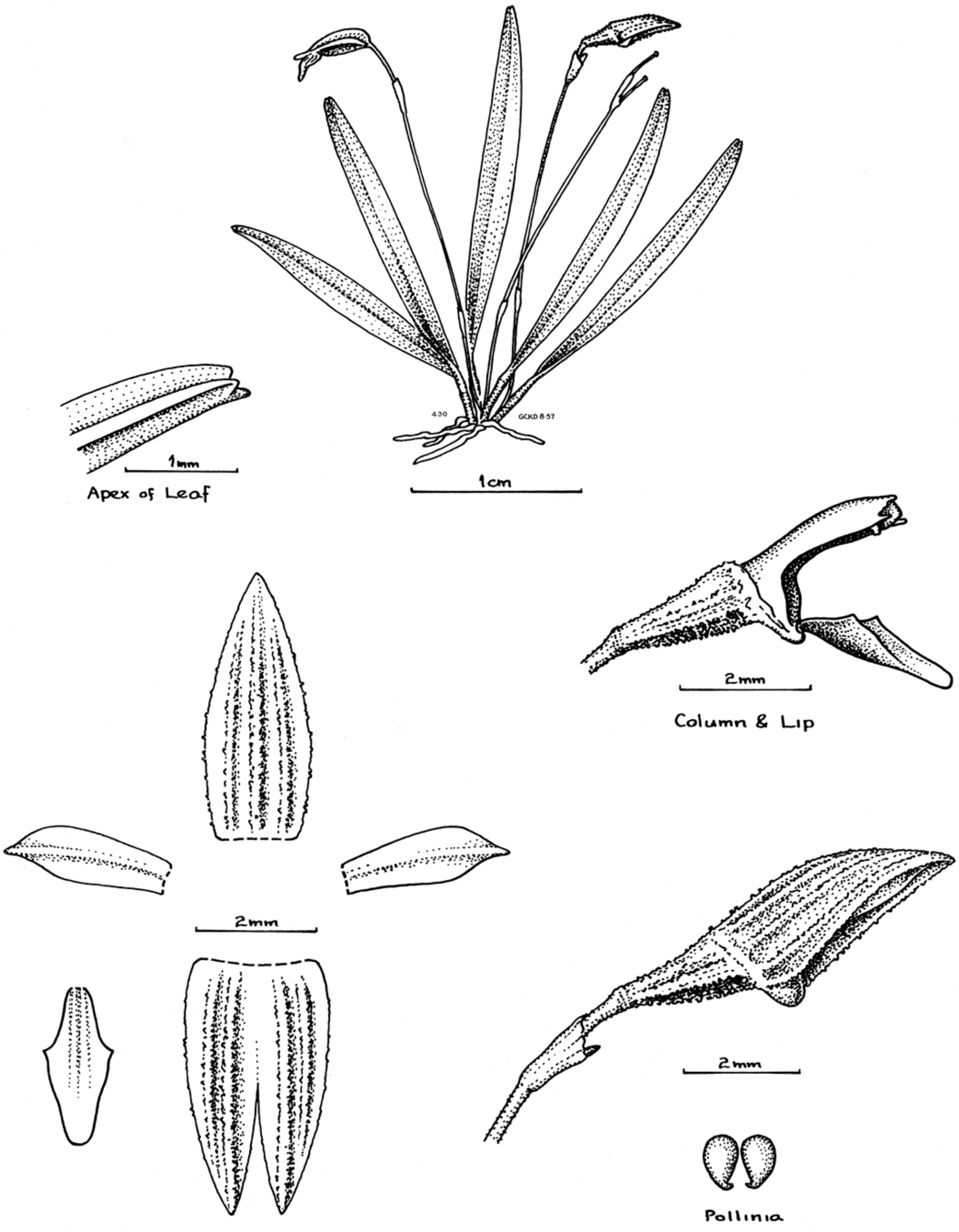


FIGURE 37. Illustration of *Specklinia pertenuis* (C.Schweinf.) Karremans & Gravend. by G.C.K. Dunsterville, based on *Dunsterville 430* (AMES) from Venezuela. Reproduced with the kind permission of the Orchid Herbarium of Oakes Ames, the Harvard University Herbaria.

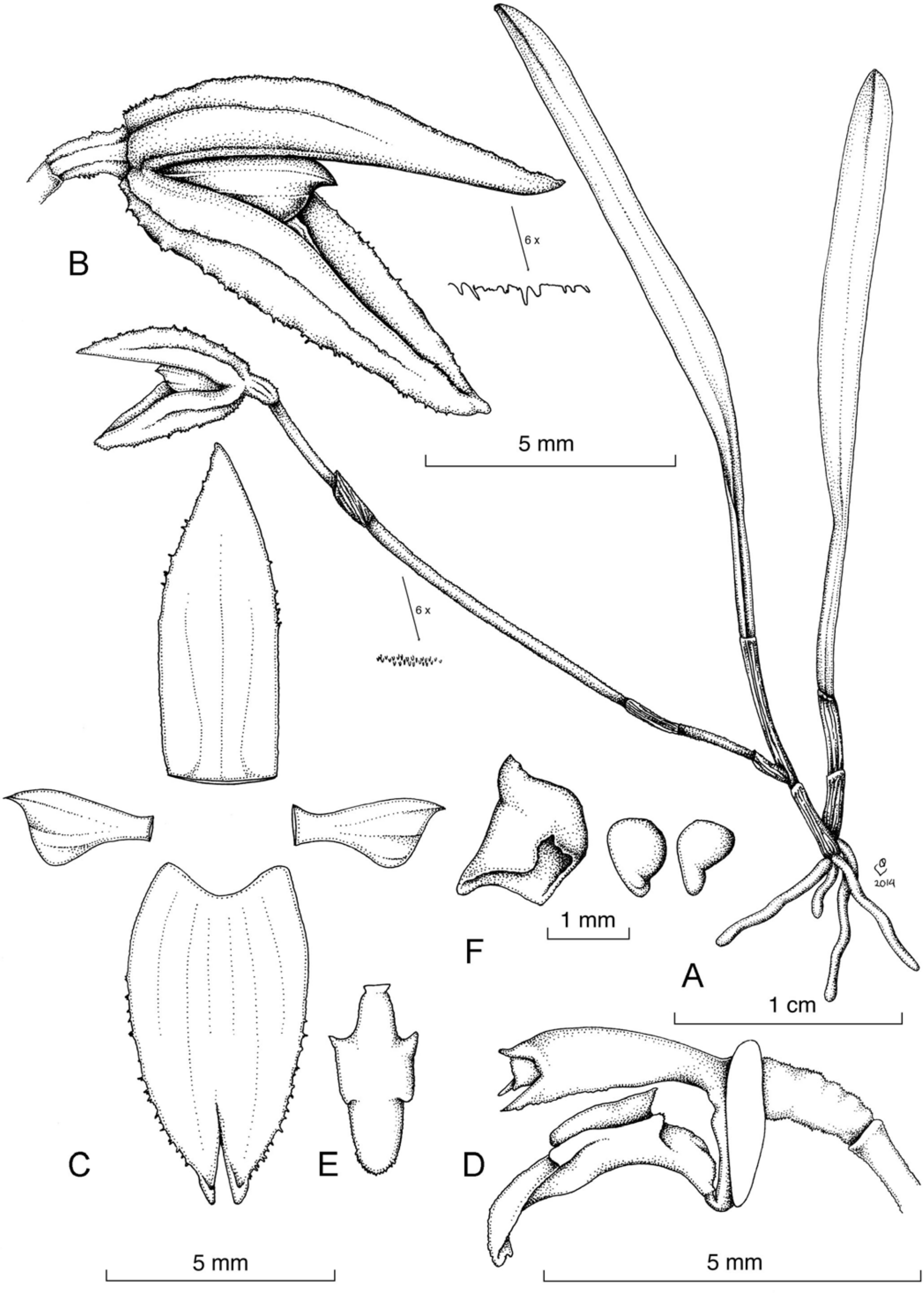


Figure 38. *Specklinia vittariifolia* (Schltr.) Pridgeon & M.W.Chase. A: habit; B: flower; C: dissected perianth; D: column and lip, lateral view; E: lip; F: anther and pollinaria. Drawn by A.P. Karremans & L. Oses from *Karremans 2945* (JBL-spirit).

11. ***Specklinia vittariifolia*** (Schltr.) Pridgeon & M.W.Chase.

Basionym: *Pleurothallis vittariifolia* Schltr. Type:—COSTA RICA. San Jerónimo. 1350 m. Flowered June 1921, *C. Wercklé no. 117* (holotype, B, destroyed; lectotype, AMES 28807!, selected by Pupulin (2010); illustration of type, AMES 28807!)

Epiphytic, caespitose, ascending, erect herb to 4.5 cm tall. Roots fibrous, flexuous, glabrous, to 1 mm in diameter. Stem abbreviated, terete, to 3.5–9.0 mm long, completely concealed by a papyraceous, subancipitous, acute sheaths to 3 mm long. Leaf narrowly linear, up to 35 × 1.5–3.0 mm, minutely and irregularly emarginate at apex, the mid-vein protruding abaxially into a small apicule, gradually tapering toward the base into a deeply conduplicate petiole, subcoriaceous. Inflorescence borne laterally from the base of the leaf, without an annulus, successively single flowered, up to 4.0–4.5 cm long, glandular; peduncle terete, to 3.0 cm long, with 1 distant, glandular, terete bract, 3 mm long. Floral bracts infundibuliform, glandular, broadly ovate, acute to subacuminate, 2.5 mm. Pedicel terete, glandular, 5 mm long, persistent. Ovary subclavate, with low, irregularly crenulate crests, 1.5–2.0 mm long, green. Flowers up to 4, reddish-orange, developed in succession. Sepals fleshy, densely glandulose on the outer surface; dorsal sepal lanceolate-elliptic, 3-veined, acute, the base semi-hyaline, flushed with orange along the veins, the distal two thirds reddish-orange with occasional transparent spots, 7.5–9.0 × 2.0–3.0 mm; lateral sepals narrowly elliptic-oblanceolate, subfalcate, 3-veined, 7.5–9.0 × 3.5–4.5 mm, connate for about three quarters of their length, the base saccate, membranaceous-hyaline, the apex acute. Petals reddish-orange, small, ligulate-falcate, 3.5–4.0 × 1.7–1.9 mm, 2-veined. Lip reddish-orange, small, longitudinally arched-convex in natural position, thinly articulate with the column foot by a hyaline claw, sagittate to oblong when expanded, obtuse, 4.5 × 1.0 mm, provided with a pair of acute, sub-trapezoid lateral lobes from just below to just above the middle, margin dentate-erose, especially apically. Column dark-red, arched, terete and slender at the base, 3.5 mm long without the foot, provided with membranous wings serrulate along the margins, the apex prolonged into a deeply cucullate, lacerate clinandrium; column foot, stout, fleshy, 1.0 mm long. Anther cap deeply cucullate, ovate, crested, 2-celled. Pollinia 2, obovate-complanate, minutely hooked at the base. *NOTE: Description based on *AK2945* and *IC1111*.

Other material examined:—COSTA RICA. San José: Vázquez de Coronado, Jesús, Parque Nacional Braulio Carrillo, Sendero La Botella, 10°09’33.9”N 83°57’14.8”W, 702 m, bosque muy húmedo tropical transición a premontano, epífitas en bosque secundario y primario, *A.P. Karremans, D. Bogarín & M. Fernández 2943* (JBL-spirit, D6069!). Idem. *A.P. Karremans, D. Bogarín & M. Fernández 2945* (JBL-spirit, D4898!, D5959!; Fig. 1f, 2d & 12). Limón: Pococí, Guápiles, Buenos Aires. Cuenca río Santa Clara, faldas del Volcán Turrialba, 10°05’25,96’’N 83°45’39,33’’W, 1190 m, 1 de octubre 2013, *I. Chinchilla 1111* (JBL-spirit!). Pococí, Guápiles, Cariari, Gerson Villalobos legit, *A.P. Karremans 5944* (JBL-spirit, D6100!). Limón: Guápiles, Río Corinto, sendero paralelo al Río Corinto y riberas del mismo, 10°19’09’’N 83°56’10’’W, 500 m, *C. Chávez 52* (MO!). Guácimo, Pocora, La Argentina, camino a la catarata del río Dos Novillos, 10°06’07.71’’ N 83°39’28.74’’ W, 591 m, bosque muy húmedo tropical transición a premontano, 25 noviembre 2013, *A.P. Karremans, D. Bogarín & G. Villalobos 6026* (JBL-spirit!). Heredia: La Selva, 1 Oct. 1985, *J.T. Atwood 85-74* (USJ!; SEL!). La Selva, on tree fall on SSE, 2 Oct. 1985, *J.T. Atwood 85-81* (SEL!). La Selva, on cacao along SOC of Annex, 4 Oct. 1985, *J.T. Atwood 85-103* (SEL!). La Selva, on tree fall on SSE, 5 Oct. 1985, *J.T. Atwood 85-123* (SEL!). La Selva, 6 Oct. 1985, *J.T. Atwood 85-127* (USJ!; SEL!). San José: Zona Protectora La Cangreja. Santa Rosa de Puriscal. Bosque primario en la márgenes del Río Negro, 09°42’28’’N 84°23’35’’W, 400 m. 20 Oct. 1992, *J.F. Morales & Q. Jiménez 891* (CR!). Costa Rica, without specific locality, *R. Lent 1762b* (CR!; SEL!). EL SALVADOR. Departamento Chalatenango, entre Dulce Nombre de María y San Fernando, km 12, a 1200 m. *H. Cl. Clason sub. Hamer 309* (SEL!; illustration by Hamer (1974)!). Cerro Campana, behind Ahuachapan-Ataco, alt. 1400 m, 12 June 1975 (SEL!). MEXICO: Chiapas, Municipio Acacoyagua, Mt. Madre Vieja, 15.450401 N 92.877612 W, 1000 m, *E. Matuda 2532* (MEXU; SEL!). Municipio Escuintla, Mt. Ovando, 15.39083 N 92.6025 W, 935 m, *E. Matuda 28541* (MEXU; AMO, illustration!). Municipio La Concordia, Finca Custepec, trail NW from Finca, 1–3 km along trail, 15.73333 N 92.73333 W, cloud forest, 1180 m, *R. J. Hamshire 1244* (MEXU). PANAMA: Bocas del Toro road, Los Gutiérrez to [Cerro] Pinola. Cultivated at Selby Botanical Gardens, SEL 78-455 ex N. Williams, Aug. 1985, *E.A. Christenson 1326* (SEL!).

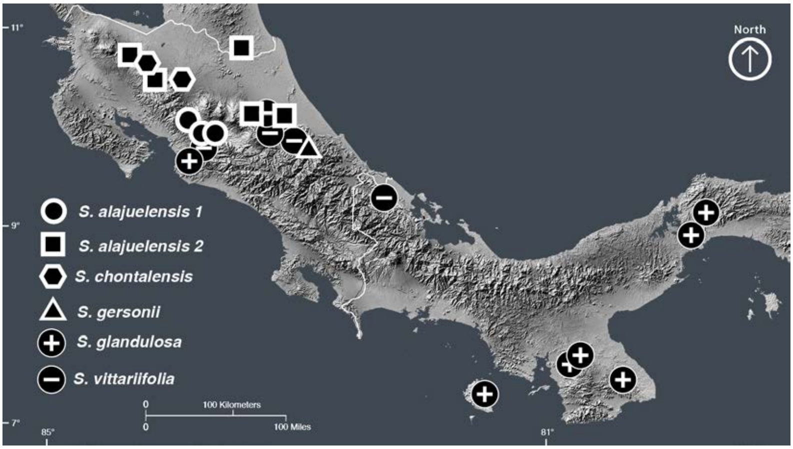


Figure 39. Extract of the distribution map of the glandulous species of *Specklinia*, with emphasis on Nicaragua, Costa Rica and Panama. Edited by D. Bogarín.

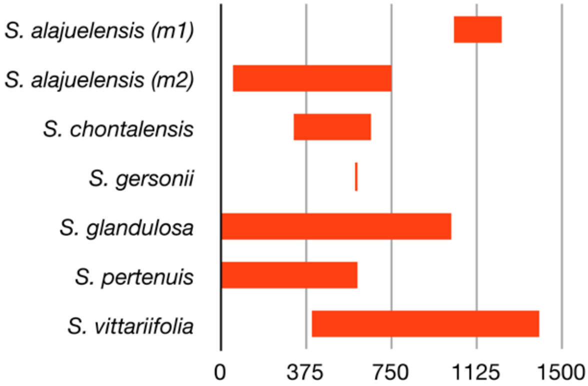


Figure 40. Known elevation distribution of the glandulous species of *Specklinia*. Based on the studied specimens cited. On the X-axis the elevations in meters are depicted.

Figure 41. Intra-specific variation of flower morphology of diverse individuals of two species of glandulous *Specklinia*. A: *S. alajuelensis* 1. B: *S. vittariifolia*. Scale bar = 1 cm. Photographs by A.P. Karremans.

Etymology:—The name alludes to the very long, thin leaves, which is reminiscent of species of the fern genus *Vittaria*.

Distribution:—Known from Mexico, El Salvador, Costa Rica and Panama. In Costa Rica and Panama it is found mostly in the Caribbean lowlands, from 400 to 1190 m elevation. In Mexico and El Salvador it is found at higher elevations, from 1000 to 1400 m (Fig. 32).

Notes:—Aside from a sterile fragment of the plant and an accompanying sketch of the type kept at AMES, nothing else remains of the type material of *Specklinia vittariifolia*. The specimen was supposedly collected in San Jerónimo, Moravia, Costa Rica. We were unable to locate any such plants in the area during a series of field trips, and doubt it did indeed come from there. It is more likely that Wercklé actually collected this plant a few km North-East along the same road, where this species is abundant. There are more collections from Wercklé that, like this species, are typically found on the warm and humid Caribbean watershed, but were allegedly collected in San Jerónimo, a dryer and colder locality in the Central Valley (Pupulin 2010). The illustration of the type and description are not very specific to any of the species of this group; however, the plant fragment and illustration clearly depict a species with long and narrow leaves and an inflorescence that barely reaches the length of the leaf.

Specklinia vittariifolia is locally abundant in certain areas in the Costa Rican Caribbean at mid to low elevations. It has been confused with the similar *S. glandulosa* in herbaria and living collections, however, it can be easily distinguished by the long leaves and relatively short inflorescences which in living material rarely exceed the leaf. The flower is conspicuously downward twisted, and its segments noticeably spreading. Although frequently appearing single flowered, the species can produce up to 4 flowers in tight succession on a single inflorescence, however only one is developed at a time. The flowers are relatively large, with sepals exceeding 7.5 mm long and a lip of 4 mm long, with a pair of conspicuous sub-trapezoid lateral lobes.

The herbarium sheet of Roy Lent's number *1762* kept at CR represents a mix of both *S. alajuelensis* and *S. vittariifolia*. We cannot be certain if both were collected together or if they come from different localities. However, the locality corresponds perfectly with that of other material of *S. alajuelensis*, and we have therefore chosen to give that material the number *Lent 1762a*. The two CR specimens with long, narrow leaves, and all of those kept at SEL are *S. vittariifolia*, and are given the number *Lent 1762b*, with unknown locality.

Discussion and conclusions

Heller & Hawkes (1966) were probably the first to note the phylogenetic relatedness of *Specklinia barbae*, *S. chontalensis*, *S. glandulosa*, *S. pertenuis* and *S. vittariifolia*, and coined the term "*S. glandulosa* alliance" for this species' group. Luer (2006) placed all of them in genus *Sarcinula* Luer, considering that they were not closely related to *Specklinia lanceola*, the type species of the latter genus. Here we have shown that the glandulous species of *Specklinia* include at least 6 species, that they do not form a monophyletic group, and that they are phylogenetically closely allied to the type species of *Specklinia* (Fig. 29).

This particular group ranges from Mexico to Venezuela and is apparently absent in the Antilles and the Andes (Fig. 32). The highest species diversity can be found in Nicaragua, Costa Rica and Panama (Fig. 39). Ecologically these species occupy similar niches and can be found growing sympatrically, which is apparently not rare for several well distinguished species of *Specklinia*. The here studied *Specklinia alajuelensis* (2), *S. gersonii* and *S. vittariifolia*, in addition to the close relatives, *S. luis-diegoi* (Luer) Luer, *S. microphylla* (Rich. & Galeotti) Pridgeon & M.W.Chase and *S. tribuloides* (Sw.) Pridgeon & M.W.Chase, were all found growing in a single locality along the Dos Novillos river in Costa Rica. Similarly, at the type locality of *S. alajuelensis* (1) in La Palma, San Ramón we have also collected *S. calyptrostele* (Schltr.) Pridgeon & M.W.Chase, *S. corniculata* (Sw.) Steudel, *S. fulgens* (Rchb.f.) Pridgeon & M.W.Chase, *S. grobyi* (Bateman ex Lindl.) Barros, *S. lanceola* and *S. tribuloides* in a single patch of a few dozen trees. The glandulous *Specklinia* have a notorious preference for mid to low elevations (Fig. 40). They can be found growing from sea level to up to about 1400 m, with all the studied species overlapping at 600 m in elevation.

The glandulous *Specklinia* share a "standard" morphological pattern, and the differences between them are not as conspicuous as their similarities (Fig. 28). Nevertheless, their intra-specific variation is low and populations are

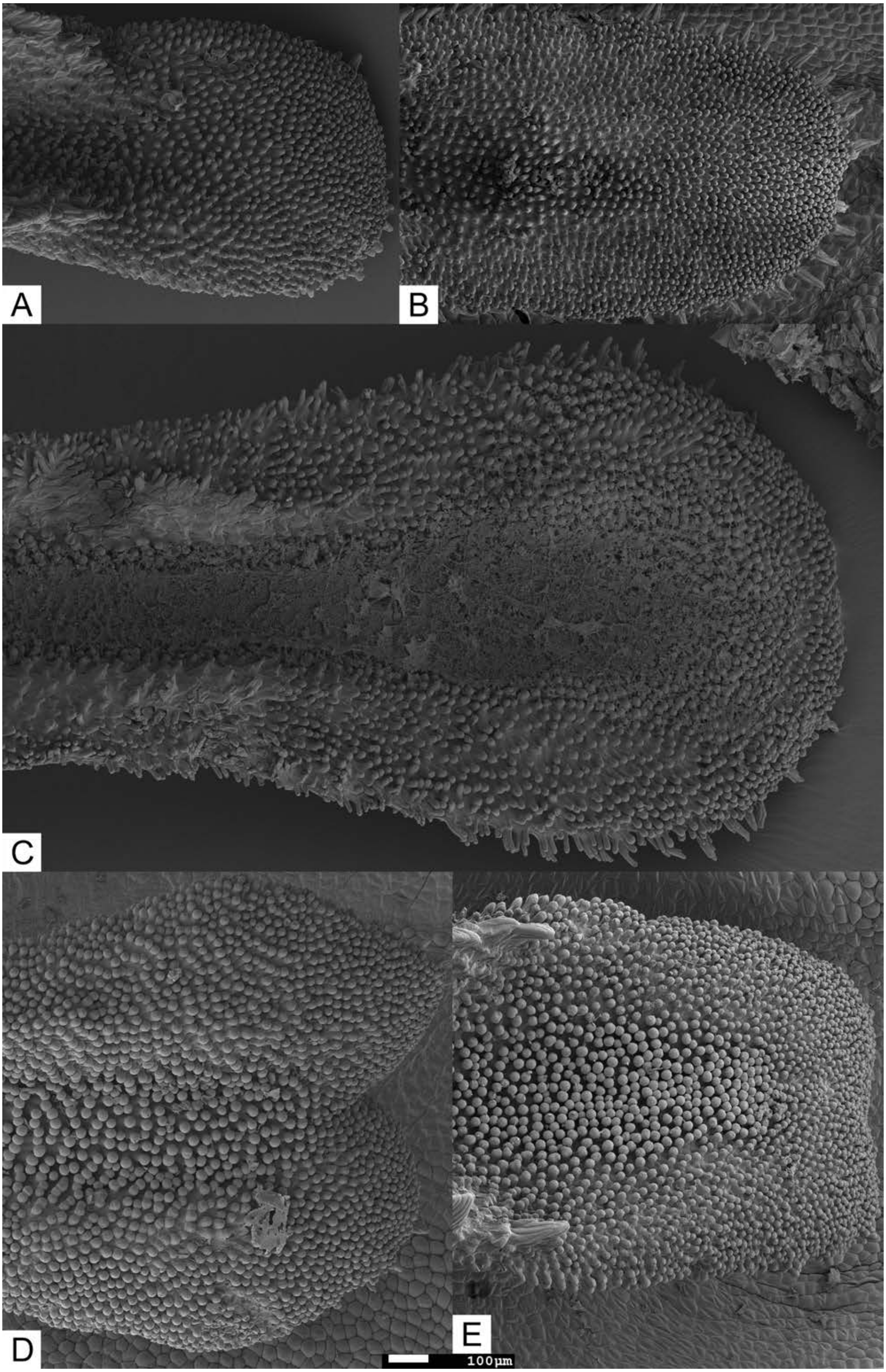


Figure 42. Micrographs of the lip apices of *Specklinia* species. A: *Specklinia alajuelensis* 1 (*Pupulin 8470*); B: *Specklinia alajuelensis* 2 (*Karremans 6460*); C: *Specklinia chontalensis* (*Pupulin 6543*); D: *Specklinia gersonii* (*Karremans 6025*); E: *Specklinia vittariifolia* (*Chinchilla 1111*). Photographs by A.P. Karremans.

morphologically stable (Fig. 41). Genetically they are not only a non monophyletic group, but are also highly differentiated (Table 4), with the nrITS sequence divergence between each species varying between 2.2% (between *S. alajuelensis* and *S. vittariifolia*) and 6.2% (between *S. alajuelensis* and *S. chontalensis*). If studied in detail, morphological differences become apparent as well. The tiny lips of species of this group are extremely delicate and deform easily with manipulation. In most illustrations they look quite similar to each other, sharing a comparable shape. They are all fully glandular and longitudinally depressed in the middle, where the glands are denser and residues are visible (Fig. 42). This common pattern is probably due to a similar pollination system in which the pollinator, following the nectar guides and steered by the lip lobes, is led to the base of the column/lip cavity. Nevertheless, they can be quite different from each other in size, shape, ornamentation, and even color. The lips of the sister species *S. alajuelensis* and *S. vittariifolia* for example are easily distinguished with micro-photography (Fig. 43).

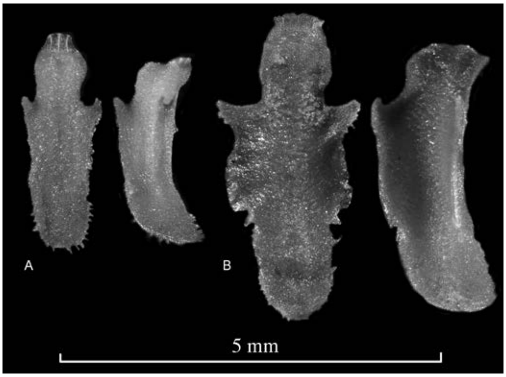


Figure 43. Comparison of the lip size, shape, ornamentation and color of two species of glandulous *Specklinia*, in natural position (right) and extended (left). A: *S. alajuelensis* 1 (*Pupulin 8470*). B: *S. vittariifolia* (*Chinchilla 1111*). Scale bar = 5 mm. Photographs by A.P. Karremans.

Sepal size, shape and color although similar also differs (Fig. 27). Even though all sepals are externally glandular, they are so in varying pattern and degrees (Fig. 44). The glands are variable in length between the different species, and are mostly conical or obconical in shape, but not capitate. The glands are placed mostly along the veins and margins, where also occasional stomata are found in all species. Those stomata are similar to those found on the inner surface of the sepals of species of the *Specklinia endotrachys* complex (Chapter 8). Additionally, and probably as in all Pleurothallidinae, sunken trichomes can be frequently observed on the outer surface of the sepals (Chapter 8).

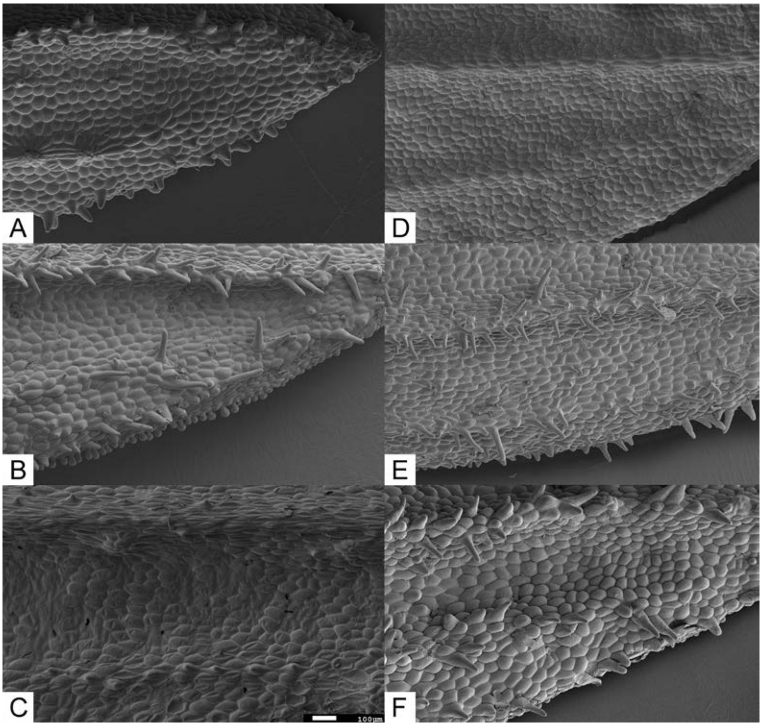


Figure 44. Micrographs of the outer surface of the sepals of *Specklinia* species. A: *Specklinia alajuelensis* 1 (*Pupulin 8470*); B: *Specklinia alajuelensis* 2 (*Karremans 6460*); C: *Specklinia chontalensis* (*Pupulin 6543*); D: *Specklinia gersonii* (*Karremans 6025*); E: *Specklinia glandulosa* (*Karremans 6306*) F: *Specklinia vittariifolia* (*Chinchilla 1111*). Photographs by A.P. Karremans.

Chapter 4

A new *Specklinia* (Orchidaceae: Pleurothallidinae) from Costa Rica and Panama

Diego Bogarín
Adam P. Karremans
Rafael Rincón
Barbara Gravendeel

A new species of *Specklinia* from the Cordillera de Talamanca in southern Costa Rica and western Panama is described and illustrated. *Specklinia absurda* most closely resembles *Specklinia fuegi*. It differs from that species in the pendent, single flowered inflorescence, whitish yellow sepals with red-pink veins and yellow apex, red petals with translucent margins, pandurate-trilobed, unguiculate lip with a distinct papillose isthmus below the anterior lobe, and reniform, erose, erect lateral lobes, with a Y-shaped thickened, hirsutulous apical callus. The affinities of this species to other *Specklinia* species are discussed.

Keywords: *Specklinia absurda*, *Sylphia*, Talamanca, taxonomy, *Trichosalpinx*, systematics.

Introduction

The orchid genus *Specklinia* Lindl. was redefined by Pridgeon and Chase (2001) to group the species of several infrageneric concepts of polyphyletic *Pleurothallis*, including sects. *Hymenodanthe* Barb.Rodr., *Tribuloides* Luer, *Muscariae* Luer, *P.* subgenus *Empusella* Luer, *P.* subgenus *Pseudoctomeria* Kraenzl., and genus *Acostaea* Schltr. In Pridgeon (2005), *Specklinia* was defined as a genus of around 200 species, ranging from Mexico and West Indies to Brazil and Bolivia. A later account of the genus by Barros and Trettel Rodrigues (2009) yielded about 420 binomials. However, a phylogenetic study by Karremans *et al.* (Chapter 6) suggests that only around 95 species can be included in the redefined concept of the genus to obtain monophyly. As such, *Specklinia* is still variable both in terms of vegetative and floral morphology, but can be recognized by the frequently small plants with ramicauls shorter than the leaves provided with an abbreviated stem with an annulus, the sepals and petals mostly membranaceous, the lateral sepals connate for at least half their length and convergent, petals mostly obtuse and entire (never acuminate or lanceolate), wider above the middle, and a linear to sub-rectangular lip hinged to the column foot; the column is provided with a toothed androclinium, and a pair of prominent rounded wings near the apex, the ventral anther and stigma, and the nude pollinaria, which are flattened towards the base and have no caudicles or viscidium.

Luer (2006) regarded the new circumscription of Pridgeon and Chase (2001) as a polyphyletic aggregation of many taxa. Instead, he proposed to split *Specklinia* in ten genera with five major groups: *Muscarella* Luer, *Pabstiella* Brieger & Senghas, *Panmorphia* Luer, *Sarcinula* Luer and *Specklinia*, and four other monospecific genera created for the rest of the morphologically “aberrant” species in addition to *Sylphia* Luer, a genus of four species with long tailed sepals and crested or spiculate ovaries. Few authors have used these segregate genera, and circumscription of most has still to be evaluated with DNA analyses.

Materials and methods

This study was conducted at Jardín Botánico Lankester (JBL) of the Universidad de Costa Rica, Naturalis Biodiversity Center, Leiden, The Netherlands and Herbario UCH of Universidad Autónoma de Chiriquí, Panamá between April 2011 and January 2013. Specimens at JBL (from living and spirit collections), and dried and spirit material available at CR, INB, JBL, L, UCH and USJ were revised. Phenological data were recorded in the field and from cultivated specimens.

Herbarium specimens were deposited at CR, JBL, PMA, UCH and USJ. The map and georeferences for specimens were obtained using a Garmin eTrex Vista GPS and Google Earth 6.1.0 ©. Ecological zones were estimated by using the Holdridge Life Zone System (Holdridge, 1987) and the Mapa Ecológico de Costa Rica by Bolaños *et al.* (2005). Sketches of specimens were drawn with a Leica MZ 9.5 stereomicroscope with a drawing tube. Color illustrations were made using an Epson Perfection 4490 Photo Scanner, a Nikon D5100 digital camera and a DFC295 Leica digital microscope color camera with Leica FireCam version 3.4.1 software. The new species was illustrated and described from living specimens. Scanning electron microscope (SEM) micrographs were taken from flowers fixed in FAPA (Ethanol 50%, Acetic Acid, Formalin at a proportion of 18:1:1). The samples were then dehydrated through a series of ethanol steps and were subjected to critical-point drying using liquid CO2. Dried samples were mounted and sputter-coated with gold and observed with a JEOL JSM-5300 scanning electron microscope, at an accelerating voltage of 10kV.

Phylogenetic analysis

Of the 35 sequences used here, 31 were downloaded from NCBI GenBank, where they were deposited by Pridgeon *et al.* (2001) and Chiron *et al.* (2012). Those sequences were used to place four sequences, which belong to an unpublished analysis of *Specklinia* (Chapter 6) (Table 5). Plants were obtained from the living collections at JBL, and their vouchers are kept in the spirit collections at JBL. Fresh leaf and flower cuttings of approximately 1 cm2 were dried with silica gel. Samples (20 mg) were pulverized and extraction was performed by following the DNEasy extraction procedure (Qiagen). The nuclear ribosomal internal transcribed spacer (nrITS) region was amplified using the methods and primers for sequencing and amplification described by Sun *et al.* (1994), while Sanger sequencing was done commercially by Macrogen on a 96-capillary 3730xl DNA Analyzer automated sequencer (Applied Biosystems, Inc.) using standard dye-terminator chemistry (Macrogen, Inc.).

The Staden *et al.* (2003) package was used for editing of the sequences. Contigs were exported as .fas files and opened in Mesquite v2.72 (Maddison & Maddison 2007), where they were checked for misalignments and adjusted manually. The ends of each data set were trimmed to eliminate possible erroneous data, and gaps were regarded as missing data (filled with Ns). *Phloeophila peperomioides* AF275690 was used as outgroup, as it was found to be the most distantly related of all included species (Pridgeon *et al.* 2001). The trees were produced with an analysis of the nrITS dataset of 35 sequences using BEAST v1.6.0. (Drummond & Rambaut 2007). Parameters were set to preset, except for substitution model GTR with 8 categories, clock model uncorrelated lognormal, tree prior Yule process, and number of generations 20,000,000. The resulting trees were combined using TreeAnnotator v1.6.0., where the first 100 trees were used as burn-in. FigTree v1.3.1. (Rambaut 2009) was used to edit the resulting tree. Branch fading is correlated to posterior probabilities of those branches.

12. *Specklinia absurda* Bogarín, Karremans & Rincón, Phytotaxa 115(2): 34. 2013.

> *The species is similar to Specklinia fuegi (Rchb.f.) Solano & Soto Arenas; however, it differs in the pendent plant, single-flowered inflorescence, whitish-yellowish sepals with red-pink veins and yellow apex, red petals with translucent margins and the pandurate-trilobed, unguiculate lip with a distinct papillose isthmus, and erect, reniform, erose lateral lobes, with a Y-shaped thickened, hirsute callus. It is also similar to Specklinia cactantha Pridgeon & M.W.Chase but differs in the erect, glabrous inflorescence, not spiculate sepals and pandurate-trilobed lip.*

Type:—COSTA RICA-PANAMÁ. Puntarenas-Chiriquí: Coto Brus-Renacimiento, línea fronteriza hacia el Cerro Pando, después del mojón N.338, 8°55’11.22” N 82°43’18.18” W, 2446 m, bosque muy húmedo montano bajo, epífita en bosque primario, “*in sylvis virginis versus montium Pando in itinere ad summum Costa Rica austro-orientalis in finibus utrimque Costa Rica et Panama*”, 19 abril 2011, *D. Bogarín 8711, Jiménez & Karremans* (holotype, CR!; isotypes, JBL!, PMA!, UCH!, USJ!; Fig. 45 & 46).

Table 5. List of the 35 taxa used in the phylogenetic analysis. The vouchers, NCBI GenBank accession number and source are given. Scientific names follow Pridgeon 2005.

Taxon	Sequence Voucher	GenBank Accession Number	Sequence Source
Dryadella edwallii (Cogn.) Luer	*MWC305*	AF262824	GenBank
Dryadella hirtzii Luer	*BGH123364*	EF079367	GenBank
Dryadella kautskyi (Pabst) Luer	CVDB1997	JQ306380	GenBank
Dryadella simula (Rchb.f.) Luer	*MWC1095*	AF262825	GenBank
Dryadella susanae (Pabst) Luer	*GC11240*	JQ306486	GenBank
Phloeophila peperomioides (Ames) Garay	*None*	AF275690	GenBank
Platystele compacta (Ames) Ames	*MWC5637*	AF262822	GenBank
Platystele misera (Lindl.) Garay	*MWC5625*	AF262823	GenBank
Platystele stenostachya (Rchb.f.) Garay	*MWC5618*	AF262821	GenBank
Scaphosepalum gibberosum (Rchb.f.) Rolfe	*MWC968*	AF262817	GenBank
Scaphosepalum grande Kraenzl.	*MWC1107*	AF262819	GenBank
Scaphosepalum swertiifolium (Rchb.f.) Rolfe	*MWC1383*	AF262818	GenBank
Scaphosepalum ursinum Luer	*BGH124283*	EF079365	GenBank
Scaphosepalum verrucosum (Rchb.f.) Pfitzer	*MWC1331*	AF262820	GenBank
Specklinia absurda Bogarín, Karremans & R.Rincón	*DB9772 (JBL-Spirit)*	KC425826	Karremans *et al.* (unp.)
Specklinia absurda Bogarín, Karremans & R.Rincón	*DB8711 (JBL-Spirit)*	KC425827	Karremans *et al.* (unp.)
Specklinia brighamii (S. Watson) Pridgeon & M.W.Chase	*SOL761*	AF262925	GenBank
Specklinia condylata (Luer) Pridgeon & M.W.Chase	*MWC6808*	AF262873	GenBank
Specklinia costaricensis (Rolfe) Pridgeon & M.W.Chase	*MWC5612*	AF262862	GenBank
Specklinia costaricensis (Rolfe) Pridgeon & M.W.Chase	*MWC5636*	AF262863	GenBank
Specklinia fimbriata (Ames & C.Schweinf.) Solano	*SOL769*	AF262924	GenBank
Specklinia fuegi (Rchb.f.) Luer	*AK 5600 (JBL-Spirit)*	KC425786	Karremans *et al.* (unp.)
Specklinia fulgens (Rchb.f.) Pridgeon &M.W.Chase	*MWC5630*	AF262872	GenBank
Specklinia grobyi (Bateman ex Lindl.) F.Barros	*GC09357*	JQ306388	GenBank
Specklinia grobyi (Bateman ex Lindl.) F.Barros	*MWC1093*	AF262860	GenBank
Specklinia grobyi (Bateman ex Lindl.) F.Barros	*GC04524*	JQ306485	GenBank
Specklinia lanceola (Sw.) Lindl.	*MWC1433*	AF262861	GenBank
Specklinia lanceola (Sw.) Lindl.	*AP s.n.*	KC425838	Pridgeon & Chase 2002
Specklinia mirifica Pridgeon & M.W.Chase	*MWC6800*	AF262865	GenBank
Specklinia montezumae Luer (Luer)	*AK229 (JBL-Spirit)*	KC425811	Karremans *et al.* (unp.)
Specklinia picta (Lindl.) Pridgeon & M.W.Chase	CVDB2146	JQ306384	GenBank
Specklinia picta (Lindl.) Pridgeon & M.W.Chase	*GC06131*	JQ306385	GenBank
Specklinia remotiflora Pupulin & Karremans	*MWC1303*	AF262859	GenBank
Specklinia subpicta (Schltr.) F.Barros	*GC11046*	JQ306389	GenBank
Specklinia tribuloides (Sw.) Pridgeon & M.W.Chase	*MWC5615*	AF262867	GenBank

Epiphytic, caespitose herb, up to 2.5 cm long. Roots flexuous, to 1 mm in diameter. Ramicauls erect, up to 1 cm long, enclosed by 2 tubular sheaths up to 5 mm long. Leaves suberect, elliptic, obtuse, emarginate, with an apicule, lenticular, 1.0–2.2 × 0.5–0.8 cm, cuneate at the base, narrowing into an indistinct petiole less than 3 mm long. Inflorescence racemose, single-flowered, pendent, glabrous, shorter or as long as the leaves, 1.0–1.5 cm long; peduncle, up to 1.1 cm long, rachis 1 mm long. Floral bracts ovate, acute, conduplicate, membranaceous, up to 1.0 mm long. Pedicels 8 mm long, persistent. Ovary to 1.5 mm long, carinate, echinate. Flowers with the sepals whitish-yellowish with red-purple veins, apex yellow, petals red with translucent margins, lip with the lateral lobes reddish, midlobe yellow with margin pinkish white and column whitish- greenish. Dorsal sepal ovate to elliptic, acuminate, entire, dorsally with three keels, concave, 9.2 × 3.5 mm. Lateral sepals connate up to 4.5 mm into an ovate, entire, dorsally keeled lamina 11.3 × 5.7 mm, the acuminate, filiform apices to 2.5 mm long. Petals spathulate-lanceolate, acute, entire, 4.3 × 1.6 mm. Lip unguiculate, pandurate-trilobed, 4.8 × 4.0 mm, with a distinct isthmus below the midlobe, lateral lobes reniform, erose, erect in natural position, 1.4 × 1.8 mm, isthmus quadrate, papillose, 0.9 × 0.9 mm, midlobe triangular, puberulent, erose, 2.8 × 2.1 mm, with a Y-shaped, thickened, hirsute callus from the middle towards the apex, attached to the column foot. Column cylindric, footed, arcuate, entire apically, with a pair of minute calli at base, anther apical and stigma ventral. Pollinia two, ovoid, without caudicles or viscidium. Anther cap rounded, cucullate. (Fig. 47A–E)

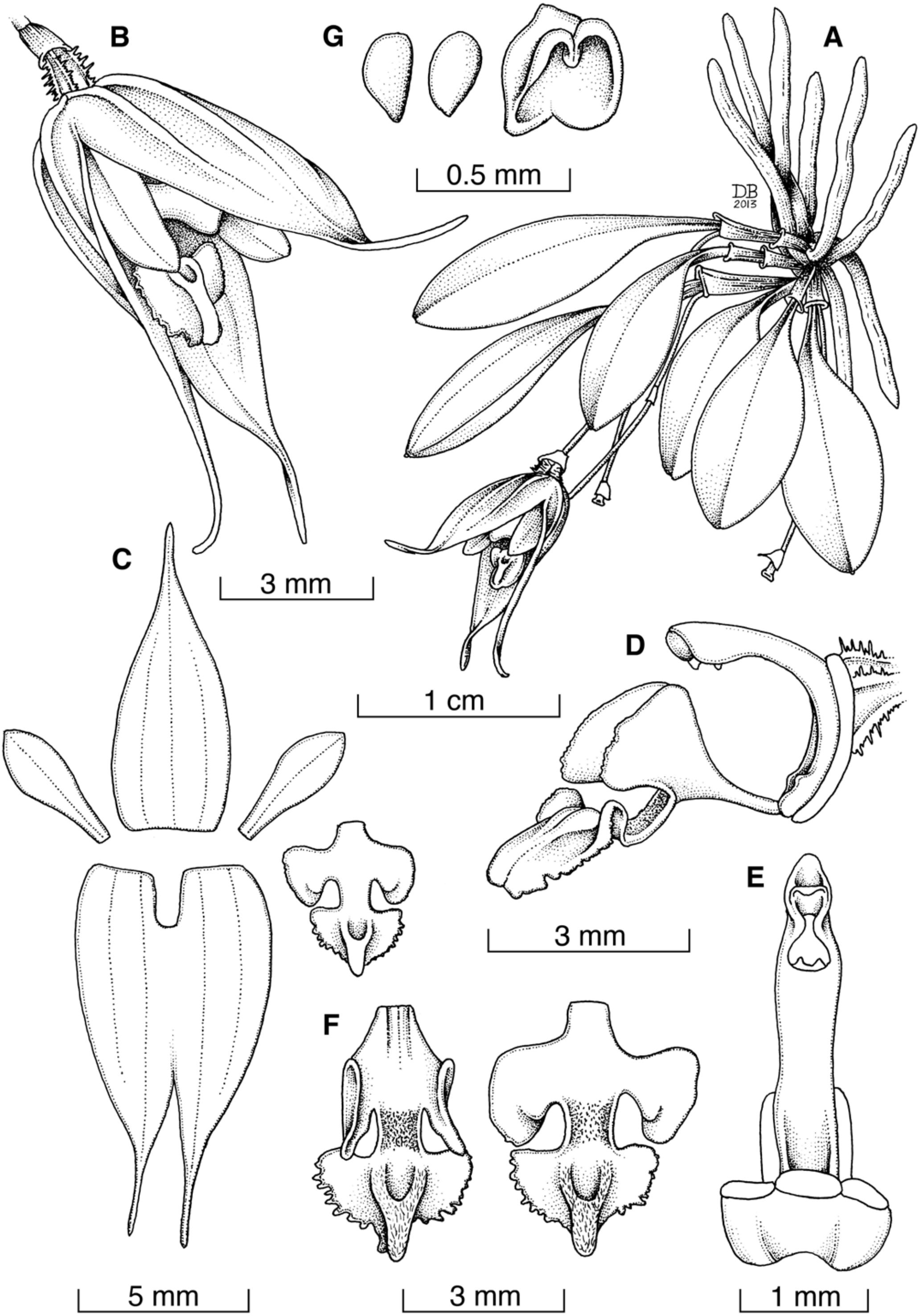


Figure 45. *Specklinia absurda*. A. Habit. B. Flower. C. Dissected perianth. D. Column and lip, lateral view. E. Column, front view. F. Lip, natural position and spread. G. Pollinarium and anther cap. Drawn from the holotype by D. Bogarín.

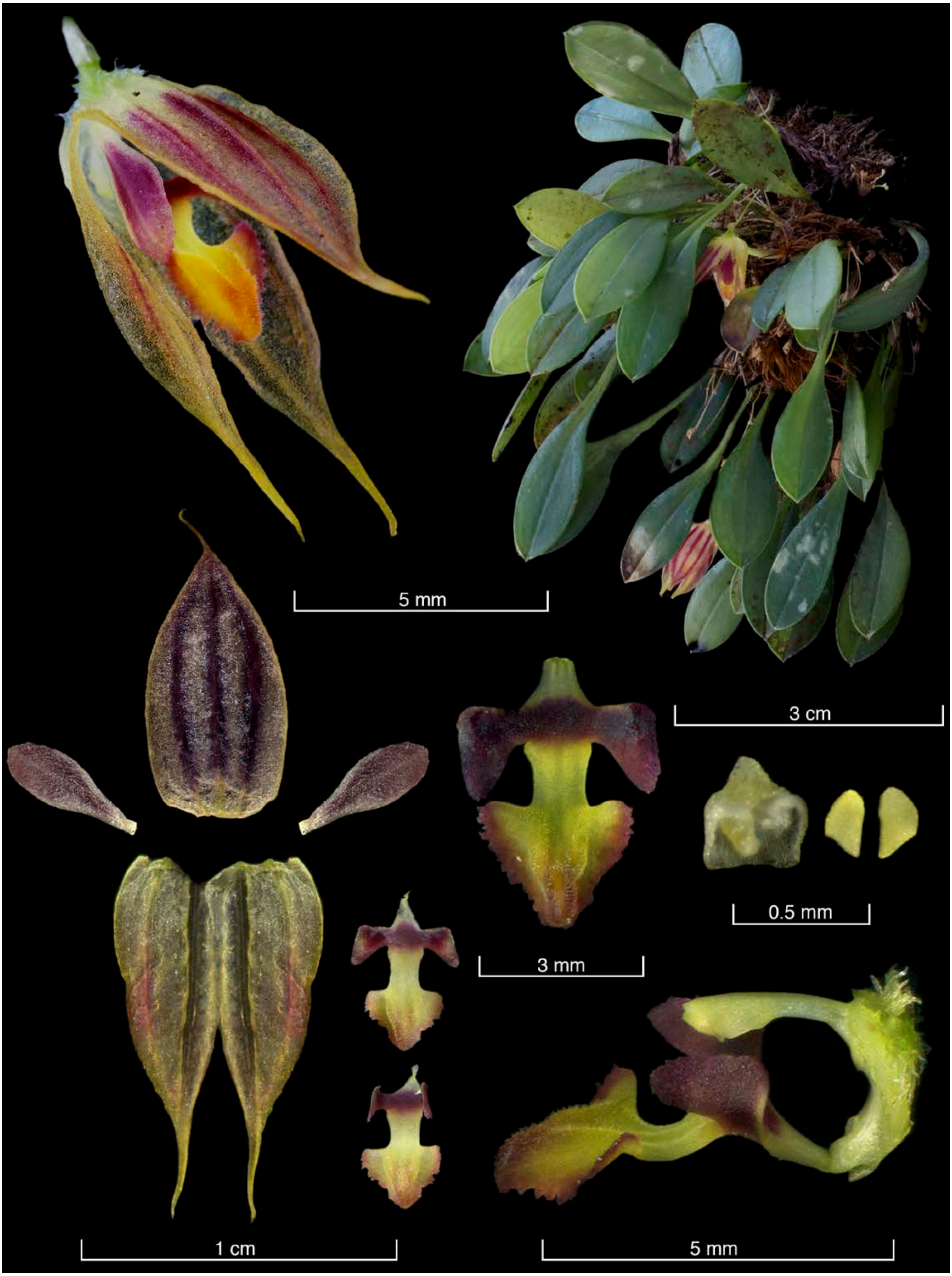


Figure 46. Lankester Composite Dissection Plate (LCDP) of *Specklinia absurda*. A. Habit. B. Flower. C. Dissected perianth. D. Column and lip, lateral view. E. Lip, spread. F. Pollinarium and anther cap. Based on photographs of *Bogarín et al. 9772* (JBL) by A.P. Karremans and D. Bogarín.

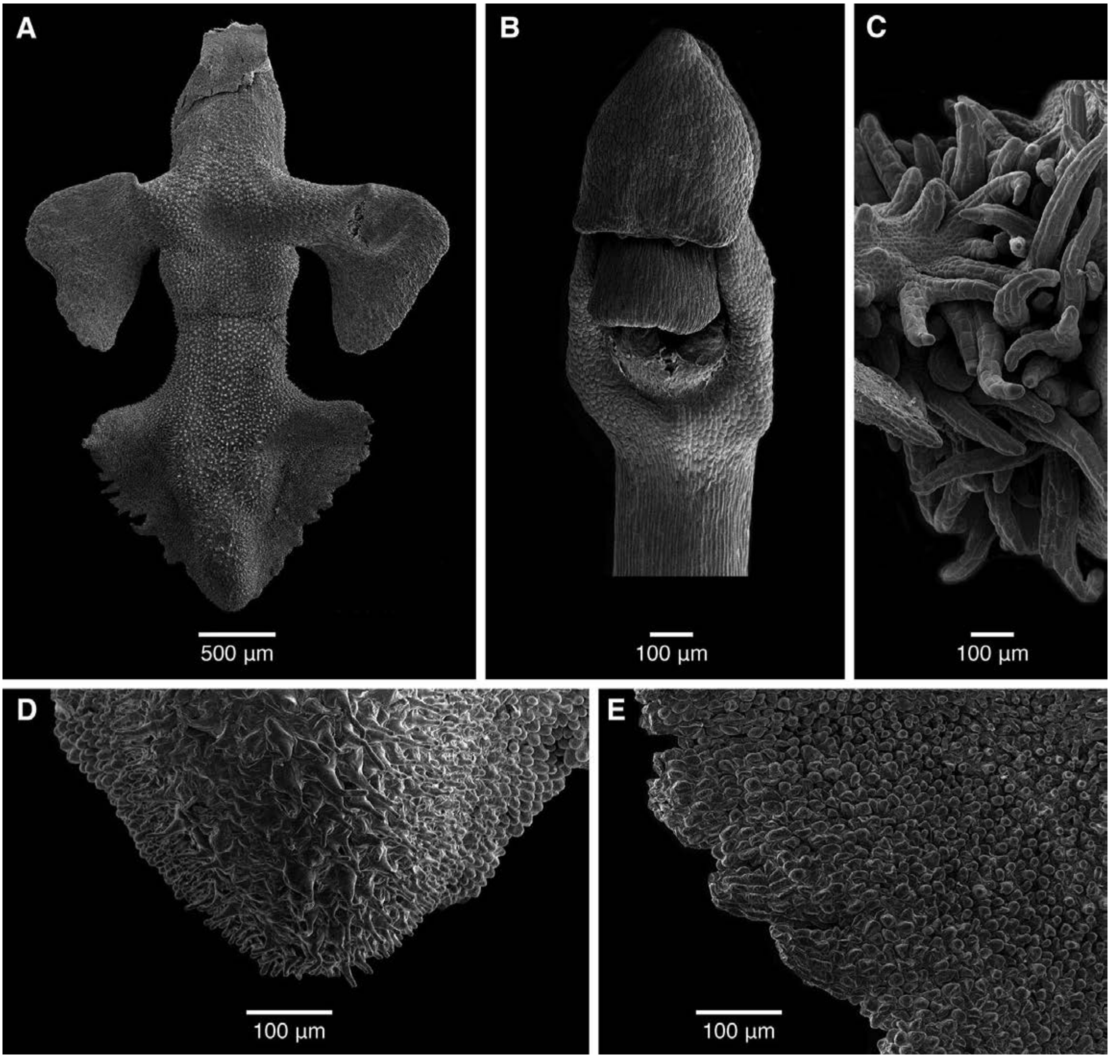


Figure 47. SEM images of micromorphology of *Specklinia absurda*. A. Lip, flattened. B. Column front view showing the anther cap, rostellum and stigma. C. Surface of the echinate ovary showing the cellular projections. D. Lip apex showing the epidermal cells of the apex of the callus. E. Basal lip lobe margin showing the shape of the papillate epidermal cells. Voucher specimen: *Bogarín et al. 9772* (JBL). By A.P. Karremans.

Distribution:—Endemic to the Cordillera de Talamanca in southern Costa Rica and western Panama (Fig. 48).

Habitat and ecology:—Epiphytes forming large colonies on main trunks in oak forest mostly in shaded spots in wet forest (Holdridge 1987) at around 2400–2550 m elevation.

Etymology:—From the Latin *absurdum*, "absurd, illogical, out of tune, contrary to common sense" in reference to the flower morphology, especially the lip, which seems exceptional when compared to its closest relatives.

Other material examined:—COSTA RICA. Limón: Talamanca, Bratsi, Parque Internacional La Amistad, Valle del Silencio, orillas del Río Terbi cerca del andarivel, 9°06'41.81" N 82°57'42.44" W, 2462 m, bosque pluvial montano, 16 agosto 2012, *Bogarín 9864, Fernández, Godínez, Karremans, Kruizinga & C. M. Smith* (JBL-spirit). Límite entre

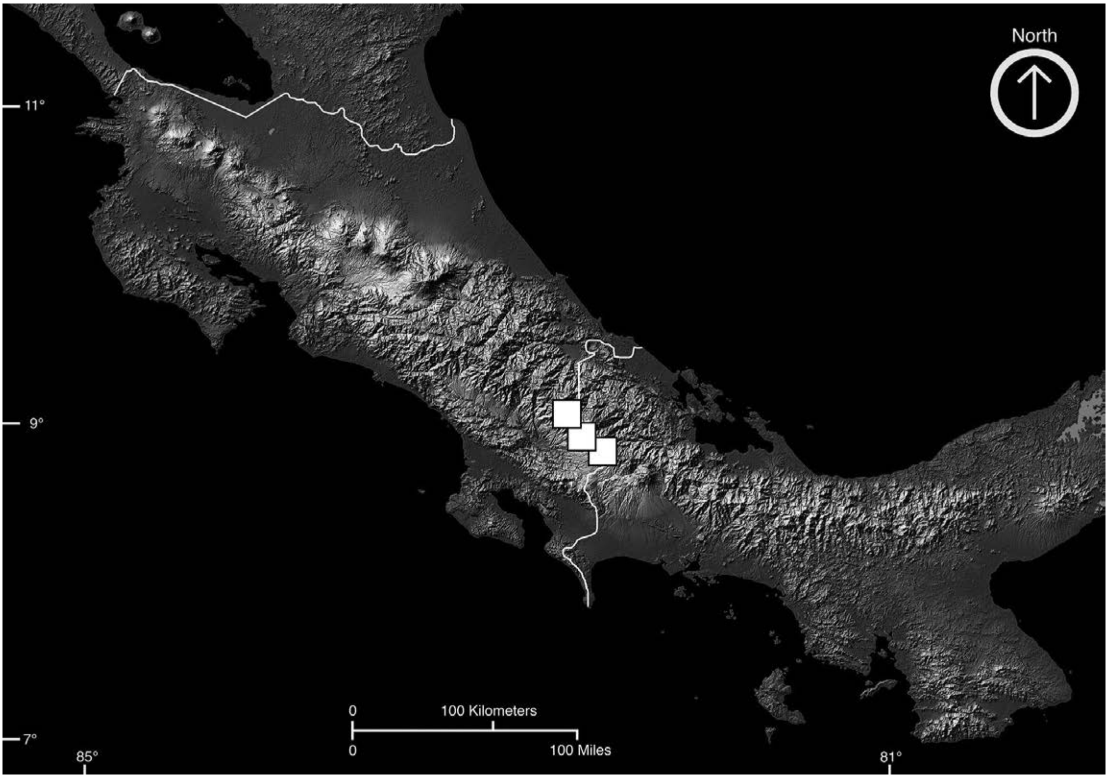


Figure 48. Distribution of *S. absurda* in Costa Rica and Panama, by D. Bogarín.

Limón y Puntarenas: Talamanca-Buenos Aires, Bratsi-Potrero Grande, Parque Internacional La Amistad, Sector Altamira, sendero al Valle del Silencio, Cerro Hoffman, sobre la divisoria de aguas, 9°05’38.2” N 82°58’37.73” W, 2553 m, bosque pluvial montano, 14 agosto 2012, *Bogarín 9772, Fernández, Godínez, Karremans, Kruizinga & Smith* (JBL-spirit).

Notes:—This species is similar to *Specklinia fuegi* (Rchb.f.) Solano & Soto Arenas. It differs mainly in the pendent, single-flowered inflorescence (rather than erect, few- flowered), whitish-yellowish sepals with red-pink veins and yellow apex, red petals with translucent margins (rather than white-cream) and pandurate-trilobed, unguiculate lip with a distinct papillose isthmus below the anterior lobe, and reniform, erose lateral lobes, erect in natural position (Fig. 47A), with a Y-shaped thickened, hirsute, papillose apical callus (Fig. 47D, E; rather than a simple, smooth lip without an isthmus). *Specklinia absurda* is also similar to *Specklinia cactantha* (Luer) Pridgeon & M.W.Chase and *S. turrialbae* (Luer) Luer. The main differences among the species related to *S. absurda* are summarized in Table 6 and can be observed in Fig. 49.

Table 6. Comparison of the species of *Specklinia* related to *S. absurda*.

Character	*S. absurda*	*S. cactantha*	*S. fuegi*	*S. turrialbae*
Inflorescence	creeping or pendent, single-flowered, glabrous	erect, single-flowered, spiculate	erect, successively flowered, glabrous	erect, single-flowered, glabrous
Sepals	entire, acuminate, white suffused with purple	spiculate externally, long acuminate, white-rose	entire, acuminate, white-cream	entire, filiform, white
Synsepal size	11.3 x 5.7 mm	11.0 x 3.5 mm	7.5 x 3.0 mm	10 x 3 mm
Petals	acute, red-purple	obtuse or truncate-retuse, pale green with rose	obtuse, greenish-white	obtuse to acute, white-yellowish
Lip	pandurate-trilobed, with a distinct isthmus, erose	oblong-ovate, without isthmus, entire	subtrilobed, without isthmus, entire	subtrilobed or elliptic, without isthmus, entire
Lip callus	Y-shaped, thickened, pilose	a pair of low carinae, entire	a pair of marginal carinae, entire	a pair of marginal carinae, entire

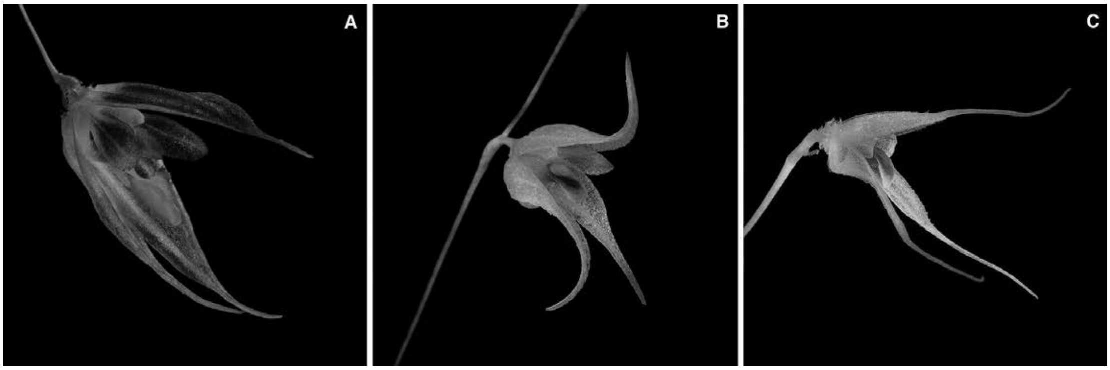


Figure 49. Flower morphology: A. *Specklinia absurda* (*Bogarín 9772*, JBL). B. *Specklinia fuegi* (*Karremans 5600*, JBL). C. *Specklinia turrialbae* (*Karremans 5635*, JBL). A-B by D. Bogarín. C by A.P. Karremans.

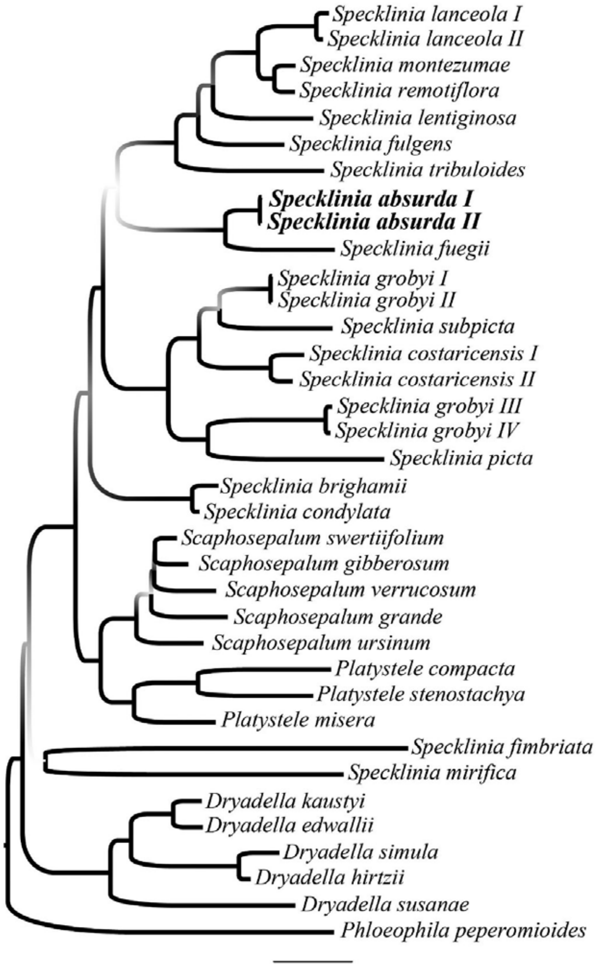


Figure 50. Phylogenetic placement of *S. absurda*. The trees were produced with an analysis of the ITS dataset of 35 sequences using BEAST v1.6.0. High posterior probabilities are viewed as “hard” branches (above 0.9), while low support can be seen as disappearing branches (below 0.5). Tree edited by A.P. Karremans using FigTree v.1.3.1.

Phylogenetic Placement:—Placing *S. absurda* among its closest relatives based on morphology proved to be a difficult task. The plant habit resembles species of the *S. grobyi* (Bateman ex Lindl.) Barros complex with elliptic-ovate or suborbicular thick leaves, whereas the pendent, single- flowered inflorescence and acuminate sepals resemble those of *S. fimbriata* (Ames & C.Schweinf.) Solano and other species placed in *Muscarella*. However, *S. absurda* lacks the erect multi- flowered inflorescence, linear lip and glabrous ovary of the *S. grobyi* complex, and the loose raceme, denticulate, fringed or fimbriate petals, and pollinaria with caudicles, which characterize species of *Muscarella*. Superficially *Trichosalpinx webbiae* Luer & Escobar is also similar, sharing the elliptic, coriaceous thick leaves, acuminate, dorsally carinate sepals, obovate petals and a trilobed lip with the lateral lobes erect in natural position; however, that species has obscurely lepanthiform bracts (rather than tubular), prostrate leaves mottled with purple beneath (rather than erect, immaculate), erect inflorescences (rather than pendent), a glabrous ovary (rather than echinate) and a simple, trilobulate lip with entire margins (rather than a pandurate-trilobed, erose lip with a distinct papillose isthmus below the anterior lobe, and a Y-shaped thickened apical callus in *S. absurda*). Further morphological and molecular study of *T. webbiae* will be useful to confirm its phylogenetic affinities. An unpublished molecular phylogenetic analysis of *Specklinia* carried out by Karremans *et al.* indicates that *S. absurda* is related to some species placed in *Sylphia* by Luer (2006), all found embedded within *Specklinia*, sister to a clade that includes species of the *S. grobyi* complex and all the orange-flowered species of *Specklinia*, including the type of the genus, *Specklinia lanceola* (Sw.) Lindl. (Fig. 50, Table 5). The new species is similar to *Specklinia cactantha*, *S. fuegi* and *S. turrialbae* in habit, ramicauls and petiolate leaves, inflorescence, echinate ovary (in the material we studied; Fig. 47C), acuminate sepals and rounded to apiculate, obovate petals, and column thickened towards the apex, and pollinaria without caudicles or viscidium. Nevertheless, the combination of unusual characters observed in the new species, especially the complex lip morphology (Fig. 47A), is not found in any of those species groups, and we have yet to find a species that we can say is sister to this species of *Specklinia*.

Blumea 59: 180-184

Chapter 5

Specklinia lugduno-batavae (Orchidaceae: Pleurothallidinae), a new species in the *S. digitalis* group

Adam P. Karremans
Diego Bogarín
Barbara Gravendeel

Specklinia lugduno-batavae from the Caribbean lowlands of Nicaragua and Costa Rica is formally described and illustrated. The new species belongs to the *Specklinia digitalis* group and can be recognised by the creeping habit, purple spotted abaxial surface of the leaf and the almost immaculate whitish-cream flowers, which are produced in succession on a very short, flexuous inflorescence. The name honours Leiden University and the Hortus botanicus Leiden. The novelty is compared with its closest relatives, *Specklinia digitalis*, *S. pisinna* and *S. succulenta*.

Keywords: Costa Rica, Nicaragua, *Specklinia digitalis*, *S. lugduno-batavae*, *S. pisinna*, *S. succulenta*

Introduction

Traditionally included in *Pleurothallis* R.Br. (Luer 1986), the genus *Specklinia* Lindl. was re-established by Pridgeon & Chase (2001), and has ever since then, with some exceptions (Luer 2006), received general acceptance by the orchid community (Pridgeon 2005; Pupulin *et al.* 2012, Chapter 1; Bogarín *et al.* 2013b, Chapter 4; Karremans *et al.* 2013a). Species in the genus are recognised by the tiny habit with ramicauls shorter than the leaf, obtuse petals, a ligulate lip, prominent column wings and naked pollinia that lack caudicles (Karremans 2014, Chapter 7).

About 100 species are recognised in the genus (Chapter 6). It is distributed from Mexico, through Central America, southwards into Bolivia and Brazil, and the Antilles. *Specklinia grobyi* (Bateman ex Lindl.) F.Barros is perhaps the best known, most widely distributed and most variable species within the genus. Many of its morphological or geographical "variants" have been named, however the difficulty of clearly delimiting those entities has led authors to prefer a broad circumscription of *S. grobyi*. It is thus best referred to as the *S. grobyi* species complex (Luer 2006).

Within the 'grobyi' complex there are nonetheless several morphologically discrete, well-recognisable and accepted species. *Specklinia digitalis* (Luer) Pridgeon & M.W.Chase and *S. pisinna* (Luer) Solano & Soto Arenas from northern Central America are good examples. Both are easily distinguished from their close relatives by the tiny habit (plants under 3 cm tall), with suborbicular leaves, spotted with purple abaxially, the relatively elongate, racemose, multi-flowered inflorescence with a single flower open at a time, the conspicuous thickening of the apex of the dorsal sepal, and the ligulate, unlobed, mostly inornate lip, which is shallowly depressed in the middle. A third species with these general morphological features was described recently from the French Guyana as *Specklinia succulenta* Bellone & Archila. Here we formally describe a fourth species within the *S. digitalis* group, within the 'grobyi' complex, from Nicaragua and Costa Rica.

Taxonomic treatment

13. ***Specklinia lugduno-batavae*** Karremans, Bogarín & Gravend., Blumea 59(3): 180. 2015.

> *The species is similar to Specklinia pisinna but can be distinguished by the prostrate habit (vs. erect habit), shorter leaves (up to 8 vs. 11 mm long), the flexuous inflorescence with up to 6 flowers (vs. straight and containing up to 3 flowers), the creamy-white flowers (vs. heavily suffused and striped with purple), and the shorter lip (up to 1.6 vs. 2.3 mm). Specklinia digitalis is also similar but the new species can be distinguished by the shorter leaves (4-8 vs. 12-15 mm), the shorter inflorescence (up to 1.5 cm vs. up to 15 cm), the shorter sepals (3-4 vs. 5 mm long), the ligulate to narrowly elliptic petals (vs. obovate) and the shorter lip (1.5-1.6 vs. 2 mm long).*

Type:—COSTA RICA. Heredia, Sarapiquí, Horquetas, unpaved road to Rara Avis, ca. km 6, 10°20’40.2”N 83°59’30.3”W, 200 m, 9 April 2009, *F. Pupulin, B. Arias, D. Bogarín & C. Ossenbach 7709* (holotype JBL-spirit; D5055; Fig. 51 & 52).

Epiphytic, caespitose, prostrate to sub-erect herb to 1 cm tall, excluding the inflorescence. Roots fibrous, flexuous, glabrous. Stem abbreviated, terete-cylindric, to 1-2 mm long, monophyllous, completely concealed by papyraceous, sheaths. Leaves coriaceous, sub-orbicular to broadly elliptic, 4-8 by 3-6 mm, densely spotted with purple abaxially. Inflorescence borne laterally from the apex of the stem, without an annulus, an erect, flexuous, distichous, successively flowered raceme, with 1-2 flowers open at once, producing up to 6 flowers per inflorescence, up to 15-20 mm long; peduncle cylindric, to 15 mm long. Floral bracts infundibuliform, broadly ovate, acute, 1 mm long. Pedicel cylindric, glabrous, persistent, 2 mm long including the subclavate ovary. Flowers whitish cream, immaculate to slightly brownish stained along the sepal veins. Sepals fleshy, glabrous; dorsal sepal elliptic, 3-veined, acute, 4.0 by 2.1-2.3 mm; lateral sepals completely fused into an elliptic synsepal, 4-veined, 4.0-4.5 by 3 mm. Petals ligulate to narrowly elliptic, obtuse, 2.1-2.2 by 0.9-1.0 mm, 1-veined. Lip ligulate, longitudinally slightly arched-convex in natural position, thinly articulate with the column foot by a hyaline claw, obtuse, longitudinally depressed in the middle, 1.5-1.6 by 0.7-0.8 mm. Column slightly arched, terete-slender at the base, 1.6-1.8 mm long without the foot, provided with low, broad membranous wings at the apex; column foot inconspicuous. Anther cap deeply cucullate, ovate, 2-celled. Pollinia 2, obovate-complanate, minutely hooked at the base, lacking caudicles. The description is based on *Pupulin 7709*, *Pupulin 7710* and *Bogarín 6761*.

Etymology:—The name honours the Academia Lugduno Batava, nowadays Leiden University, and its Hortus Academicus Lugduno-Batavus, the current Hortus botanicus Leiden.

Other material examined:—Costa Rica, Heredia, Sarapiquí. OET, La Selva, Surá trail, 350 m, *R. Aguilar 8729* (LSCR), 16 Apr. 2004; CES trail, *O. Vargas 1264* (LSCR), 13 Jun. 2005. Unpaved road to Rara Avis, ca. km 6, 10°20’40.2”N 83°59’30.3”W, 200 m, *F. Pupulin, B. Arias, D. Bogarín & C. Ossenbach 7707* (JBL-spirit; D3465), 9 April 2009; *F. Pupulin et al. 7708* (JBL-spirit; D3752), 9 April 2009; *F. Pupulin et al. 7710* (JBL-spirit; D3126), 9 April 2009; *D. Bogarín, B. Arias, C. Ossenbach & F. Pupulin 6761* (JBL-spirit; D2921; Fig. 2b), 9 Abril 2009.

Other photographical material examined:—Nicaragua, Refugio de Vida Silvestre Los Guatusos, photographed by Fabricio Díaz Santos [photographic voucher, 108 in ‘Orquídeas del Río San Juan‘ Díaz Santos (2010)]. Nicaragua, Guatusos Reserve, photographed by Dick Culbert (digital voucher, www.dixpix.ca/meso_america/Flora/orchids/052_platystele.html).

Distribution & Ecology:—The species is known only from the tropical wet forest of the Caribbean lowlands occurring in Nicaragua and Costa Rica at elevations between 200-350 m.

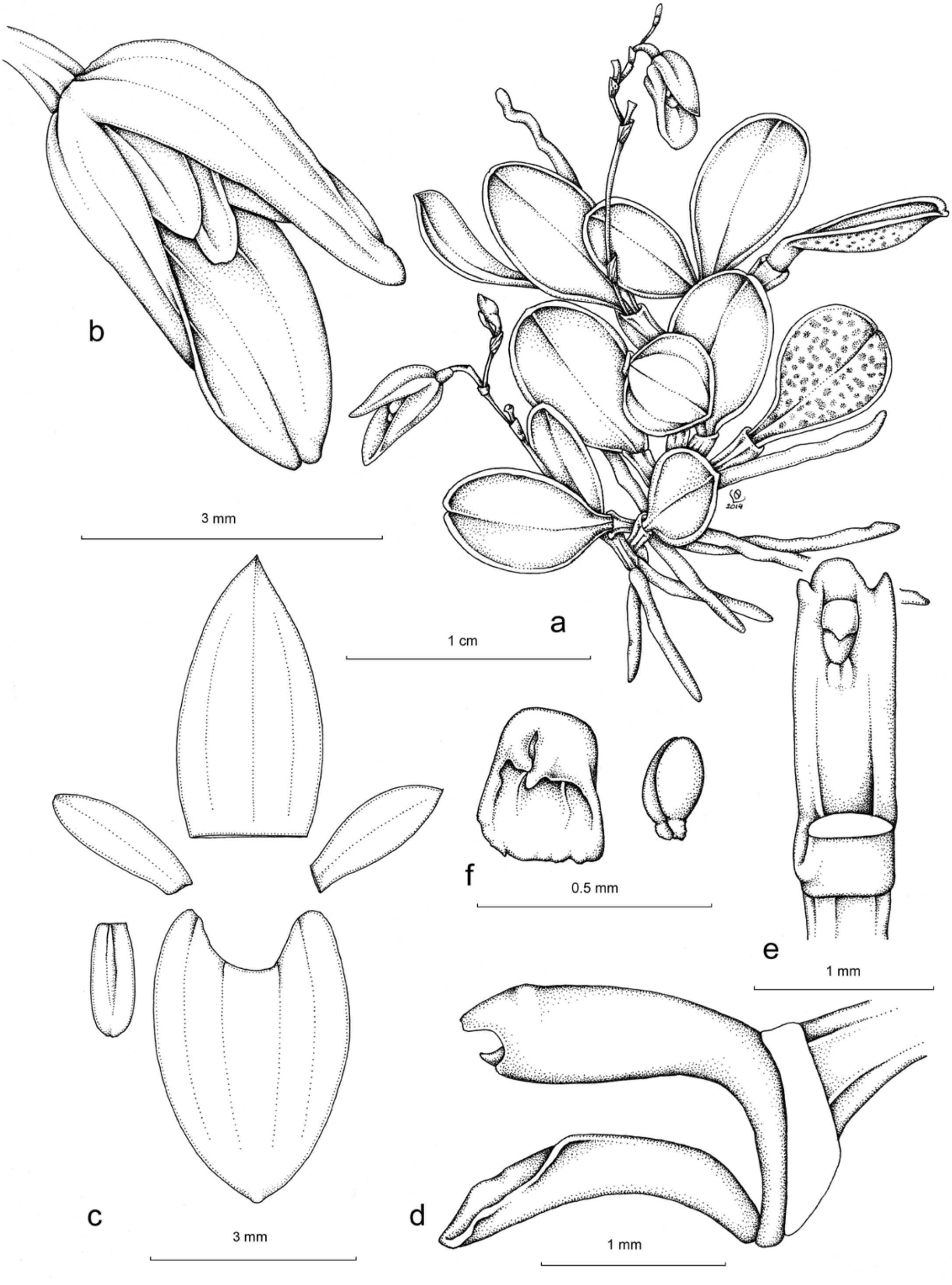


Figure 51. Specklinia lugduno-batavae Karremans, Bogarín & Gravendeel. a. Habit. b. Flower. c. Dissected perianth; d. Column and lip, lateral view; e. Column, ventral view; f. Anther cap and pollinia (*Pupulin 7709*, JBL-spirit). Drawn by D. Bogarín and inked by L. Oses.

Figure 52. *Specklinia lugduno-batavae* Karremans, Bogarín & Gravendeel. a. Closeup on a flower (*Pupulin 7709*, JBL-spirit); B. Showing the habit (*Bogarín 6761*, JBL-spirit) — Photographs by A.P. Karremans (A) and D. Bogarín (B).

Figure 53.The close relatives of *Specklinia lugduno-batavae* Karremans, Bogarín & Gravendeel. a. & b. *Specklinia digitalis* (Luer) Pridgeon & M.W.Chase (*Karremans 5737*, L-spirit); c. & d. *Specklinia pisinna* (Luer) Solano & Soto Arenas (*Karremans 4797*, L-spirit); e. & f. *Specklinia succulenta* Bellone & Archila (*Bellone 680*, LY) — Photographs by W. Driessen (A - D) and G. Chiron (E - F), reproduced with their kind permission.

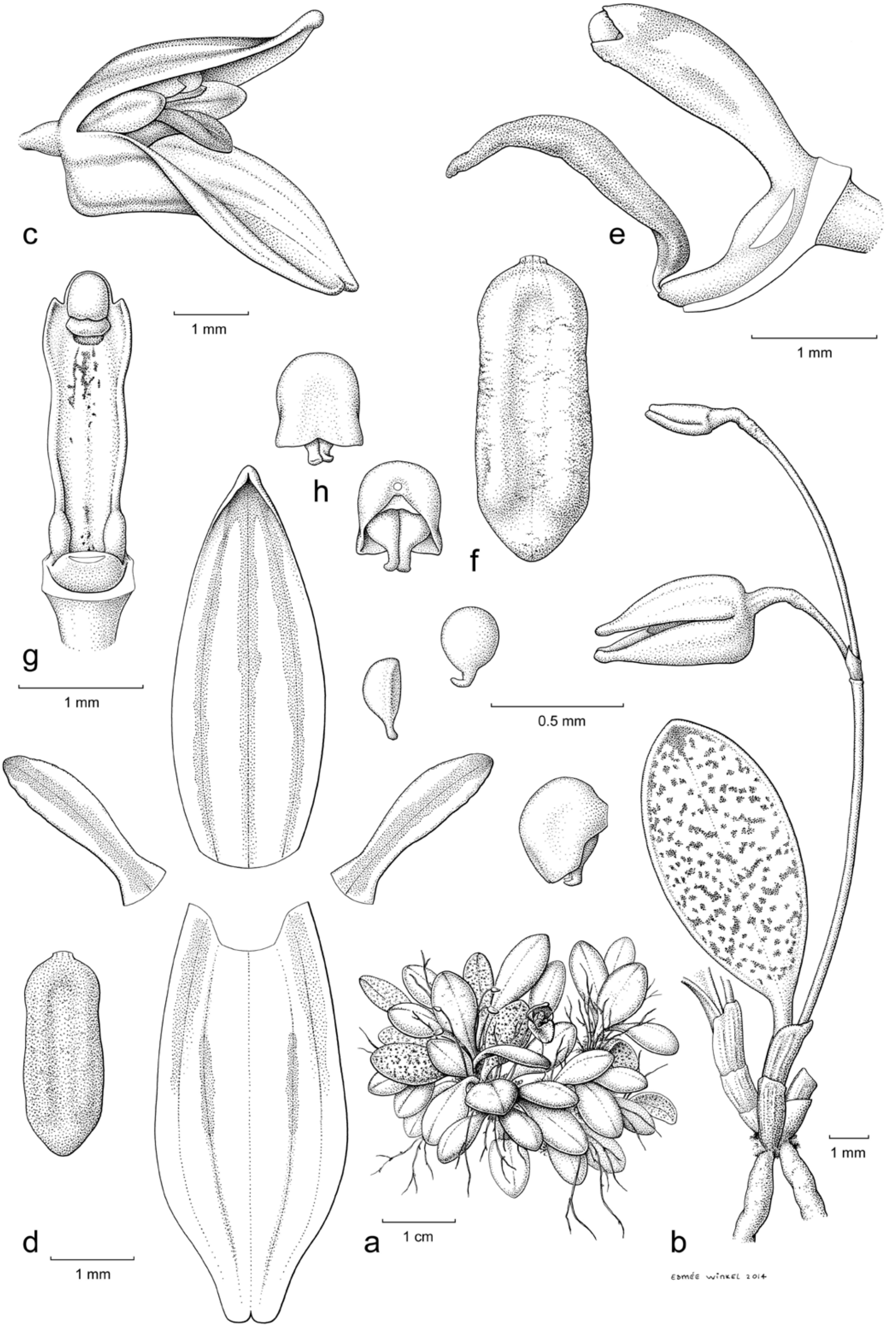


Figure 54. *Specklinia pisinna* (Luer) Solano & Soto Arenas. a. Habit. b. A leaf and inflorescence. c. Flower. d. Dissected perianth. e. Column and lip, lateral view; f. lip; g. Column, ventral view; h. Anther cap and pollinia (*Karremans 4749*, L-spirit). Drawn by E. Winkel.

Note:—The short plant up to 1 cm tall, the suborbicular leaf with purple spots on the abaxial surface, the flexuous, successive racemose inflorescence with a single flower open at a time, the conspicuous thickening of the apex of the dorsal sepal, and the ligulate, unlobed, mostly inornate lip, which is shallowly depressed longitudinally in the middle places *S. lugduno-batavae* in the *S. digitalis* species group (Fig. 53). The few tiny flowers and short inflorescence are similar to that of *S. pisinna* (Fig. 54), a species known to occur in Mexico, Guatemala and Honduras. However, the new species can be distinguished by the prostrate habit (vs. erect habit), with shorter leaves, up to 8 mm long (vs. 11 mm), the flexuous inflorescence containing up to 6 flowers (vs. straight, containing up to 3 flowers), the creamy-white flowers (vs. heavily suffused and striped with purple) and the shorter lip (up to 1.6 vs. 2.3 mm). From the Mexican endemic *S. digitalis*, it can be distinguished by the smaller prostrate habit with shorter leaves, 4-8 mm long (vs. 12-15 mm) and shorter inflorescence (up to 2 vs 15 cm long) the ligulate to narrowly elliptic petals (vs. obovate). *Specklinia succulenta* from French Guyana is also similar, but the new species can be distinguished by the prostrate habit (vs. erect), the short inflorescence (up to 2 vs. 10 cm long), the whitish cream flowers (vs. greenish-yellow) and the immaculate lip (vs. a lip with two purple stripes).

Figure 55. *Hortus Academicus Lugduno-Batavus* as depicted in the *Index Plantarum Horti Lugduno Batavi* (Boerhaave 1710). The chapel in the background is still part of the Hortus botanicus in Leiden today.

Phylogenetic reassessment of *Specklinia* and its allied genera

Chapter 6

Genetic similarity versus morphological divergence: phylogenetics of *Specklinia* (Orchidaceae)

Adam P. Karremans
Federico J. Albertazzi
Freek T. Bakker
Diego Bogarín
Marcel C.M. Eurlings
Alec Pridgeon
Franco Pupulin
Barbara Gravendeel

The phylogenetic relationships within *Specklinia*, a recently re-established genus of the Orchidaceae (Pleurothallidinae), and related genera are re-evaluated using Bayesian analyses of nuclear ITS and chloroplast *matK* sequence data of a wide sampling of species. *Specklinia* is found basically biphyletic in the DNA based trees, with species alternatively assigned to *Muscarella* proven distinct, monophyletic and well recognizable. *Muscarella* is therefore recognized as distinct. *Specklinia* as such includes about 95 morphologically highly variable species. Their phenotypic differences had prompted the creation of up to eleven generic names within this relatively small group. Here we show not only that these morphologically divergent species are closely related, but also that they can still be recognized by certain conserved morphological traits. The genera *Acostaea*, *Areldia*, *Empusella*, *Cucumeria*, *Gerardoa*, *Pseudoctomeria*, *Sarcinula*, *Sylphia*, *Tribulago* and *Tridelta* are found imbedded within Specklinia, and therefore placed in synonymy. *Specklinia* is confirmed sister to a clade that includes *Platystele*, *Scaphosepalum* and *Teagueia*. Five well supported subgenera are proposed for *Specklinia* and are characterized both geographically and morphologically. The species belonging to each subgenus are listed. *Incaea* is synonymized with *Dryadella*, and *Rubellia* is reduced under *Platystele*. New combinations for several species of *Dryadella*, *Muscarella*, *Platystele* and *Specklinia* are proposed.

Keywords: molecular phylogeny; morphology; Pleurothallidinae; *Specklinia*; systematics; taxonomy

Introduction

How to adequately circumscribe a genus is still highly debatable. Genera, as well as other above species-level groupings, are frequently considered arbitrary groups of species. Arbitrariness is reduced by the implementation of objective methodologies that result in the establishment of biologically significant groups. Recent systematic work, especially such that include molecular data, tends to result in more inclusive generic delimitations, whereas work based on morphological data tends to result in narrower generic delimitations. Humphreys and Linder (2009) suggested that "good genera are predictive and stable", which can be attained assessing, for example, their morphological recognisability, monophyly and reproductive isolation (Scopece *et al*. 2010).

Traditionally, *Specklinia* Lindl. (Orchidaceae: Pleurothallidinae) had been considered a synonym of *Pleurothallis* R.Br. (Luer 1986). However, the generic limits of the mammoth genus *Pleurothallis* were recircumscribed (Pridgeon & Chase 2001) on the basis of molecular studies by Pridgeon *et al*. (2001). The authors presented new evidence to re-establish *Specklinia*, recognizing 86 species. Both in the bootstrap consensus trees of the *matK*/*trnL-F* dataset and the most parsimonious tree from the combined *matK*/*trnL-F*/ITS DNA dataset a morphologically highly heterogeneous set of taxa, including *Dryadella simula* (Rchb.f.) Luer, *Pleurothallis costaricensis* Rolfe, *P. lentiginosa* Lehmann

& Kraezlin, *P. endotrachys* Rchb.f., *Acostaea costaricensis* Schltr., and species of the genera *Platystele* Schltr. and *Scaphosepalum* Pfitzer, are found together in a clade. In the tree obtained from the nrITS DNA matrix, based on a larger sampling, *P. lanceola* (Sw.) Sprengel —the type species of the genus *Specklinia*— was found together with *P. endotrachys*, *P. fulgens* Rchb.f., *P. lateritia* Endrés ex Rchb.f., *P. lentiginosa*, and *P. tribuloide*s (Sw.) Lindl., forming a distinct subclade treated by the authors as the "core" *Specklinia*.

The recircumscribed *Specklinia* included species of Pleurothallis subgen. *Specklinia* [*P*. sects. *Hymenodanthae* Barb.Rodr., *Tribuloides* Luer, *Muscariae* Luer], subgen. *Empusella*, subgen. *Pseudoctomeria* and *Acostaea* Schltr., showing low levels of sequence divergence (Pridgeon & Chase 2001). Among the morphological features useful to define *Specklinia*, the authors indicated the, usually, small plants with a short stem with an annulus, the variously connate sepals, and the hinged lip; the sepals and petals of *Specklinia* mostly membranous; the column with a toothed apex; and ventral anther and stigma. However, even with the removal of the basal *Dryadella* Luer and the derived *Platystele* and *Scaphosepalum*, the resulting circumscription of *Specklinia* is variable both in terms of vegetative and floral morphology (Luer 2006; Pupulin *et al*. 2012; Bogarín *et al.* 2013b, Chapter 4; Karremans *et al.* 2013b).

Specklinia is thus difficult to characterize on the basis of a particular set of distinguishing morphological features (Karremans 2014, Chapter 7), promoting the creation of several new genera, expressly designed to fit one or more morphologically aberrant species of *Specklinia* (Luer 2004; 2006). Due to the different interpretations of the circumscription of *Specklinia*, it had been difficult to estimate the actual number of species belonging to the genus. Pridgeon (2005) accounted for 200 species, but one year later Luer (2006) reduced the genus to some 40 species. Most recently Barros & Trettel Rodrigues (2009) accounted for 420 binomials, about five times the original number transferred by Pridgeon & Chase (2001).

Here we have chosen to re-evaluate phylogenetic relationships among the species with Specklinia affinity using a wide range of evidence. Our approach is to combine a molecular phylogeny covering about half of the species that belong to the genus, with a morphological and geographical characterization, as well as the establishment of a subgeneric classification. Our main goal is to understand relationships among species of all the proposed genera within this species group: *Acostaea*, *Areldia* Luer, *Cucumeria* Luer, *Dryadella*, *Gerardoa* Luer, *Incaea* Luer, *Muscarella* Luer, *Platystele*, *Pseudoctomeria* Kraenzl., *Rubellia* (Luer) Luer, *Sarcinula* Luer, *Scaphosepalum*, *Specklinia*, *Sylphia* Luer, *Teagueia* (Luer) Luer, *Tribulago* Luer, *Tridelta* Luer, *Trigonanthe* (Schltr.) Brieger and *Verapazia* Archila.

Materials and methods

Specimens were field-collected or obtained from the living collections at Lankester Botanical Garden (JBL), University of Costa Rica, the Hortus botanicus in Leiden (L), or from the private collections of G. Villalobos in Costa Rica, G. Vierling in Germany, and W. Driessen, P. Dubbeldam, T. Sijm and J. Wubben in the Netherlands. Selection of material was done on the basis of availability and interspecific variation. At least one representative of the genera, subgenera, or other groupings accepted in the alternative classification systems was included in the sampling when available. Many of the species included are Costa Rican in distribution, reflecting the prevailing nature of the JBL collections; however, specimens from a wide geographical range have been included as well. Putative species are represented by more than a single accession whenever possible, in order to assure better species delimitation, reducing risks of laboratory mix-ups and in accounting for sequencing error. Vouchers of specimens used are kept in the liquid collections at JBL or L, unless specified otherwise (Table 7).

DNA sequences of *Masdevallia hornii* Königer (= *Phloeophila yupanki* (Luer & R.Váquez) Pridgeon & M.W.Chase), *Platystele catiensis* Karremans & Bogarín, *Platystele tica* Karremans & Bogarín, *Specklinia absurda* Bogarín, Karremans & R.Rincón, *Specklinia acoana* Bogarín, *Specklinia berolinensis* Bogarín, *Specklinia remotiflora* Pupulin & Karremans and *Specklinia succulenta* Bellone & Archila were obtained from the plants that served as type material (Chiron *et al.* 2012; Pupulin *et al.* 2012, Chapter 1; Bogarín *et al.* 2013b, Chapter 4; Bogarín *et al.* 2014; Fernández *et al.* 2014).

Table 7. List of vouchers and GenBank number used in the phylogenetic analyses. Scientific names mostly follow Pridgeon (2005).

Taxon	Sequence Voucher	GenBank Accession Number ITS	GenBank Accession Number *matK*	Sequence Source
Anathallis grayumii (Luer) Luer (1)	*Karremans 2747*	KC425730	-	Karremans 2014
Anathallis grayumii (Luer) Luer (2)	*Pupulin 3794*	KC425731	KP012494	Karremans 2014
Anathallis lewisiae (Ames) Solano & Soto Arenas	Bogarín 1056	KC425733	KC425858	Karremans 2014
Anathallis pabstii (Garay) Pridgeon & M.W.Chase	*Karremans 4821*	KC425737	KC425859	Karremans 2014
Anathallis rabei (Foldats) Luer	*Karremans 4794*	KC425738	KC425860	Karremans 2014
Dryadella albicans (Luer) Luer	*Karremans 4861*	KC425742	KC425863	This Study
Dryadella aviceps (Rchb. f.) Luer	*van den Berg 1989*	JQ306381	-	GenBank
Dryadella edwallii (Cogn.) Luer	*Chase 305*	AF262824	AF265454	Pridgeon *et al.* 2001
Dryadella guatemalensis (Schltr.) Luer	*Karremans 3642*	KC425743	-	This Study
Dryadella hirtzii Luer	*BGH-123364*	EF079367	EF079327	GenBank
Dryadella kautskyi (Pabst) Luer	*van den Berg 1997*	JQ306380	-	GenBank
Dryadella simula (Rchb. f.) Luer	*Chase 1095*	AF262825	AF265453	Pridgeon *et al.* 2001
Dryadella susanae (Pabst) Luer	*Chiron 11240*	JQ306486	-	GenBank
Echinosepala aspasicensis (Rchb.f.) Pridgeon & M.W.Chase	*Chase 971*	AF262905	-	Pridgeon *et al.* 2001
Echinosepala aspasicensis (Rchb.f.) Pridgeon & M.W.Chase	*Bogarín 1945*	-	EU214340	GenBank
Lankesteriana barbulata (Lindl.) Pridgeon & M.W. Chase	*Bogarín 8606*	KC425726	KC425856	Karremans 2014
Lepanthopsis apoda (Garay & Dunst.) Luer	*Pridgeon 126*	KF747841	-	This Study
Pabstiella parvifolia (Lindl.) Luer (1)	*Karremans 2680*	KC425812	KP012497	This Study
Pabstiella parvifolia (Lindl.) Luer (2)	*Karremans 2680*	KC425813	-	This Study
Phloeophila nummularia (Rchb. f.) Garay (1)	*Karremans 5959*	KF747839	KP012380	This Study
Phloeophila nummularia (Rchb. f.) Garay (2)	*Karremans 5982*	-	KP012381	This Study
Phloeophila nummularia (Rchb. f.) Garay (3)	*Stenzel 896*	KC425841	-	Stenzel 2004
Phloeophila pelecaniceps (Luer) Pridgeon & M.W. Chase	*Chase 1128*	AF262810	AF265450	Pridgeon *et al.* 2001
Phloeophila peperomioides (Ames) Garay (1)	*None*	AF275690	AF291103	Pridgeon *et al.* 2001
Phloeophila peperomioides (Ames) Garay (2)	*Bogarín 7112*	KC425745	-	This Study
Phloeophila pleurothallopsis (Kraenzl.) Pridgeon & M.W. Chase (1)	*Chase 978*	AF262812	-	Pridgeon *et al.* 2001
Phloeophila pleurothallopsis (Kraenzl.) Pridgeon & M.W. Chase (2)	*Chase 5638*	AF262811	AF265451	Pridgeon *et al.* 2001
Phloeophila pleurothallopsis (Kraenzl.) Pridgeon & M.W. Chase (3)	*Karremans 4818*	KC425746	KP012495	This Study
Phloeophila pleurothallopsis (Kraenzl.) Pridgeon & M.W. Chase (4)	*Karremans 4856*	KC425747	KP012496	This Study
Phloeophila yupanki (Luer & R.Vásquez) Pridgeon & M.W.Chase (1)	*Karremans 4858*	KC425748	KP012498	This Study
Phloeophila yupanki (Luer & R.Vásquez) Pridgeon & M.W.Chase (2)	Karremans 5706a	KF747776	KP012382	This Study
Phloeophila yupanki (Luer & R.Vásquez) Pridgeon & M.W.Chase (3)	*Karremans 5706b*	KF747777	-	This Study
Platystele acicularis Luer & Hirtz	*Karremans 5785*	KF747778	KP012383	This Study
Platystele aurea Garay (1)	*Karremans 4807*	KC425762	-	This Study
Platystele aurea Garay (2)	*Karremans 5707b*	-	-	This Study
Platystele aurea Garay (3)	*Karremans 5707a*	KF747779	-	This Study
Platystele beatricis P. Ortiz	*Karremans 4801*	KC425749	KP012499	This Study
Platystele catiensis Karremans & Bogarín	*Bogarín 9661*	-	KP012384	This Study
Platystele caudatisepala (C.Schweinf.) Garay	*Bogarín 10230*	-	KP012385	This Study
Platystele compacta (Ames) Ames	*Karremans 4088*	KC425750	-	This Study
Platystele consobrina Luer	*Karremans 4835*	KC425751	-	This Study
Platystele gyroglossa Luer	*Karremans 4834*	KC425752	-	This Study
Platystele hirtzii Luer	*Karremans 5755*	KF747780	-	This Study
Platystele lancilabris (Rchb.f.) Schltr.	*Bogarín 10593*	-	KP012386	This Study
Platystele microtatantha (Schltr.) Garay	*Bogarín 8022*	KF747781	-	This Study
Platystele minimiflora (Schltr.) Garay	*Karremans 5980*	KF747782	KP012387	This Study
Platystele misasiana P. Ortiz	*Karremans 5768*	KF747783	KP012388	This Study
Platystele misera (Lindl.) Garay (1)	*Karremans 5749*	KF747784	KP012389	This Study
Platystele misera (Lindl.) Garay (2)	*Chase 5625*	AF262823	AF265470	Pridgeon *et al.* 2001
Platystele ovatilabia (Ames & C. Schweinf.) Garay	*Bogarín 3941*	KC425753	-	This Study
Platystele oxyglossa (Schltr.) Garay	*Karremans 4253*	KC425754	KP012500	This Study
Platystele oxyglossa (Schltr.) Garay aff.	*Karremans 5407*	KC425755	-	This Study
Platystele propinqua (Ames) Garay	*C.M. Smith 500*	KF747785	KP012390	This Study
Platystele reflexa Luer aff.	*Karremans 5733*	KC425756	-	This Study
Platystele schmidtchenii Schltr.	*Karremans 5995*	KF747786	-	This Study
Platystele stenostachya (Rchb.f.) Garay (1)	*Bogarín 5806*	KF747787	-	This Study
Platystele stenostachya (Rchb.f.) Garay (2)	*Pupulin 7919*	KC425759	KP012501	This Study
Platystele stenostachya (Rchb.f.) Garay (3)	*Chase 5618*	AF262821	-	Pridgeon *et al.* 2001

Table 7. Continued.

Taxon	Sequence Voucher	GenBank Accession Number ITS	GenBank Accession Number *matK*	Sequence Source
Platystele tica Karremans & Bogarín	*Karremans 5829A*	KP012458	KP012391	This Study
Platystele ximenae Luer & Hirtz	*Karremans 4865*	KC425760	KP012502	This Study
Scaphosepalum anchoriferum (Rchb.f.) Rolfe	*Bogarín 5418*	KP012459	KP012392	This Study
Scaphosepalum gibberosum (Rchb.f.) Rolfe	Chase 968	AF262817	AF265458	Pridgeon *et al.* 2001
Scaphosepalum grande Kraenzl.	*Chase 1107*	AF262819	-	Pridgeon *et al.* 2001
Scaphosepalum medinae Luer & J. Portilla (1)	*Karremans 4810a*	KC425763	-	This Study
Scaphosepalum medinae Luer & J. Portilla (2)	*Karremans 4810b*	KF747788	-	This Study
Scaphosepalum microdactylum Rolfe	*Pupulin 7897*	KP012460	KP012393	This Study
Scaphosepalum ovulare Luer	*Karremans 4809*	KC425764	KP012503	This Study
Scaphosepalum swertiifolium (Rchb.f.) Rolfe	*Chase 1383*	AF262818	-	Pridgeon *et al.* 2001
Scaphosepalum swertiifolium (Rchb.f.) Rolfe aff.	*Karremans 4811*	KC425765	KP012504	This Study
Scaphosepalum ursinum Luer (1)	*Karremans 4817*	KC425766	-	This Study
Scaphosepalum ursinum Luer (2)	*BGH-124283*	EF079365	-	GenBank
Scaphosepalum verrucosum (Rchb.f.) Pfitzer (1)	*Karremans 4812*	KC425767	KP012505	This Study
Scaphosepalum verrucosum (Rchb.f.) Pfitzer (2)	*Chase 1331*	AF262820	-	Pridgeon *et al.* 2001
Specklinia absurda Bogarín, Karremans & Rincón (1)	*Bogarín 9772*	KC425826	-	This Study
Specklinia absurda Bogarín, Karremans & Rincón (2)	*Bogarín 8711*	KC425827	KP012506	This Study
Specklinia acanthodes (Luer) Pridgeon & M.W.Chase	*Pridgeon 232*	KF747842	-	This Study
Specklinia acicularis (Ames & C.Schweinf.) Pridgeon & M.W.Chase	*Pupulin 5232*	KF747789	-	This Study
Specklinia acoana Bogarín	*A. Rojas 7718*	KF747800	-	This Study
Specklinia acrisepala (Ames & C.Schweinf.) Pridgeon & M.W.Chase (1)	*Karremans 3770*	KC425768	-	This Study
Specklinia acrisepala (Ames & C.Schweinf.) Pridgeon & M.W.Chase (2)	*M. Fernández 604*	KF747790	-	This Study
Specklinia alajuelensis Karremans & Pupulin (1)	*Karremans 5501*	KC425792	-	This Study
Specklinia alajuelensis Karremans & Pupulin (2)	*Karremans 3268*	KP012455	KP012411	This Study
Specklinia alajuelensis Karremans & Pupulin (3)	*Bogarín 2895*	KP012454	KP012412	This Study
Specklinia alajuelensis Karremans & Pupulin (4)	*Karremans 3265*	KC425791	-	This Study
Specklinia alata (A.Rich. & Galeotti) Solano & Soto Arenas	*Karremans 4840*	KC425806	-	This Study
Specklinia alta (Luer) Luer	*Karremans 5721*	KF747791	KP012394	This Study
Specklinia aristata (Hook.) Luer	*Stenzel 996*	KC425842	-	Stenzel 2004
Specklinia barbae (Schltr.) Luer (1)	*Karremans 5396*	KC425770	-	This Study
Specklinia barbae (Schltr.) Luer (2)	*Karremans 4853*	KC425771	-	This Study
Specklinia barbae (Schltr.) Luer (3)	Karremans 3928	KC425769	-	This Study
Specklinia barbae (Schltr.) Luer (4)	*M. Fernández 646*	KP012461	KP012395	This Study
Specklinia blancoi (Pupulin) Soto Arenas & Solano ano	*Karremans 5701*	KC425772	-	This Study
Specklinia brighamii (S.Watson) Pridgeon & M.W.Chase (1)	*Karremans 4799*	KC425773	-	This Study
Specklinia brighamii (S.Watson) Pridgeon & M.W.Chase (2)	*JBL-00887*	KC425774	-	This Study
Specklinia cabellensis (Rchb.f.) Karremans (1)	*Karremans 5712*	KF747792	KP012396	This Study
Specklinia cabellensis (Rchb.f.) Karremans (2)	*Karremans 5712*	KF747793		This Study
Specklinia cabellensis (Rchb.f.) Karremans (3)	*Karremans 5712*	KF747794		This Study
Specklinia cactantha (Luer) Pridgeon & M.W.Chase (1)	*Karremans 5965*	KF747795	KP012397	This Study
Specklinia cactantha (Luer) Pridgeon & M.W.Chase (2)	*Karremans 5979*	KF747796	-	This Study
Specklinia calyptrostele (Schltr.) Pridgeon & M.W.Chase (1)	*Pupulin 7060*	KC425775	KP012507	This Study
Specklinia calyptrostele (Schltr.) Pridgeon & M.W.Chase (2)	*Pupulin 7724*	KF747798	KP012398	This Study
Specklinia chontalensis (A.H.Heller & A.D.Hawkes) Luer (1)	*Pupulin 6543*	KC425776	-	This Study
Specklinia chontalensis (A.H.Heller & A.D.Hawkes) Luer (2)	*Pupulin 6543*	KF747799	KP012399	This Study
Specklinia claviculata (Luer & Hirtz) Luer	*Karremans 4827*	KC425777	-	This Study
Specklinia colombiana (Garay) Pridgeon & M.W.Chase aff.	*Karremans 4942*	KC425825	-	This Study
Specklinia colombiana (Garay) Pridgeon & M.W.Chase (1)	*Karremans 3235*	KC425809	-	This Study
Specklinia colombiana (Garay) Pridgeon & M.W.Chase (2)	*M. Fernández 481*	KC425810	-	This Study
Specklinia condylata (Luer) Pridgeon & M.W.Chase (1)	*Bogarín 7855*	KP012462	-	This Study
Specklinia condylata (Luer) Pridgeon & M.W.Chase (2)	*M. Fernández 170*	KP012463	-	This Study
Specklinia condylata (Luer) Pridgeon & M.W.Chase aff.	*Chase 6808*	AF262873	-	Pridgeon *et al.* 2001
Specklinia corniculata (Sw.) Steud. (4)	*Karremans 5180*	KF747801	KP012400	This Study
Specklinia corniculata (Sw.) Steud. (5)	*JBL-02240a*	KF747802	KP012401	This Study
Specklinia corniculata (Sw.) Steud. (6)	*JBL-02240b*	KF747803	KP012402	This Study
Specklinia corniculata (Sw.) Steud. (1)	*JBL-02227*	KC425781	-	This Study
Specklinia corniculata (Sw.) Steud. (2)	*Karremans 4782*	KC425782	-	This Study
Specklinia corniculata (Sw.) Steud. (3)	*Stenzel 889*	KC425844	-	Stenzel 2004

Table 7. Continued.

Taxon	Sequence Voucher	GenBank Accession Number ITS	GenBank Accession Number *matK*	Sequence Source
Specklinia costaricensis (Rolfe) Pridgeon & M.W.Chase (1)	*Chase 5636*	AF262863	-	Pridgeon *et al*. 2001
Specklinia costaricensis (Rolfe) Pridgeon & M.W.Chase (2)	*Bogarín 5643*	KC425783	-	This Study
Specklinia costaricensis (Rolfe) Pridgeon & M.W.Chase (3)	*Chase 5612*	AF262862	AF265459	Pridgeon *et al*. 2001
Specklinia cucumeris (Luer) Karremans (1)	Karremans 5757a	KF747804	KP012403	This Study
Specklinia cucumeris (Luer) Karremans (2)	*Karremans 5757b*	KF747805	-	This Study
Specklinia digitalis (Luer) Pridgeon & M.W.Chase	*Karremans 5737*	KF747806	KP012404	This Study
Specklinia displosa (Luer) Pridgeon & M.W.Chase (1)	*Karremans 5713b*	KF747807	KP012405	This Study
Specklinia displosa (Luer) Pridgeon & M.W.Chase (2)	*Karremans 5713c*	KF747808	-	This Study
Specklinia dodii (Garay) Luer	*Karremans 5963*	KF747809	KP012406	This Study
Specklinia dunstervillei Karremans, Pupulin & Gravend. (1)	*Karremans 5966*	KP012456	-	This Study
Specklinia dunstervillei Karremans, Pupulin & Gravend. (2)	*Karremans 5899*	-	KP012423	This Study
Specklinia endotrachys (Rchb.f.) Pridgeon & M.W.Chase (1)	*Blanco 961a*	KC425784	KP012508	This Study
Specklinia endotrachys (Rchb.f.) Pridgeon & M.W.Chase (2)	*Blanco 961b*	KF747810	KP012407	This Study
Specklinia fimbriata (Ames & C. Schweinf.) Luer	*Karremans 3718*	KC425785	-	This Study
Specklinia fuegi (Rchb.f.) Solano & Soto Arenas (1)	*Karremans 5600*	KC425786	KP012408	This Study
Specklinia fuegi (Rchb.f.) Solano & Soto Arenas (2)	*Karremans 5600*	KF747811	-	This Study
Specklinia fulgens (Rchb.f.) Pridgeon & M.W.Chase (1)	*Chase 5630*	AF262872	-	Pridgeon *et al*. 2001
Specklinia fulgens (Rchb.f.) Pridgeon & M.W.Chase (2)	*Karremans 3284*	KC425800	-	This Study
Specklinia fulgens (Rchb.f.) Pridgeon & M.W.Chase (3)	*JBL-001675*	KC425790	-	This Study
Specklinia fulgens (Rchb.f.) Pridgeon & M.W.Chase (4)	*Karremans 4843*	KC425788	-	This Study
Specklinia fulgens (Rchb.f.) Pridgeon & M.W.Chase (5)	*Karremans 3593*	KC425787	KP012409	This Study
Specklinia gersonii Bogarín & Karremans	*Karremans 6025*	KP012457	KP012424	This Study
Specklinia gracillima (Lindl.) Pridgeon & M.W.Chase (1)	*Karremans 4831*	KC425793	-	This Study
Specklinia gracillima (Lindl.) Pridgeon & M.W.Chase (2)	*Karremans 5999*	KF747812	-	This Study
Specklinia grisebachiana (Cogn.) Luer	*Stenzel 619*	KC425846	-	Stenzel 2004
Specklinia grobyi (Bateman ex Lindl.) F.Barros (1)	*Karremans 5463*	KF747813	-	This Study
Specklinia grobyi (Bateman ex Lindl.) F.Barros (2)	*JBL-10285*	KF747814	-	This Study
Specklinia grobyi (Bateman ex Lindl.) F.Barros (3)	*Pupulin 8187*	KC425799	-	This Study
Specklinia grobyi (Bateman ex Lindl.) F.Barros (4)	*Chiron 09357*	JQ306388	-	GenBank
Specklinia grobyi (Bateman ex Lindl.) F.Barros (5)	*Chase 1093*	AF262860	-	Pridgeon *et al*. 2001
Specklinia grobyi (Bateman ex Lindl.) F.Barros (6)	*Karremans 4220*	KC425794	-	This Study
Specklinia grobyi (Bateman ex Lindl.) F.Barros (7)	Karremans 3759	KC425796	-	This Study
Specklinia grobyi (Bateman ex Lindl.) F.Barros aff. (1)	*Karremans 4833*	KC425798	-	This Study
Specklinia grobyi (Bateman ex Lindl.) F.Barros aff. (2)	*Chiron 04524*	JQ306485	-	GenBank
Specklinia grobyi (Bateman ex Lindl.) F.Barros aff. (3)	*Karremans 5958*	KF747829	KP012413	This Study
Specklinia guanacastensis (Ames & C.Schweinf.) Pridgeon & M.W. Chase	*Karremans 6018*	KP012464	KP012414	This Study
Specklinia hastata (Ames) Pridgeon & M.W.Chase	*Bogarín 4910*	KF747773	-	This Study
Specklinia helenae (Fawc. & Rendle) Luer	*Stenzel 766*	KC425847	-	Stenzel 2004
Specklinia herpestes (Luer) Luer (1)	*Karremans 4082a*	KC425801	-	This Study
Specklinia herpestes (Luer) Luer (2)	*Karremans 4082b*	KC425802	-	This Study
Specklinia icterina Bogarín	*Bogarín 8767*	KC425778	-	This Study
Specklinia lanceola (Sw.) Lindl. (1)	*Karremans 5503*	KC425803	-	This Study
Specklinia lanceola (Sw.) Lindl. (2)	*Pridgeon s.n.*	KC425838	-	Pridgeon & Chase 2002
Specklinia lanceola (Sw.) Lindl. (3)	*Chase 1433*	AF262861	-	Pridgeon *et al*. 2001
Specklinia lentiginosa (F.Lehm. & Kraenzl.) Pridgeon & M.W.Chase (1)	*None*	AF275692	-	Pridgeon *et al*. 2001
Specklinia lentiginosa (F.Lehm. & Kraenzl.) Pridgeon & M.W.Chase (2)	*Karremans 3011*	KC425804	-	This Study
Specklinia lichenicola (Griseb.) Pridgeon & M.W.Chase	*Stenzel 452*	KC425845	-	Stenzel 2004
Specklinia llamachoi (Luer) Luer	*Stenzel 545*	KC425848	-	Stenzel 2004
Specklinia longilabris (Lindl.) Luer	*Stenzel 895*	KC425849	-	Stenzel 2004
Specklinia lugduno-batavae Karremans, Bogarín & Gravend. (1)	*Pupulin 7709*	KC425824	-	This Study
Specklinia luis-diegoi (Luer) Luer (1)	*Karremans 5500*	KC425835	-	This Study
Specklinia luis-diegoi (Luer) Luer (2)	*Karremans 5500*	KF747815	-	This Study
Specklinia macroblepharis (Rchb. f.) Luer	*Karremans 4860*	KC425805	-	This Study
Specklinia megalops (Luer) Luer	*Karremans 4792*	KC425807	-	This Study
Specklinia microphylla (A.Rich. & Galeotti) Pridgeon & M.W.Chase (1)	*Bogarín 9394*	KC425808	-	This Study
Specklinia microphylla (A.Rich. & Galeotti) Pridgeon & M.W.Chase (2)	*JBL-00968*	KP012465	-	This Study
Specklinia montezumae (Luer) Luer (1)	*Karremans 229*	KC425811	KP012509	This Study

TABLE 7. Continued.

Taxon	Sequence Voucher	GenBank Accession Number ITS	GenBank Accession Number *matK*	Sequence Source
Specklinia montezumae (Luer) Luer (2)	*Karremans 5751*	KF747816	-	This Study
Specklinia morganii (Luer) Luer (1)	*Karremans 5728a*	KF747817	KP012415	This Study
Specklinia morganii (Luer) Luer (2)	*Karremans 5728b*	KF747818	-	This Study
Specklinia mucronata (Lindl. ex Cogn.) Karremans	*Stenzel 478*	KC425850	-	Stenzel 2004
Specklinia obliquipetala (Acuña & C.Schweinf.) Karremans	*Stenzel 789*	KC425851	-	Stenzel 2004
Specklinia pfavii (Rchb.f.) Pupulin & Karremans (1)	Karremans 4825	KC425814	KP012510	This Study
Specklinia pfavii (Rchb.f.) Pupulin & Karremans (2)	*Karremans 3656*	KF747819	-	This Study
Specklinia pfavii (Rchb.f.) Pupulin & Karremans (3)	*JBL-11086*	KF747820	-	This Study
Specklinia picta (Lindl.) Pridgeon & M.W.Chase (1)	*van den Berg 2146*	JQ306384	-	GenBank
Specklinia picta (Lindl.) Pridgeon & M.W.Chase (2)	*Karremans 4836*	KC425815	-	This Study
Specklinia picta (Lindl.) Pridgeon & M.W.Chase aff.	*Chiron 06131*	JQ306385	-	GenBank
Specklinia pisinna (Lindl.) Solano & Soto Arenas (1)	*Karremans 4797*	KC425795	-	This Study
Specklinia pisinna (Lindl.) Solano & Soto Arenas (2)	*Karremans 4839*	KC425797	-	This Study
Specklinia psichion (Luer) Luer (1)	*Bogarín 8299*	KC425816	-	This Study
Specklinia psichion (Luer) Luer (2)	*Karremans 5955*	KF747821	-	This Study
Specklinia quinqueseta (Ames) Luer	*Karremans 3940*	KC425817	-	This Study
Specklinia recula (Luer) Luer (1)	*Karremans 5300a*	KF747822	KP012416	This Study
Specklinia recula (Luer) Luer (2)	*Karremans 5300b*	KF747823	KP012417	This Study
Specklinia recula (Luer) Luer (3)	*Karremans 5832*	KF747824	KP012418	This Study
Specklinia recula (Luer) Luer (4)	*Karremans 5823*	KP012466	-	This Study
Specklinia remotiflora Pupulin & Karremans (1)	*Karremans 4798a*	KC425818	KP012511	This Study
Specklinia remotiflora Pupulin & Karremans (2)	*Karremans 4798b*	KC425819	-	This Study
Specklinia remotiflora Pupulin & Karremans (3)	*Karremans 4854*	KC425820	-	This Study
Specklinia remotiflora Pupulin & Karremans aff.	*Chase 1303*	AF262859	AF265456	Pridgeon *et al.* 2001
Specklinia schaferi (Ames) Luer	*Stenzel 453*	KC425852	-	Stenzel 2004
Specklinia scolopax (Luer & R.Escobar) Pridgeon & M.W.Chase	*Karremans 4820*	KC425821	KP012512	This Study
Specklinia segregatifolia (Ames & C.Schweinf.) Solano & Soto-Arenas	*Bogarín 7990*	KC425822	-	This Study
Specklinia simmleriana (Rendle) Luer	*Karremans 4205*	KC425823	-	This Study
Specklinia sp. (1)	*Karremans 5988*	KF747774	KP012419	This Study
Specklinia sp. (2)	*Karremans 5989*	KF747775	KP012420	This Study
Specklinia sp. (3)	*Bogarín 9668*	KF747832	-	This Study
Specklinia sp. (4)	*Karremans 5962*	KF747828	KP012421	This Study
Specklinia sp. (5)	*Karremans 5997a*	KF747825	-	This Study
Specklinia sp. (6)	Karremans 5997b	KF747826	-	This Study
Specklinia sp. (7)	*Karremans 5996*	KF747827	KP012422	This Study
Specklinia sp. (8)	*Karremans 4823*	KC425779	KP012513	This Study
Specklinia spectabilis (Ames & C.Schweinf.) Pupulin & Karremans (1)	*Karremans 5250*	KC425829	-	This Study
Specklinia spectabilis (Ames & C.Schweinf.) Pupulin & Karremans (2)	*Bogarín 7401*	KC425830	-	This Study
Specklinia spectabilis (Ames & C.Schweinf.) Pupulin & Karremans (3)	*Karremans 5699*	KC425828	-	This Study
Specklinia strumosa (Ames) Luer	*Karremans 4359*	KC425831	-	This Study
Specklinia subpicta (Schltr.) F.Barros	*Chiron 11046*	JQ306389	-	GenBank
Specklinia succulenta Bellone & Archila	*Bellone 680*	JQ306383	-	GenBank
Specklinia tribuloides (Sw.) Pridgeon & M.W.Chase (1)	*Chase 5615*	AF262867	-	Pridgeon *et al.* 2001
Specklinia tribuloides (Sw.) Pridgeon & M.W.Chase (2)	*Stenzel 634*	KC425853	-	Stenzel 2004
Specklinia tribuloides (Sw.) Pridgeon & M.W.Chase (3)	*Karremans 3276*	KC425834	-	This Study
Specklinia tribuloides (Sw.) Pridgeon & M.W.Chase (4)	*Karremans 4804a*	KC425832	-	This Study
Specklinia tribuloides (Sw.) Pridgeon & M.W.Chase (5)	*Karremans 4804b*	KC425833	-	This Study
Specklinia trichyphis (Rchb.f.) Luer	*Stenzel 620*	KC425854	-	Stenzel 2004
Specklinia trilobata (Luer) Pridgeon & M.W.Chase	*Pridgeon 112*	KF747843	-	This Study
Specklinia truncicola (Rchb.f.) F.Barros & L.R.S.Guim.	*JG 4131*	JQ306391	-	GenBank
Specklinia turrialbae (Luer) Luer (1)	*Karremans 5635*	KF747830	KP012425	This Study
Specklinia turrialbae (Luer) Luer (2)	*Karremans 5601*	KF747831	-	This Study
Specklinia vierlingii Baumbach	*Pupulin 2894*	KC425780	-	This Study
Specklinia vittariifolia (Schltr.) Pridgeon & M.W.Chase (1)	*Karremans 2945*	KP012452	KP012410	This Study
Specklinia vittariifolia (Schltr.) Pridgeon & M.W.Chase (2)	*Karremans 5944*	KP012453	-	This Study
Specklinia wrightii (Rchb.f.) Luer	*Stenzel 733*	KC425855	-	Stenzel 2004
Teagueia tentaculata Luer & Hirtz	*Pridgeon 142*	KF747844	-	This Study
Trichosalpinx notosibirica (T. Hashim.) Luer	*Pridgeon 225*	KF747845	-	This Study

DNA extraction and sequencing:—Fresh leaf and flower cuttings of about 1 cm² were obtained from all the selected individuals of each species. Each individual sample was put into a polypropylene bag with silica gel to dry for about a week after which the silica was removed and new dry silica was added. Twenty mg of every individual sample was pulverized in liquid nitrogen with a Retsch MM 300 shaker for 5 min using three bullets/glass beads. Extraction was performed following the DNEasy Plant Mini Kit extraction protocol (QIAGEN). DNA concentration for each sample was adjusted to 10 µmol/l using a NanoDrop Spectrophotometer (ND 1000).

The nuclear ribosomal internal transcribed spacer (ITS) region was amplified using the methods and primers 17SE (ACGAATTCATGGTCCGGTGAAGTGTTCG) and 26SE (TAGAATTCCCCGGTTCGCTCGCCGTTAC) for sequencing and amplification, described by Sun *et al.* (1994). The chloroplast gene matK was amplified and sequenced using the Kew *matK* primers 2.1aF (ATCCATCTGGAAATCTTAGTTC) and 5R (GTTCTAGCACAAGAAAGTCG). Amplification was done by preparing each sample with a PCR mix composed of genomic DNA, Dream Taq Buffer, dNTPs, both primers, Dream Taq, water, and the extracted DNA. Samples were amplified in a MJ Research PTC-200 Pelthier Thermal Cycler, using a temperature profile of 94°C/5 min, followed by 34 cycles of 94°C/30 s, 55°C/30 s, and 72°C/2 min, and finally 72°C/10 min. Sanger sequencing was performed by Macrogen (http://www.macrogen.com) or BaseClear (http://www.baseclear.com) on an ABI 3730xl (Applied Biosystems).

Building the data sets:—The STADEN (Staden *et al.* 2003) package was used for editing the sequences. Where more than one base pair was equally probable among the Sanger tracers, the Unicode nomenclature (IUPAC) was used. In a few cases the two reads for one sample were too short and there was no overlap, so Pregap was unable to build a contig. In these cases, the forward and reverse sequences were merged by filling in missing positions with Ns. Sequences were aligned manually in Mesquite v2.72 (Maddison & Maddison 2007). The ends of each data set were trimmed and sequences were edited manually.

After the alignments had been edited, additional sequences were obtained from Hagen Stenzel (Stenzel 2004), and from NCBI GenBank, the latter using nBLAST. *Echinosepala aspasicensis* (Rchb.f.) Pridgeon & M.W.Chase was used as outgroup in all cases, as this taxon has been suggested to be the most earliest-branching lineage of all included species (Pridgeon *et al.* 2001).

Phylogenetic analysis:—The nrITS, *matK* and nrITS+*matK* data sets were analyzed using the Find Model web server (available at http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html) which uses MODELTEST [a program designed to compare different nested models of DNA substitution in a hierarchical hypothesis-testing framework (Posada & Crandall 1998)] to calculate the model scores, based on the AIC criterion. Gaps were small and scarce and therefore treated as missing data or eliminated from the data set. Phylogenetic inference with the maximum likelihood method was done using the randomized accelerated maximum likelihood (RAxML; Stamatakis, 2006). The nrITS+*matK* data set was analyzed using RAxML v8.1.11 (Stamatakis 2014), available on the CIPRES Science Gateway (Miller *et al.* 2010), with the GTR + CAT model. The program Bayesian Evolutionary Analysis and Sampling of Trees (BEAST; Drummond & Rambaut 2007) was used to analyze nrITS (results not shown), *matK* (results not shown), and nrITS + *matK* combined matrices. BEAST estimates rooted, time-measured phylogenies inferred using strict or relaxed molecular clock models, and was therefore preferred over Bayesian analyses methods. It is also a framework for testing evolutionary hypotheses without relying on a single tree topology. Substitution and clock models were set as unlinked. The GTR + Γ model included estimated frequencies, and 10 rate categories were used to model Γ distribution for both nrITS + *matK*. A relaxed clock model was used for both partitions; however, the model used for nrITS was Lognormal, while for *matK* it was set to Exponential, a better fit for the data. The used tree prior was speciation - yule birth, and the number of generations of the Markov Chain was set to 30,000,000.

Concatenating gene sequences for phylogenetic analysis can lead to artifacts, especially when discrepancies are found between the individual gene trees (Edwards *et al.* 2007; Kubatko & Degnan 2007). Therefore we tested whether strongly supported incongruence existed between our nrITS and *matK*-based trees. In the concatenated data set, nrITS sequences are directly followed by the *matK* sequence. In some cases one of the two sequences was not available but these were then equally analyzed as missing data. This was proven not to interfere with the final results when sampling size is large enough (Wiens 2006; Karremans 2010; Karremans *et al.* 2013a). Trees were visualized in FigTree v.1.3.1 (Rambaut 2009). Posterior probability (PP) values and bootstraps were added to the branches of the trees using the labeling option. Branches were re-ordered decreasingly.

Morphological characterization:—The morphological dissimilarities among species of *Specklinia* has led to a proliferation of generic concepts, proposing the segregation of several small species groups from the genus. With 95 species in a broad sense, *Specklinia* includes at least the type species of the genera *Acostaea* Schltr., *Cucumeria* Luer, *Empusella* Luer, *Pseudoctomeria* Kraenzl., *Sarcinula* Luer, *Sylphia* Luer, and *Tribulago* Luer (Pridgeon 2005). The type of the monotypic genus *Gerardoa* Luer was also transferred to *Specklinia* (Luer 2004), and morphological similarity would suggest that the monotypic *Areldia* Luer and *Tridelta* Luer might also belong in a broad concept of *Specklinia*. Lastly, Luer (2006) segregated species of *Pleurothallis* R.Br. subgen. *Specklinia* (Lindl.) Garay sect. *Muscariae* Luer into *Muscarella* Luer, a genus that has been mostly considered a synonym of *Specklinia*. For discussion and characterization purposes the most frequently taxonomically used morphological characters were manually added to a “per clade” summarized tree. This was done by collapsing the node subtending each clade in the consensus tree obtained from the combined nrITS+*matK* dataset in the BEAST analysis, using FigTree v.1.3.1.

Scanning Electron Microscopy (SEM):—Tissue samples of floral parts were prepared for SEM observation by harvesting tissue from flowers up to 48 h after the beginning of anthesis, fixing in FAA (ethanol 50%, acetic acid, formalin at a proportion of 18:1:1 v/v), and dehydration through a series of ethanol steps and critical-point drying using liquid CO_2. Dried samples were mounted and sputter-coated with gold and observed with a JEOL JSM-5300 scanning electron microscope, at an accelerating voltage of 10 kV. All images were processed digitally.

Macrophotography:—Color illustrations of whole flowers and pollinaria were made using a Nikon® D5100, D5300 or D7100 digital camera, a DFC295 Leica® digital microscope color camera with Leica FireCam version 3.4.1 software, and an Epson® V370 Photo Scanner. Adobe Photoshop® was used for editing images and stacking whenever necessary.

Geographical distribution patterns:—For discussion and characterization purposes, geographical labels were manually added to a “per clade” summarized tree. This was done by collapsing the node subtending each clade in the consensus tree obtained from the combined nrITS+*matK* dataset in the BEAST analysis, using FigTree v.1.3.1. Geographical (Table 8) data were taken from known distributions reported in the literature, especially by Luer (1988; 1990; 1991; 2005; 2006). Only the two countries where the species of each clade were most represented are shown. A comparison of clade composition in four distant and well-botanized regions —Antilles, Brazil, Ecuador, and Mexico— is given for comparative purposes.

Results

Nomenclature:—Taxon names follow Pridgeon (2005) unless indicated otherwise. Clades have been coded from A to J to simplify description of some species groups (Fig. 56).

The diverse analyses:—Six different analyses are presented here. Bayesian and likelihood methods are used to analyze the nrITS, *matK* and combined nrITS+*matK* matrices. The resulting consensus tree of the Bayesian and Likelihood analysis of the combined nrITS and *matK* matrices have been used to establish the clades (Fig. 56); those clades were not found back in all the tree topologies retrieved. The two support values from those analyses are given for each clade discussed here-forth. The resulting trees from the individual datasets can be found as supplementary files, their results are not presented here in detail. A summary of all the support values is given (Table 9). Differences between the separate analyses of the plastid *matK* and nuclear ITS matrices were found. Nevertheless, this is mostly due to the low resolution of the *matK* analyses and do not represent “hard” incongruences. The combined matrix mostly resulted in higher clade support and more consistent results and is thus preferred for the discussion. Concatenation of sequences was not always possible as fewer *matK* sequences were available.

Table 8. Absolute numbers of species belonging to each clade reported per country. The figures are based largely on Luer (1988; 1990; 1991; 2005; 2006).

Country	*Clade A*	*Clade B*	*Clade C*	*Clade D*	*Clade E*	*Clades A to E (Speck.)*	*Clade F (Platy.)*	*Clade G (Scaph.)*	*Clade H (Teag.)*	*Clades F to H*	*Clade I (Musca.)*	*Clades A to I*	*Clade J (Dryad.)*	*Clades A to J (Total)*
Belize	2	0	2	0	1	5	6	0	0	6	1	12	1	13
Bolivia	0	0	2	0	1	3	6	2	0	8	6	17	5	22
Brazil	0	0	2	0	0	2	4	0	0	4	2	8	17	25
Colombia	2	0	4	3	8	17	35	20	3	58	12	87	15	102
Costa Rica	16	2	6	2	6	32	16	4	0	20	7	59	5	64
Cuba	2	0	5	0	1	8	2	0	0	2	5	15	0	15
Dominican Rep.	0	0	7	0	0	7	0	0	0	0	2	9	0	9
Ecuador	1	0	10	2	4	17	56	35	10	101	29	147	18	165
Guatemala	3	1	3	0	2	9	14	1	0	15	5	29	3	32
Guyana	3	0	3	0	1	7	2	1	0	3	2	12	0	12
Haiti	2	0	6	0	1	9	0	0	0	0	2	11	0	11
Honduras	3	1	2	0	2	8	5	1	0	6	2	16	2	18
Jamaica	3	0	1	0	1	5	0	0	0	0	2	7	0	7
Mexico	4	1	4	0	1	10	8	1	0	9	2	21	3	24
Nicaragua	4	1	2	0	1	8	4	1	0	5	2	15	2	17
Panama	11	2	6	2	7	28	18	4	0	22	0	50	6	56
Peru	0	0	1	0	0	1	7	2	1	10	5	16	9	25
Puerto Rico	0	0	0	0	0	0	0	0	0	0	1	1	0	1
Venezuela	2	0	1	0	1	4	9	5	0	14	6	24	1	25

The *Specklinia* clade (Fig. 2 & 3; P.P.=61; Bp=56) is sister to a clade that includes accessions of the genera *Platystele*, *Scaphosepalum* and *Teagueia*. It can be subdivided into several subclades:

Clade A (Fig. 1 & 2; *Specklinia* subgen. *Specklinia*; P.P.=1; Bp=84) includes all species of *Specklinia* with reddish orange to greenish orange stained flowers. It includes the accessions of *Specklinia alajuelensis*, *S. barbae*, *S. blancoi*, *S. chontalensis*, *S. corniculata*, *S. displosa*, *S. dunstervillei*, *S. endotrachys* (type species of *Empusella*), *S. fulgens*, *S. gersonii*, *S. guanacastensis*, *S. lanceola* (type species of *Specklinia*), *S. lentiginosa* (type species of *Pseudoctomeria*) *S. montezumae* (type species of *Gerardoa*), *S. pfavii*, *S. psichion*, *S. remotiflora*, *S. spectabilis*, *S. tribuloides* (type species of *Tribulago*) and *S. vittariifolia*.

Clade B (Fig. 2 & 3; *Specklinia* subgen. *Sylphia*; P.P.=1; Bp=99) is sister to Clade A and contains the accessions of *Specklinia absurda*, *S. cucumeris* (type species of *Cucumeria*), *S. fuegi* (type species of *Sylphia*), and *S. turrialbae*.

Clade C (Fig. 2 & 3; *Specklinia* subgen. *Hymenodanthae*; P.P.=1; Bp=100) is sister to a clade including Clade A and Clade B (P.P.=0.97; Bp=39). It includes all species of *Specklinia* related to *S. grobyi*. The flowers of this group are characteristically whitish to yellowish, never stained orange. This includes *S. alta, S. calyptrostele, S. costaricensis, S. digitalis, S. dodii, S. gracillima, S. grobyi, S. grisebachiana, S. lichenicola, S. lugduno-batavae, S. microphylla, S. morganii, S. picta, S. pisinna, S. schaferi, S. subpicta, S. succulenta, S. trichyphis, S. truncicola* and *S. wrightii*.

Clade D (Fig. 2 & 3; *Specklinia* subgen. *Acostaea*; P.P.=1; Bp=81) contains *Specklinia cactantha*, *S. luis-diegoi*, *S. colombiana* (type species of *Acostaea*), *S. recula* and *S. trilobata*.

Clade E (Fig. 2 & 3; *Specklinia* subgen. *Sarcinula*; P.P.=1; Bp=100) includes the accessions of *Specklinia acoana, S. acrisepala, S. berolinensis, S. brighamii, S. condylata, S. scolopax, S. simmleriana* and *S. vierlingii*.

Clade F (Fig. 2 & 3; *Platystele*; P.P.=0.72; Bp=54) includes the accessions of *Platystele aurea* (type species of genus *Rubellia*), which are sister to the type clade (P.P.=0.91; Bp=72), which includes the accession of *Platystele beatricis, P. catiensis, P. caudatisepala, P. compacta* (type species of *Platystele*), *P. consobrina, P. gyroglossa, P. hirtzii, P. microtatantha, P. minimiflora, P. misasina, P. misera, P. ovatilabia, P. oxyglossa, P. propinqua, P. schmidtchenii, P. stenostachya, P. tica* and *P. ximenae*.

Clade G (Fig. 2 & 3; *Scaphosepalum*; P.P.=0.87; Bp=52) includes the accessions of *Scaphosepalum anchoriferum, S. clavellatum, S. gibberosum, S. grande, S. microdactylum, S. ovulare, S. swertiifolium, S. ursinum* and *S. verrucosum* (type species of genus *Scaphosepalum*).

Table 9. Support values for selected clades obtained in the six different phylogenetic reconstructions made from the nrITS, *matK* and combined (nrITS+*matK*) matrices. Each matrix was analyzed by using Bayesian (BEAST) and Likelihood (RAxML) methods. Values are presented in the for of posterior probabilities (P.P.) in case of the BEAST analyses and bootstrap values (Bp) in the case of the RAxML analyses. Not Applicable (NA) is indicated when a clade consists of a single sequence. Unsupported (UN) is indicated when a clade is not found back.

	nrITS BEAST	*nrITS RAxML*	*matK BEAST*	*matK RAxML*	*Combined BEAST*	*Combined RAxML*
Clade A	1	75	UN	UN	1	84
Clade B	0.99	78	0.98	58	1	98
Clade C	1	100	1	94	1	100
Clade D	1	86	NA	NA	1	81
Clade E	1	100	0.99	50	1	100
Specklinia (A-E)	0.98	81	UN	UN	0.65	56
Clade F	0.88	52	0.92	56	0.88	54
Clade G	0.88	62	0.61	UN	0.87	52
Clade H	NA	NA	NA	NA	NA	NA
Clade I	0.48	UN	0.98	63	0.98	89
Clade J	1	100	0.99	69	1	98
Phloeophila	1	91	UN	UN	1	88

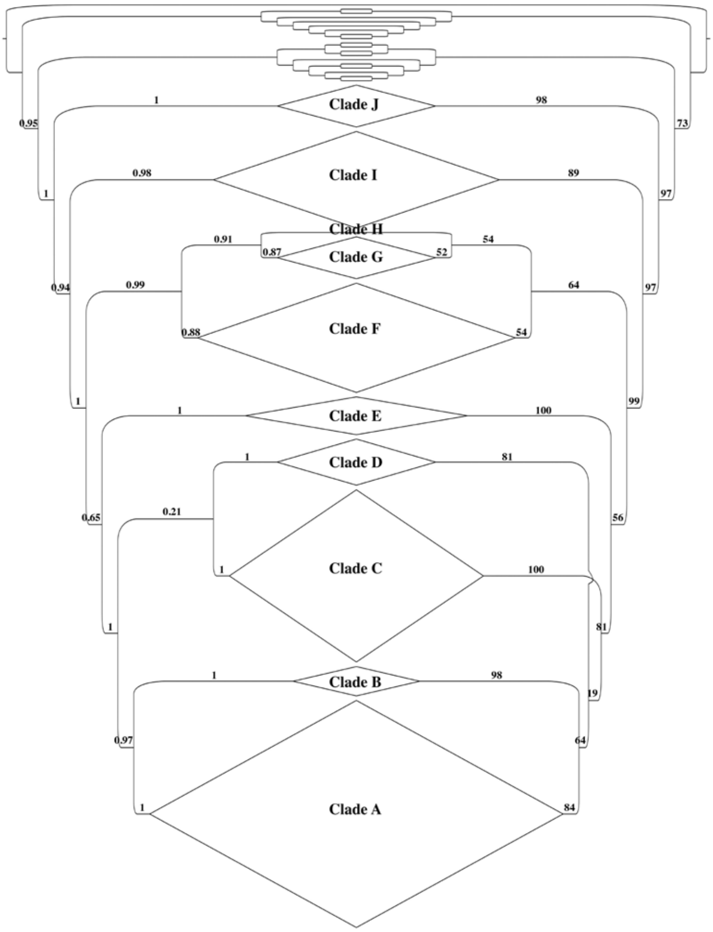


Figure 56. Phylogenetic relationship amongst the species of *Specklinia* and relatives inferred from the combined nrITS+*matK* dataset, summarized by clades. A. Using BEAST v1.6.0., where node values are posterior probabilities. B. using RAxML v8.1.11., where node values are bootstraps.

Clade H (Fig. 2 & 3; *Teagueia*) includes only the accession of *Teagueia tentaculata*. It is found sister to Clade *Scaphosepalum* (P.P.=0.74; Bp=54).

Clade I (Fig. 2 & 3; *Muscarella*; P.P.=0.98; Bp=89). It is sister to a highly supported clade (P.P.=1; Bp=99), which includes *Platystele*, *Scaphosepalum*, *Specklinia* and *Teagueia*. *Muscarella* includes the accessions of *Pabstiella parvifolia*, which are sister to the highly supported type clade (P.P.=1; Bp=99), that including *Specklinia alata, S. aristata* (type species of *Muscarella*), *S. cabellensis, S. claviculata, S. fimbriata, S. hastata, S. helenae, S. herpestes, S. llamachoi, S. longilabris, S. macroblepharis, S. marginata, S. megalops, S. mucronata, S. obliquipetala, S. quinqueseta, S. segregatifolia*, and *S. strumosa*.

Clade J (Fig. 2 & 3; *Dryadella*; P.P.=1; Bp=98) is sister to a highly supported clade (P.P.=0.94; Bp=97) including *Muscarella*, *Platystele*, *Scaphosepalum*, *Specklinia* and *Teagueia*. *Dryadella* includes the accessions of *Dryadella albicans, D. aviceps, D. edwallii, D. guatemalensis, D. hirtzii, D. kautskyi, D. simula, D. susanae* and *Phloeophila yupanki* (type species of *Incaea*).

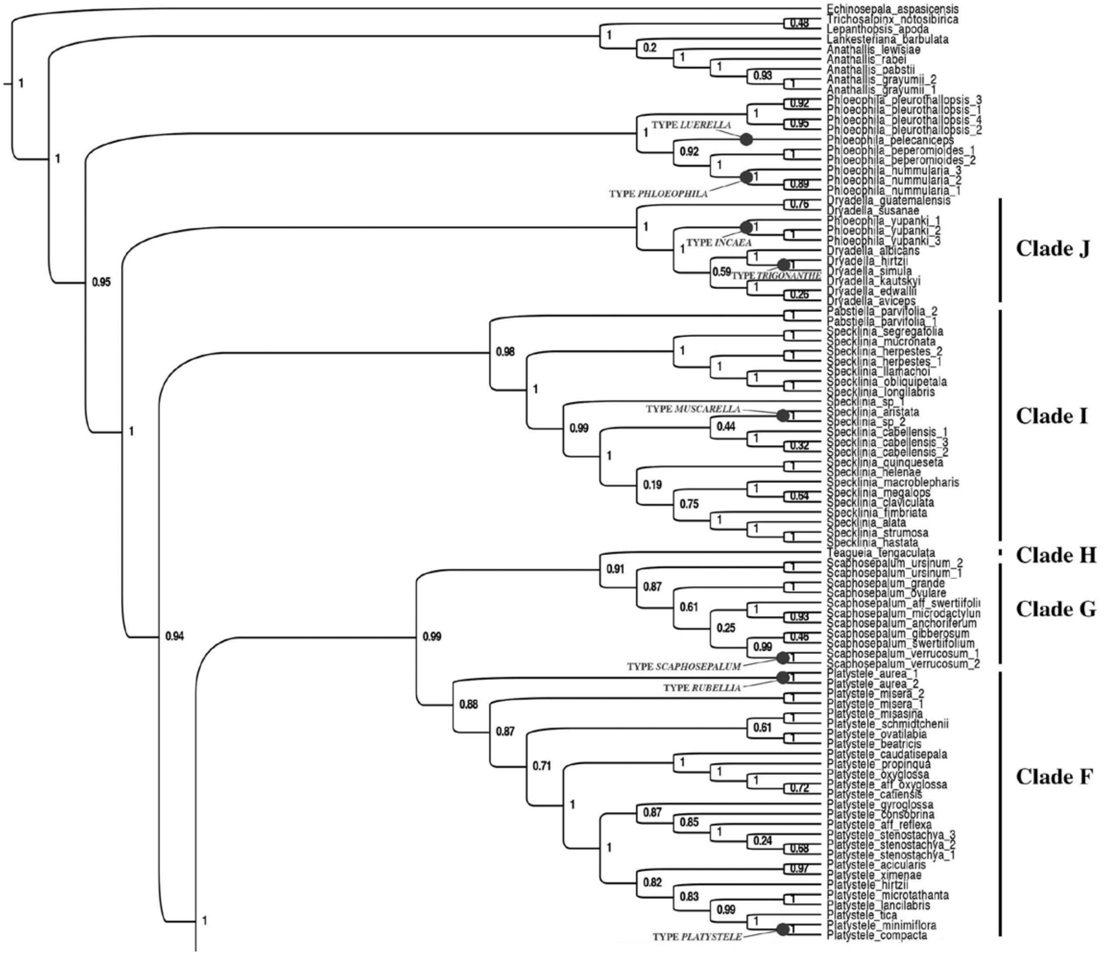


Figure 57. Phylogenetic relationship amongst the species of *Specklinia* based on a combined nrITS + *matK* dataset, using BEAST v1.6.0. Node values are posterior probabilities. A. Tree with branches transformed to be of equal length. B. Branch lengths relate to the relative number of changes.

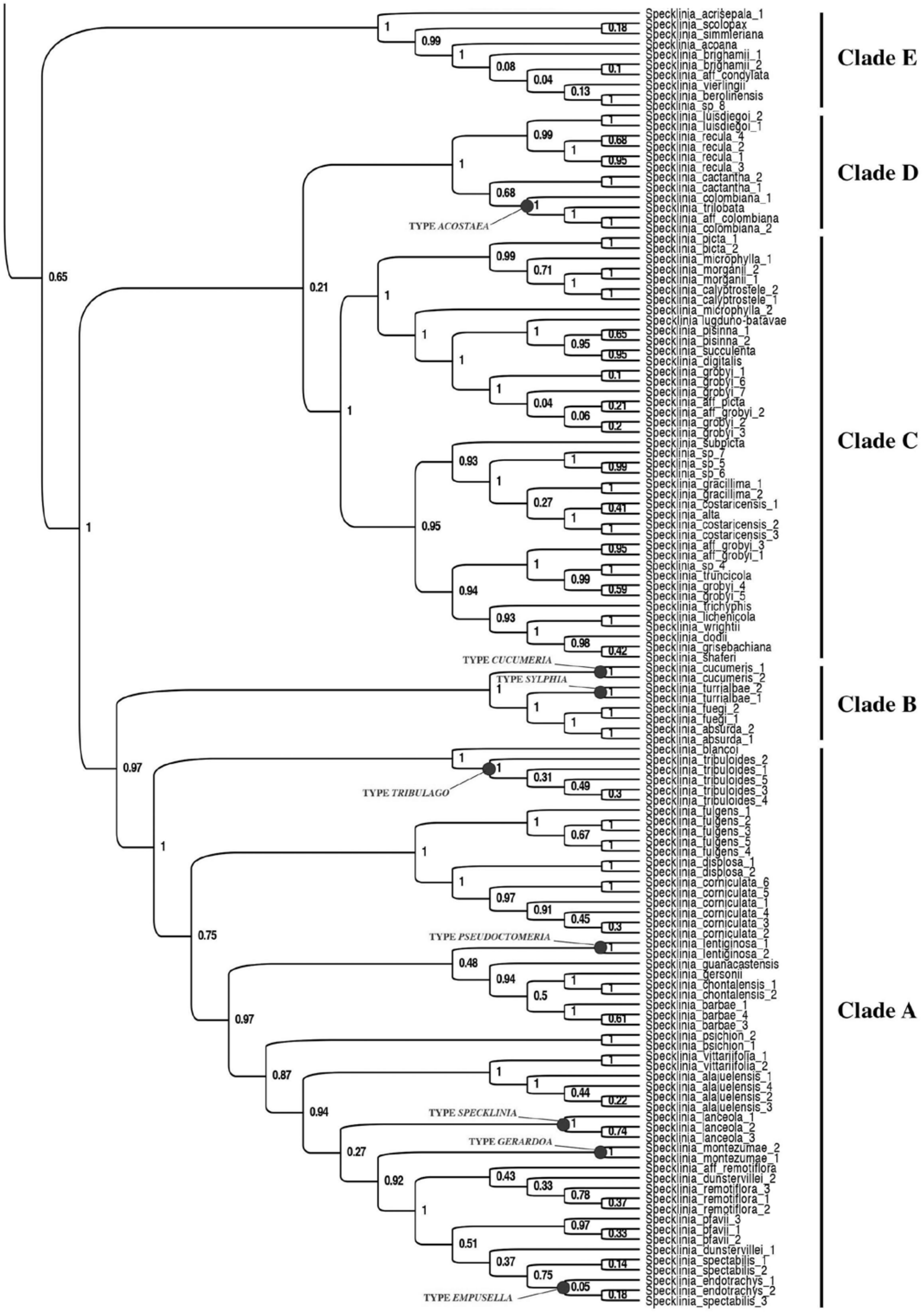


FIGURE 57. Continued

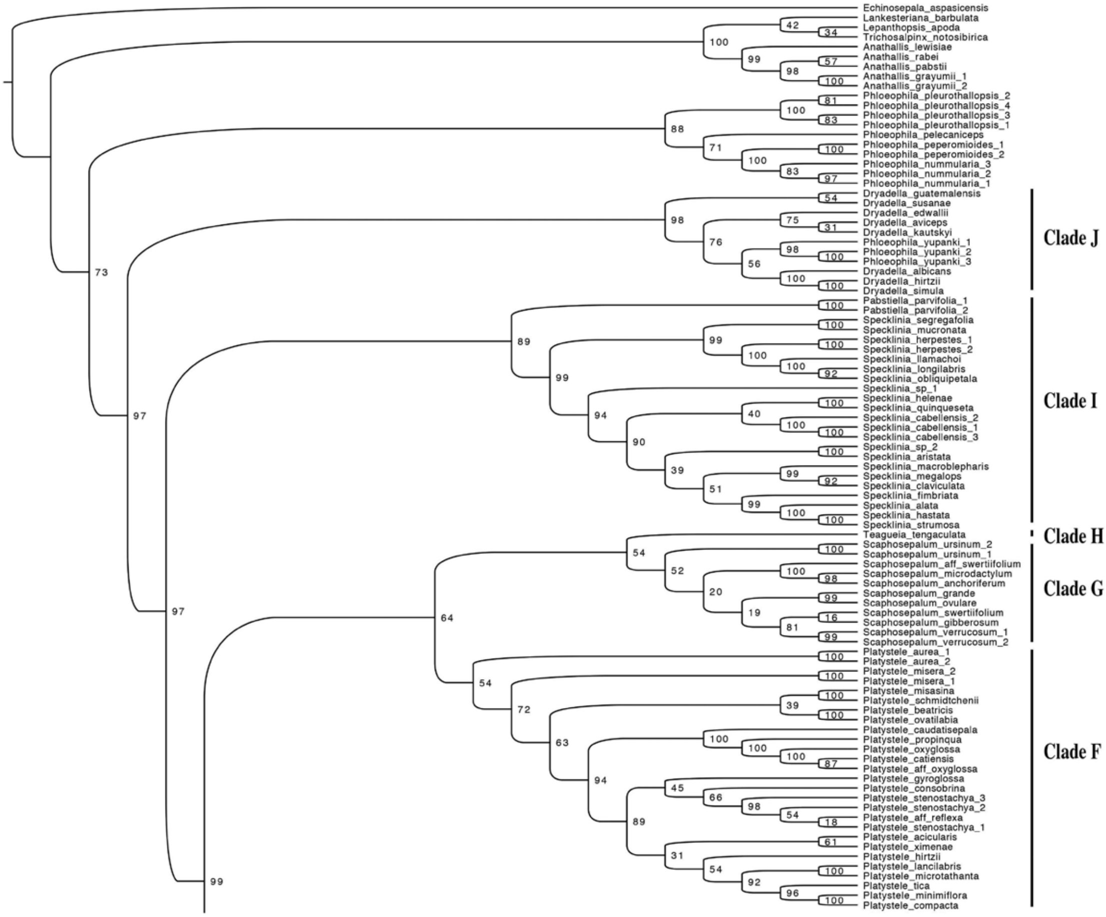


FIGURE 58. Phylogenetic relationship amongst the species of *Specklinia*. The tree was produced with an analysis of a combined nrITS + *matK* dataset using RAxML v8.1.11. Node values are bootstraps.

TABLE 10. Morphological recognition of the diverse clades within the *Specklinia* group.

Taxon	*Inflorescence*	*Flower Color*	*Pollinaria*
Dryadella (Clade J)	Successive, a single flower open at the same time.	Greenish yellow with purple spots, streaks of stains.	Pollinia + Caudicles
Muscarella (Clade I)	Successive, a single flower open at the same time.	Greenish yellow with purple spots, streaks of stains.	Pollinia + Caudicles
Teagueia (Clade H)	Simultaneous, several flowers open at once.	Monochrome, color varying.	Pollinia + Viscidium
Scaphosepalum (Clade G)	Successive, a single flower open at the same time.	Greenish yellow with purple spots, streaks of stains.	Pollinia (naked)
Platystele (Clade F)	Varying from successively single-flowered to simultaneous.	Monochrome, color varying.	Pollinia + Viscidium
Specklinia subgen. *Specklinia* (Clade A)	Successive, a single flower open at the same time.	Monochrome reddish orange or yellowish orange.	Pollinia (naked)
Specklinia subgen. *Sylphia* (Clade B)	Successive, a single flower open at the same time.	Mostly whitish with some purple streaks.	Pollinia (naked)
Specklinia subgen. *Hymenanthae* (Clade C)	Simultaneous, several flowers open at once.	Mostly monochrome purple, yellow, green or whitish.	Pollinia (naked)
Specklinia subgen. *Acostaea* (Clade D)	Successive, a single flower open at the same time.	Color varying.	Pollinia (naked)
Specklinia subgen. *Sarcinula* (Clade E)	Successive, a single flower open at the same time.	Greenish yellow with purple spots, steaks of stains.	Pollinia (naked)

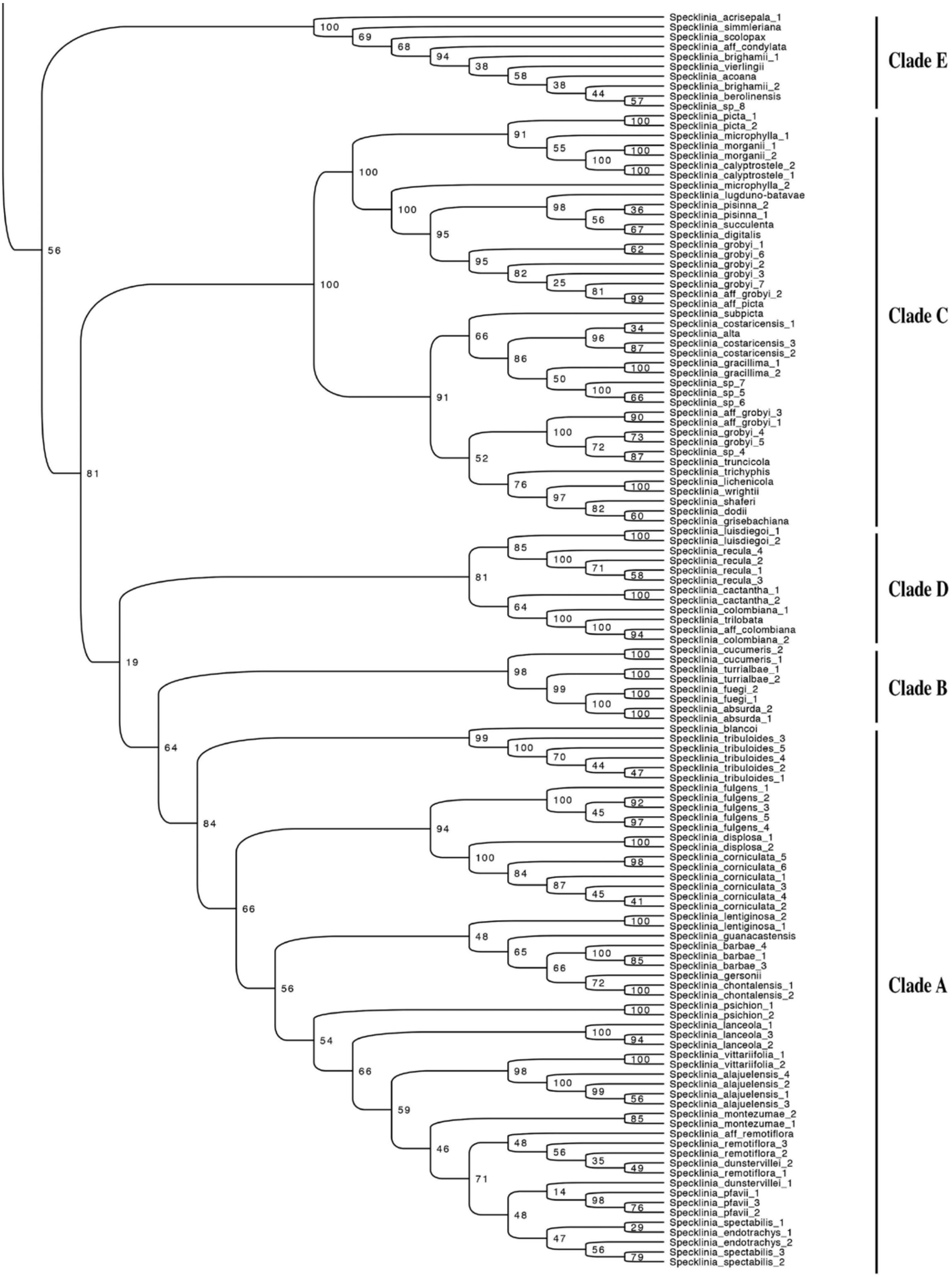


FIGURE 58. Continued

Morphology:—Morphological characterization of clades (Fig. 59; Table 10) was achieved by evaluating the available plant material or, when no entire voucher was available, by relying on the cited literature, mostly Luer (2006). Most species of *Specklinia* (Clades A though E) do share a short stem (much shorter than the leaves), obtuse petals and a ligulate-oblong lip; however, a single synapomorphy is shared by all species —the pollinia are nude. The lack of a caudicle and viscidium in *Specklinia* and *Scaphosepalum* allows for each pollinium to be free, albeit adjacent (Fig. 60). In species of *Dryadella* and *Muscarella*, pollinia are linked by a flat, granular, bilobed caudicle (whale-tail type pollinarium). In *Platystele* and *Teagueia*, pollinia lack caudicles but are linked by a drop-like viscidium (bubble-like pollinarium). The latter is associated with the apical disposition of the anther and stigma in the column (Fig. 61).

Other characters that proved most consistently distinct among the clades were inflorescence type (Fig. 62), flower coloration patterns and lip and column features. Characters such as resupination (=orientation of the flowers in such a way that the labellum is in abaxial position), so-called fasciculate inflorescences associated with a reduction in the length of the rachis, long-apiculate sepals, and prominently winged columns seem to have evolved several times independently. A sensitive lip evolved several times independently in Pleurothallidinae, but in the *Specklinia* clade it evolved only once (subgen. *Acostaea*).

Inflorescence (Fig. 62). Successively developing inflorescences, with one or few flowers open at once, are found in clades A, B, D, E, F, G and I. Simultaneously developing inflorescences, typically with several flowers open at the same time, are found in clades C, F and H. An extremely reduced rachis on which the pedicels are clustered (so-called fascicled inflorescences) is found in clades A, E, F and I.

Resupination (Fig. 62). In general species of this group have resupinate flowers, with a few exceptions per clade. Notably, for clade G non-resupination is typical.

Flower color (Fig. 63 & 64). Species of most clades have white to green flowers diversely spotted, striped or suffused with purple. Exceptions are found in clades A, C, F and H, of which the flowers are diversely colored, but mostly monochrome. Reddish orange to yellowish orange flowers are characteristic of clade A.

Lateral sepals (Fig. 63 & 64). Lateral sepals are generally convergent, forming an obtuse to acute synsepal; exceptions are found in clades B, F, H and I where the lateral sepals are free and divergent, and frequently long-apiculate. In clade G, the lateral sepals form a basally concave synsepal and are apically narrowed and thickened, usually with thickened calli on the distal portion.

Petals (Fig. 63 & 64). Simple, obtuse to acute petals are found throughout all clades except for clade I, where the petals are characteristically fimbriate and acute to caudate.

Lip. The lip of species in clades A and C is simple, ligulate-oblong. The lip of species of clade E is similar but provided with a pair of basal lobules. The lip of species in clade B is unguiculate. The lip of species of clade D has a series of complex lobes and calli, in several species it is extremely sensitive to touch. In clades F and H the lip is ovate-cordate, and in the latter it embraces the column.

Column (Fig. 61). The column of the species belonging to clades A, B, C, D, E, G, I and J is elongate and slender, with an incumbent anther and a ventral stigma. The column of species of clade F and H is short and stout, and the anther and stigma are apical. The column of species of clade C and D have a pair of prominent, rounded wings near the apex and a pair of orbicular glands at the base. In clade I the column is characteristically inornate.

Pollinia (Fig. 60 & 61). The "whale-tail" type pollinia, connected by a dry, granulose, bilobate caudicle, are only found in clades I and J. In clades F and H the pollinia are minuscule, lack caudicles and are provided with a drop-like viscidium at the base. In clade A, B, C, D, E and G the pollinia lack caudicles and a viscidium.

Geographical distribution:—The genus *Specklinia* is widespread, extending from Mexico to Bolivia and Brazil, through Central America and the Antilles. Nevertheless, geographical patterns of clade diversity can be seen in the resulting phylogenetic trees (Table 8; Fig. 65). Clades A and B are predominantly Costa Rican and Panamanian in distribution. Clade C has two disjunct centers of diversity, one in Hispaniola (Haiti and Dominican Republic) and another in Ecuador. Clade D is best represented in Colombia, while Clade E has the highest species diversity in Costa Rica and Panama. In general terms, *Specklinia* (Clades A to E) is most diverse in Costa Rica and Panama, followed by Ecuador and Colombia with about half the species. The sister genera, in clades F, G, H and I are mostly Andean in distribution, all with the highest diversity in Ecuador and Colombia. Finally, Clade J has two disjunct centers of diversity, one in Ecuador/Colombia (Andes) and another in Brazil.

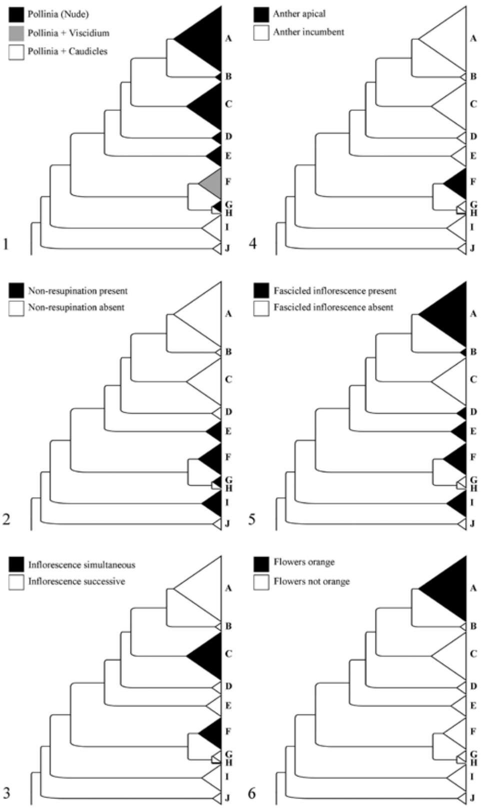


FIGURE 59. The combined nrITS + *matK* based phylogeny with the clades collapsed showing. 1: Pollinarium type. 2: Non-resupination. 3: Multi-flowered inflorescence 4: Apical anther. 5: Fascicled inflorescence. 6: Orange flowers.

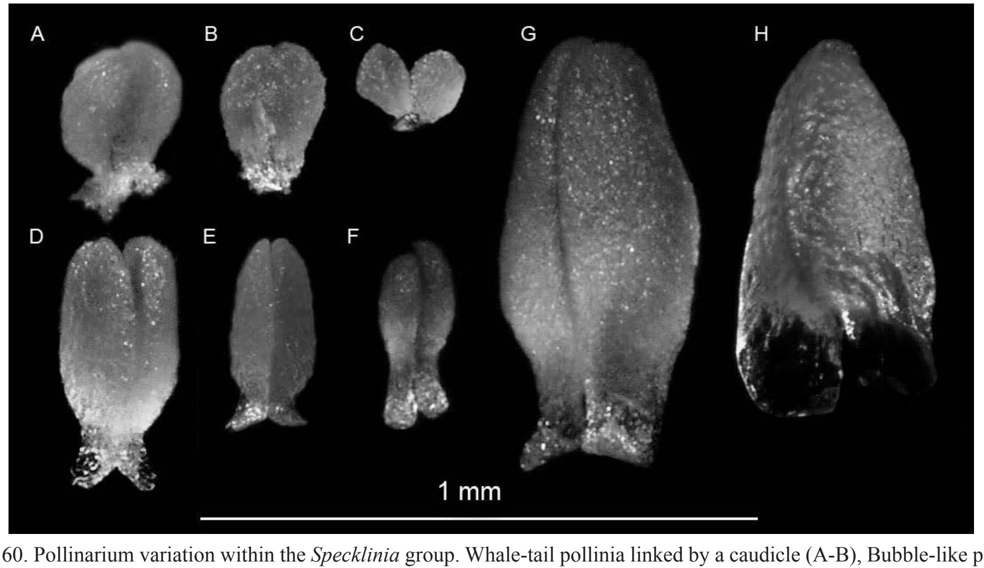


FIGURE 60. Pollinarium variation within the *Specklinia* group. Whale-tail pollinia linked by a caudicle (A-B), Bubble-like pollinia, brought together by a drop-like liquid viscidium (C), naked pollinia, adjacent but free (D-H). A: *Dryadella* (*AK6180*). B: *Muscarella strumosa* (*AK6450*). C: *Platystele* aff. *oxyglossa* (*MF789*). D: *Scaphosepalum microdactylum* (*DB10529*). E: *Scaphosepalum clavellatum* (*DB9218*). F: *Specklinia colombiana* (*DB8826*). G: *Specklinia condylata* (*MF173*). H: *Specklinia* aff. *endotrachys* (*AK5899*). Photographs by A.P. Karremans.

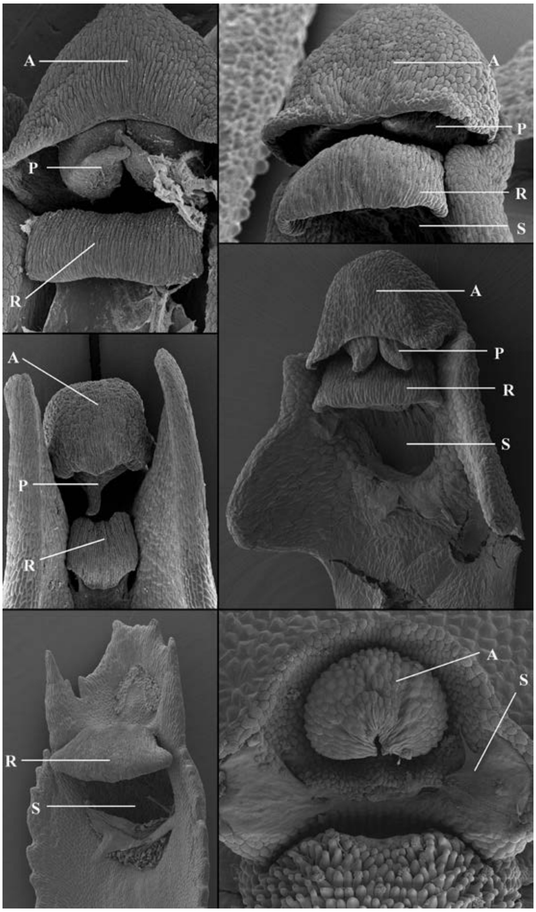


Figure 61. Column variation within the *Specklinia* group. Incumbent anther, ventral stigma covered by a large bubble-shaped rostellum, pollinia free (A-E), apical anther and stigma, rostellum reduced (F). A: *Specklinia barbae* (Clade A; *DB6483*). B: *Specklinia absurda* (Clade B; *DB9772*). C: *Specklinia grobyi* (Clade C; *AK4217*). D: *Specklinia recula* (Clade D; *AK5300*). E: *Specklinia berolinensis* (Clade E; *AK5806*). F: *Platystele* aff. *reflexa* (*AKsn*). Figure nomenclature is: A - anther cap, P - pollinia, R - rostellum, S - stigma. Photographs by A.P. Karremans.

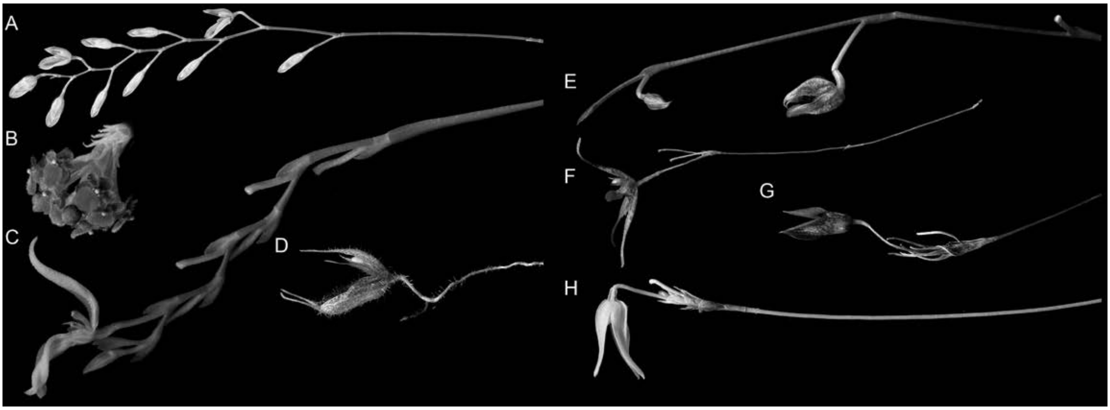
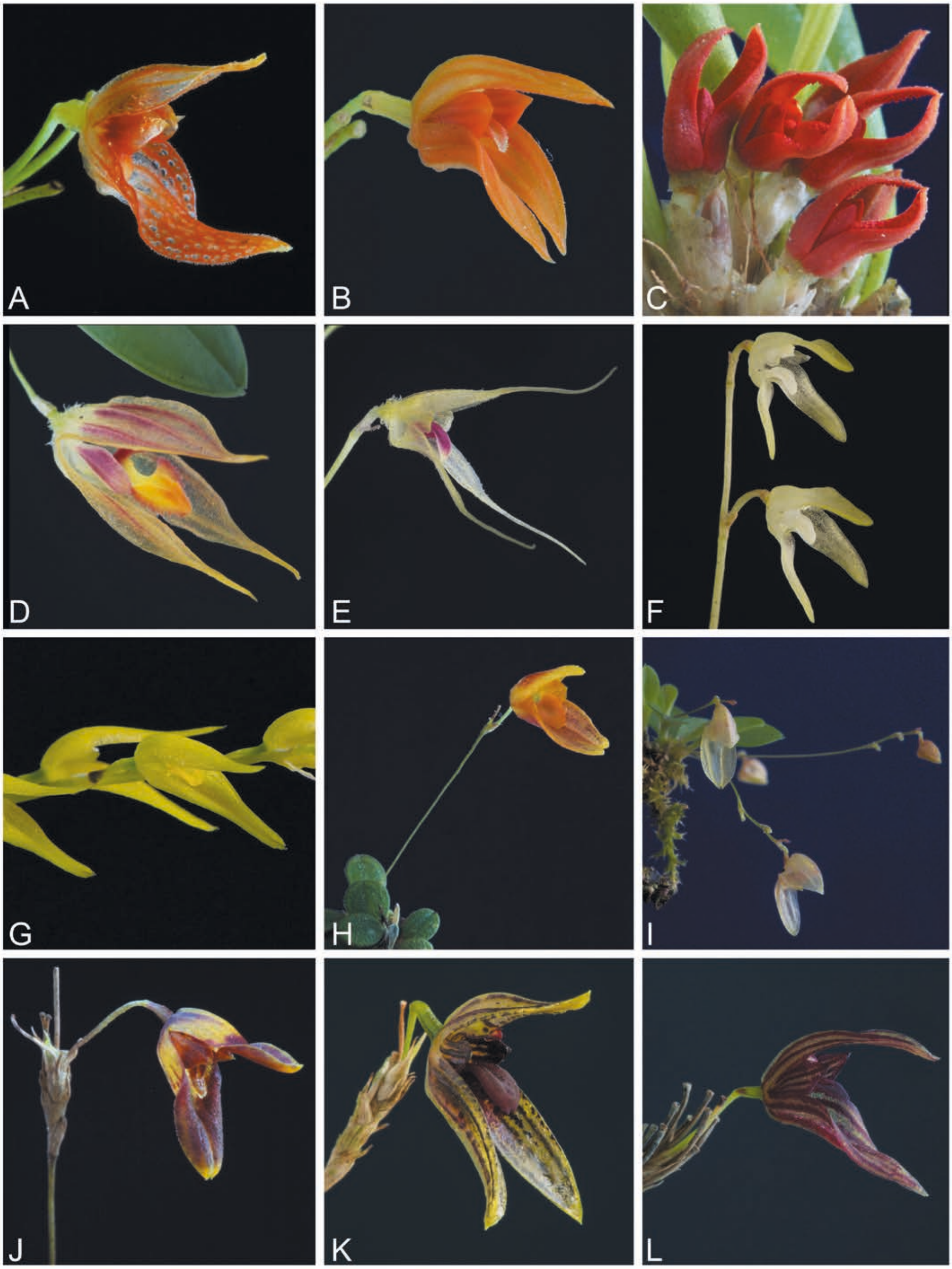
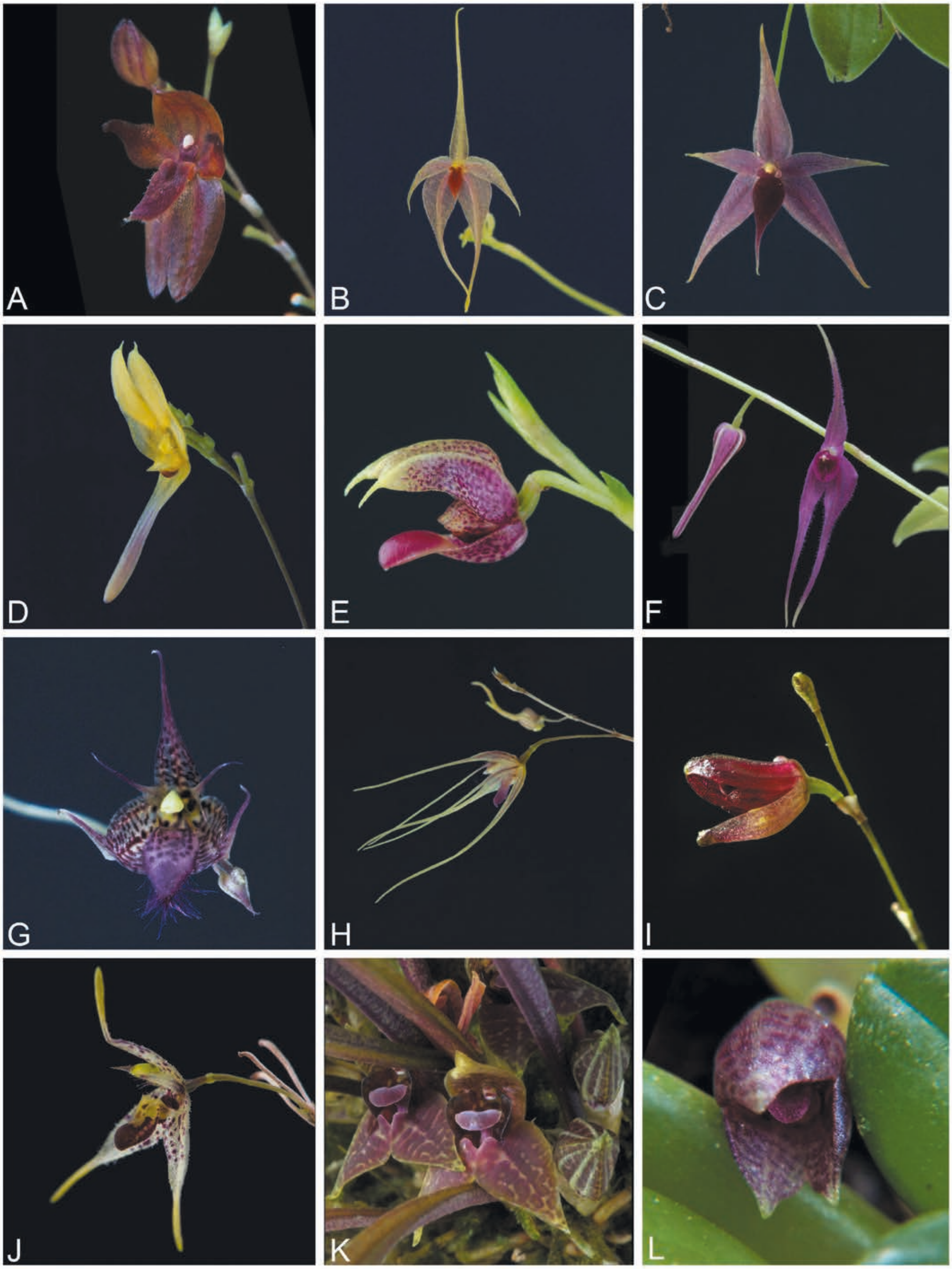


Figure 62. Inflorescence variation within the *Specklinia* group. Inflorescence simultaneous and elongate (A), simultaneous and fasciculate (B), successive and elongate (C-E), successive and fasciculate (F-H). A: *Specklinia grobyi*. B: *Platystele umbellata.* C: *Specklinia pfavii*. D: *Muscarella fimbriata*. E. *Scaphosepalum microdactylum*. F. *Muscarella strumosa*. G: *Specklinia acrisepala*. H: *Specklinia fulgens*. Photographs by A.P. Karremans, except for B, which was made by W. Driessen.



A - Specklinia barbae (AK3928)
B - Specklinia guanacastensis (AK6018)
C - Specklinia tribuloides (AK3276)
D - Specklinia absurda (DB9772)
E - Specklinia turrialbae (AK5635)
F - Specklinia calyptrostele (DB2419)
G - Specklinia costaricensis (JBL-06854)
H - Specklinia luis-diegoi (AK5500)
I - Specklinia colombiana (JBL s.n.)
J - Specklinia acrisepala (AK s.n.)
K - Specklinia berolinensis (AK5806)
L - Specklinia simmleriana (AK4205)

FIGURE 63. Representative species of each of the five clades of *Specklinia*. A-C: *Specklinia* subgen. *Specklinia* (Clade A). D-E: *S*. subgen. *Sylphia* (Clade B). F-G: *S*. subgen. *Hymenodanthae* (Clade C). H-I: *S*. subgen. *Acostaea* (Clade D). J-L: *S*. subgen. *Sarcinula* (Clade E). Photographs by A.P. Karremans.



A - Platystele aurea (Driessen s.n.)
B - Platystele caudatisepala (DB10230)
C - Platystele propinqua (AK4086)
D - Scaphosepalum clavellatum (FP2665)
E - Scaphosepalum microdactylum (FP8576)
F - Teagueia rex (Driessen s.n.)
G - Muscarella herpestes (AK4082)
H - Muscarella quinqueseta (AK3940)
I - Muscarella segregatifolia (DB10439)
J - Muscarella strumosa (DB10011)
K - Dryadella guatemalensis (AK3642)
L - Dryadella yupanki (AK5706)

Figure 64. Representative species of each of the genera sister to *Specklinia*. A-C: *Platystele* (Clade F). D-E: *Scaphosepalum* (Clade G). F: *Teagueia* (Clade H). G-J: *Muscarella* (Clade I). K-L: *Dryadella* (Clade J). All photographs were made by A.P. Karremans, except for A, G & L, which were made by W. Driessen.

Overall distinct presence and absence patterns of species of each clade are also evident (Fig. 66). Clades A and B are absent from Brazil and Ecuador, whereas only B is absent from the Antilles. Clade C is present in all the evaluated areas, the Antilles, Brazil, Ecuador and Mexico. Clade D is absent from all except Ecuador. Clade E is absent from Brazil, rare in the Antilles and Mexico, but present in Ecuador. Clade F is rare in the Antilles and Brazil. Clade G is absent in the Antilles and rare in Brazil and Mexico. Clade H is absent from all areas except Ecuador. Clade I is present in all, but rare in Brazil. Clade J is absent from the Antilles, and present in all others. No distribution is given for Costa Rica and Panama because all clades are present except for clade H, which is endemic to the Andes.

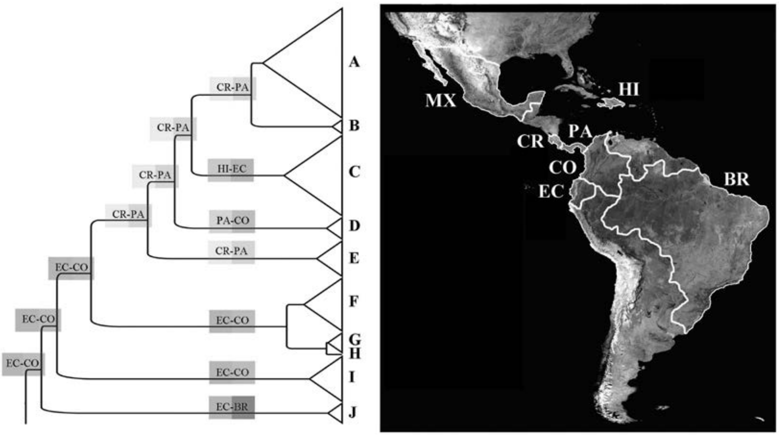


FIGURE 65. The combined ITS+*matK* based phylogeny with the clades collapsed showing the number one and two countries with most species of each clade, respectively. BR = Brazil, CO = Colombia, CR = Costa Rica, EC = Ecuador, HI = Hispaniola (Dominican Republic + Haiti), MX = Mexico and PA = Panama.

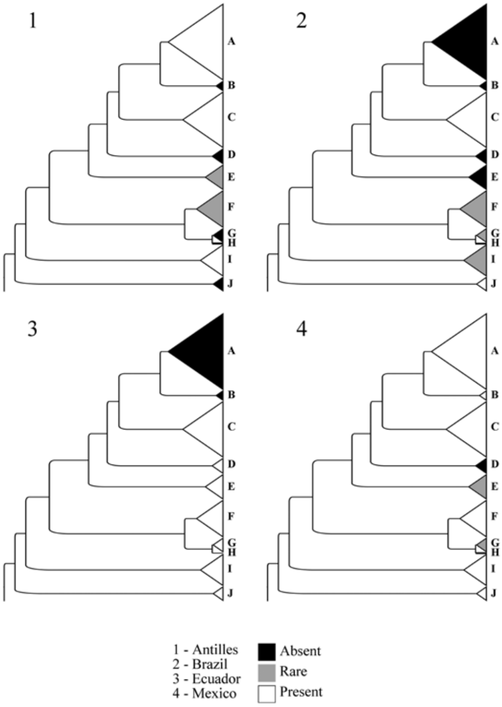


FIGURE 66.The combined nrITS+*matK* based phylogeny with the clades collapsed showing the presence/absence of species of each clade in reference regions, the Antilles, Brazil, Ecuador and Mexico. A clade is considered rare if 5% or less of its species are present.

Discussion

Our analysis with a broad sampling of *Specklinia* species proves that the genus by any current definition (Pridgeon & Chase 2001; Pridgeon 2005; Luer 2006; Barros & Trettel Rodrigues 2009) is not monophyletic, and is in need of re-circumscription. Similar issues have been encountered in most analyses of individual genera in the Pleurothallidinae (Karremans 2010; Chiron *et al*. 2012; Karremans *et al*. 2013a). The morphological dissimilarities among species of *Specklinia* led to a proliferation of generic concepts, and to the proposal of segregating several small species groups from the genus. Clade A, which includes the type species of *Specklinia*, together with clades B, C, and D forms a highly supported monophyletic group in all our analyses (P.P.=1; Bp=81). The of clades A, B, C, D and E (here *Specklinia* clade) received much higher support in the nrITS only analyses (P.P.=0.98; Bp=81) vs the combined analyses (P.P.=0.65; Bp=56), this is due to the fact that the clade is not supported by the *matK* data. Conservatively, we have chosen also to include clade E within our concept of *Specklinia*, because even though that received low support, species belonging to that clade are hardly distinguishable morphologically from other *Specklinia*. Recognizing them as a separate genus is not only not supported by our data, but would also make *Specklinia* almost undiagnosable.

In this sense, *Specklinia* includes 95 species, amongst which are the type species of the genera *Acostaea*, *Areldia*, *Cucumeria*, *Empusella*, *Gerardoa*, *Pseudoctomeria*, *Sarcinula*, *Sylphia*, *Tribulago* and *Tridelta*. Recognizing these genera reduces *Specklinia* to just a few species and requires the recognition of quite a large number of additional generic names. As *Specklinia* in a broad sense has a manageable number of species and can be easily recognized morphologically we feel it unnecessary to recognize additional segregate generic concepts. Nevertheless, we believe the five clades here included within *Specklinia* (A, B, C, D and E) are distinct enough to warrant subgeneric recognition. They all form highly supported clades (P.P.≥95; Bp≥80) and are placed on well separated branches. They are composed by morphologically similar species with unique distribution patterns, and have been mostly recognized at one time or another as distinct units (4 out of 5 have been given at least one generic name).

Clades F, G and H include the type species of the genera *Platystele*, *Scaphosepalum* and *Teagueia* respectively (Fig. 1 & 2). The three are always found together in a well supported clade (P.P.=0.99; Bp=64) that is sister to *Specklinia*. The type species of genus *Rubellia*, *R. rubella* (=*Platystele aurea*), was found sister with moderate support (P.P.=0.88; Bp=54) to a well supported clade (P.P.=0.87; Bp=72) which includes all other species of *Platystele*. Morphologically *Platystele aurea* is quite similar to other species of the genus, the plant habit being indistinguishable from other *Platystele* species, and it also share the typical apical anther and stigma. We therefore believe it best not to recognize this monotypic genus as separate. *Rubellia*, which was previously unplaced (Pridgeon 2005), is therefore placed under synonymy of *Platystele*. Genus *Teagueia*, which had been previously associated with *Platystele* (Luer 1990), was found sister to *Scaphosepalum* instead (P.P.=0.91; Bp=54). Flower morphology of *Teagueia* species is similar to some *Platystele*. Nevertheless the plant habit, which is not under the pollinator's selective pressure, is indeed more similar to *Scaphosepalum*. A broader sampling of *Teagueia* species might clear up their phylogenetic relationships in the future. The *Scaphosepalum* clade had moderate support (P.P.=0.87; Bp=52), it includes of the accessions of species of the genus, including its type.

From *Specklinia* we do exclude the species found in clade I. The clade, which includes the type species of genus *Muscarella*, was found well supported in our analyses (P.P.=0.98; Bp=89). *Muscarella* was always found sister to a clade that includes *Platystele*, *Scaphosepalum*, *Specklinia* and *Teagueia*, and thus its inclusion within *Specklinia*, as proposed by Pridgeon & Chase (2001) and Pridgeon (2005) would make the genus paraphyletic.

Clade J includes the type species of genus *Dryadella*, in a highly support (P.P.=1; Bp=98) which includes all other species ascribed to the genus. The type species of *Incaea*, a monospecific genus that was previously unplaced, is here found embedded within *Dryadella*. The two are therefore synonymized, with *Dryadella* having priority.

Incongruences between nrITS and *matK*:—The nuclear ITS and plastid *matK* are the most commonly used genetic regions for phylogenetic reconstruction in Pleurothallidinae (Pridgeon *et al.* 2001; Chiron *et al.* 2012; Karremans *et al.* 2013a; Karremans 2014, Chapter 7). Nevertheless, those studies clearly show that the faster evolving nrITS has much higher resolution than the more conserved *matK*, especially at generic level or below. In the particular case of our study, the phylogenetic reconstruction based solely on *matK* suffered from the low sequence variation and therefore had little resolution. *Specklinia* was not retrieved as monophyletic, and within Specklinia, clade A was also not retrieved. Nevertheless, all the other clades evaluated here (B through J) were diversely supported. One noteworthy difference is that clade E was found sister to clade G (P.P.=0.62; Bp 19) instead of it being sister to the rest of *Specklinia* (clades A, B, C and D) as was found in all nrITS and combined analyses. Even though the relationship between clade E and G is not highly supported, it also not very highly supported as a member of *Specklinia*, and it should be considered in future studies if the inclusion of clade E within *Specklinia* is adequate. Morphologically the species belonging to clade E are very difficult to set apart from other Specklinia, and it would not be advantageous to segregate them for the time being.

The phylogenetic reconstructions based solely on nrITS were very similar in structure to the combined analyses. The most noteworthy difference between the nrITS and combined analyses is that the *Specklinia* clade (sum of clades A, B, C, D and E) has a much higher support when *matK* is excluded (Bp=81 vs. Bp 56). This would be expected as it was mentioned previously that the *matK* data finds affinity of clade E with clade G instead of with the *Specklinia* clades. There are other seldom incongruences between nrITS and *matK*, but they can be considered "soft", as none have high support (most nodes collapse using a threshold 50 for the bootstrap support).

Differences between Bayesian and ML:—Between the Bayesian and ML analyses it is more accurate to talk about differences rather than incongruences. Although not directly comparable, support was overall lower in the RAxML (presented as bootstrap values) vs BEAST (presented as posterior probabilities) analyses. The main nodes discussed here, clades A through J, and the *Specklinia* clade (A through E), were all retrieved with the same species composition in both analyses. One slight difference is that sister to clades A and B in the RAxML analysis is clade D (Bp=19), while in the BEAST analysis it is clade C (P.P.=0.49); both with very low support. Some differences are found amongst species groups within each of the main clades. However, these too are not highly supported (P.P.≤0.8; Bp≤60), and have no impact on the discussion here.

Recognition of groups at generic and subgeneric level:—A common misconception amongst modern authors is that DNA data will in itself resolve taxonomic issues. DNA data albeit less subjective, is also subject to the correct application of names, data reading mistakes, and adequate interpretation of the observed variation (Karremans *et al*. 2015b, Chapter 2). In our view genera should be monophyletic, but also diagnosable and informative, and at the same time should both reflect past proposals in order to keep a stable classification.

Genetically it is difficult to establish a cut off value to recognize genera. Nevertheless, genetic distance, measured by the length of branches in the phylogenetic reconstructions can be a good point of comparison. Branch lengths in other genera presented here, for instance *Dryadella*, *Muscarella*, *Platystele* and *Phloeophila*, are similar or even longer than those observed within *Specklinia*, and only those of *Scaphosepalum* are significantly shorter (Fig. 57). It is also possible to compare sequence diversity as a measure of relative number of variable sites in the sequences belonging to each clade (Table 11). The combined nrITS + *matK* matrix includes 1576 characters. After excluding the outgroups (*Echinosepala* Pridgeon & M.W.Chase, *Anathallis* Barb.Rodr., *Lankesteriana* Karremans and *Trichosalpinx* Luer) the combined matrix shows variation in 637 characters corresponding to about 40% of the total characters analyzed. *Specklinia* by itself, which includes 57 of the 95 species attributable to the genus, shows variation in 28% of the total characters analyzed. *Platystele*, of which we analyzed less than a fifth of the known species, shows variation in 20% of the characters. *Muscarella*, with about one third of the species included, shows variation in 18% of all its characters. It is likely that, with a larger sampling of *Muscarella* and *Platystele* species, both genera will have similar sequence variations as those observed in *Specklinia* or even more.

Within *Specklinia*, the lowest number of variable sites was found in clade E, with only 3%, while the highest is found in clade A, with 15%. This is undoubtably in part explained by the total and relative number of species analyzed, for clade E we analyzed only 8 species (44% of the total species that belong to the clade) while for clade A we analyzed 20 species (77% of the total). Nevertheless, not all the variation is explained by species number. In clade A for example, the ITS sequences of sister species can differ from 2% to up to 6% (Karremans *et al.* 2015b, Chapter 2; Karremans *et al.* 2015c, Chapter 3).

Table 11. Species belonging to each representative clade; comparison of the here analyzed species, the total species known to belong the that particular clade, and the percentage of analyzed species as to the total. Variable sites in the combined nrITS+*matK* dataset; variable sites amongst all sequences of specimens within each clade, the variable sites in relation to the total number of sites (base pairs in the combined matrix = 1576). Not Applicable (NA) indicates clades with a single sequence.

	Analyzed Species	*Total Species*	*Analyzed vs Total (%)*	*Variable Sites*	*Variable vs Total (%)*
Clade A	20	26	77	236	15
Clade B	4	5	80	109	7
Clade C	20	32	63	212	13
Clade D	5	12	42	169	11
Clade E	8	18	44	51	3
Specklinia (A to E)	57	95	60	445	28
Clade F	19	100	19	311	20
Clade G	9	52	17	96	6
Clade H	1	14	7	NA	NA
Clade I	19	53	36	278	18
Clade J	9	55	16	135	9
Phloeophila	4	9	44	134	9

Geographical patterns:—As defined here *Specklinia* includes 95 species found growing from Mexico to Bolivia and Brazil, through Central America and the Antilles (Fig. 65). The highest species diversity can be found in Costa Rica and Panama, and it is also there where most clade diversity is found. Species of *Specklinia* are commonly found in Ecuador, but species from subgen. *Specklinia* (clade A) and subgen. *Sylphia* (clade B) are absent or rare. Several *Specklinia* species are known from the Antilles, with the notable exception of species from subgen. *Acostaea* (clade D) and subgen. *Sylphia* (clade B). The combination of geographical and genetic data allows for the interpretation that *Specklinia* has a north-Andean (Ecuador and Colombia) ancestry and that it diversified in southern Central America (Costa Rica and Panama) and the Antilles later on. Based on the similarity of species groups, the radiation into the Antilles most likely occurred through the North of Middle America (Mexico and Guatemala) rather than through South America (Venezuela).

Platystele, *Teagueia* and *Scaphosepalum*, the sister taxa of *Specklinia* (Fig. 65), are all of north-Andean ancestry. The Andes is also the center of diversity of these three genera (*Teagueia* being endemic); only a few species venturing into Central America. Those genera are, not surprisingly, almost absent from the Antilles. The whole clade is not well represented in Brazil either, strengthening the north-Andes to south-Central America speciation pattern of this group.

Species of other genera that have been placed in *Specklinia*:—Many Brazilian endemics have been treated as *Specklinia* (Luer 2004; Barros & Trettel Rodrigues 2009), but most of those actually belong to the genera *Anathallis* and *Pabstiella* (Luer 2007; 2009; Chiron *et al.* 2012). *Specklinia* species although uncommon do occur in Brazil, but it is only members of subgen. *Specklinia* that are found there. Those species can be recognized by multi-flowered inflorescences with whitish to yellowish flowers, a linear lip (vs. trilobate in *Pabstiella*), obtuse petals (vs. acute in *Anathallis*), a prominently winged column (vs. wingless in *Pabstiella*) with a toothed apex (vs. prominently fringed in *Anathallis*) and naked pollinaria (vs. pollinaria with granular caudicles in both *Anathallis* and *Pabstiella*).

Species of *Lankesteriana* Karremans have also been treated as *Specklinia* (Luer 2004). Nevertheless, Karremans (2014, Chapter 7) showed that these species are relatives of *Trichosalpinx* and *Zootrophion* instead, and are therefore only distant relatives of *Specklinia*. *Lankesteriana* species have linear to lanceolate petals (vs. elliptic in *Specklinia*) and the androclinium is conspicuously fimbriate (vs. androclinium erose or inornate), and pollinia with caudicles (vs. without caudicles in *Specklinia*).

A few dozen species previously placed in *Pleurothallis* subgen. *Acuminatia* Luer and *Pleurothallis* subgen. *Effusia* Luer were transferred to *Specklinia* by Luer (2004). Nevertheless these species are morphologically quite different from *Specklinia* species, and DNA data shows that they belong in *Stelis* (Karremans *et al.* 2013a).

Taxonomic consequences

***Specklinia* Lindl., Gen. Sp. Orch. PI., 8. 1830**:—Lectotype: *Epidendrum lanceola* Sw., Nov. Gen. Sp. Prodr., 123. 1788 (selected by Garay & Sweet, J. Arnold Arb. 53: 528. 1972).

Synonyms:
Acostaea Schltr., Repert. Spec. Nov. Regni Veg., Beih. 19: 283. 1923.
Areldia Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 255. 2004.
Cucumeria Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 257. 2004.
Empusella Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 258. 2004.
Gerardoa Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 86. 2006.
Pseudoctomeria Kraenzl., Bull. Misc. Inform. Kew 1925(3): 116. 1925.
Sarcinula Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 201. 2006.
Sylphia Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 227. 2006.
Tribulago Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 265. 2004.
Tridelta Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 232. 2006.

Species of *Specklinia* can be recognized by having ramicauls shorter than the leaves, an abbreviated stem with an annulus, sepals and petals mostly membranaceous, lateral sepals connate for at least half their length and convergent, petals mostly obtuse and entire (never acuminate or lanceolate), wider above the middle, and a linear to sub-rectangular lip hinged to the column foot. The column has a toothed androclinium, a pair of prominent rounded wings near the apex, ventral anther and stigma. The most unique feature shared between all members of *Specklinia* are the pollinaria that are flattened towards the base and that lack both caudicles and a viscidium.

Specklinia subgen. ***Acostaea*** (Schltr.) Karremans.
Bas. *Acostaea* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 19: 22, 102, 283. 1923. Type: *Acostaea costaricensis* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 19: 22, 102, 284. 1923. Lectotype designated by Summerhayes (1967).
Syn. *Areldia* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 255. 2004. Bas. *Pleurothallis* subgen. *Dresslera* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 38. 1986. Type: *Pleurothallis dressleri* Luer, Selbyana 3(1-2): 98-100, f. 152. 1976.

Specklinia subgen. *Acostaea* (Clade D) was highly supported and contains the species assigned to *Acostaea*, plus a few species of *Specklinia* and of *Sylphia*. The species are rare and regional, with the notable exceptions of *Specklinia* colombiana and *Specklinia recula*. They all share a tiny plant size, frequently creeping habit, elongate inflorescences and a column with prominent wings at the apex and a pair of glands on the column foot. It includes 12 species endemic to Costa Rica, Panama, Colombia and Ecuador, with a peak of diversity in Panama and Colombia.

Specklinia bicornis (Luer) Pridgeon & M.W.Chase
Bas. *Acostaea bicornis* Luer, Phytologia 54: 379. 1983.
Specklinia campylotyle (P.Ortiz) Pridgeon & M.W.Chase
Bas. *Acostaea campylotyle* P.Ortiz, Orquideología 13: 240. 1979.
Specklinia colombiana (Garay) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
Bas. *Acostaea colombiana* Garay, Orquideología 9: 112. 1974.
Syn. *Specklinia mirifica* Pridgeon & M.W.Chase, Lindleyana 16: 258. 2001.
Bas. *Acostaea costaricensis* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 19: 284. 1923.
The best-known species of *Acostaea*, *A. costaricensis*, was renamed *Specklinia mirifica* by Pridgeon and Chase (2001) when *Acostaea* was placed under the synonymy of *Specklinia*. Nevertheless if *Specklinia colombiana* is considered a synonym then it would have priority. If they are considered different then the next name to be applicable to this concept would be *Acostaea glandulata* P.Ortiz and not *S. mirifica*.
Specklinia coronula (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 259. 2004.
Bas. *Pleurothallis coronula* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 171. 1999.

Specklinia cactantha (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
Bas. *Pleurothallis cactantha* Luer, Selbyana 3: 72. 1976.
Specklinia cycesis (Luer & R.Escobar) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 260. 2004.
Bas. *Pleurothallis cycensis* Luer & R.Escobar, Orquideología 20: 49. 1996.
Specklinia dressleri (Luer) Bogarín & Karremans, Lankesteriana 14(3): 262. 2014.
Bas. *Pleurothallis dressleri* Luer, Selbyana 3: 98. 1976.

No DNA data were available for *S. dressleri*, the type species of the monotypic genus *Areldia*, for this study. Nevertheless, plant and flower morphology suggest affinity with subgen. *Acostaea*. A creeping plant with a relatively long inflorescence with a single flower open at once is reminiscent of *S. luis-diegoi*, whereas the broad column wings and callus of the lip suggest affinity with *S. colombiana*.

Specklinia luis-diegoi (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 262. 2004.
Bas. *Pleurothallis luis-diegoi* Luer, Revista Soc. Boliv. Bot. 3: 55. 2001.
Specklinia recula (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 263. 2004.
Bas. *Pleurothallis recula* Luer, Lindleyana 11: 92. 1996.
Specklinia tenax (Luer & R.Escobar) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
Bas. *Acostaea tenax* Luer& R.Escobar, Orquideologia 15: 123. 1982.
Specklinia trilobata (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
Bas. *Acostaea trilobata* Luer, Selbyana 1(3): 216. 1975.
Specklinia unicornis (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
Bas. *Acostaea unicornis* Luer, Phytologia 54: 379. 1983.

Specklinia subgen. ***Hymenodanthae*** (Barb.Rodr.) Karremans.

Bas. *Pleurothallis* sect. *Hymenodanthae* Barb.Rodr., Gen. Sp. Orchid. 2: 9. 1882. Lectotype: *Pleurothallis grobyi* Bateman ex Lindl., Edwards's Bot. Reg. 21: t. 1797. 1835. Lectotype designated by Luer (1986).

Syn. *Lepanthes* sect. *Longicaulae* Barb.Rodr., Gen. Sp. Orchid. 2: 40. 1882. Type: *Pleurothallis trilineata* Barb. Rodr., Gen. Sp. Orchid. 1: 6--7. 1877. Lectotype designated by Luer (1986).

Syn. *Pleurothallis* subsect. *Longicaulae* (Barb.Rodr.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 86. 1986. Bas. *Lepanthes* sect. *Longicaulae* Barb.Rodr., Gen. Sp. Orchid. 2: 40. 1882. Type: *Pleurothallis trilineata* Barb.Rodr., Gen. Sp. Orchid. 1: 6--7. 1877. Lectotype designated by Luer (1986).

Specklinia subgen. *Hymenanthae* (Clade C) is a highly supported clade that includes the species of the *Specklinia* grobyi-picta complex. Species belonging to this clade can be recognized as species of *Specklinia s.l.* by their convergent lateral sepals, the obtuse petals, ligulate lip and pollinaria without caudicles or viscidium, and within *Specklinia* by the inflorescence that is frequently elongate, exceeding the leaves, racemose, multi-flowered, with several flowers open at once, the flowers mostly monochrome purple, yellow, green or whitish, never orange, a column with a pair of prominent, rounded wings near the apex and a pair or orbicular glands at the base, and a linear-ligulate lip. This subgenus of 32 species has the widest distribution in the genus. It is the only clade of *Specklinia* found in all areas from Mexico, through Central America and the Antilles, south to Bolivia and Brazil. The most variable and widespread of all species of the genus, *S. grobyi*, belongs to this group. All species of *Specklinia* from Brazil, as well as most species of *Specklinia* from the Antilles, Ecuador and Mexico belong to this subgenus.

Specklinia acutiflora (Ruiz & Pav.) Pupulin, Anales Jard. Bot. Madrid 69(2): 167. 2012.
Bas. *Humboldtia acutiflora* Ruiz & Pav., Syst. Veg. Fl. Peruv. Chil. 1: 236. 1798.
Specklinia alta (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 258. 2004.
Bas. *Pleurothallis alta* Luer, Lindleyana 11(3): 143-144, f. 4. 1996.
Specklinia acutidentata (Cogn.) Luer = *Specklinia grobyi*
Specklinia barbosana (De Wild.) Campacci, Bol. CAOB 69-70: 27. 2008.
Bas. *Pleurothallis barbosana* De Wild, Gard. Chron. 39. 244. 1906.
Specklinia biglandulosa (Schltr.) Pridgeon & M.W.Chase = ***Specklinia grobyi***
Specklinia bipapularis (Dod) Luer = ***Specklinia schaferi***

Specklinia blepharoglossa (Luer) Luer = ***Specklinia grisebachiana***
Specklinia calyptrostele (Schltr.) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
  Bas. *Pleurothallis calyptrostele* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 19: 23. 1923.
Specklinia costaricensis (Rolfe) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
  Bas. *Pleurothallis costaricensis* Rolfe, Bull. Misc. Inform. Kew 1917(2): 80. 1917.
Specklinia curtisii (Dod) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
  Bas. *Pleurothallis curtisii* Dod, Moscosoa 3: 111. 1984.
Specklinia digitalis (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
  Bas. *Pleurothallis digitalis* Luer, Orquídea (Mexico City), n.s. 6(1): 3-4. 1976.
Specklinia dodii (Garay) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 260. 2004.
  Bas. *Pleurothallis dodii* Garay, J. Arnold Arbor. 50: 463. 1969.
Specklinia feuilletii Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 103: 311. 2005.
Specklinia florulenta (Linden & Rchb.f.) Pridgeon & M.W.Chase = ***Specklinia picta***
Specklinia flosculifera (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 260. 2004.
  Bas. *Pleurothallis flosculifera* Luer, Lindleyana 14: 113. 1999.
Specklinia formondii (Dod) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
  Bas. *Pleurothallis formondii* Dod, Moscosoa 3: 116. 1984.
Specklinia gracillima (Lindl.) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
  Bas. *Pleurothallis gracillima* Lindl., Fol. Orchid. 9: 35. 1859.
Specklinia grisebachiana (Cogn.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 260. 2004.
  Bas. *Pleurothallis grisebachiana* Cogn. Symb. Antill. 6: 409. 1909.
Specklinia grobyi (Bateman ex Lindl.) F.Barros, Hoehnea 10: 110. 1983 (1984).
  Bas. *Pleurothallis grobyi* Bateman ex Lindl., Edwards's Bot. Reg. 21: t. 1797. 1835.
Specklinia jesupii (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 261. 2004.
  Bas. *Pleurothallis jesupii* Luer, Lindleyana 14: 116. 1999.
Specklinia lichenicola (Griseb.) Pridgeon & M.W.Chase, Lindleyana 16: 258. 2001.
  Bas. *Pleurothallis lichenicola* Griseb., Cat. Pl. Cub.: 259. 1866.
Specklinia lugduno-batavae Karremans, Bogarín & Gravend., Blumea 59: 180. 2015.
Specklinia marginalis (Rchb.f.) F.Barros, Hoehnea 10: 110. 1983 [1984].
  Bas. *Pleurothallis marginalis* Rchb.f., Bonplandia (Hannover) 3(15-16): 224-225. 1855.
Specklinia microphylla (A.Rich. & Galeotti) Pridgeon & M.W.Chase, Lindleyana 16: 258. 2001.
  Bas. *Pleurothallis microphylla* A.Rich. & Galeotti, Ann. Sci. Nat., Bot., sér. 3, 3: 17. 1845.
Specklinia mitchellii (Dod) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 262. 2004.
  Bas. *Pleurothallis mitchellii* Dod, Moscosoa 3: 109. 1984.
Specklinia morganii (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 262. 2004.
  Bas. *Pleurothallis morganii* Luer, Lindleyana 11: 171. 1996.
Specklinia mornicola (Mansf.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 262. 2004.
  Bas. *Pleurothallis mornicola* Mansf., Ark. Bot. 22A(8): 13. 1929.
Specklinia pectinifera Luer & Hirtz, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 61. 2006.
Specklinia picta (Lindl.) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
  Bas. *Pleurothallis picta* Lindl., Edwards's Bot. Reg. 21: t. 1797. 1835.
Specklinia pisinna (Luer) Solano & Soto Arenas, Icon. Orchid. 5--6: xi. 2002 (2003).
  Bas. *Pleurothallis pisinna* Luer, Lindleyana 6(2): 105, f. 1991.
Specklinia producta (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
  Bas. *Pleurothallis producta* Luer, Selvyana 3: 176. 1976.
Specklinia schaferi (Ames) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 263. 2004.
  Bas. *Pleurothallis schaferi* Ames, Orchidaceae 7: 119. 1922.
Specklinia stillsonii (Dod) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
  Bas. *Pleurothallis stillsonii* Dod, Moscosoa 3: 107. 1984.

Specklinia subpicta (Schltr.) F.Barros, Orchid Memories: 19. 2004.
Bas. *Pleurothallis subpicta* Schltr., Anexos Mem. Inst. Butantan, Secc. Bot. 1(4): 42. 1922.
Specklinia trichyphis (Rchb.f.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 264. 2004.
Bas. *Pleurothallis trichyphis* Rchb.f., Flora 48: 276. 1865.
Specklinia viridiflora (Seehawer) F.J. de Jesus, R.Miranda & Chiron, Richardiana 14: 284-285.
Bas. *Pleurothallis viridiflora* Seehawer, Die Orchidee 50: 637. 1999.
Specklinia wrightii (Rchb.f.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 265. 2004.
Bas. *Pleurothallis wrightii* Rchb.f., Flora 48: 276. 1865.

Specklinia subgen. ***Sarcinula*** Karremans.
Type: *Pleurothallis condylata* Luer, Selbyana 3:80. 1976.
Syn. *Sarcinula* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 201. 2006. Bas. *Pleurothallis acicularis* Ames & C.Schweinf., Sched. Orch. 10: 21-23. 1930.

Specklinia subgen. *Sarcinula* (Clade E) was found to be a highly supported clade, basically including the non-orange-flowered species of Luer's *Sarcinula*. The exact phylogenetic position of *Specklinia acicularis*, the type species of *Sarcinula*, remains unclear. With its narrow leaves it is an outlier amongst the other members of *Sarcinula*. However, floral coloration pattern also do not suggest affinity with subgen. *Specklinia*. Because of this uncertainty we prefer to describe subgenus *Sarcinula* with a different type species, one that is also "typical" for the group but ending up consistently in the same clade in all analyses.

Leaves are linear to narrowly obovate, the inflorescence is longer than the leaf, successive, with a single flower open at once, the rachis is reduced making the pedicels appear fasciculate, the flowers are yellowish to greenish diversely suffused, dotted or striped with purple or brown, and the lip has a pair of basal lobules. Eighteen species are distributed across Central America, Colombia and Ecuador, with the highest diversity in Costa Rica and Panama. A single species extends into Mexico and the Antilles, and one species is reported from Bolivia and another from the Guyanas. No species are known from Peru and Brazil.

Specklinia acanthodes (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 256. 2001.
Bas. *Pleurothallis acanthodes* Luer, Selbyana 1(3): 222, f. 46. 1975.
Specklinia acicularis (Ames & C.Schweinf.) Pridgeon & M.W.Chase, Lindleyana 16: 256. 2001.
Bas. *Pleurothallis acicularis* Ames & C.Schweinf., Sched. Orch. 10: 21-23. 1930.
Specklinia acoana Bogarín, Lankesteriana 13(3). 2013.
Specklinia acrisepala (Ames & C.Schweinf.) Pridgeon & M.W.Chase, Lindleyana 16: 256. 2001.
Bas. *Pleurothallis acrisepala* Ames & C.Schweinf., Sched. Orch. 8: 22-23. 1925.
Specklinia alexii (A.H.Heller) Pridgeon & M.W.Chase, Lindleyana 16: 256. 2001.
Bas. *Pleurothallis alexii* A.H.Heller, Phytologia 14(1): 8-9, t. 4. 1966.
Specklinia areldii (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 256. 2001.
Bas. *Pleurothallis areldii* Luer, Selbyana 2(4): 383-384. 1978.
Specklinia berolinensis Bogarín, Lankesteriana 13(3). 2013.
Specklinia brighamella (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 256. 2001.
Bas. *Pleurothallis brighamella* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 171, f. 22a. 1999.
Specklinia brighamii (S.Watson) Pridgeon & M.W.Chase, Lindleyana 16: 256. 2001.
Bas. *Pleurothallis brighamii* S.Watson, Proc. Amer. Acad. Arts 23(2): 285-286. 1888.
Specklinia calderae (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 259. 2004.
Bas. *Pleurothallis calderae* Luer, Orquideología 22(1): 53-56. 2001.
Specklinia condylata (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
Bas. *Pleurothallis condylata* Luer, Selbyana 3:80. 1976.
Specklinia icterina Bogarín, Lankesteriana 13(3). 2013.
Specklinia purpurella (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
Bas. *Pleurothallis purpurella* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 176, f. 31a. 1999.

Specklinia rinkei (Luer) J.M.H.Shaw, Orchid Rev. 122(1308): 77. 2014.

Bas. *Sarcinula rinkei* Luer, Selbyana 30: 18, f. 35. 2009.

Specklinia scolopax (Luer & R.Escobar) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.

Bas. *Pleurothallis scolopax* Luer, Orquideología 14(2): 172. 1981.

Specklinia simmleriana (Rendle) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 263. 2004.

Bas. *Pleurothallis simmleriana* Rendle, J. Bot. 38(451): 274-275. 1900.

Specklinia striata (H.Focke) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 264. 2004.

Bas. *Pleurothallis striata* H.Focke, Tijdschr. Wis-Natuurk. Wetensch. Eerste Kl. Kon. Ned. Inst. Wetensh. 4: 63-64. 1851.

Specklinia vierlingii Baumbach, Orchideen (Hamburg) 63(5): 405-406. 2012.

Specklinia subgen. ***Specklinia***.

Type: *Epidendrum lanceola* Sw., Prodr. 123. 1788.

Syn. *Empusella* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 258. 2004. Bas. *Pleurothallis* subgen. *Empusella* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 41. 1986. Type: *Pleurothallis endotrachys* Rchb.f., Linnea 41: 95. 1876.

Syn. *Gerardoa* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 86. 2006. Bas. *Pleurothallis montezumae* Luer, Lindleyana 11(2): 83, f. 20. 1996.

Syn. *Pleurothallis* sect. *Apodae-caespitosae* Lindl., Fol. Orchid. ~*Pleurothallis*~ 35. 1859. Type: *Epidendrum corniculatum* Sw., Prodr. 123. 1788. Lectotype designated by Luer (1986).

Syn. *Pleurothallis* subsect. *Apodae-caespitosae* (Lindl.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 84. 1986. Type: *Epidendrum corniculatum* Sw., Prodr. 123. 1788. Lectotype designated by Luer (1986).

Syn. *Pleurothallis* subgen. *Empusella* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 41. 1986. Type: *Pleurothallis endotrachys* Rchb.f., Linnea 41: 95. 1876.

Syn. *Pleurothallis* subgen. *Pseudoctomeria* (Kraenzl.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 67. 1986. Bas. *Pseudoctomeria* Kraenzl., Bull. Misc. Inform. Kew 1925(3): 116. 1925. Type. *Pleurothallis lentiginosa* F.Lehm. & Kraenzl., Bot. Jahrb. Syst. 26(3--4): 446. 1899.

Syn. *Pleurothallis* sect. *Tribuloides* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 91. 1986. Bas. *Epidendrum tribuloides* Sw. Prodr. 123. 1788.

Syn. *Pseudoctomeria* Kraenzl., Bull. Misc. Inform. Kew 1925(3): 116. 1925. Bas. *Pleurothallis lentiginosa* F.Lehm. & Kraenzl., Bot. Jahrb. Syst. 26(3--4): 446. 1899.

Syn. *Tribulago* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 265. 2004. Bas. *Pleurothallis* sect. *Tribuloides* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 91. 1986. Type. *Epidendrum tribuloides* Sw. Prodr. 123. 1788.

Specklinia subgen. *Specklinia* (Clade A) includes morphologically highly diverse species, which is reflected in the number of generic names proposed for such a relatively low number of species. Nonetheless they can be recognized as species of the *Specklinia s.l.* clade by their convergent lateral sepals, obtuse petals, ligulate lip and pollinaria lacking caudicles and a viscidium, and within *Specklinia* particularly for their reddish-orange stained flowers. Orange-stained flowers are rare in the other clades of *Specklinia s.l.* The inflorescence is successive, rarely with more than one flower per inflorescence open at once. Such an inflorescence is also found in species assigned to subgen. *Sarcinula* (Clade E), but the pedicels of the flowers of species in subgen. *Specklinia* remain green (vs. papery) and can further be distinguished by the lack of a pair of basal lobes at the base of the lip.

This clade consists of 27 species distributed in Central America, Colombia, Venezuela, the Guyanas and the Antilles. The highest diversity is found in Costa Rica and Panama, which together account for 23 reported species. Two species are known from Mexico, and two from the Antilles. No species of this group seem to be present in Ecuador, Peru, Bolivia and Brazil.

Specklinia alajuelensis Karremans & Pupulin, Phytotaxa 218(2): 108. 2015.

Specklinia barbae (Schltr.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 259. 2004.

Bas. *Pleurothallis barbae* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 19: 104. 1923.

Specklinia barboselloides (Schltr.) Pridgeon & M.W.Chase = ***Specklinia corniculata***

Specklinia blancoi (Pupulin) Soto Arenas & Solano, Icon. Orchid. 5--6: t. 669. 2002 (2003).

Bas. *Pleurothallis blancoi* Pupulin, Caesiana 15: 1-4, f. 1-2. 2000.

Specklinia chontalensis (A.H.Heller & A.D.Hawkes) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 259. 2004.

Bas. *Pleurothallis chontalensis* A.H.Heller & A.D.Hawkes, Phytologia 14(1): 10-11. 1966.

Specklinia corniculata (Sw.) Steud., Nomencl. Bot., ed. 2, 2: 489. 1841.

Bas. *Epidendrum corniculatum* Sw., Prodr. 123. 1788.

Specklinia displosa (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.

Bas. *Pleurothallis displosa* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 172, f. 24a. 1999.

Specklinia emarginata Lindl., Gen. Sp. Orchid. Pl. 8-9. 1830. = ***Specklinia corniculata***

Specklinia dunstervillei Karremans, Pupulin & Gravend., PLoS ONE 10(7): e131971(5). 2015.

Specklinia endotrachys (Rchb.f.) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.

Bas. *Pleurothallis endotrachys* Rchb.f., Linnea 41: 95. 1876.

Specklinia exilis (C.Schweinf.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 260. 2004.

Bas. *Pleurothallis exilis* C.Schweinf., Fieldiana, Bot. 28(1): 1951.

Specklinia fulgens (Rchb.f.) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.

Bas. *Pleurothallis fulgens* Rchb.f., Gard. Chron., n.s. 4(95): 516. 1875.

Specklinia gersonii Bogarín & Karremans, Phytotaxa 218(2): 112. 2015.

Specklinia glandulosa (Ames) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.

Bas. *Pleurothallis glandulosa* Ames, Sched. Orch. 6: 60-61. 1923.

Specklinia guanacastensis (Ames & C.Schweinf.) Pridgeon & M.W.Chase, Lindleyana 16: 258. 2001.

Bas. *Pleurothallis guanacastensis* Ames & C.Schweinf., Sched. Orch. 10: 27-29. 1930.

Specklinia juddii (Archila) Pupulin & Karremans, Orchidee (Hamburg) 64(6): 480. 2013.

Bas. *Empusella judii* Archila, Revista Guatemal. 15(1): 99. 2012.

Specklinia lanceola (Sw.) Lindl., Gen. Sp. Orchid. Pl.: 8. 1830.

Bas. *Epidendrum lanceola* Sw., Prodr. 123. 1788.

Specklinia lentiginosa (F.Lehm. & Kraenzl.) Pridgeon & M.W.Chase, Lindleyana 16: 258. 2001.

Bas. *Pleurothallis lentiginosa* F.Lehm. & Kraenzl., Bot. Jahrb. Syst. 26(3--4): 446. 1899.

Specklinia leptantha (Schltr.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 261. 2004.

Bas. *Pleurothallis leptantha* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 7: 107. 1920.

Specklinia minuta (Ames & C.Schweinf.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 262. 2004.

Bas. *Pleurothallis minuta* Ames & C.Schweinf., Sched. Orch. 10: 30-32. 1930.

Specklinia montezumae (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 262. 2004.

Bas. *Pleurothallis montezumae* Luer, Lindleyana 11(2): 83, f. 20. 1996.

Syn. Nov.: *Kraenzlinella rinkei* Luer, Harvard Pap. Bot. 16(2): 326. 2011.

We were originally going to transfer *K. rinkei* to *Specklinia* based on the description and illustration. The short stem, long, petiolate leaves, short, successive inflorescences, lamellate ovaries, orange flowers, a pair of lobes at the base of the column foot, the lip with an apiculum beneath the tip, the disc with a pair of low, serrated calli and a conspicuous, acute anther, all suggested affinity with both *S. montezumae* and *S. fulgens*. The main difference being that the flowers of *K. rinkei* are non-resupinate. In the meantime we were able to obtain photographs of the specimen from which the type material was prepared from Bryon Rinke, and those show resupinate flowers of something which we believe is conspecific with *S. montezumae*.

Specklinia pertenuis (C.Schweinf.) Karremans & Gravend., Phytotaxa 218(2): 116. 2015.

Bas. *Pleurothallis pertenuis* C.Schweinf. Bot. Mus. Leafl. 8: 83. 1935.

Specklinia pfavii (Rchb.f.) Pupulin & Karremans, Phytotaxa 63: 8. 2012.

Bas. *Pleurothallis pfavii* Rchb.f., Flora 69(34): 555. 1886.

Specklinia psichion (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 263. 2004.

Bas. *Pleurothallis psichion* Luer, Lindleyana 11(2): 89, f. 24. 1996.

Specklinia remotiflora Pupulin & Karremans, Phytotaxa 63: 11. 2012.

Specklinia spectabilis (Ames & C.Schweinf.) Pupulin & Karremans, Phytotaxa 63: 15. 2012).
Bas. *Pleurothallis spectabilis* Ames & C.Schweinf., Sched. Orch. 8: 34-35. 1925.
Specklinia tribuloides (Sw.) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
Bas. *Epidendrum tribuloides* Sw., Prodr. 123. 1788.
Specklinia vittariifolia (Schltr.) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
Bas. *Pleurothallis vittariifolia* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 19: 26. 1923.

Specklinia subgen. ***Sylphia*** (Luer) Karremans.

Bas. *Sylphia* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 227. 2006. Type: *Pleurothallis turrialbae* Luer, Lindleyana 6(2): 105, 106--108, f. 1991.

Syn. *Cucumeria* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 257. 2004. Bas. *Pleurothallis* sect. *Cucumeres* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 81. 1986. Type. *Pleurothallis cucumeris* Luer, Selbyana 5(2): 162--163. 1979.

Specklinia subgen. *Sylphia* (Clade B). The inflorescence is successive, with one flower per inflorescence open at once. Flowers are resupinate, transparent whitish to greenish, diversely suffused with purple. The lateral sepals are divergent, free, and long-apiculate. Petals are obtuse. The lip is unguiculate. Pollinia lack caudicles and a viscidium.

This little group contains five species found in Costa Rica and Panama. A single species extends northward into Guatemala and Mexico. The type species of the polyphyletic *Sylphia*, *S. turrialbae*, is included in this clade. Together with the morphologically similar *S. absurda*, *S. echinata* and *S. fuegi* they form a natural group. The type of the monotypic *Cucumeria*, *S. cucumeris*, is included in this subgenus based on DNA data. However it is different morphologically from all other members. Future studies might reveal it does not belong here. Nevertheless, all of these species are morphologically "typical" within *Specklinia*, even *S. cucumeria*, which resembles *S. lentiginosa*.

Specklinia absurda Bogarín, Karremans & Rincón, Phytotaxa 115(2): 34. 2013.
Specklinia cucumeris (Luer) Bogarín & Karremans, Lankesteriana 14(3): 261. 2014.
Bas. *Pleurothallis cucumeris* Luer, Selbyana 5(2): 162-163. 1979.
Specklinia echinata (L.O.Williams) Soto Arenas & Solano, Icon. Orchid. (Mexico) 5-6: t. 670. 2002 (2003).
Bas. *Pleurothallis fuegii* var. *echinata* L.O.Williams, Ann. Missouri Bot. Gard. 33(1): 120. 1946.
Specklinia fuegi (Rchb.f.) Solano & Soto Arenas, Icon. Orchid. 5-6: x. 2002 (2003).
Bas. *Pleurothallis fuegi* Rchb.f., Beitr. Orchid.-K.C.Amer. 97-98, t. 10. f. 11-15. 1866.
Specklinia turrialbae (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 264. 2004.
Bas. *Pleurothallis turrialbae* Luer, Lindleyana 6(2): 105, 106-108, f. 1991.

Unplaced names:

Specklinia mazei (Urb.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 262. 2004.
Bas. *Pleurothallis mazei* Urb., Repert. Spec. Nov. Regni Veg. 15: 1004. 1917.

This is another morphologically aberrant species. We have been unable to study any living material or obtain DNA sequences of this species. There are several morphological features that would indicate an affinity to *Specklinia* rather than to *Anathallis*, including the short stem, the non-apiculate, short petals, the ligulate, hairless lip, and the pollinia lacing caudicles and a viscidium. Without further information we cannot place it more specifically.

Specklinia aurantiaca (Dod) Karremans, comb. nov.
Bas. *Cryptophoranthus aurantiacus* Dod, Moscosoa 1(1): 50. 1976.

We have been unable to study any living material or obtain a DNA sequence of this aberrant species. It was designated as type species of the monospecific genus *Tridelta* Luer. Its phylogenetic placement is currently unknown. In the drawing and description we find some similarities with other species of *Specklinia* such as the broad column wings, almost linear lip and orange-colored flowers, and without further information we cannot place it more specifically.

Excluded names:

Pabstiella integripetala (E.Pessoa & F.Barros) Karremans.
Bas. *Specklinia integripetala* E.Pessoa & F.Barros, Nordic J. Bot. 32(2): 129, 131, f.1A-E. 2014.
The authors of this species compared it to *Muscarella semperflorens* (Lindl.) Luer [as *Specklinia semperflorens* (Lindl.) Pridgeon & M.W.Chase], and distinguished it by the "acute sepals, petals with entire margin and column with a clinandrium with an entire margin". Those characters, although rare in *Muscarella* are standard within *Pabstiella*, where this species clearly belongs.

Pabstiella brasilica Luer & Toscano, Harvard Pap. Bot. 17(2): 310, 312, f.5. 2011.
Syn.: *Specklinia ianthina* E.Pessoa & F.Barros, Nordic J. Bot. 32(2): 131, 132, f.1F-J. 2014.

The illustrations of *S. ianthina* and *P. brasilica* are extremely similar and the types come from neighboring localities. No explanation as to how these species can be distinguished from each other was provided by the authors, and therefore the names are here considered synonyms. The exact phylogenetic position of *Pabstiella brasilica* and its close relative *Anathallis spiculifera* (Lind.) Luer is still not resolved (to our knowledge). We believe both are related to *Madisonia kerrii* (Braga) Luer, a monospecific genus that is yet unplaced. Despite all these uncertainties, they certainly do not belong in *Specklinia*.

Specklinia alata (A.Rich. & Galeotti) Solano & Soto Arenas = ***Muscarella marginata***
Bas. *Pleurothallis alata* A.Rich. & Galeotti, Ann. Sci. Nat., Bot., sér. 3, 3: 17. 1845.

Specklinia bulbophylloides (Schltr.) Luer = ***Muscarella zephyrina***
Bas. *Pleurothallis bulbophylloides* Schltr., Repert. Spec. Nov. Regni Veg. 27: 50. 1929.

Specklinia discalis (Luer & J.Portilla) Luer = ***Muscarella trullifera***
Bas. *Pleurothallis discalis* Luer & J.Portilla, Selbyana 23: 35. 2002.

***Dryadella* Luer, Selbyana 2(2-3): 207. 1978.**:— Type: Masdevallia elata Luer, Phytologia 39(4): 199. 1978.

Synonym:
Incaea Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 87. 2006. Type: *Pleurothallis yupanki* Luer & R.Vásquez, Phytologia 55(3): 203. 1984.

Dryadella as defined by Luer (2005) and Pridgeon (2005) is accepted. As such it includes 55 species, distributed from Mexico to Bolivia and Brazil, through Central America. They are absent from the Antilles. Vegetatively they are tufted little plants with narrow fleshy leaves. The flowers are frequently yellowish spotted with brown or purple. The sepals are caudate, and connate basally. The lip is bicallous, and hinged to the column foot by a slender claw. The column is broadly winged, with a ventral anther and stigma. The pollinia are "whale-tail" type, with a pair of flat caudicles. The genus is here modified only by the inclusion of the following species:
Dryadella yupanki (Luer & Vasquez) Karremans.
Bas. *Pleurothallis yupanki* Luer & R.Vásquez, Phytologia 55: 203. 1984.
The monospecific genus *Incaea* was previously unplaced in the Pleurothallidinae. In the analyses presented here its type species is placed amongst members of *Dryadella*. Morphologically *D. yupanki* is in fact similar to other species of this genus.

***Muscarella* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 94. 2006.**:— Type: *Pleurothallis aristata* Hook. Ann. Nat. Hist. 2(1): 329--330, pl. 15. 1839.

Synonyms:
Verapazia Archila, Rev. Guatemalensis 2(3): 32--33, f. 1. 1999. This name is invalid for lack of indication of the type species under articles 9 and 10.
Pleurothallis R.Br. subgen. *Specklinia* (Lindl.) Garay sect. *Muscariae* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 89. 1986.

Species of *Specklinia* sect. *Muscariae* (Luer 1986), which later formed the genus *Muscarella* (Luer, 2006), have been mostly accepted as part of *Specklinia* (Pridgeon & Chase 2001; Pridgeon 2005). However, the genus forms a well-defined clade, which cannot be included within *Specklinia*. Species of *Muscarella* can be recognized by having a stem shorter than the leaves, inflorescences that are frequently lax-flexuous but can vary from elongate to fasciculate, always develop successively, and have one or rarely a few flowers open at the same time. Flowers are resupinate. Sepals are usually caudate, the petals fimbriate and acute to caudate. The column is elongate, without prominent wings or ornamentation. The pollinia are of the "whale-tail" type, with a dry, granulose, bilobate caudicle. *Muscarella* as defined by Luer (2006) is accepted. It then included 48 species, five species are added here to bring the total number up to 53.

Muscarella cabellensis (Rchb.f.) Karremans, comb. nov.
Bas. *Pleurothallis cabellensis* Rchb.f., Linnaea 22: 832 (1850).
Muscarella hastata (Ames) Karremans, comb. nov.
Bas. *Pleurothallis hastata* Ames, Orchidaceae 2: 268. 1908.
Muscarella mucronata (Lindl. ex Cogn.) Karremans, comb. nov.
Bas. *Pleurothallis mucronata* Lindl. ex Cogn. in I.Urban, Symb. Antill. 6: 424. 1909.
Muscarella obliquipetala (Acuña & C.Schweinf.) Karremans, comb. nov.
Bas. *Pleurothallis obliquipetala* Acuña & C.Schweinf., Bot. Mus. Leafl. 6: 3. 1938.
Muscarella segregatifolia (Ames & C.Schweinf.) Karremans, comb. nov.
Bas. *Pleurothallis segregatifolia* Ames & C.Schweinf., Sched. Orchid. 8: 33. 1925.

The accessions of *Pabstiella parvifolia* Lindl. that were included here showed affinities with *Muscarella* rather than *Pabstiella*. However, the type specimen of *P. parvifolia* is Brazilian and morphologically different from Costa Rican material. We do not venture into making a combination in *Muscarella* because it might well be that the type of *P. parvifolia* is a true *Pabstiella*, whereas what we are calling by that name might be another species.

Platystele **Schltr., Repert. Spec. Nov. Regni Veg. 8: 565. 1910.**:— Type: *Platystele bulbinella* Schltr., Repert. Spec. Nov. Regni Veg. 8(191-195): 565. 1910.

Synonym:

Rubellia Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 258. 2004. Bas. *Pleurothallis* subgen. *Rubellia* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 73. 1986. Type: *Pleurothallis rubella* Luer, Selbyana 3(3-4): 378-379, f. 289. 1977.

Platystele as defined by Luer (1990) and Pridgeon (2005) is accepted. As such *Platystele* includes 100 species that are found distributed from Mexico to Brazil and Bolivia, through Central America and the Antilles. Most species diversity is found in the northern Andes, especially Ecuador. Platystele species can be recognized by the small plants, the tiny flowers which are frequently flat with free and spreading segments, a simple lip, a short column with an apical anther and stigma. The genus is here modified only by the inclusion of the following species:

Platystele aurea Garay, Orquideología 8(3): 182. 1973.
Syn. *Pleurothallis rubella* Luer, Selbyana 3(3-4): 378-379, f. 289. 1977.
Syn. *Rubellia rubella* (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 258. 2004.

The monospecific genus *Rubellia* was previously unplaced in Pleurothallidinae. In the analyses presented here, its type species is placed sister to *Platystele* (Fig. 1 & 2). Its morphological similarities with species of *Platystele* had already been noted by Garay (1973) when he described *Platystele aurea*, a name frequently placed in synonymy of *Pleurothallis rubellia*. In our view, *Platystele aurea* and *Pleurothallis rubella* might represent two closely related yet different species. However, if considered synonyms, Garay's name has priority.

The genus *Rubellia* could have been kept separate from *Platystele* using the evidence presented here. However, the plants are similar to other members of the genus and the flowers share the apical anther and stigma and the presence of a glenion. Keeping *Rubellia* separate would not present any advantages.

***Scaphosepalum* Pfitzer, Nat. Pflanzenfam. 2(6): 136, 139. 1889[1888].**:— Type: *Masdevallia ochthodes* Rchb.f., Bonplandia 3: 70. 1855.

Scaphosepalum as defined by Luer (1988), Pridgeon (2005) and Endara (2011) is accepted. We are able to account for 52 species in the genus, with a distribution from Costa Rica to Bolivia and the Guyana Shield, and the highest diversity in the northern Andes of Colombia and Ecuador. They are distinguished especially by the non-resupinate flowers and the lateral sepals forming a basally concave synsepal and that are apically narrowed and thickened, usually with thickened calli on the distal portion

Chapter 7

Lankesteriana, a new genus in the Pleurothallidinae (Orchidaceae)

Adam P. Karremans

We estimated phylogenetic relationships within *Anathallis* and related genera using Bayesian analyses of nrITS sequence data. The genus is biphyletic in the molecular trees. A novel generic concept, *Lankesteriana*, is proposed for the species *Anathallis barbulata* and 19 close relatives. The genus is more closely related to some species of *Trichosalpinx* and *Zootrophion* than to *Anathallis* s.s. Species previously transferred from *Pleurothallis* subgen. *Acuminatia* sect. *Acuminatae* to *Anathallis*, are here transferred to *Stelis*, to which they are related phylogenetically. A few additional transfers to *Anathallis* are made. *Lankesteriana* is described and characterized, and the necessary taxonomic transfers are made.

Keywords: *Anathallis*, *Lankesteriana*, *Specklinia*, *Stelis*, phylogenetics, systematics

Introduction

The most recent reorganization of the generic classification of the Pleurothallidinae proposed by Pridgeon and Chase (2001) was largely based on the results of the molecular phylogenetic studies of the subtribe (Pridgeon *et al.* 2001). The initial analyses were made on a representative set of species and their results were extrapolated to the whole subtribe by correlation with the classification previously proposed by Luer (1986), based on morphological similarities. The circumscription of each genus was discussed and refined by Pridgeon (2005).

Subsequent molecular studies have shown that several of the genera of Pleurothallidinae still require a modified circumscription in order to comply with the monophyly criterion. *Anathallis* Barb.Rodr. is no exception. In the phylogenetic trees of Pridgeon *et al.* (2001), species of *Pleurothallis* R.Br. subgen. *Acuminatia* Luer (Luer 1999), including the type species of genus Anathallis, formed a clade together with species of *Pleurothallis* subgen. *Specklinia* sect. *Muscosae* Lindl. The clade was found sister to a clade which includes *Trichosalpinx* Luer and *Lepanthes* Sw., among others, and a broad concept of genus *Anathallis* was re-established (Prodgeon & Chase 2001; Pridgeon 2005).

However, Pridgeon's data set included only species of *Pleurothallis* subgen. *Acuminatia* sect. *Alatae* Luer and did not include representatives of sect. *Acuminatae* Lindl. had been initially analyzed. Karremans (2010) noted that species belonging to sect. *Acuminatae* were not related to those of sect. *Alatae*, but instead were found embedded within *Stelis* Sw. (*sensu* Pridgeon 2005), and suggested that, based on morphology, the same would be true for all other species in the section. The studies by Chiron *et al.* (2012) and Karremans *et al.* (2013a) confirmed that additional species of the sect. *Acuminatae* belonged in Stelis. The first set of authors even proposed a new combination for *Anathallis rubens* (Lindl.) Pridgeon & M.W.Chase in *Stelis*, but neglected to transfer all other species of the section.

Luer (2006) later segregated species of *Pleurothallis* subgen. *Specklinia* (Lindl.) Garay sect. *Muscosae* Lindl. into *Panmorphia* Luer resulting in a genus of 73 highly heterogeneous species with "*Specklinia*-like habit and *Anathallis*-like flowers". Luer later decided that the variation within *Panmophia* graded into the concept of *Anathallis*, and he reduced his *Panmophia* as a synonym of the latter (Luer 2009). Analyses of molecular data by Stenzel (2004) demonstrated that species of *Panmorphia* (including the type) were embedded within *Anathallis*. This conclusion was confirmed by Chiron *et al*. (2012), who included a broad representation of *Anathallis* species in their analyses.

One *Anathallis* species, the broadly distributed and highly variable *Anathallis barbulata* (Lindl.) Pridgeon & Chase, was shown to be distinct from all the other species (Chiron *et al.* 2012). It is probably the most well known species of the group here discussed. In Luer's subgeneric classification of genus *Pleurothallis* R.Br., *A. barbulata* and a few close relatives were placed in *Pleurothallis* subgen. *Specklinia* sect. *Muscosae* Lindl. (Luer 1986). Later on, they were transferred to *Anathallis* by Pridgeon and Chase (2001) and *Panmorphia* by Luer (2006). We present nrITS analyses showing that most species of *Panmorphia*, including the type species, *Anathallis sertularioides* (Sw.) Pridgeon & Chase, are embedded within *Anathallis*. Our data also show that *Anathallis barbulata* and a few sister species are not closely related to other *Anathallis* and require generic recognition to maintain monophyly.

Most of these *Specklinia*-like species of *Anathallis* have also been treated as species of *Specklinia* Lindl. at some point or another. A more extensive molecular phylogenetic analysis of *Specklinia* (Karremans *et al.* unp, Chapter 6), excludes the species here treated as *Anathallis* (Pupulin *et al.* 2012, Chapter 1; Bogarín *et al.* 2013b, Chapter 4; Karremans *et al.* 2013b), requiring the circumscription of those genera in the present manuscript. It becomes necessary as well to propose the systematic modifications required in order to attain monophyly within *Anathallis*, *Specklinia*, and *Stelis* and to propose a segregated generic concept for the *A. barbulata* and its close relatives.

Material and Methods

This study was conducted at Jardín Botánico Lankester (JBL) of the Universidad de Costa Rica and Naturalis Biodiversity Center - Leiden University, between October 2011 and October 2013. Living material was studied at Lankester Botanical Garden and the Hortus Botanicus in Leiden, while dried and spirit material was deposited at CR, JBL-spirit and L-spirit. Taxon names mostly follow Pridgeon (2005).

Photography:—Color illustrations of complete flowers were made using a Nikon D5100 digital camera, while photographs of the columns and pollinaria were taken using a DFC295 Leica digital microscope color camera with Leica FireCam version 3.4.1 software. Scanning electron microscope (SEM) micrographs were taken from flowers fixed in FAA (formalin 10%, glacial acetic acid 5%, water 35%, ethanol 50%). The floral samples were then dehydrated through a series of ethanol steps and subjected to critical-point-drying using liquid CO_2. Dried samples were mounted and sputter-coated with gold and observed with a JEOL JSM-5300 scanning electron microscope at an accelerating voltage of 10kV.

Phylogenetic Analysis:—The data matrix included 56 individuals (Table 12), 18 of which were produced in this study. The remaining data were obtained from GenBank (Pridgeon *et al.* 2001; Chiron *et al.* 2012; Karremans *et al.* 2013a). Plants were obtained from living collections at Lankester Botanical Garden in Costa Rica, the Hortus botanicus in Leiden, and private collections. Vouchers were deposited in spirit collections at JBL and L. Fresh leaf and flower cuttings of approximately 1 cm^2 were dried with silica gel. Samples (20 mg) were pulverized and extraction performed following the DNEasy procedure (Qiagen). The nuclear ribosomal internal transcribed spacer (nrITS) region was amplified using the methods and primers for sequencing and amplification described by Sun *et al.* (1994), and Sanger sequencing was done commercially by Macrogen on a 96-capillary 3730xl DNA Analyzer automated sequencer (Applied Biosystems, Inc.) using standard dye-terminator chemistry (Macrogen, Inc.).

The Staden *et al.* (2003) package was used for editing of the sequences. Contigs were exported as .fas files and opened in Mesquite v2.72 (Maddison & Maddison 2007), where they were checked for base calling errors, the matrix was aligned manually. The ends of each data set were trimmed to eliminate possible erroneous data, and gaps were regarded as missing data (filled with Ns). The data matrix is deposited in the Dryad Digital Repository (Heneghan *et al.* 2011). *Echinosepala aspasicensis* was used as the outgroup, as it was found to be one of the most distantly related of all included species (Pridgeon *et al.* 2001). The trees were produced with an analysis of the nrITS dataset of 43 sequences using BEAST v1.6.0. (Drummond & Rambaut 2007). Parameters were set to preset, except for substitution model GTR with 10 categories, clock model uncorrelated lognormal, tree prior Yule process, and number of generations 20,000,000. The resulting trees were combined using TreeAnnotator v1.6.0., where the first 2000 trees were used as burn-in. FigTree v1.3.1. (Rambaut 2009) was used to edit the resulting tree. Posterior probabilities are given for each node in decimal form.

Table 12. List of vouchers and GenBank number used in the phylogenetic analyses. Scientific names mostly follow Pridgeon 2005.

Taxon	Voucher collector and number	GenBank number	Source
Anathallis adenochila (Loefgr.) F.Barros (1)	*van den Berg 2148 (HUEFS)*	JQ306490	Chiron *et al.* 2012
Anathallis adenochila (Loefgr.) F.Barros (2)	*Karremans 4871 (L)*	KC425725	This Study
Anathallis angustilabia (Schltr.) Pridgeon & M.W.Chase	Manning 890604 (K)	AF262868	Pridgeon *et al.* 2001
Anathallis aristulata (Lindl.) Luer	*van den Berg 2042 (HUEFS)*	JQ306338	Chiron *et al.* 2012
Anathallis barbulata (Lindl.) Pridgeon & M.W. Chase (1)	*Chiron 11071 (HUEFS)*	JQ306457	Chiron *et al.* 2012
Anathallis barbulata (Lindl.) Pridgeon & M.W. Chase (2)	*Bogarín 8606 (JBL)*	KC425726	This Study
Anathallis barbulata (Lindl.) Pridgeon & M.W. Chase (3)	*Karremans 5750 (L)*	KF747834	This Study
Anathallis bolsanelloi Chiron & V.P.Castro	*van den Berg 2000 (HUEFS)*	JQ306342	Chiron *et al.* 2012
Anathallis burzlaffiana (Luer & Sijm) Luer	*Karremans 4857 (L)*	KC425727	This Study
Anathallis citrina (Schltr.) Pridgeon & M.W.Chase	*van den Berg 2086 (HUEFS)*	JQ306498	Chiron *et al.* 2012
Anathallis corticicola (Schltr. ex Hoehne) Pridgeon & M.W.Chase	*Hermans 3685 (K)*	AF262870	Pridgeon *et al.* 2001
Anathallis cuspidata (Luer) Pridgeon & M.W. Chase	*Bogarín 9619 (JBL)*	KF747835	This Study
Anathallis depauperata (Cogn.)	*Karremans 4808 (L)*	KC425735	This Study
Anathallis duplooyi (Luer & Sayers) Luer	*Karremnas 4888 (JBL)*	KF747836	This Study
Anathallis fractiflexa (Ames & C. Schweinf.) Luer (1)	*Bogarín 8988 (JBL)*	KC425728	This Study
Anathallis fractiflexa (Ames & C. Schweinf.) Luer (2)	*Bogarín 8988 (JBL)*	KC425729	This Study
Anathallis grayumii (Luer) Luer (1)	*Karremans 2747 (JBL)*	KC425730	This Study
Anathallis grayumii (Luer) Luer (2)	*Pupulin 3794 (JBL)*	KC425731	This Study
Anathallis heterophylla Barb.Rodr.	*van den Berg 2031 (HUEFS)*	JQ306339	Chiron *et al.* 2012
Anathallis kautskyi (Pabst) Pridgeon & M.W.Chase	*van den Berg 2051 (HUEFS)*	JQ306340	Chiron *et al.* 2012
Anathallis lewisiae (Ames) Solano & Soto Arenas	*Bogarín 1056 (JBL)*	KC425733	This Study
Anathallis linearifolia (Cogn.) Pridgeon & M.W.Chase	*Hrmans 2336 (K)*	AF262869	Pridgeon *et al.* 2001
Anathallis microgemma (Schltr. ex Hoehne) Pridgeon & M.W.Chase	*Manning 940319 (K)*	AF262894	Pridgeon *et al.* 2001
Anathallis minutalis (Lindl.) Pridgeon & M.W.Chase	*Jimenez-M. 1044 (UNAM)*	AF262922	Pridgeon *et al.* 2001
Anathallis nanifolia (Foldats) Luer	*Karremans 4793 (L)*	KC425736	This Study
Anathallis nectarifera Barb.Rodr.	*van den Berg 2078 (HUEFS)*	JQ306458	Chiron *et al.* 2012
Anathallis obovata (Lindl.) Pridgeon & M.W.Chase (1)	*Kollmann 6092 (MBML)*	JQ306497	Chiron *et al.* 2012
Anathallis obovata (Lindl.) Pridgeon & M.W.Chase (2)	*Stenzel 840 (CU)*	JF934822	Stenzel 2004
Anathallis obovata (Lindl.) Pridgeon & M.W.Chase (3)	*Karremans 4796 (L)*	KF747797	This Study
Anathallis ourobranquensis Campacci & Menini	*Chiron 11220 (HUEFS)*	JQ306459	Chiron *et al.* 2012
Anathallis pabstii (Garay) Pridgeon & M.W.Chase	Karremans 4821 (L)	KC425737	This Study
Anathallis pachyphyta (Luer) Pridgeon & M.W.Chase	*Karremans 4795 (L)*	KC425734	This Study
Anathallis peroupavae (Hoehne & Brade) F. Barros	*Karremans 5759 (L)*	KF747837	This Study
Anathallis petropolitana (Hoehne) Luer & Toscano	*van den Berg 2089 (HUEFS)*	JQ306491	Chiron *et al.* 2012
Anathallis piratiningana (Hoehne) F.Barros	*van den Berg 2066 (HUEFS)*	JQ306344	Chiron *et al.* 2012
Anathallis pubipetala (Hoehne) Pridgeon & M.W.Chase	*van den Berg 2106 (HUEFS)*	JQ306460	Chiron *et al.* 2012
Anathallis rabei (Foldats) Luer	*Karremans 4794 (L)*	KC425738	This Study
Anathallis radialis (Porto & Brade) Pridgeon & M.W.Chase	*Chiron 10144 (HUEFS)*	JQ306345	Chiron *et al.* 2012
Anathallis rudolfii (Pabst) Pridgeon & M.W.Chase	*van den Berg 2127 (HUEFS)*	JQ306461	Chiron *et al.* 2012
Anathallis sertularioides (Sw.) Pridgeon & M.W.Chase	*Solano 807 (UNAM)*	AF262871	Pridgeon *et al.* 2001
Anathallis welteri (Pabst) F.Barros	*van den Berg 2009 (HUEFS)*	JQ306341	Chiron *et al.* 2012
Echinosepala aspasicensis (Rchb. f.) Pridgeon & M.W. Chase	*Hermans 2160 (K)*	AF262905	Pridgeon *et al.* 2001
Frondaria caulescens (Lindl.) Luer	*Luer 18778 (K)*	AF262914	Pridgeon *et al.* 2001
Lepanthes felis Luer & R. Escobar	*Hermans 2899 (K)*	AF262891	Pridgeon *et al.* 2001
Lepanthes steyermarkii Foldats	*Hermans 2682 (K)*	AF262889	Pridgeon *et al.* 2001
Lepanthes woodburyana Stimson	*Hermans 2931 (K)*	AF262890	Pridgeon *et al.* 2001
Lepanthopsis astrophora Garay	*Manning 941040 (K)*	AF262893	Pridgeon *et al.* 2001
Lepanthopsis floripecten (Rchb. f.) Ames	*van den Berg 2063 (HUEFS)*	JQ306336	Chiron *et al.* 2012
Trichosalpinx arbuscula (Lindl.) Luer	*Hermans 1266 (K)*	AF262888	Pridgeon *et al.* 2001
Trichosalpinx berlineri (Luer) Luer	*Hermans 1605 (K)*	AF262900	Pridgeon *et al.* 2001
Trichosalpinx blaisdellii (S.Watson) Luer	*Kew 1997-7412 (K)*	AF262887	Pridgeon *et al.* 2001
Trichosalpinx dependens (Luer) Luer	*van den Berg 2011 (HUEFS)*	JQ306456	Chiron *et al.* 2012
Trichosalpinx orbicularis (Lindl.) Luer	*Hermans 1349 (K)*	AF262886	Pridgeon *et al.* 2001
Zootrophion atropurpureum (Lindl.) Luer (1)	*Kew 1997-7414 (K)*	AF262898	Pridgeon *et al.* 2001
Zootrophion atropurpureum (Lindl.) Luer (2)	*van den Berg 2056 (HUEFS)*	JQ306415	Chiron *et al.* 2012
Zootrophion serpentinum Luer	*Manning 921030 (K)*	AF262899	Pridgeon *et al.* 2001

Results

The consensus gene tree (Fig. 67) was obtained from a BEAST analysis of a matrix of 56 ITS sequences (Table 12), including 41 individuals belonging to 34 different species of genus *Anathallis*. The resulting tree includes two highly supported clades of *Anathallis* species; the first is coded clade *Anathallis* and the second clade has been coded *Lankesteriana*.

Clade *Lankesteriana* (P.P. = 0.98) includes the accessions of the species *Anathallis barbulata*, *A. cuspidata, A. duplooyi* and *A. fractiflexa*. A clade including *Trichosalpinx* berlineri and *T. dependens* (*Trichosalpinx* II) is highly supported (P.P. = 1) as sister to the *Lankesteriana* clade. Sister to both is a clade including species of *Zootrophion* with high support (P.P. = 0.94).

Clade *Anathallis* is highly supported (P.P. = 1) and includes all accessions of genus *Anathallis* with the exception of those found in clade *Lankesteriana*. Clade *Anathallis* includes *A. obovata*, type species of the genus, and *A. sertularioides*, type species of genus *Panmorphia*. A clade including *Trichosalpinx blaisdellii* and *T. orbicularis* (*Trichosalpinx* I) is found with low support (P.P. = 0.35) sister to the *Anathallis*. Altogether they are sister, with medium support (P.P.=0.66), to a highly supported (P.P. = 1) clade which includes the accessions of *Frondaria* Luer, *Lepanthes* Sw. and *Lepanthopsis* (Cogn.) Ames.

Both mentioned clades are sister to each other, and in turn to an accession of *Trichosalpinx arbuscula* (*Trichosalpinx* III), with low support (P.P. = 0.44). High support (P.P. = 1) is found for a clade which includes all the accessions of *Anathallis*, *Frondaria*, *Lepanthes*, *Lepanthopsis*, *Trichosalpinx* and *Zootrophion* Luer.

Branch length varies greatly within the whole group. The length of accessions of clade *Lankesteriana* double or triple those of *Anathallis*, the latter having accumulated many more nucleotide changes.

Discussion

The DNA based evidence obtained here supports the results of Chiron *et al.* (2012), showing that *Anathallis* is non-monophyletic. The addition of other accessions of the variable *A. barbulata*, and of its close relatives *A. duplooyi*, *A. cuspidata* and *A. fractiflexa* confirms that this species group as a whole should be excluded from *Anathallis*. The two highly supported clades of *Anathallis* are not sister to each other. Most of these species had already been segregated from *Anathallis* into *Panmorphia* by Luer, together with several others. However, *Panmorphia* is not monophyletic. The type species of *Panmorphia* is a member if *Anathallis s.s.*, necessitating a novel generic concept for the remaining species of the former *Panmorphia*. When describing *Panmorphia*, Luer (2006) suggested that he could find a "continuum of variations among them", however, he did mention that "several affinities among the species can be recognized". One of those affinities was likely this little group. In fact, this species group can also be easily distinguished from other species of the genus on morphological grounds, and they are therefore recognized as a segregate genus here forth.

Lankesteriana Karremans, Lankesteriana 13(3): 321. 2014.
Type:—*Pleurothallis barbulata* Lindl. Folia Orch. *Pleurothallis* 40. 1859. Replaced name for *Pleurothallis barbata* H.Focke, Bot. Zeitung (Berlin) 11(13): 227. 1853 (non *Pleurothallis barbata* Westc., Phytologist 1: 54. 1841).

> *Species of Lankesteriana are somewhat similar to Anathallis but can be distinguished by the tri-alate ovary (vs. cylindrical), the bilabiate flowers with lateral sepals convergent and usually fused to above the middle (vs. sepals free and spreading), the deeply depressed midline of the lip (vs. not or superficially depressed), the bilobed, helmet-shaped rostellum (vs. ligulate, not bilobed). Additionally, none of the known species of Lankesteriana have: 1) a habit that exceeds 3 cm tall (excluding the inflorescence), 2) ramicauls longer than the leaf, 3) multiple flowers open simultaneously on an inflorescence; 4) whitish to greenish flowers; all of which are commonly found in Anathallis.*

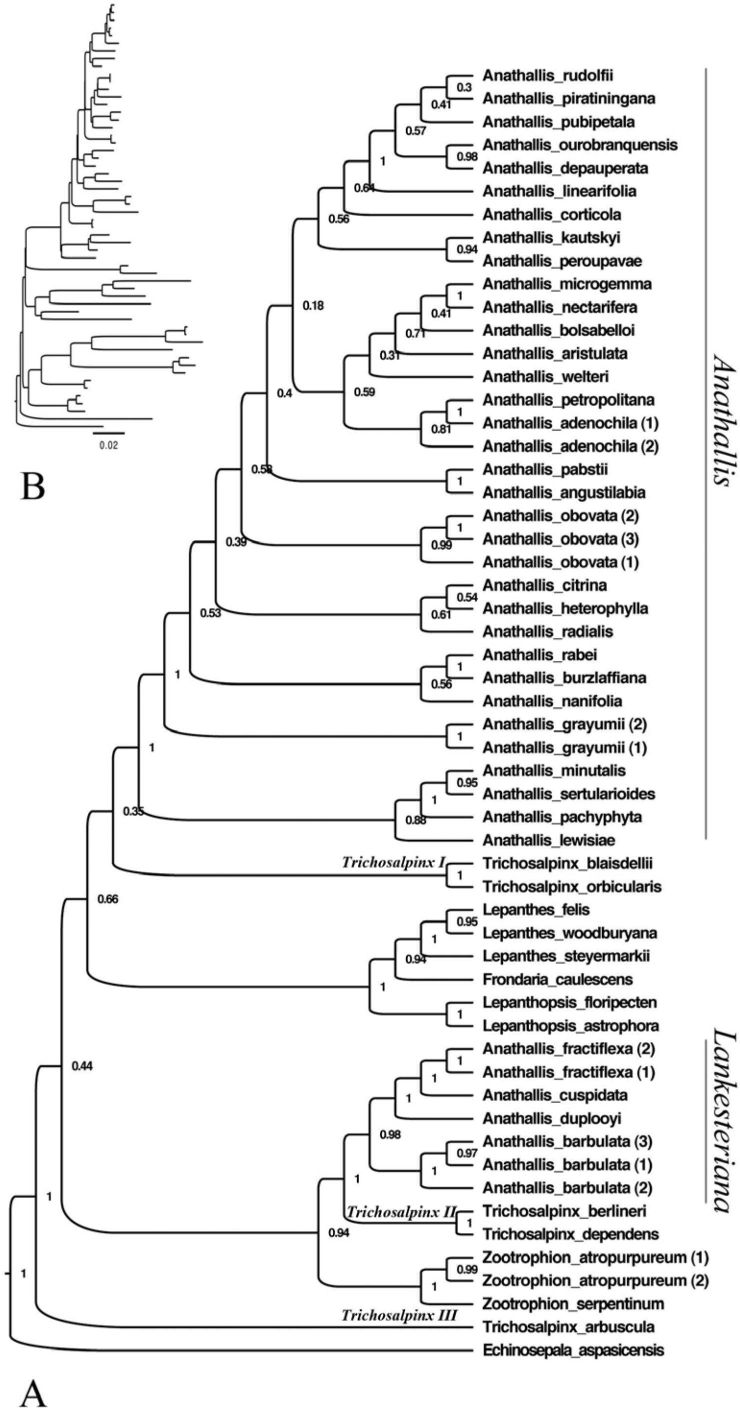


Figure 67. Consensus tree from a BEAST analysis of a matrix of 56 ITS sequences. The analysis ran for 20,000,000 generations. A. Branch length transformed to be equal for each species. Values on the nodes are Posterior Probabilities. Species names for each terminal is included. B. Relative branch lengths maintained, showing amount of evolutionary changes. Scale equals a 2% change. Posterior probability values and species names are excluded, but are equal to those of A. Trees edited by A.P. Karremans using FigTree.

Description:—Plants very small, 0.5-3 cm tall (excluding the inflorescence), epiphytic, caespitose. Ramicauls ascending, shorter than the leaf, never proliferating, with 1-3 imbricating, tubular, glandular to microscopically glandular sheaths. Leaf erect to prostrate. Inflorescence elongate, frequently exceeding the leaves, successive, with one flower open at a time. Flowers usually brownish-purple, sepals glabrous to ciliate. Ovary trialate. Sepals elliptic, acute, the lateral ones fused to above the middle or least convergent, forming a synsepal. Petals lanceolate to ovate-elliptic, widest near the middle, obtuse or acute, to acuminate, sometimes caudate. Lip oblong, to more or less pandurate, with a pair of basal sub-orbicular lobes, with a deep linear middle depression. Column winged, androclinium fimbriate-dentate, rostellum helmet-shaped, with prominent lateral lobes. Anther helmet-shaped. Pollinia in pairs, with reduced, granulose, whale-tail shaped caudicles (Fig. 68 & 69).

Etymology:—The name honors both the Lankester Botanical Garden of the University of Costa Rica, which is celebrating 40 years of existence, and also honors the homonymous scientific journal Lankesteriana, International Journal on Orchidology.

Distribution and ecology:—Nineteen species of ***Lankesteriana*** Karremans are recognized here, however as is frequent with other tiny pleurothallids, species of this genus tend to be overlooked in the field and lumped together into broad and variable species concepts. Species of *Lankesteriana* are distributed from southern Mexico, through Central America, the Andes, and all the way down to Bolivia and Brazil (Fig. 70). Costa Rica, Ecuador and Colombia contain the largest number of species, whereas Brazil, the center of diversity of sister genus *Anathallis*, has just a few *Lankesteriana*; they are notably absent from the Antilles. They occur between 280 and 2800 m in elevation, but most are found at mid elevations between 600 and 2000 m.

Luer (1986) had noted that flowers of species here treated as *Lankesteriana* were similar to some species of *Trichosalpinx* subgen. *Trichosalpinx* (*Trichosalpinx* I & II in Fig. 67). In fact, they resemble species of *Trichosalpinx* much more than *Anathallis*. *Trichosalpinx* was established by Luer for a group of species which shared the lepanthiform bracts of the stem and which did not fit well in either *Draconanthes* (Luer) Luer, Lepanthes or *Lepanthopsis* (Luer 1997), however that meant that they did not share a particular synapomorphy, and may not represent a natural grouping. The inclusion of a few species of *Trichosalpinx* in the DNA studies of Pridgeon *et al.* (2001) evidenced the polyphyly of the genus. A phylogenetic analysis of genus *Trichosalpinx*, including many more additional species, further evidences the need for a complete re-circumscription of this highly polyphyletic genus, which is diversely interrelated with all other genera in the clade (Fernández *et al.* unpublished).

Subgenus *Trichosalpinx* is biphyletic in the analysis presented here (Fig. 67), with a clade including the type of the genus (*Trichosalpinx* I), sister to *Anathallis*, and a second clade (*Trichosalpinx* II), sister to *Lankesteriana*. A reconsideration of *Trichosalpinx* will be a hazardous task that falls outside of the scope of this study. It suffices to say that we consider sister genera *Anathallis* and *Trichosalpinx* (*Trichosalpinx* I) distinct enough to keep them as separate genera and that the clade which includes *Lankesteriana* and *Trichosalpinx* II was until now unnamed. When revising *Trichosalpinx* in the future it can be re-considered if it is advantageous to include the few species belonging to *Trichosalpinx* II in a broadened *Lankesteriana*, however, based on morphology and genetic distance, such a move is in our view unfavorable.

With species of subgen. *Trichosalpinx* they share the fused sepals (with a few exceptions), the usually purplish-brown flowers, the extremely sensitive linear lip, with a pair of rounded lobes at the base, and a midline depression and the helmet-shaped rostellum. These traits suggest that both groups share a similar pollinator group. Species of subgen. *Trichosalpinx* however can be easily distinguished from those of *Lankesteriana* by the much larger plants, with long ramicauls covered with lepanthiform bracts and the simultaneously multi-flowered inflorescences.

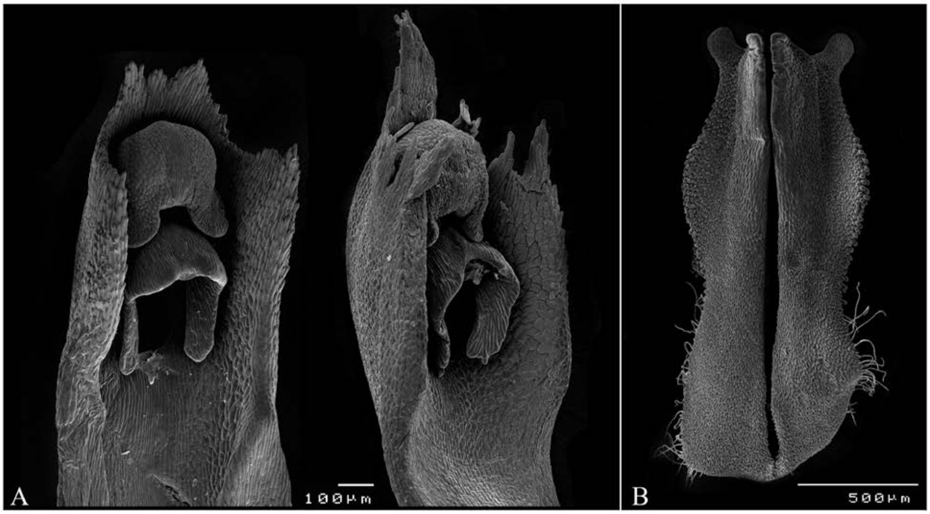


Figure 68. SEM images of micromorphology of Lankesteriana species. A. Column ventral view showing the androclinium, anther cap, helmet-like rostellum and stigma. B. The flattened lip, showing the midline depression, the basal sub-orbicular lobes and the glandular hairs near the apex. Specimens are *Lankesteriana cuspidata* (A-left & B; *Bogarín 9619*; JBL-spirit) an*d Lankesteriana barbulata* (A-right; *Karremans 5444*; JBL-spirit). Photographs by A.P. Karremans.

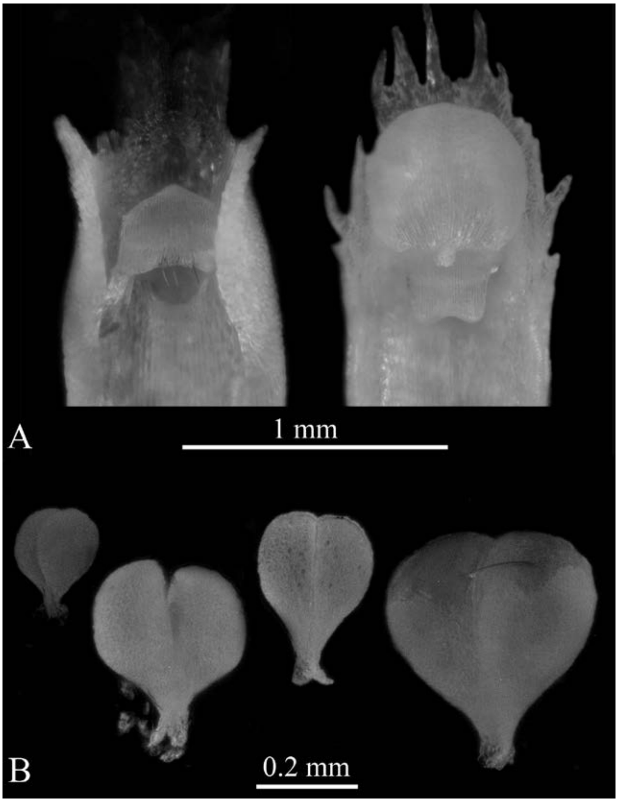


Figure 69. Micrographs taken with the Leica stereo microscope. A. Apex of the column in ventral view, from left to right, of *Lankesteriana cuspidata* (*Fernández 69*5; JBL-spirit) and *Anathallis polygonoides* (*JBL-28237*; JBL-spirit). B. Pollinaria, from left to right, of *Lankesteriana cuspidata* (*Fernández 695*; JBL-spirit), *Anathallis polygonoides (JBL-28237*; JBL-spirit), *Anathallis lewisae* (*Bogarín 1056*; JBL-spirit) an*d Trichosalpinx blaisdellii* (*Pupulin 1092*; JBL-spirit). Photographs by A.P. Karremans.

Key to the genera with Specklinia-like habit

1. Inflorescence frequently lax-flexuous, sepals usually caudate, petals fimbriate, acute to caudate, column inornate to narrowly winged .... *Muscarella*
- Inflorescence mostly congested-straight, sepals usually not caudate, petals entire to minutely denticulate, infrequently caudate, column ornate 2

2. Petals linear to lanceolate, acute to acuminate, column wings quadrate to triangular, androclinium conspicuously fimbriate 3
- Petals elliptic to spathulate, obtuse, column wings rounded, androclinium erose or inornate 4

3. Inflorescence single or simultaneously multi-flowered. Flowers star-shapped, lateral sepals free, flowers mostly white, green or yellow, lip lacking a deep mid-line depression, rostellum ligulate .... *Anathallis*
- Inflorescence successively single flowered. Flowers bilabiate, lateral sepals fused, flowers brownish-purple, lip with deep a midline depression, rostellum helmet-like bilobate .... *Lankesteriana*

4. Lip mostly linear-ligulate, column wings prominent, pollinia without caudicles .... *Specklinia*
- Lip trilobed, with a pair of suborbicular lobes close to the middle, column inconspicuously ornate or inornate, pollinia with caudicles .... *Pabstiella*

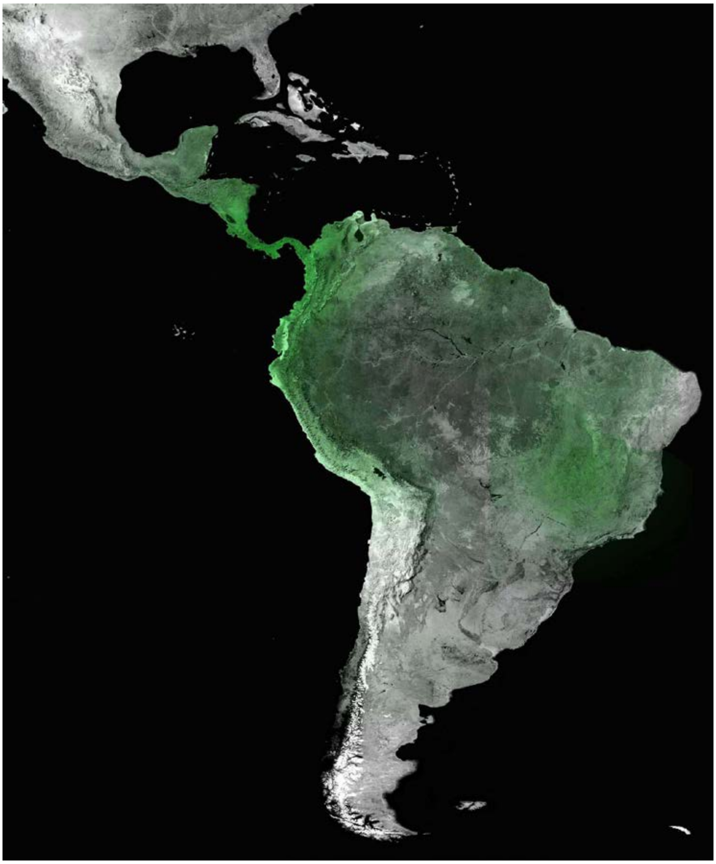


FIGURE 70. Distribution map (in green) of the 19 known species of *Lankesteriana* Karremans. The highest diversity of the genus in found from Costa Rica to Colombia and Ecuador.

Lankesteriana abbreviata (Schltr.) Karremans, Lankesteriana 13(3): 326. 2014.
Bas. *Pleurothallis abbreviata* Schltr., Repert. Spec. Nov. Regni Veg. 10: 352. 1912.
Lankesteriana barbulata (Lindl.) Karremans, Lankesteriana 13(3): 326. 2014.
Bas. *Pleurothallis barbulata* Lindl. Folia Orch. *Pleurothallis* 40. 1859. Replacement name for *P. barbata* H.Focke, 1853.
Note: *Specklinia pereziana* Kolan. published in 2011 from Colombia, is virtually indistinguishable from *Lankesteriana barbulata*, a common, widely distributed, variable species with several heterotypic synonyms. As *L. barbulata* was not even mentioned by the author there is no evidence to separate the two.
Lankesteriana casualis (Ames) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. *Pleurothallis casualis* Ames, Sched. Orch. 9: 30, 1925.
Lankesteriana caudatipetala (C.Schweinf.) Karreman, Lankesteriana 13(3): 327. 2014.
Bas. *Pleurothallis caudatipetala* C.Schweinf. Bot. Mus. Leafl. 10: 175. 1942.
Lankesteriana comayaguensis (Ames) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. *Pleurothallis comayaguensis* Ames, Bot. Mus. Leafl. 4: 31, 1936.
Lankesteriana cuspidata (Luer) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. *Pleurothallis cuspidata* Luer, Selbyana 3: 282, 1977.
Lankesteriana duplooyi (Luer & Sayers) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. *Pleurothallis duplooyi* Luer & Sayers. Rev. Soc. Bol. Bot. 3: 48, 2001.
Lankesteriana edmeiae (F.J. de Jesus, Xim. Bols. & Chiron) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. *Anathallis edmeiae* F.J. de Jesus, Xim. Bols. & Chiron, Richardiana 13: 296. 2013.
Lankesteriana escalarensis (Carnevali & Luer) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. *Pleurothallis escalarensis* Carnevali & Luer, Novon 13: 414. 2003.
Lankesteriana fractiflexa (Ames & C.Schweinf.) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. *Pleurothallis fractiflexa* Ames & C.Schweinf., Sched. Orch. 10: 26, 1930.
Lankesteriana haberi (Luer) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. *Pleurothallis haberi* Luer, Selbyana 23: 36. 2002.
Lankesteriana imberbis (Luer & Hirtz) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. *Pleurothallis imberbis* Luer & Hirtz, Lindleyana 11: 163, 1996.
Lankesteriana inversa (Luer & R.Vásquez) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. Pleurothallis inversa Luer & R.Vásquez, Rev. Soc. Bol. Bot. 3: 50. 2001.
Lankesteriana involuta (L.O.Williams) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. *Pleurothallis involuta* L.O.Williams, Bot. Mus. Leafl. 12: 239. 1946.
Lankesteriana millipeda (Luer) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. *Pleurothallis millipeda* Luer, Orquideología 20: 216. 1996.
Lankesteriana minima (C.Schweinf.) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. *Pleurothallis minima* C.Schweinf., Bot. Mus. Leafl. 3: 82. 1935.
Lankesteriana muricaudata (Luer) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. *Pleurothallis muricaudata* Luer, Selbyana 7: 119. 1982.
Lankesteriana rubidantha (Chiron & Xim.Bols.) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. *Specklinia rubidantha* Chiron & Xim.Bols., Richardiana 9: 125. 2009.
Lankesteriana steinbuchiae (Carnevali & G.A.Romero) Karremans, Lankesteriana 13(3): 327. 2014.
Bas. *Pleurothallis steinbuchiae* Carnevali & G.A.Romero, Novon 4: 90. 1994.

Anathallis Barb.Rodr., Gen. Sp. Orch. Nov. 1: 23. 1877.
Type:—*Anathallis fasciculata* Barb.Rodr., Gen. Sp. Orch. Nov. 1: 23. 1877.
This relatively old genus remained mostly unused until it was re-established by Pridgeon and Chase (2001), and re-defined by Pridgeon (2005). It was not clear how many and which species actually belonged to the concept, but initially about 90 species were transferred. About 90 more names were added by other authors since then (mostly transfers from other genera, but also new species). If we exclude the species that belong to *Lankesteriana* and *Stelis*, we end up just shy of 140 species, a number which seems reasonable.

Species of *Anathallis* are distributed from southern Mexico through Central America, the Antilles and all South America down to Argentina. They are most diverse in Brazil at low to mid elevations. They are easily recognized by the more or less star-shaped flower, with linear to lanceolate, acute to acuminate petals that are similar to the sepals. The lip is horizontally placed and very sensitive, its general shape is linear-ligulate but frequently it has small lobes at the base and/or middle. The column is sharply winged and prominently fimbriate. The pollinaria come in pairs and have reduced whale-tail shaped caudicles.

One species before treated as Specklinia is transferred here to *Anathallis* based on those morphological features.

Anathallis napintzae (Luer & Hirtz) Karremans, Lankesteriana 13(3): 328. 2014.
Bas. *Pleurothallis napintzae* Luer & Hirtz, Lindleyana 11: 173. 1996.

Stelis Sw., J. Bot. (Schrader) 2: 239. 1799.
Lectotype:—*Epidendrum ophioglossoides* Jacq., Enum. Pl. Carib., 29. 1760.
Although this genus has been traditionally accepted (Karremans *et al.* 2013a), it was greatly modified by Pridgeon and Chase (2001) and Pridgeon (2005). As such the genus was broadened from its classic definition (Luer 2009) to include several species groups before placed in *Pleurothallis*. *Stelis* in its broad sense was phylogenetically analyzed and extensively discussed by Karremans (2010) and Karremans *et al*. (2013a), and was proven largely monophyletic if the species of *Pleurothallis* subgen. *Acuminatia* sect. *Acuminatae* were transferred to it. That species group was found to be closely related to the species of Stelis in a strict sense (Luer 2009). It will suffice to say here that although smaller, better defined and informative generic concepts are preferred by the author, these species are transferred to a broad sense of *Stelis* where they are more accurately placed than previously.

In any other scenario this species group would require generic recognition, however, several other genera would have to be recognized and/or re-circumscribed as well. This might be possible at a later stage when the species belonging to each of those other groupings are well understood. The species transferred here were in any case already proven non-monophyletic as a group by Karremans *et al.* (2013a), however, all still within the broad concept of *Stelis*.

Stelis ariasii (Luer & Hirtz) Karremans, Lankesteriana 13(3): 328. 2014.
Bas. *Pleurothallis ariasii* Luer & Hirtz, Lindleyana 12: 42. 1997.
Stelis asperilinguis (Rchb.f. & Warsz.) Karremans,Lankesteriana 13(3): 328. 2014.
Bas. *Pleurothallis asperilinguis* Rchb.f. & Warsz., Bonplandia (Hannover) 2: 114. 1854.
Stelis aurea (Lindl.) Karremans, Lankesteriana 13(3): 328. 2014.
Bas. *Pleurothallis aurea* Lindl., Ann. Mag. Nat. Hist. 12: 397. 1843.
Replaced synonym: *Dendrobium acuminatum* Kunth in F.W.H.von Humboldt, A.J.A.Bonpland & C.S.Kunth, Nov. Gen. Sp. 1: 357. 1816 = *Anathallis acuminata* (Kunth) Pridgeon & M.W. Chase.
Note: The name *Dendrobium acuminatum* has priority over *P. aurea*, however *Stelis acuminata* Luer & Hirtz occupies the combination in *Stelis*. The heterotypic synonyms of this species, if not proven distinct and if not occupied in genus *Stelis*, have priority in the necessity of a new name. Therefore *Stelis aurea* is proposed for this species.
Stelis candida (Luer & Hirtz) Karremans, Lankesteriana 13(3): 328. 2014.
Bas. *Pleurothallis candida* Luer & Hirtz, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 107. 1999.
Stelis catenata Karremans, Lankesteriana 13(3): 328. 2014.
Replaced synonym: *Pleurothallis ramulosa* Lindl., Fol. Orchid. 9: 33. 1859.
Ety.: From the Latin *catenatus* referring to the chains of ramicauls formed.
Note: The name *Stelis ramulosa* Luer & Dalström (2004) occupies the combination in *Stelis* required for *Pleurothallis ramulosa* [=*Anathallis ramulosa* (Lindl.) Pridgeon & M.W. Chase]. Its heterotypic synonym *Pleurothallis superposita* Schltr. (1916) can't be combined in *Stelis* either as *Stelis superposita* Schltr. (1915) is also occupied. A new name for the species is therefore proposed.
Stelis coripatae (Luer & R.Vásquez) Karremans, Lankesteriana 13(3): 328. 2014.
Bas. *Pleurothallis coripatae* Luer & R.Vásquez, Phytologia 46: 362. 1980.

Stelis dimidia (Luer) Karremans, Lankesteriana 13(3): 328. 2014.

Bas. *Pleurothallis dimidia* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 109. 1999.

Stelis jesupiorum (Luer & Hirtz) Karremans, Lankesteriana 13(3): 329. 2014.

Bas. *Pleurothallis jesupiorum* Luer & Hirtz, Lindleyana 11: 164. 1996.

Stelis lagarophyta (Luer) Karremans, Lankesteriana 13(3): 329. 2014.

Bas. *Pleurothallis lagarophyta* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 112. 1999.

Stelis lamprophylla (Schltr.) Karremans, Lankesteriana 13(3): 329. 2014.

Bas. *Pleurothallis lamprophylla* Schltr., Repert. Spec. Nov. Regni Veg. 15: 205. 1918.

Replaced synonym: *Pleurothallis dolichopus* Schltr., Repert. Spec. Nov. Regni Veg. 10: 394. 1912 = *Anathallis dolichopus* (Schltr.) Pridgeon & M.W. Chase.

Note: The name *Pleurothallis dolichopus* has priority over *P. lamprophylla*, however *Stelis dolichopus* Schltr. occupies the combination in *Stelis*. The heterotypic synonyms of this species, if not proven distinct and if not occupied in genus *Stelis*, have priority in the necessity of a new name. Therefore *Stelis lamprophylla* is proposed for this species.

Stelis lauta Karremans, Lankesteriana 13(3): 329. 2014.

Replaced Synonym: *Pleurothallis concinna* Luer & R.Vásquez, Revista Soc. Boliv. Bot. 2: 133. 1999.

Ety.: From the Latin *lautus*, elegant, fine, as a replacement for the also Latin adjective *concinnatus* used in the original description of this species.

Note: The name *Stelis concinna* Lindl. (1834) occupies the combination in *Stelis* required for *Pleurothallis concinna* [=*Anathallis concinna* (Leur & R.Vásquez) Pridgeon & M.W. Chase]. A new name for the species is proposed.

Stelis lennartii Karremans, Lankesteriana 13(3): 329. 2014.

Replaced Synonym: *Pleurothallis anderssonii* Luer, Lindleyana 11: 145. 1996.

Ety.: The name honors Lennart Andersson, to whom the species was originally dedicated.

Note: The name *Stelis anderssonii* Luer & Endara occupies the combination in *Stelis* required for *Pleurothallis anderssonii* [=*Anathallis anderssonii* (Luer) Pridgeon & M.W. Chase]. A new name for the species is proposed.

Stelis maguirei (Luer) Karremans, Lankesteriana 13(3): 329. 2014.

Bas. *Pleurothallis maguirei* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 113. 1999.

Stelis mediocarinata (C.Schweinf.) Karremans, Lankesteriana 13(3): 329. 2014.

Bas. *Pleurothallis mediocarinata* C.Schweinf., Fieldiana, Bot. 33: 26. 1970.

Stelis melanopus (F.Lehm. & Kraenzl.) Karremans, Lankesteriana 13(3): 329. 2014.

Bas. *Pleurothallis melanopus* F.Lehm. & Kraenzl., Bot. Jahrb. Syst. 26: 443. 1899.

Replaced synonym: *Pleurothallis stenophylla* Lehm. & Kraenzl., Bot. Jahrb. Syst. 26: 442. 1899 = *Anathallis stenophylla* (Lehm. & Kraenzl.) Pridgeon & M.W. Chase.

Note: The name *Pleurothallis stenophylla* has priority over *P. melanopus*, however *Stelis stenophylla* Rchb.f. occupies the combination in *Stelis*. The heterotypic synonyms of this species, if not proven distinct and if not occupied in genus *Stelis*, have priority in the necessity of a new name. Therefore *Stelis melanopus* is proposed for this species.

Stelis meridana (Rchb.f.) Karremans, Lankesteriana 13(3): 329. 2014.

Bas. *Pleurothallis meridana* Rchb.f., Linnaea 22: 826. 1850.

Stelis montserratii (Porsch) Karremans, Lankesteriana 13(3): 329. 2014.

Bas. *Pleurothallis montserratii* Porsch, Oesterr. Bot. Zeitsch. 158. 1905.

Replaced synonym: *Pleurothallis rubens* Lindl., Edwards's Bot. Reg. 21: t. 1797. 1835.

Note: The name *Pleurothallis rubens* has priority over *P. montserratii*, however as *Stelis rubens* Schltr. (1910) occupies the combination in *Stelis*, a new name has to be proposed in that genus. Chiron *et al.* (2012) proposed *Stelis neorubens* Chiron, however the heterotypic synonyms of this species, if not proven distinct and if not occupied in genus *Stelis*, have priority in the necessity of a new name. Therefore *Stelis montserratii* is proposed for this species and has priority over *S. neorubens*, unless it is proven a distinct species.

Stelis papuligera (Schltr.) Karremans, Lankesteriana 13(3): 329. 2014.

Bas. *Pleurothallis papuligera* Schltr., Repert. Spec. Nov. Regni Veg. 10: 453. 1912.

Stelis regalis (Luer) Karremans, Lankesteriana 13(3): 329. 2014.
Bas. *Pleurothallis regalis* Luer, Selbyana 5: 178. 1979.
Stelis scariosa (Lex.) Karremans, Lankesteriana 13(3): 330. 2014.
Bas. *Dendrobium scariosum* Lex. in P.de La Llave & J.M.de Lexarza, Nov. Veg. Descr. 2 (Orchid. Opusc.): 39. 1825.
Stelis schlimii (Luer) Karremans, Lankesteriana 13(3): 330. 2014.
Bas. *Pleurothallis schlimii* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 120. 1999.
Stelis sclerophylla (Lindl.) Karremans, Lankesteriana 13(3): 330. 2014.
Bas. *Pleurothallis sclerophylla* Lindl., Edwards's Bot. Reg. 21: t. 1797. 1835.
Stelis soratana (Rchb.f.) Karremans, Lankesteriana 13(3): 330. 2014.
Bas. *Pleurothallis soratana* Rchb.f., Xenia Orchid. 3: 25. 1881.
Stelis spathilabia (Schltr.) Karremans, Lankesteriana 13(3): 330. 2014.
Bas. *Pleurothallis spathilabia* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 27: 56. 1924.
Stelis spathuliformis (Luer & R.Vásquez) Karremans, Lankesteriana 13(3): 330. 2014.
Bas. *Pleurothallis spathuliformis* Luer & R.Vásquez, Revista Soc. Boliv. Bot. 2: 137. 1999.
Stelis unduavica (Luer & R.Vásquez) Karremans, Lankesteriana 13(3): 330. 2014.
Bas. *Pleurothallis unduavica* Luer & R.Vásquez, Phytologia 46: 372. 1980.
Stelis vasquezii (Luer) Karremans, Lankesteriana 13(3): 330. 2014.
Bas. *Pleurothallis vasquezii* Luer, Phytologia 49: 220. 1981.

Conclusions

High species diversity and the many cases of convergence and parallelism make the systematics of the Pleurothallidinae quite hazardous. Morphological features are often congruent with phylogenetic hypotheses based on DNA data, but homoplasy can occur in morphological traits; similar morphological features may not always reflect a similar evolutionary history. Molecular data provide an independent data set that can be used to evaluate morphological homoplasy. Several modifications to the genera *Anathallis*, *Specklinia* and *Stelis* have been proposed here in an effort to circumscribe genera that are both monophyletic and diagnosable using morphological characters. With the exclusion of the species belonging to *Lankesteriana* and *Stelis*, the recircumscribed *Anathallis* is monophyletic based on all available data.

It must be stressed that the present work does not intend to be a molecularly based phylogenetic study of *Anathallis* and *Lankesteriana*. Instead, a systematic re-circumscription of those genera is proposed using an all evidence approach in which clear morphological patterns are correlated with available DNA evidence. The analyses of additional genetic regions and of a broader species set might refine the phylogenetic relationships among these species, however, as already evidenced in several earlier studies the basic phylogenetic reconstruction produced using a representative number of nrITS sequences is mostly found unchanged (Pridgeon & Chase 2001; Karremans 2010; Karremans *et al.* 2013a), especially when the found clades have been thoroughly characterized morphologically (Luer 2002; Karremans 2010).

Lankesteriana (Fig. 71) is a well supported and defined genus of some 19 species. They are widely distributed in the Neo tropics with the noteworthy exception of the Antilles. The genus is phylogenetically closely related to some species of *Trichosalpinx* and *Zootrophion*, however, the tiny habit with an extremely reduced ramicaul with adpressed inconspicuous bracts, and the relatively long successively single flowered inflorescences resemble species of *Anathallis* and *Specklinia* much more closely. On the other hand, the frequently purplish flowers with usually fused lateral sepals and an extremely sensitive lip are once again reminiscent of some species of *Trichosalpinx* subgen. *Trichosalpinx*.

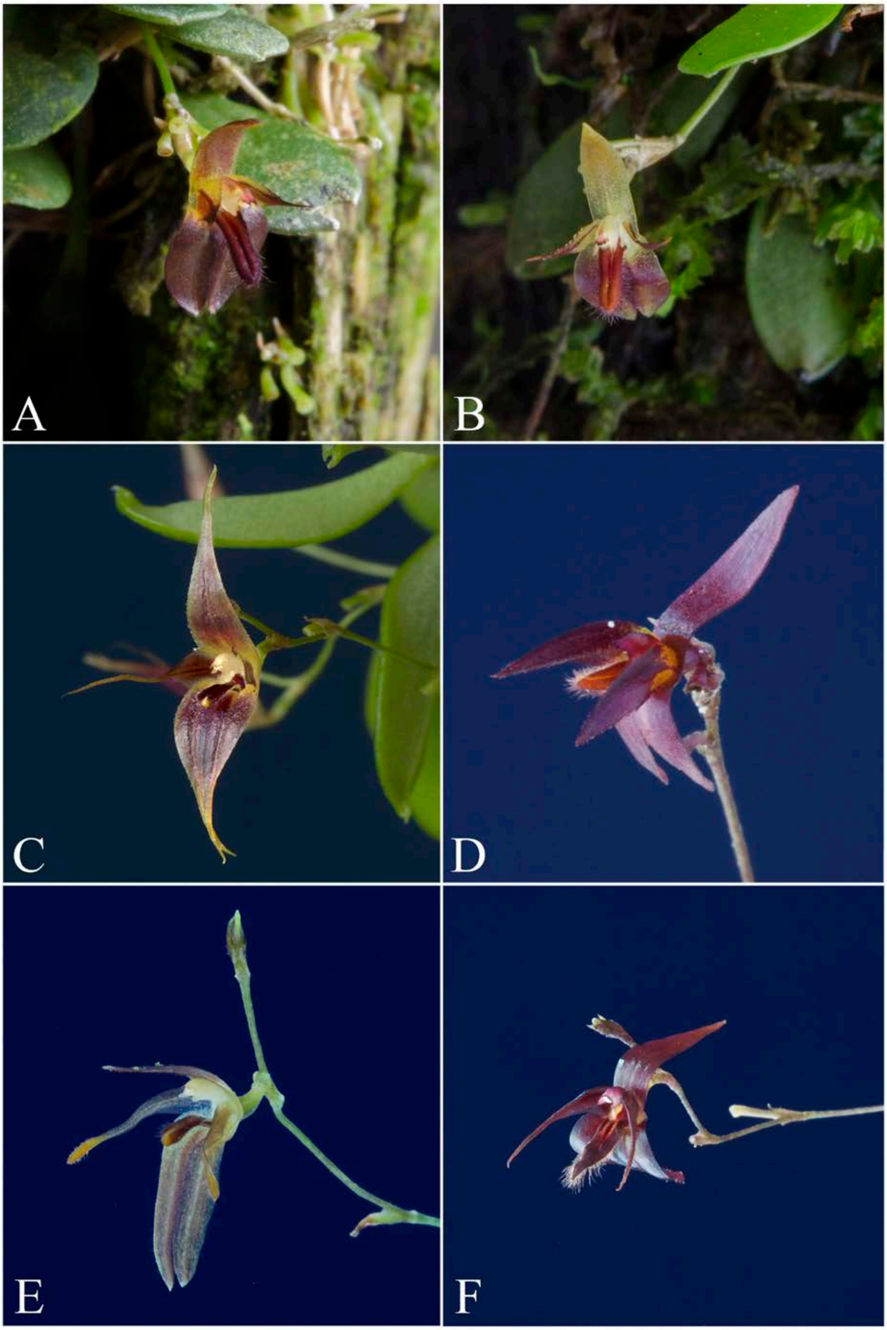


FIGURE 71. Representative species of genus *Lankesteriana*. A. *Lankesteriana barbulata* (*Karremans 5187*; JBL-spirit). B. A. *Lankesteriana barbulata* (*Karremans 5447*; JBL-spirit) C. *Lankesteriana cuspidata* (*Bogarín 9619*; JBL-spirit). D. *Lankesteriana duplooyi* (*Karremans 4888*; JBL-spirit). E. *Lankesteriana fractiflexa* (B*ogarín 8988*; JBL-spirit). F. *Lankesteriana* sp.nov. (*Karremans 4900*; JBL-spirit). Photographs by A.P. Karremans.

Assessing the pollination mechanisms of *Specklinia*

Annals of Botany 116(3): 437-455

Chapter 8

Pollination of *Specklinia* by nectar feeding *Drosophila*: first reported case of a deceptive syndrome employing aggregation pheromones in Orchidaceae

Adam P. Karremans
Franco Pupulin
David Grimaldi
Kevin Beentjes
Roland Butôt
Gregorio E. Fazzi
Karsten Kaspers
Jaco Kruizinga
Peter Roessingh
Erik Smets
Barbara Gravendeel

Background and Aims: The first documented observation of pollination in Pleurothallidinae was that of Augustus Endrés who noticed that the “viscid sepals” of *Specklinia endotrachys* were visited by a “small fly”. Mark Chase would later identify the visiting flies as being of the genus *Drosophila*. Here, we document and describe how species of the *Specklinia endotrachys* complex are pollinated by different *Drosophila* species.

Methods: Specimens of *Specklinia* and *Drosophila* were collected in the field in Costa Rica and preserved at JBL and L. Flies were photographed, filmed and observed for several days during a 2-year period and were identified by a combination of noninvasive DNA barcoding and anatomical surveys. Tissue samples of the sepals, petals and labellum of *Specklinia* species were observed and documented with SEM, LM and TEM. EAG experiments were done on *Drosophila hydei*, using the known aggregation pheromones ethyl tiglate, methyl tiglate and isopropyl tiglate. Floral compounds were analysed with GC-MS using those same pheromones as standards.

Key Result: We find that flowers of *Specklinia endotrachys*, *S. pfavii*, S. remotiflora and *S. spectabilis* are visited and pollinated by several different but closely related *Drosophila* species. The flies are arrested by aggregation pheromones, including ethyl tiglate, methyl tiglate and isopropyl tiglate, released by the flowers, and to which at least *D. hydei* is very sensitive. Visible nectar drops on the adaxial surface of sepals are secreted by nectar secreting stomata; encouraging the *Drosophila*, both males and females, to linger on the flowers for several hours at a time. The *Drosophila* frequently show courtship behaviour; occasionally copulating. Several different species of *Drosophila* can be found on a single species of *Specklinia*.

Conclusions: Species of the *Specklinia endotrachys* group share a similar pollination syndrome. There seems to be no species-specific relationships between the orchids and the flies. We do not expect the *Specklinia* species to hybridise naturally as their populations do not overlap geographically. The combination of pheromone attraction and nectar feeding is likely to be a generalised pollination syndrome in Pleurothallidinae.

Keywords: aggregation pheromones, courtship, deceit, *Drosophila repleta* group, nectar secreting stomata, Pleurothallidinae, reward, *Specklinia endotrachys*, *Specklinia pfavii*, *Specklinia spectabilis*, *Specklinia remotiflora*.

Introduction

Epiphytism is likely to be the major contributor to the species richness of the Orchidaceae family, more specifically Epidendroideae (Gravendeel *et al.* 2004). Nonetheless, pollinator adaptation might be the driving force of the remarkable floral diversification in orchids. Jersáková *et al.* (2006) argue that this adaptation is likely to be unilateral, without change in the pollinator (Williams 1982). Co-evolution between orchids and their pollinators is apparently uncommon (Szentesi 2002). Orchids frequently exploit existing plant-pollinator relationships or even sexual systems of insects, exemplified by species that achieve pollination through deception, not offering floral rewards (Ackerman 1986; Jersáková *et al*. 2006; Ramírez *et al.* 2011).

Pollination by deceit is well known among orchids and has been frequently considered another key innovation contributing to the high species richness of the family (van der Pijl & Dodson 1966; Cozzolino & Widmer 2005). Food deception has evolved repeatedly in different angiosperm groups, but is mostly restricted to a few species per family (Renner 2005), while estimates suggest that a third of all orchids might be food deceptive (Ackerman, 1986), where it seems to have arisen many times independently. Sexual deception has been reported in several phylogenetically unrelated orchid clades (van der Pijl & Dodson 1966; Adams & Lawson, 1993; Ayasse *et al.* 2003; Singer 2002; Singer *et al.* 2004; Blanco & Barboza 2005; Ciotek *et al.* 2006; Phillips *et al*. 2009; Peakall *et al.* 2010). If confirmed to be a generalised syndrome in those species rich species' groups, sexual deceit might well represent up to 10% of the pollination syndromes in the Orchidaceae.

Together, those percentages would suggest that deceitful pollination could represent close to half of all pollination syndromes in the orchids. However, considering that only few orchid-pollinator relationships have been studied in detail, and several of those have found "non-obvious" floral rewards being offered to pollinators, including scents, triterpenoid resins, pseudopollen, lipid-rich substances, and low amounts of nectar and oils (Chase *et al.* 2009; Davies and Turner 2004; Mickeliunas *et al*. 2006; Pansarin & Amaral 2006; Whitten *et al.* 2007; Pansarin *et al.* 2008; Stpiczyńska & Davies 2008; Pansarin & Pansarin 2011; Pansarin *et al*. 2013; Papadopulous *et al*. 2013; Davies *et al.* 2014), non-obvious floral rewards might be overestimating the cases in which orchids offer no reward at all. Such a case is that of the *Specklinia endotrachys* (Rchb.f.) Pridgeon & M.W.Chase species complex (Pleurothallidinae).

Pleurothallidinae includes more than 4100 species (Pridgeon 2005), likely making it the largest subtribe among the orchids and one of the largest amongst flowering plants in general. Myophily, or fly pollination, seems to be the general in all the genera of the subtribe, with few exceptions. Myophily is the second largest pollination syndrome in the Orchidaceae, with an estimated 15-25% of the whole family being pollinated by flies (van der Pijl & Dodson 1966; Christensen 1994; Borba & Semir 2001). However, aside from research on *Acianthera* Scheidw. (Borba and Semir 2001; de Melo *et al.* 2010), *Dracula* Luer (Endara *et al.* 2010) *Lepanthes* (Blanco & Barboza 2005), *Octomeria* R.Br. (Barbosa *et al.* 2009), *Pleurothallis* R.Br. (Duque-Buitrago *et al*. 2014) and Stelis Sw. (Albores and Sosa 2006), few pollination syndromes in the Pleurothallidinae have been studied in depth and are yet fully described. Considering the high species and floral morphology diversity it is quite likely that a plethora of different pollination syndromes are present within these fly pollinated orchids.

Endrés, in 1878, noted that flies were attracted to the nectar present in the flowers of *S. endotrachys* (Pupulin *et al.* 2012, Chapter 1). Chase (1985) observed *Drosophila immigrans* visiting and pollinating *Specklinia spectabilis* (Ames & C.Schweinf.) Pupulin & Karremans. He noted that flowers emitted a faint rotten-fruit odor, but did not report the presence of nectar. Nectar production could not be confirmed by Pupulin *et al.* (2012, Chapter 1), but the authors did find that flowers of *Specklinia endotrachys*, *S. pfavii* (Rchb.f.) Pupulin & Karremans, *S. remotiflora* Pupulin & Karremans and *S. spectabilis* were all visited frequently and for long periods of time by Drosophiloid flies at Lankester Botanical Garden in Costa Rica, so they suspected a reward.

Orchidaceae show great adaptability in the rewards offered to potential pollinators, ranging from perfume, to oil, nectar and pollen (Smets *et al*. 2000). Unlike most other Asparagales, and numerous other monocots, Orchidaceae do not possess gynopleural or septal nectaries (Smets & Cresens 1988; Smets *et al*. 2000). Nectar secretion has been observed on the perianth parts (more specifically on the labellum) in some cases but perigonal nectaries are not that common in Orchidaceae as in Liliales where this feature can be considered synapomorphic (Smets *et al.*

2000). Floral fragrances are produced by osmophores (scent glands) occurring in a large group of plants (Vogel 1990; Dressler 1993). In orchids, osmophores may be located on the sepals, petals and labellum (Dressler 1993); the shape seems to vary from unicellular trichomes (Curry *et al*. 1991), pear-shaped or spherical unicellular hairs with irregular cuticle (Stpiczyńska 1993), dome-shaped papillae (Ascensãno *et al.* 2005), papillose cells with smooth cuticle (de Melo *et al.* 2010), to a rugose surface with a sculptured cuticle or wrinkled surface with a smooth cuticle (Antoń *et al*. 2012). The morphology of osmophores in fly-pollinated orchids has been examined only in a few species of Pleurothallidinae. Those studies have shown that osmophores are generally found on the sepals (Vogel 1990; Teixeira *et al*. 2004; de Melo *et al.* 2010).

In this paper we report the outcomes of a multidisciplinary study on the ecology, biology and phylogeny of the *Specklinia endotrachys* species group and allies, and of their pollinators of the *Drosophila repleta* species group. We address two specific questions: (1) how does pollination occur; (2) is pollination of *Specklinia* Lindl. species-specific. To answer these questions we collected plants and flies in the wild, made video documenting pollination and orchid-insect interaction, carried out LM, SEM and TEM observation, used DNA barcoding, and conducted EAG and GC-MS experiments.

Materials and methods

Living material:—Specimens of Specklinia species were field collected in Costa Rica and cultivated at the greenhouses of the Lankester Botanical Garden, University of Costa Rica and the Hortus botanicus of Leiden University (Leiden, The Netherlands), from 2012 till 2014. Voucher specimens of the plants were prepared from cultivated material and deposited at JBL (spirit), L (spirit) and CR.

Flies were photographed, filmed and observed for a total of 30 days during a 2-year period at the open-air greenhouses at Lankester Botanical Garden, in Costa Rica. Observations were mostly made between 06:00 and 18:00 h, with five observations extending that period overnight for all Specklinia species. Flies were identified by a combination of noninvasive (sample rescue after lysis) DNA barcoding of the 660 bp long COI marker by KB and anatomical surveys by DG. Only visitors that would linger on flowers and were highly interactive with sepals and lip (interacting with flower parts for more than 60 min) and/or that carried pollinaria were considered as putative pollinators. Vouchers for the insects were prepared from both field collected and greenhouse collected specimens and are kept at L (spirit) and AMNH.

Phylogenetics:—*Specklinia*. The phylogenetic concept of *Specklinia* follows Pridgeon *et al.* (2001). Those authors found that *Specklinia endotrachys* was closely related to *Specklinia lanceola* (Sw.) Lindl., the type species of the genus *Specklinia*, and a few other mainly orange-flowered species including *S. fulgens* (Rchb.f.) Pridgeon & M.W.Chase, *S. lentiginosa* (F.Lehm. & Kraenzl.) Pridgeon & M.W.Chase and *S. tribuloides* (Sw.) Pridgeon & M.W.Chase (Pupulin *et al.* 2012, Chapter 1). The species belonging to the *S. endotrachys* (*sensu* Pupulin *et al.* 2012, Chapter 1) complex are here treated as a monophyletic group within *Specklinia* based both on morphological similarities and additional unpublished molecular data (Bogarín *et al.* 2013b, Chapter 4; Karremans *et al*. 2013a).

Drosophila. Whole specimens were used for non-destructive extraction, using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's protocol. Elution was performed in 150 µl buffer AE. To obtain standard animal DNA barcode fragment of the mitochondrial cytochrome c oxidase subunit COI gene (Hebert *et al*. 2003), PCR was performed using a primer cocktail containing primers LCO1490 and HCO2198 (Folmer *et al*. 1994), and Lep-F1 and Lep-R1 (Hebert *et al.* 2004). PCR reactions contained 18.75 µl mQ water, 2.5 µl 10x PCR buffer CL, 1.0 µl 10mM of each primer, 0.5 µl 2.5 mM dNPTs and 0.25 µl 5 U Qiagen Taq. The PCR protocol consisted of an initial denaturation step of 180 s at 94 °C, followed by 40 cycles of 15 s at 94 °C, 30 s at 50 °C and 40 s at 72 °C, with a final extension of 300 s at 72 °C and a pause at 12 °C. Sanger sequencing was performed by Macrogen (http://www.macrogen.com) or BaseClear (http://www.baseclear.com) on an ABI 3730xl (Applied Biosystems).

The Staden *et al.* (2003) package was used for editing of the sequences. Contigs were exported as .fas files and opened in Mesquite v2.72 (Maddison and Maddison 2007), where they were checked for base calling errors, the matrix was aligned manually. *Drosophila melanogaster* was used as outgroup. The trees were produced with an analysis of the COI dataset using BEAST v1.6.0. (Drummond & Rambaut 2007). Parameters were set to preset, except for substitution model GTR with 10 categories, clock model uncorrelated exponential, tree prior Yule process, and number of generations 20,000,000. The resulting trees were combined using TreeAnnotator v1.6.0., using the first 3000 trees as burn-in. FigTree v1.3.1. (Rambaut 2009) was used to edit the resulting tree. Posterior probabilities are given for each node in decimal form. Sequences have been made available through BOLD.

Photo/Video-camera documentation:—*Video Recording*. The videos of the fly visitation were taken with the video option of a Nikon D5100 digital camera and a HD 720p Autofocus Logitech web cam.

Macrophotography. Colour illustrations of flowers and flies were made using a Nikon D5100 digital camera, a DFC295 Leica digital microscope colour camera with Leica FireCam version 3.4.1 software, and a Zeiss SteREO Discover V12 stereomicroscope using the AxioVision stacking software.

Scanning Electron Microscopy (SEM):—Tissue samples of the sepals, petals and labellum were prepared for SEM observation by harvesting tissue from the flowers up to 48 h after the beginning of anthesis, fixing in FAPA (ethanol 50%, acetic acid, formalin at a proportion of 18:1:1 v/v), and dehydration through a series of ethanol steps and critical-point drying using liquid CO_2. Dried samples were mounted and sputter-coated with gold and observed with a JEOL JSM-5300 scanning electron microscope, at an accelerating voltage of 10 kV. All images were processed digitally.

Light Microscopy (LM):—Tissue samples of the sepals of *S. pfavii* were prepared for LM observation by harvesting flowers up to 48 h after the beginning of anthesis, fixing in Ethanol 70%, dehydration through a graded series of ethanol 70%, 96%, 100% and xylene, impregnation with paraffin 60 C, and embedding in paraffin. Sections of 7 µm were cut using a Jung Biocut 2035 rotary microtome. To prepare for staining the samples were de-paraffinated in xylene, rehydrated through a series of ethanol step, and stained by placing in 1% alcian blue for 10 min. The samples were then rinsed in tapwater and demiwater, stained with nuclear fast red for 5 min, rinsed in demiwater, dehydrated through a graded series of ethanol and washed with xylene. Finally a coverslip with Entellan was placed on the sample and photographs were taken with a Zeiss Axioskop connected to a Leica DFC490 camera.

Transmission electron microscopy (TEM):—Freshly collected flowers were fixed for 3 h in a modified Karnovsky fixative (2.5% glutaraldehyde, 2% formaldehyde) and washed in a 0.1 M sodium cacodylate buffer (pH 7.4). After washing in 0.1 M sodium cacodylate buffer the material was postfixed for 2 h in 1% osmium tetroxide and then washed in distilled water. After dehydration in a series of ethanol and propylene oxide, the pieces were infiltrated with Epon by submerging them in a mixture of propylene oxide and Epon (1:1) for 1 hour. After overnight evaporation of the remaining propylene oxide, the material was embedded in fresh Epon and polymerized at 60 ºC for 48 h. Ultrathin sections were cut with an LKB ultratome, mounted on film-coated copper slot grids and poststained with uranyl acetate and lead citrate (Reynolds 1963). The sections were examined with a Jeol 1010 TEM.

Analysis of floral compounds with GC MS:—Floral compounds were extracted by two different methods. The first one consisted of rinsing the flowers in 5 ml heptane for up to 1 min; after removal of the flowers the heptane was concentrated to approximately 0.5 ml using a gentle stream of nitrogen. The second method consisted of trapping odours from open flowers with a volatile collector trap in which air was circulated by a membrane pump in a closed system. After each passage through the membrane-pump, the air was cleaned by a carbon filter. The volatiles were trapped on 50 mg of Porapak Porous Polymer Adsorbent (Sigma Aldrich) in a glass tube. After collecting (typically 3 h) the volatiles were eluted from the adsorbent with 3 ml of pure pentane. The pentane was subsequently concentrated to approximately 0.5 ml using a gentle stream of nitrogen. All samples were stored at -20 °C in anticipation for further analysis.

All extracts were analysed on a Thermo Scientific Trace 1300 gas chromatograph coupled to a Thermo Scientific DSQII mass spectrometer. A Restek Rxi-5ms capillary column (30 m x 0.25 mm, 0.25 mm film thickness) was used.

The initial oven temperature was 80 °C. After 2 min the temperature was increased to 120 °C (10 °C/ min). The final temperature was maintained for 7 min. Helium was used as carrier gas (1.2 ml/min). The split injection mode was used (injection volume 1 ml, inlet temperature 220 °C, split ratio 1:30). Mass spectra were taken in electron ionisation (EI) mode (at 70 eV) in the range of m/z 30-200 (500 amu/s). The ion source temperature and the interface line temperature were set to 250 °C and 200 °C, respectively. Compounds were identified by comparison of their mass spectra and retention times with those of commercially purchased reference samples.

Analysis of the floral droplets:—Drops produced on the adaxial sepal surface were collected with a fine glass pipet point and stored in a glass vial at -20 °C. Fehling's reagent was used to detect sugar presence in the collected drops. Two solutions were prepared and mixed immediately before use, forming a deep blue solution containing a cupric ion. Solution "A" was composed of 17.32 g of hydrated copper sulphate crystals in 250 ml of water, and solution "B" of 86.5 g of sodium potassium tartrate and 35 g of sodium hydroxide in 250 ml of water.

Electrophysiology:—*Fly Culturing and odour stimuli*. *Drosophila hydei* eggs were obtained from a commercial grower and reared at 23 °C, 50% rh and 16:8 L/D cycle. Flies were picked randomly 4-7 days after emergence from the eggs.

Moats *et al.* (1987) and Symonds and Wertheim (2005), reported several aggregation pheromones for D. hydei. Ethyl tiglate, methyl tiglate and isopropyl tiglate (98% purity, Sigma Aldrich) were selected. A volume of 1 µL of hexane diluted pure compounds (10-1, 10-2, 10-3 and 10-4 v/v) was pipetted on 5mm×50mm filter paper. After at least 60 sec to allow the hexane to evaporate, the strip was placed inside a Pasteur pipette. Z-3-Hexen-1-ol (diluted 10-1 v/v) was used as an external standard (positive control) and an empty Pasteur pipette as negative control. Stock solutions were freshly prepared before the experiments and kept at -20 °C in 1.5 ml bottles closed with Teflon lined caps. Pasteur stimulus pipettes were prepared daily.

Insect preparation and Electroantennogram (EAG) recording. Both male and female *D. hydei* were used in the experiments as no behavioural differences have been reported (Bartelt *et al*. 1985; 1986; 1988). A total of 14 animals were tested. Individual flies were cooled, immobilized at 4 °C for approximately 30 minutes and pushed into a plastic pipette just wide enough the catch the head. Recordings were made with a high impedance amplifier (IDAC-4) and EAG2000 software (Syntech, Kirchzarten, Germany), using glass capillaries filled with insect ringer. The recording electrode was inserted at the base of the antenna, the reference electrode only contacted the tip ("surface contact recording", den Otter *et al.* 1980). The preparations lifetime was several hours.

The insect was positioned 1 cm in front of the outlet of a charcoal filtered and humidified airstream (2 l/min). The chemical stimuli from the Pasteur pipettes were injected into the this flow (1 second, 2.5 ml odour pulses) with 60 sec intervals in random order. All EAG responses were expressed relative to the external standard (Z-3-Hexen-1-ol).

Statistics. To evaluate the effects of stimulus compound and concentration on the EAG responses a linear mixed models (Grüber *et al.* 2011) was constructed with Gaussian error function and log link function. The response variable was the standardized EAG amplitude described above. Explanatory variables we the stimulus compound (ethyl tiglate, methyl tiglate and isopropyl tiglate) and the stimulus concentration (dilutions from 10-1 to 10-4 v/v in hexane). To account for the variation caused by differences between individual flies individual was included as a random factor. The input for the models were 162 EAG amplitudes measured in 14 individual insects. To validate the model we visually inspected the fit using a qqplot and also plotted the residuals against the fitted values. No obvious patterns were present. The residuals did not differ from a normal distribution (Shapiro test, W = 0.9942, p = 0.767) and were homoscedastic (Bartlett test (for compounds), K2 = 3.094, df = 2, p = 0.21). All statistical tests were conducted in R (version 3.0.1; R Core Team 2013).

Results

Plant Biology:—The orchid species studied, *S. endotrachys*, *S. pfavii*, *S. remotiflora* and *S. spectabilis*, belong to a group of species which noticeably share a reddish-orange color of the perianth parts, especially of the sepals (Fig. 72a-d). These species produce long-lived multi-flowered successive inflorescences. Large plants may have 10+ flowers simultaneously opened, however, only one per single inflorescence. The four species have a tendency of flowering all year round in greenhouse conditions, but in field they do have flowering peaks. At least during 6 months all four species were flowering simultaneously in the greenhouses, and capsules were formed eventually. We have not found these species at many different localities in the field; however, when present they are commonly found in large colonies, and we have observed dozens of plants of *S. pfavii* and *S. remotiflora* growing in dense groups (Fig. 72e & f). Fruit-set was observed in both greenhouse and field conditions only in Costa Rica, not in The Netherlands. None of the documented plants of any of the species showed autogamy. In a wild population of *S. pfavii* (Table 13), 40% of the plants had capsules, however only 20% of the inflorescences had a capsule, and 8% of the produced flowers were pollinated. Those plants produced 1 to 7 flowers per inflorescence (can be more +20 under greenhouse conditions), and never more than a single capsule per inflorescence. Capsules are always found on the apex of the inflorescence, suggesting flowering succession is detained after frutification. Drops are produced after anthesis on the rugose areas of the sepals of all four species (Fig. 73). The drops keep growing and accumulate unless removed; they are fed upon by flies, ants and other floral visitors. If not removed they persist even after the flower withers. The drops are transparent and semi-liquid at ambient temperature; they change from liquid and transparent to pasty and opaque with increasing temperatures.

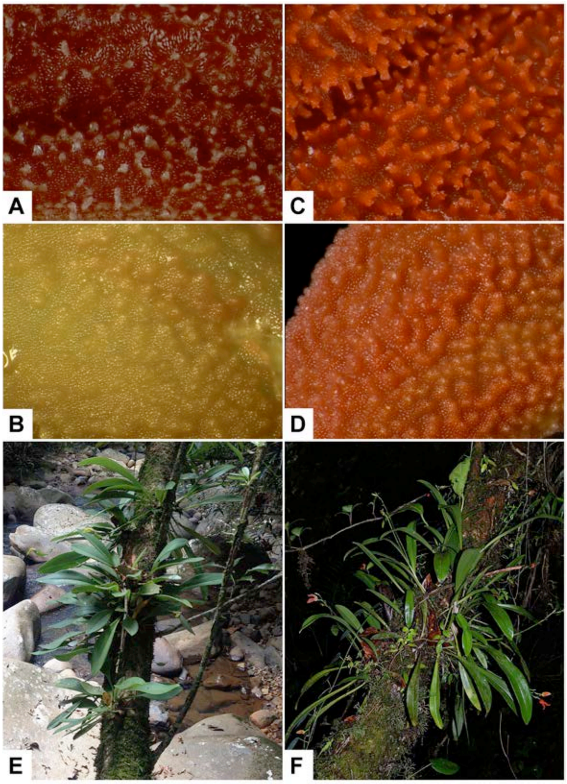


Figure 72. A-D. Adaxial surface of the sepals of diverse members of the orange-flowered *Specklinia* showing the structural and coloration diversity. A. *S. endotrachys* (*Blanco 961*). B. *S. pfavii* (*JBL-11098*). C. *S. remotiflora* (*AK4023*). D. *S. spectabilis* (*JBL-02535*). E-F. *Specklinia* species as found growing in field conditions in Costa Rica. E. *S. pfavii*, growing at a river's edge at 650 m elevation. F. *S. remotiflora*, in the cloud forest at around 2000 m elevation. Vouchers kept at JBL (spirit). Photographs by APK (A-E) and Joszef Geml (F).

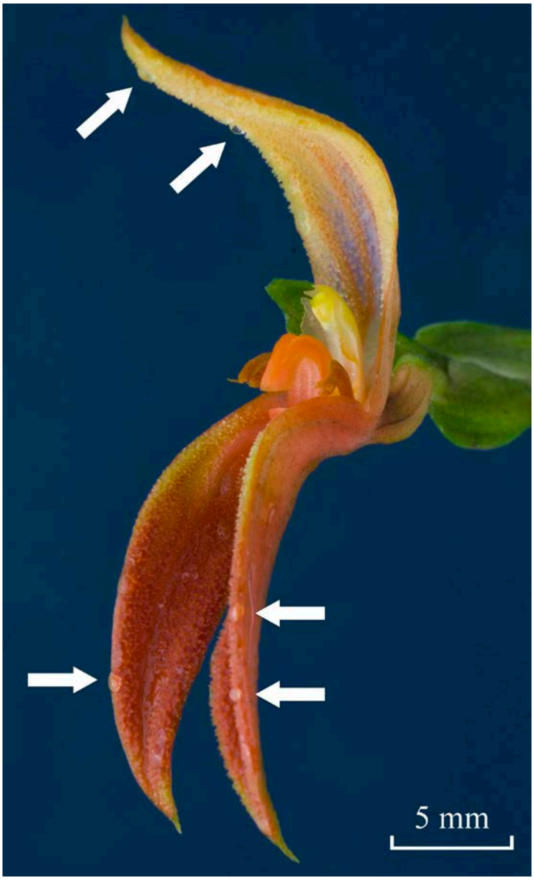


Figure 73. Nectar drops on the sepals of *Specklinia endotrachys* (*Blanco 961*). Photograph by Melania Fernández at Lankester Botanical Garden.

Table 13. Reproductive success relative to flowering in a wild population of *Specklinia pfavii* in Costa Rica.

Population	*Plants*	*Inflorescences*	*Flowers (total)*	*Fruits*
1	16	33	54	7
2	14	27	63	4
3	4	6	21	1
4	5	12	39	3
5	4	5	19	1
Total	43	83	196	16

Interaction	*Value*
Inflorescences per Plant	1.93
Flowers per Plant	4.56
Fruits per Plant	0.37
Fruits per Inflorescence	0.19
Fruits per Flower	0.08
Flowers per Inflorescence	2.36

Figure 74. A-B. *Drosophila* spp. sucking on the nectar secreting stomata on the apex of the papillae on the sepals of *Specklinia remotiflora*. A. Showing several flies at once. B. Shows a single fly and an area of the sepals where the stomata have been depredated by slugs. C. *Drosophila* spp. still attracted to a severed lateral sepal of *S. remotiflora* a few minutes after removal from the flower. D. *Drosophila* sp. on the lateral sepals of *Specklinia pfavii* with a drop on its mouthparts. It is likely to be a nuptial gift, regurgitated after having collected the nectar drops which are also still evident on the sepals. Arrows show the nectar droplets still present on the sepals. Photographs by APK (A-C) and FP (D) at Lankester Botanical Garden.

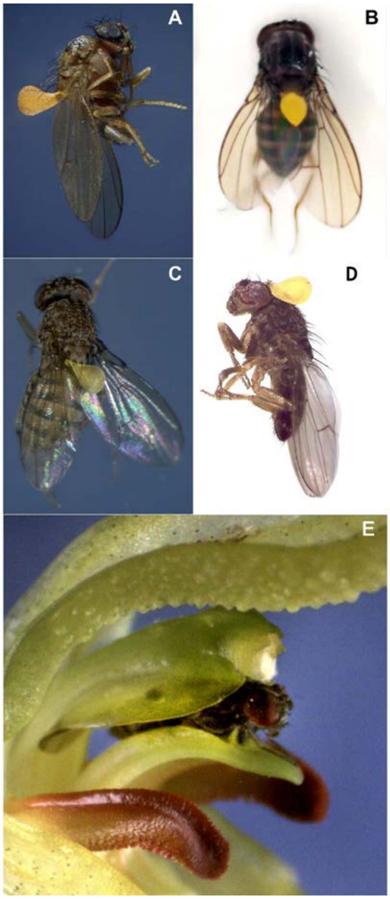


Figure 75. A-D. *Drosophila* flies with the pollinaria of *Specklinia* on the scutellum. A. *Drosophila* sp. with the pollinaria of *S. spectabilis* (*JBL-02643*). B. *Drosophila hydei* with the pollinaria of *S. remotiflora* (*Bogarín 8181*). C. *Drosophila mercatorum* (KB262-02) with the pollinaria of *S. remotiflora*. D. *Drosophila hydei* with the pollinia of *S. pfavii*. E. *Drosophila* sp. trapped between the lip and column of *S. pfavii*. It must be noted that the fly illustrated here is oriented the other way around from what is normally observed; it got the pollinia stuck to the head and not scutellum as would be expected (shown in 13d). Photographs by FP (A, B) and APK (C-E). A and B are copyrighted images reproduced from Phytotaxa 63: 1-20, with permission.

Pollinator Biology:—Flies visit the flowers for up to at least 24 h at a time, during that time they mostly remain on the flowers but occasionally leave for a few minutes and return. Visitation can happen anytime in greenhouse conditions, however it is more frequent in the early morning and late afternoon, possibly when temperatures are lower. With time passing by the visiting flies' motility is greatly reduced, becoming slower and less aware of their surroundings. They can visit singly or in groups of up to 7 individuals (possibly more). The flies move around and "inspect" the entire flower but spend most time (just above 90% of the time spent) on the papillae rich areas in the adaxial surface of the sepals (Fig. 74a & b), on which they suck during the entire time they are there [Supplementary Information - Video 1]. The attraction for the *Specklinia*'s sepals is so strong that even after immediate removal of one of them from the flower the flies still inspect it (Fig. 74c). The removal of all three sepals completely prevents flies from visiting the flowers. Amongst the most observed behaviours were: 1) fencing with the forelegs, occurring once every three minutes; 2) wing flapping and following of other flies, which are done constantly; 3) abdomen bending, about twice in three minutes [Supplementary Information - Video 2]. Two additional events were observed very rarely: 1) copulation, seen twice during the whole study period [Supplementary Information - Video 3]; and a fly with a regurgitated drop in its mouthparts, seen only once during the study period (Fig. 74d). The flies wander from sepal to sepal, frequently stepping on the movable lip. There they explore the conical rugose papillae and when placed in the right position, tilt the lip and are adpressed against the viscid rostellum (Fig. 75). The pollinia (which lack caudicles) are flattened and curved towards the base, and normally grasp the scutellum of the fly whilst the animal tries to leave the column/lip cavity in reverse [Supplementary Information - Video 4]; it can take the fly 20-30 min to liberate itself.

Pollinator Identities:—Fifty-six (56) flies were caught at the greenhouses at Lankester Botanical Garden in Costa Rica, 2 were field collected, and 2 were collected in a private garden (Table 14; Fig. 76). A total of 20 were collected on flowers of *S. remotiflora*, 20 on *S. spectabilis*, 14 on *S. pfavii*, 5 on *S. endotrachys* and 3 on *S*. sp. The flies caught all belonged to the genus *Drosophila* (Drosophilidae), except for one that belonged to genus *Hydrotaea* (Muscidae) and another to the Lauxaniidea. Of the specimens caught, 54 belong to the *Repleta* species group, 2 to the *Coffeata* group, 2 to the *Immigrans* group and 2 to an unknown species group. The *Drosophila* species found were *D. hydei* (35 samples), *D. mercatorum* (7 samples), *D*. aff. *repleta* 1 (2 samples), *D*. aff. *repleta* 2 (4 samples), D. ananassae (2 sample), *D. fuscolineata* (2 samples), *D. immigrans* (2 sample), *D*. aff. *bifurca* (1 sample) *D. nigrohydei* (1 sample), and *D*. spp. (4 different species, a sample each). *Drosophila hydei* was collected on four out of the five species of *Specklinia*, whilst the *D*. aff. *repleta* was found on 3 out of 5. *Drosophila fuscolineata*, *D. immigrans* and *D. mercatorum* were found on two out of the five species of *Specklinia*. All other *Drosophila* species were collected only on one species of *Specklinia*, and the single specimens of *Hydrotaea* (Muscidae) and Lauxaniidae were collected on *S. spectabilis*. Among the flies caught we identified 36 males and 24 females (Table 15).

Floral volatiles and droplets:—A mix of ethyl tiglate, methyl tiglate and isopropyl tiglate was analysed and used as a standard. The signal for the three standards was found back at 3.11 min, 2.48 min and 3.45 min respectively (Fig. 77a). The analysis of individual flowers shows a greater variety of signals, most of which have not been identified. Nonetheless, it is safe to say that ethyl tiglate, methyl tiglate and isopropyl tiglate can be found in both *Specklinia pfavii* (*JBL-11086*) and *S. spectabilis* (*Bogarín 7401*) as strong signals can be found extremely close to the standard times (Fig. 77b & d). In the samples of *S. remotiflora* (*Karremans 4846*) only signals similar to those of the standards of ethyl tiglate and methyl tiglate, and not isopropyl tiglate were found back (Fig. 77c).

The solution of drops collected on the adaxial surface of the sepals turned bright orange with the addition of the Fehling's reagents, evidencing the high sugar content of the drops.

Microstructures:—*SEM*. Lip - The adaxial surface of the lip is completely covered with scale-like epidermal cells. The scales are rounded and flattened towards the apex of the lip, whereas towards the base they are sharply angled and uplifted. The cuticle is somewhat rugose, however without pores or signs of ruptures of any kind. The basal scales are filamentous, and those filaments are capitate (Fig. 78a & b). Petals - Both surfaces of the petals are warty, especially near the apex. The cuticle is smooth, not ornamented, and without pores or signs of ruptures of any kind.

Table 14. Diptera specimens caught on the flowers of the *Specklinia endotrachys* species complex.

Specimen	Sex	Genus	Species	Subgenus	Orchid Species	Origin	BOLD
14003-34	Male	*Drosophila*	*ananassae*	unknown	*S. spectabilis*	JBL	ORCPL050-14
13026-17	Female	*Drosophila*	*ananassae*	unknown	*S. remotiflora*	JBL	ORCPL017-14
14003-14	Male	*Drosophila*	*bifurca aff.*	*repleta*	*S. sp.*	Private	ORCPL031-14
13026-19	Female	*Drosophila*	*fuscolineata*	*coffeata*	*S. pfavii*	JBL	-
13026-10	Male	*Drosophila*	*fuscolineata*	*coffeata*	*S. remotiflora*	Field	ORCPL010-14
13026-01	Female	*Drosophila*	*hydei*	*repleta*	*S. endotrachys*	JBL	ORCPL001-14
14003-17	Male	*Drosophila*	*hydei*	*repleta*	*S. endotrachys*	JBL	ORCPL034-14
14003-18	Male	*Drosophila*	*hydei*	*repleta*	*S. endotrachys*	JBL	ORCPL035-14
14003-26	Male	*Drosophila*	*hydei*	*repleta*	*S. endotrachys*	JBL	ORCPL043-14
14003-35	Male	*Drosophila*	*hydei*	*repleta*	*S. endotrachys*	JBL	ORCPL051-14
13026-11	Male	*Drosophila*	*hydei*	*repleta*	*S. pfavii*	JBL	ORCPL011-14
13026-18	Female	*Drosophila*	*hydei*	*repleta*	*S. pfavii*	JBL	-
14003-07	Female	*Drosophila*	*hydei*	*repleta*	*S. pfavii*	JBL	ORCPL024-14
14003-10	Male	*Drosophila*	*hydei*	*repleta*	*S. pfavii*	JBL	ORCPL027-14
14003-11	*?*	*Drosophila*	*hydei*	*repleta*	*S. pfavii*	JBL	ORCPL028-14
14003-12	Male	*Drosophila*	*hydei*	*repleta*	*S. pfavii*	JBL	ORCPL029-14
14003-13	Male	*Drosophila*	*hydei*	*repleta*	*S. pfavii*	JBL	ORCPL030-14
14003-16	Male	*Drosophila*	*hydei*	*repleta*	*S. pfavii*	JBL	ORCPL033-14
14003-19	Male	*Drosophila*	*hydei*	*repleta*	*S. pfavii*	JBL	ORCPL036-14
14003-20	Male	*Drosophila*	*hydei*	*repleta*	*S. pfavii*	JBL	ORCPL037-14
14003-21	Male	*Drosophila*	*hydei*	*repleta*	*S. pfavii*	JBL	ORCPL038-14
14003-22	Male	*Drosophila*	*hydei*	*repleta*	*S. pfavii*	JBL	ORCPL039-14
14003-23	Male	*Drosophila*	*hydei*	*repleta*	*S. pfavii*	JBL	ORCPL040-14
13026-12	Female	*Drosophila*	*hydei*	*repleta*	*S. remotiflora*	JBL	ORCPL012-14
13026-14	Male	*Drosophila*	*hydei*	*repleta*	*S. remotiflora*	JBL	ORCPL014-14
13026-20	Male	*Drosophila*	*hydei*	*repleta*	*S. remotiflora*	JBL	-
13026-22	Female	*Drosophila*	*hydei*	*repleta*	*S. remotiflora*	JBL	-
13026-23	Male	*Drosophila*	*hydei*	*repleta*	*S. remotiflora*	JBL	-
13026-24	Female	*Drosophila*	*hydei*	*repleta*	*S. remotiflora*	JBL	-
-	Male	*Drosophila*	*hydei*	*repleta*	*S. remotiflora*	JBL	-
13026-03	Male	*Drosophila*	*hydei*	*repleta*	*S. spectabilis*	JBL	ORCPL003-14
13026-25	Male	*Drosophila*	*hydei*	*repleta*	*S. spectabilis*	JBL	-
13026-26	Female	*Drosophila*	*hydei*	*repleta*	*S. spectabilis*	JBL	-
13026-27	Female	*Drosophila*	*hydei*	*repleta*	*S. spectabilis*	JBL	-
13026-29	Female	*Drosophila*	*hydei*	*repleta*	*S. spectabilis*	JBL	-
13026-30	Female	*Drosophila*	*hydei*	*repleta*	*S. spectabilis*	JBL	-
-	Female	*Drosophila*	*hydei*	*repleta*	*S. spectabilis*	JBL	-
14003-09	Female	*Drosophila*	*hydei*	*repleta*	*S. spectabilis*	JBL	ORCPL026-14
14003-27	Male	*Drosophila*	*hydei*	*repleta*	*S. spectabilis*	JBL	ORCPL044-14
14003-39	Male	*Drosophila*	*hydei*	*repleta*	*S. spectabilis*	JBL	ORCPL055-14
14003-15	Female	*Drosophila*	*immigrans*	*immigrans*	*S. sp.*	Private	ORCPL032-14
13026-21	Male	*Drosophila*	*immigrans*	*immigrans*	*S. remotiflora*	JBL	-
13026-13	Male	*Drosophila*	*mercatorum*	*repleta*	*S. remotiflora*	JBL	ORCPL013-14
13026-15	Male	*Drosophila*	*mercatorum*	*repleta*	*S. remotiflora*	JBL	ORCPL015-14
13026-16	Female	*Drosophila*	*mercatorum*	*repleta*	*S. remotiflora*	JBL	ORCPL016-14
14003-25	Male	*Drosophila*	*mercatorum*	*repleta*	*S. spectabilis*	JBL	ORCPL042-14
14003-28	Male	*Drosophila*	*mercatorum*	*repleta*	*S. spectabilis*	JBL	ORCPL045-14
14003-30	Male	*Drosophila*	*mercatorum*	*repleta*	*S. spectabilis*	JBL	ORCPL047-14
14003-38	Male	*Drosophila*	*mercatorum*	*repleta*	*S. spectabilis*	JBL	ORCPL054-14
13026-08	Male	*Drosophila*	*nigrohydei*	*repleta*	*S. remotiflora*	JBL	ORCPL008-14
13026-07	Female	*Drosophila*	*repleta aff. 1*	*repleta*	*S. remotiflora*	JBL	ORCPL007-14
13026-04	Female	*Drosophila*	*repleta aff. 1*	*repleta*	*S. remotiflora*	JBL	ORCPL004-14
14003-24	Male	*Drosophila*	*repleta aff. 2*	*repleta*	*S. sp.*	JBL	ORCPL041-14
13026-09	Female	*Drosophila*	*repleta aff. 2*	*repleta*	*S. remotiflora*	Field	ORCPL009-14
14003-08	*?*	*Drosophila*	*repleta aff. 2*	*repleta*	*S. spectabilis*	JBL	ORCPL025-14
14003-31	Male	*Drosophila*	*repleta aff. 2*	*repleta*	*S. spectabilis*	JBL	ORCPL048-14
13026-02	Female	*Drosophila*	*sp. 1*	*repleta*	*S. remotiflora*	JBL	ORCPL002-14
13026-05	Male	*Drosophila*	*sp. 2*	*repleta*	*S. remotiflora*	JBL	ORCPL005-14
13026-06	Female	*Drosophila*	*sp. 3*	*repleta*	*S. remotiflora*	JBL	ORCPL006-14
14003-33	Female	*Drosophila*	*sp. 4*	*repleta*	*S. spectabilis*	JBL	ORCPL049-14
13026-28	Female	Lauxaniidae	*unknown*	unknown	*S. spectabilis*	JBL	-
14003-29	Female	Hydrotaea	*unknown*	unknown	*S. spectabilis*	JBL	ORCPL046-14

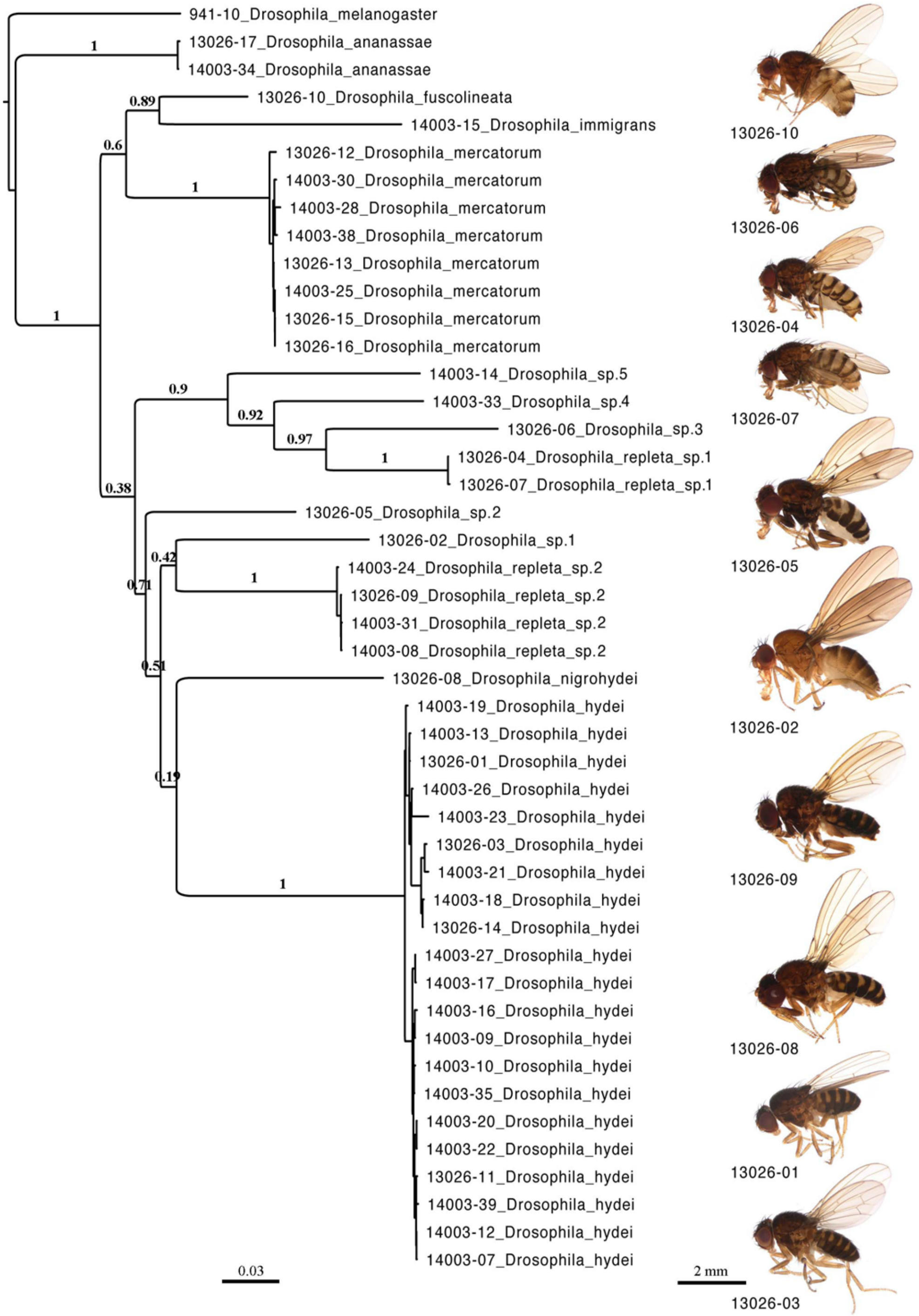


FIGURE 76. Phylogenetic relationship amongst the collected fly specimens. The trees were produced with an analysis of the COI dataset using BEAST v1.6.0. Parameters were set to preset, except for substitution model GTR with 10 categories, clock model uncorrelated exponential, tree prior Yule process, and number of generations 20,000,000. The resulting trees were combined using TreeAnnotator v1.6.0., were the first 3000 trees were used as burn-in. Node values are posterior probabilities. Edited by APK using using FigTree v.1.3.1. Photographs by KB.

Table 15. Diptera species caught summarised per orchids species and sex.

		Orchid Species					
		S. endotrachys	*S. pfavii*	*S. remotiflora*	*S. spectabilis*	*S. sp.*	**Total**
Fly species	*D. ananassae*			1Female	1 Male		1 Male 1 Female
	D. bifurca aff.					1 Male	1 Male
	D. fuscolineata		1 Female	1 Male			1 Male 1 Female
	D. immigrans			1 Male		1 Female	1 Male 1 Female
	D. hydei	4 Male 1 Female	1 + 10 Male 2 Female	4 Male 3Female	4 Male 6 Female		1 + 22 Male 12 Female
	D. mercatorum			2Male 1 Female	4 Male		6 Male 1 Female
	D. nigrohydei			1 Male			1 Male
	D. repleta aff. 1			2 Female			2 Female
	D. repleta aff. 2			1 Female	1 + 1 Male	1 Male	1 + 2 Male 1 Female
	D. sp. 1			1 Female			1 Female
	D. sp. 2			1 Male			1 Male
	D. sp. 3			1Female			1 Female
	D. sp. 4				1Female		1 Female
	unknown				2Female		2 Female

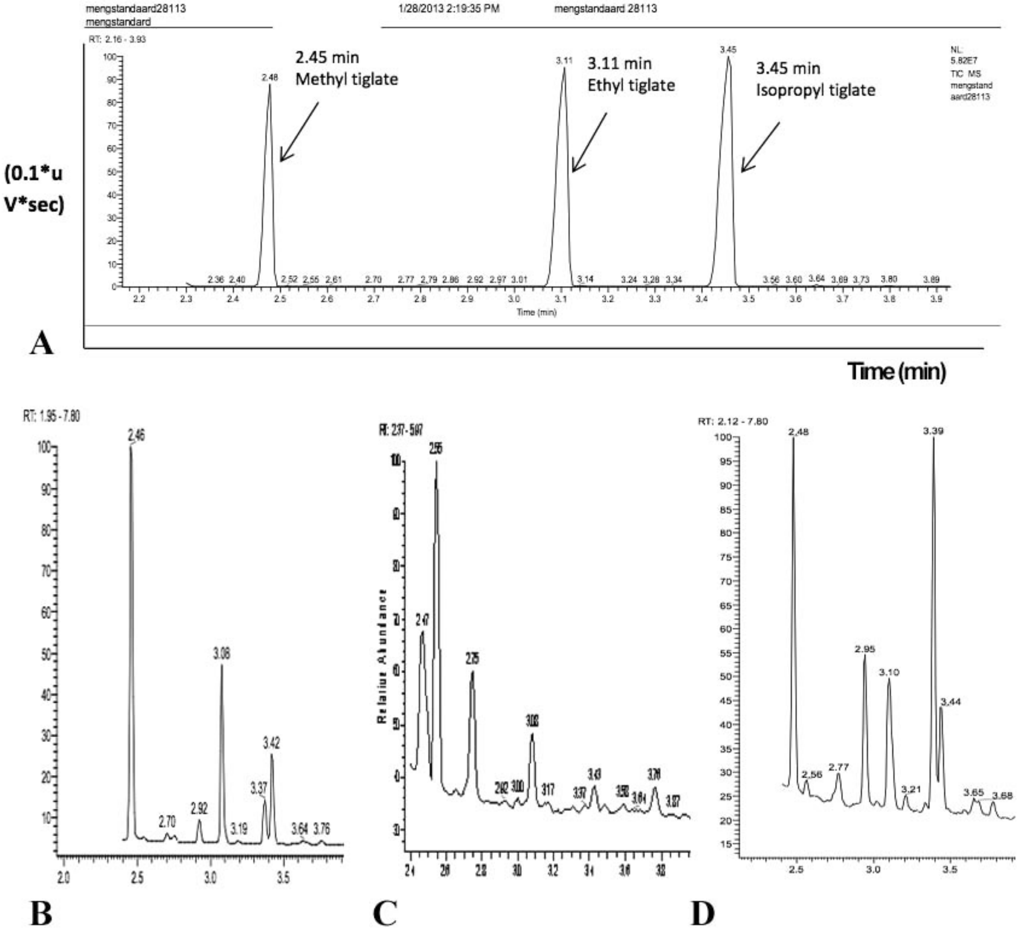


Figure 77. Standard mix of ethyl tiglate, methyl tiglate and isopropyl tiglate measured with GCMS-ITD. A. The graph shows the selected ion monitoring (SIM) signal over time. B. *Specklinia pfavii* (*JBL-11086*) C. *Specklinia remotiflora* (*Karremans 4846*). D. *Specklinia spectabilis* (*Bogarín 7410*). X axis = Time (min); Y axis = Signal (0.1*uV*sec). Figures by Misli Kaya.

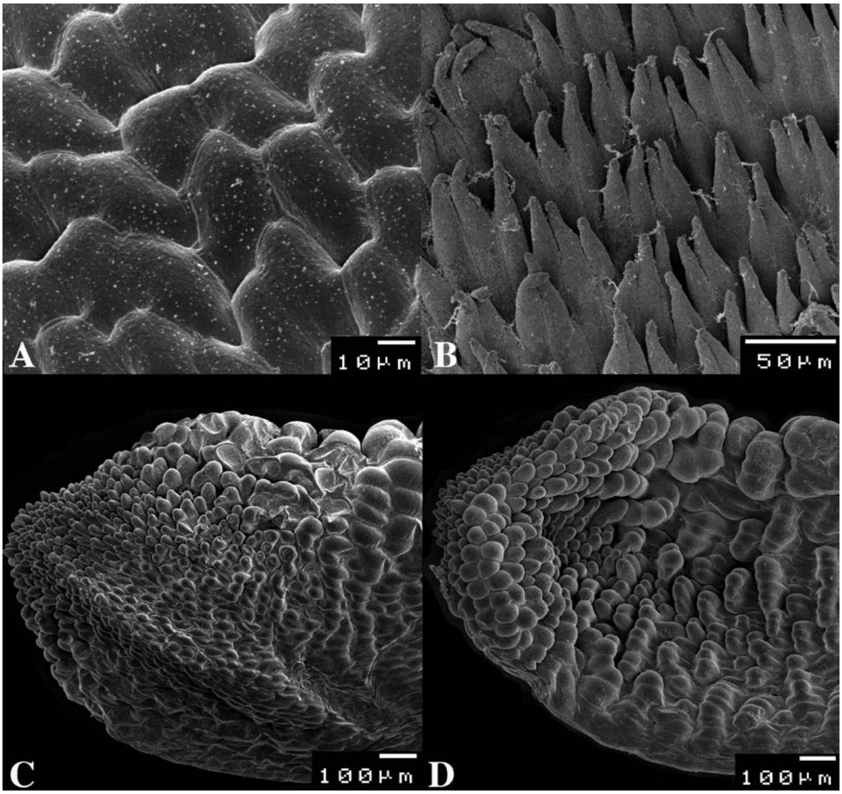


Figure 78. Micrographs of *Specklinia pfavii (JBL-11086)*: Scales cover the adaxial surface of the lip; they are flattened and rounded near the apex (A) and elevated and filamentous-capitate closer to the base (B); warts cover the outer (C) and inner (D) surfaces of the petals, especially apically. Photographs by APK.

(Fig. 78c & d). Sepals - The adaxial epidermis of all three sepals is densely rugose and covered with warts, except basally. The apex of each wart carries stomata. The stomata have wide pores and 5-6 somewhat inflated subsidiary cells. The cuticle is somewhat sculptured, not ornamented, and without pores or signs of ruptures of any kind. The stomata were permanently open and no movements were observed (Fig. 79a-d). The abaxial epidermis is smooth and mostly constantly flat, except for rare depressed areas were a sunken trichome is located; this trichome is apically irregular (Fig. 79e & f).

LM. The transversal section of the lip of *S. remotiflora* shows mostly large, rounded parenchyma cells and smaller scaly or pyriform epidermis cells on the adaxial surface (Fig. 80a & b). The petals of *S. pfavii* (Fig. 80c) and S. remotiflora (Fig. 80d) show irregularly, enlarged secretory parenchyma cells, though without apparent openings. The transversal section of the sepal shows two basic cells types, ground parenchyma near the abaxial surface (underpart in fig. 80e), which are larger and sub-rectangular, and secretory parenchyma close to and in the adaxial epidermis (upper part in fig. 80e), which are smaller and rounded. The vascular bundles are visible. The adaxial epidermis is irregular and frequently has stomata, which can be seen in both *S. pfavii* (Fig. 80f) and S. remotiflora (Fig. 80g) as prominent protrusions with an apical opening. The abaxial epidermis in turn is inornate except for the occasional sunken trichomes that can be spotted perforating the surface (Fig. 80h).

TEM. The pores of the stomata are commonly spotted in the transversal section of the adaxial epidermis of the sepals of *S. pfavii*; the subtending guard cells can be distinguished from the subsidiary cells basically by a thicker cell wall. Nevertheless their cytoplasms share the presence of mainly a nucleus with nucleolus, large vacuoles, and a high starch content, with the cells surrounding the subsidiary cells (Fig. 81).

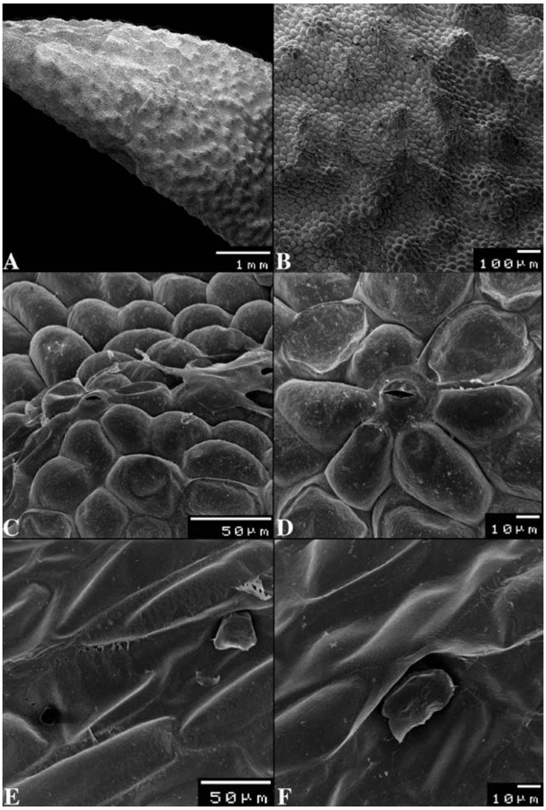


Figure 79. Micrographs of the sepals of *Specklinia pfavii* (*JBL-11086*): A median segment of a lateral sepal showing corrugation of the adaxial surface (A); the elevated cells form papillae, corrugating the adaxial surface (B); the apices of the papillae are formed by nectar secreting actinocytic stomata (C), formed by guard cells and six subsidiary cells (D); the abaxial surface is formed by flattened cells, with occasional depressions that contain a sunken trichome (E & F). Photographs by APK.

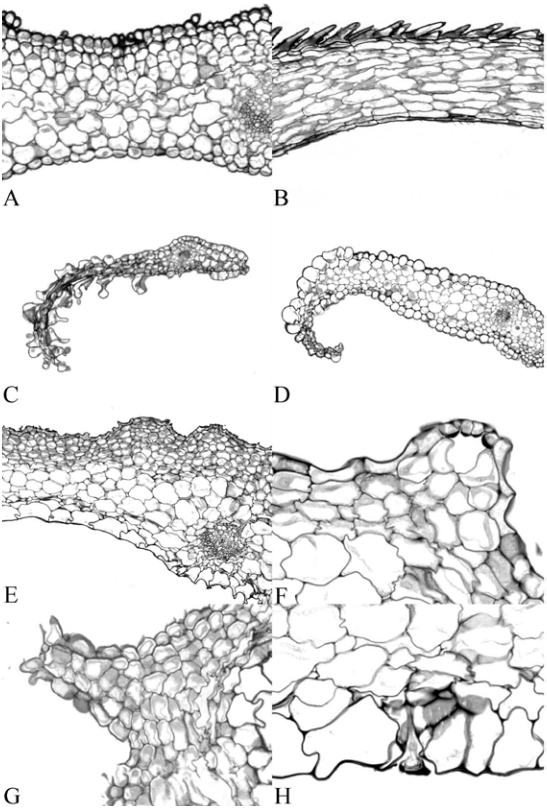
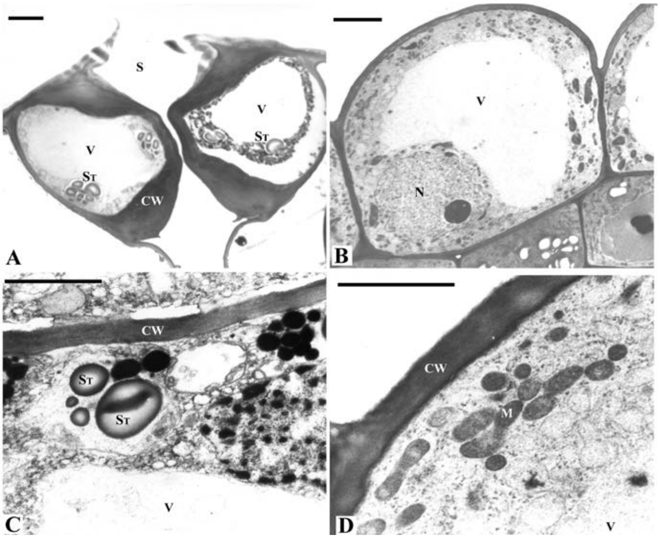


Figure 80. Light Micrographs of *S. pfavii* - *AK4835* (C, E, F, H) and *S. remotiflora* - *AK4798* (A, B, D, G): Transversal section of the lip, showing vascular bundles and keels (A); transversal section of one of the keels, showing the scale-like cells on the adaxial surface (B); large, irregular cells on the transversal section of the petal (C & D); vascular bundles and stomata visible on transversal section of the sepal (E); secretory stomata on the adaxial epidermis (F & G); sunken trichomes on the abaxial epidermis (H). Taken at 200×, 100×, 50×, 100×, 100×, 400×, 200×, 400× respectively.



Left, figure 81. Transmission Electron Micrographs (TEM) of the transversal section of the adaxial epidermis of the sepals of *S. pfavii*, showing the stomata's opened guard cells (A), their subsidiary cells (B), showing the common starch grains (C) and variously sized vesicles (E). Scale bars: A & B = 2 µm, C & D = 1 µm. CW, Cell Wall; M, Mitochondrion; N, Nucleus; S, Stomata; St, Starch; V, vacuole. Photographs by Rob Langelaan.

Electroantennography study:—*Drosophila hydei* is highly sensitive to the stimuli ethyl tiglate, methyl tiglate and isopropyl tiglate and clear dose response relations were found (Fig. 82) The highest response measured was -6.563 mV for the positive control Z-3-Hexen-1-ol (100 % by definition), while the highest values for isopropyl, ethyl and methyl tiglate were -4.462 mV, -4.361 mV and -3.328 mV, respectively. To investigate the effects of concentration, a generalised linear mixed model was used that contained in addition to the random factor 'individual' both the explanatory variables compound and concentration, as well as their interaction (Table 16a). The coefficients for the effects of these factors (Table 16b) showed a highly significant concentration effect, as expected for biological relevant stimuli. Ethyl tiglate (the reference in the linear model) gave a significantly stronger response than methyl tiglate (indicated by the negative coefficient for methyl tiglate), but does not show an interaction, i.e. the slope of the dose response curve was similar for both compounds. In contrast isopropyl tiglate did show an interaction, and the slope of the dose response curve was significantly less steep than that for ethyl tiglate (Fig. 82).

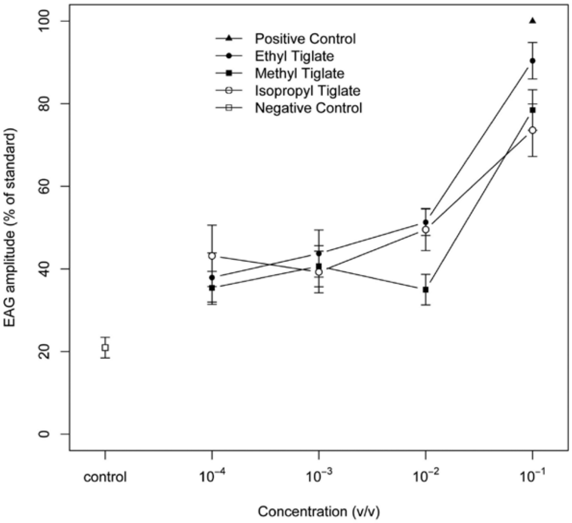


FIGURE 82. Dose response relationships for the electro antennogram measurements in 14 *Drosophila hydei* flies reacting to ethyl tiglate, methyl tiglate and isopropyl tiglate. All amplitudes are expressed as percentage of the external standard (positive control) Z-3-Hexen-1-ol.

TABLE 16a. Overview of the tested mixed models ordered by their corrected Aikake information criterium (AICc) values. The best model (top line) contained both concentration and compound as explanatory variables and their interaction.

Model structure	*K*	*AICc*	*Delta_AICc*	*AICcWt*	*Cum.Wt*	*LL*
stimulus * concentration	8	1382.23	0	0.62	0.62	-682.64
stimulus + concentration	6	1383.49	1.26	0.33	0.95	-685.47
concentration	4	1387.36	5.13	0.05	1	-689.55
Null model	3	1503.01	120.78	0	1	-748.43
stimulus + insect	5	1503.59	121.36	0	1	-746.6

TABLE 16b. Summary of the coefficients in the best model from table 3a. The intercept gives the estimate of the coefficient for ethyl tiglate, the other estimates are indicating the changes in relation to this reference. A highly significant contribution of stimulus concentration was found. Except concentration, all of the coefficients are negative, indicating that ethyl tiglate stimulated the flies significantly better than methyl tiglate The negative coefficient for the interaction of isopropyl tiglate with concentration indicates that the response differed from ethyl tiglate in a concentration dependent way (see also Fig. 82).

Coefficient	*Estimate*	*se*	*Wald z*	*P*	*Sig.*
Intercept	42.55	3.7627	11.3082	0.0000	***
methyl tiglate	-7.2331	3.4729	-2.0828	0.0373	*
Concentration	47.4570	4.9990	9.4932	0.0000	***
isopropyl tiglate : Concentration	-16.5305	7.0646	-2.3399	0.0193	*
methyl tiglate : Concentration	-5.0388	7.0597	-0.7137	0.4754	
Variance (1|insect)	112.48				
Variance (|Residual)	227.31				

Discussion

In Pupulin *et al.* (2012, Chapter 1), we established that under the name *Specklinia endotrachys* at least four similar, yet distinct, recognisable species should be treated. Our findings show that the pollination syndrome of *S. endotrachys*, *S. pfavii*, *S. spectabilis* and *S. remotiflora* is basically the same one. Both male and female flies are arrested by pheromones liberated from the flower sepals. Once on the abaxial surface the pollinators "walk" from sepal to sepal "sucking" on the warty surface of the sepals, where nectar drops have formed on the apex of the stomatal pore. The flies can be seen in groups and spend up to +24 hours, reducing their overall motility, but continuously feeding on the flowers. They display a variety of behaviours including fencing with the forelegs, flapping their wings, following other flies, bending their abdomen, and occasionally copulating. Whilst wandering from sepal to sepal the flies explore the column/lip cavity. When placed in the right position, the fly makes the movable lip tilt and is then adpressed against the viscid rostellum. The pollinia are removed whilst the fly attempts to escape from the cavity.

Biology of the *Specklinia* species studied:—Species of the *S. endotrachys* complex are found in large colonies of dozens of plants. They produce long-lived multi-flowered successive inflorescences, with up to +20 flowers over time. Each plant may be flowering for several months at a time. Overall frutification was found to be low, both in the field and in the greenhouse, making it likely that large colonies and long term flowering are necessary to attain fruitset. *Specklinia endotrachys*, *S. pfavii*, *S. remotiflora* and *S. spectabilis* have all been found growing in Costa Rica, nevertheless never sympatrically (Pupulin *et al*. 2012, Chapter 1; Fig. 83). *Specklinia endotrachys* is a mid-elevation species found only in the north of the country, *S. pfavii* and *S. spectabilis* are lowland species, growing on the pacific and caribbean watersheds respectively, of the Central and Talamanca mountain ranges, whilst *S. remotiflora* is only found in the highland cloud forests close to the continental divide in the south of the Talamanca mountain range. The +2000 m high mountain range serves as a barrier separating the populations of the four species. Allopatry facilitates divergence by both interrupting gene flow and allowing local adaptation without the necessity of high floral divergence or for that matter pollinator shifts (Harder & Johnson 2009).

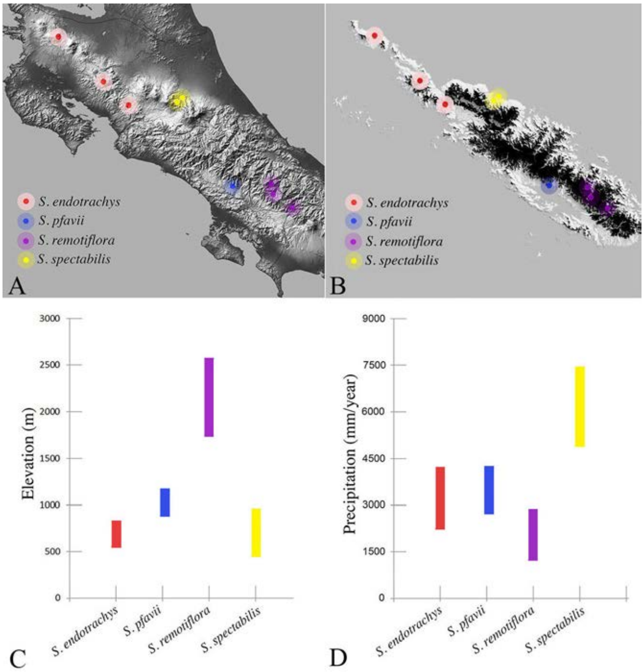


Figure 83. Distribution and ecological preferences of the *Specklinia endotrachys* group in Costa Rica. A. Actual known distribution. B. Distribution with elevations below 400 m converted to sea and elevations above 1500 m blackened. C. Distribution of elevation of found specimens. D. Distribution of precipitation in the areas where the specimens have been found.

Reddish-orange coloured flowers are characteristic of species of the *S. endotrachys* group and close relatives. Although not unique within the Orchidaceae, those colour patterns are uncommon in the family and are notoriously rare in subtribe Pleurothallidinae. Oliveira *et al.* (2012) found that species of the *Drosophila repleta* group, including *D. hydei* and *D. mercatorum*, predominantly use *Opuntia* fruits, which are commonly reddish-orange in colour, for feeding and breeding. It is likely that there is selective pressure on these *Specklinia* species to have and maintain similar colour patterns.

Nectar drops accumulate on the adaxial surface of the sepals of all species of the *S. endotrachys* complex (Fig 73 & 74d). The drops have a pasty consistency, high sugar content, and are persistent unless removed. Practically the entire surface of the sepals is covered with actinocytic stomata which are found elevated on the apex of each one of those warts as can be appreciated in the SEM photographs (Fig. 79). The transversal sections of those stomata, taken with the LM (Fig. 80) and TEM (Fig. 81), evidence high cellular activity in the stomatal guard and subsidiary cells. Starch grains, which are likely to be used as energy source for the production of nectar, are commonly observed. No clear drops nor evidence of nectar secreting stomata are found on the petals and lip, however they are found entirely covered by papillae. Those papillae are morphologically similar to secretory papillae found by Stpiczyńska and Matusiewicz (2001) in the nectary of *Gymnadenia*, and de Melo *et al.* (2010) on the lip of *Acianthera*. The high cellular activity in addition to the presence of nectar residues on the rugose surfaces (Fig. 8 & 9) might well be indicatory of secretory papillae of the lip and petals here as well but this needs further studies.

Using GC-MS we have been able to determine that ethyl tiglate, methyl tiglate and isopropyl tiglate, all of which have been cited as aggregation pheromones for *Drosophila hydei* (Moats *et al.* 1987), are being produced by the flowers of the *Specklinia endotrachys* complex (Fig. 77). The aggregation pheromones, albeit not the only substances produced by the flowers, are likely being released from the sepals, which have been cited to produce and release volatiles (Antoń *et al.* 2012; Kowalkowska *et al.* 2014).

Biology of the *Drosophila* species studied:—Aggregative behaviour in *Drosophila* is mediated by pheromones that can act in concert with odours of the habitat of the flies and indicate a suitable habitat for mating and oviposition (Moats *et al.* 1987; Markow & O'Grady 2005). The pheromones are produced by males and attract flies of both sexes (Bartelt *et al.* 1985, 1986, 1988), as also found here (Table 14 & 15). Aggregation pheromones of *Drosophila* are generally volatile esters, ketones or unsaturated hydrocarbons (Bartelt *et al.* 1985; Hedlund *et al.* 1996). Using EAG experiments we confirmed that *D. hydei* is sensitive to ethyl tiglate, methyl tiglate and isopropyl tiglate, and responds to concentrations as low as $1.0{\times}10^{-5}$ of the pure substance (Fig. 82; Table 16a, b). The measured concentration of the tiglates in the flowers was about 1µg/L.

Once on the flower the flies wander around feeding on the nectar drops accumulated on the sepals, and displaying courtship behaviours. Following the female, orienting towards her, tapping her with his forelegs, contacting her genitalia with his mouthparts, singing a species-specific courtship song, and bending his abdomen, are commonly cited as courtship behaviour for several *Drosophila* species (Greenspan & Ferveur 2000; Villella & Hall 2008). In *Drosophila subobscura*, nuptial gifts in the sense of males gifting their crop contents in the form of a regurgitated drop have been suggested to play an important role in sexual selection (Steele 1986; Immonen *et al*. 2009). Copulation, albeit rare, was also observed on the *Specklinia* flowers. No oviposition events nor eggs or larvae were ever found. Markow and O'Grady (2005) point out that for any given species mating takes place at particular locations and at specific times of the year and/or day. Markow (1988) found that *Drosophila* species exhibit distinct behaviour patterns on different pieces of fruits. In that study the author found that males of *D. melanogaster* court females on the feeding site (decaying fruit), while females of *D. nigrospiracula* would fly to non-resource-based male territories where the majority of copulations occur; oviposition was found to occur on newly exposed flesh and not elsewhere (Markow 1988).

About 85% of the caught specimens, including samples of both *D. hydei* and *D. repleta*, belong to the *Drosophila repleta* species group. Males of the *Repleta* group have a tendency to court behind the females, suggesting that male visual displays are not the primary form of sexual signalling as in other taxa, which is consistent with having almost no sexual dimorphism in coloration, wing pattern and other morphological traits (Markow & O'Grady, 2005). Adults of most species will feed on a range of food sources, however, ovipositions and larval development are typically more restricted (Carson 1974). The *Repleta* group includes many cosmopolitan species with a neartic and neotropical distribution, which reportedly use both fruits and cacti as breeding sites (Markow 1988; Markow & O'Grady 2005; Markow & O'Grady 2008; Oliveira *et al*. 2012). A particular species of *Drosophila* may feed and breed exclusively in a resource such as flowers (Brncic 1983; Markow & O'Grady 2008). However, together with the lack of observed oviposition events, absence of eggs and larvae, and the short lived flowers, it is safe to say that the flowers of *Specklinia* are a feeding site but not a breeding site for these flies.

Conclusions

We find that *Specklinia endotrachys*, *S. pfavii*, *S. spectabilis* and *S. remotiflora* share not only the same basic pollination syndrome but are also pollinated by the same species of the *Drosophila repleta* group of flies. Species of several unrelated genera of Pleurothallidinae, including *Acianthera*, *Dracula*, *Masdevallia*, *Specklinia* and *Stelis* (*sensu* Pridgeon 2005), share a similar system in which the pollinia removal occurs when a fly is pushed against the column once it walks over the lip; whilst exiting in reverse, the pointed scutellum is smeared with a viscid substance found in the rostellum, and the pollinia are removed by touching their twisted base. In those genera the observed pollen removal is reported to be done mostly by flies of the families Chloropidae, Drosophilidae and/or Phoridae (Chase 1985; Duque 1993; Borba & Semir 2001; Albores & Sosa 2006; Endara *et al*. 2010; de Melo *et al.* 2010). It is thus essentially how the fly is guided to visit the column/lip cavity that differs between these different pleurothallid species' groups.

Pheromones are likely to play an important role in initially aggregating Diptera species to pleurothallid flowers. Blanco and Barboza (2005) supposed that species of *Lepanthes*, which are pollinated by pseudocopulation, attracted male fungus gnats using sexual pheromones. Here we have been able to confirm for the first time that aggregation pheromones are being released from the sepals of *Specklinia* species to attract pollinators. The use of pheromones, be it sexual or aggregation, might be generalised in Pleurothallidinae considering that a wide range of species have secretory structures. Scent is likely to play an important role in specific pollinator attraction thus mediating reproductive isolation (Peakall *et al.* 2010).

Nectar guides are also commonly used by pleurothallids to guide the pollinators to the lip/column cavity. Many studies seem to report no "measurable" or "obvious" rewards, however evidence for nectar guides is frequently found in more detailed pollination studies in the pleurothallids (Borba & Semir 2001; Barbosa *et al*. 2009; de Melo *et al*. 2010; Duque-Buitrago *et al*. 2014). Smith (2010) found that the appearance of nectary glands lead to an increase in reproduction success. Pollination efficiency was found to be significantly lower in food deceptive orchids as compared to rewarding species (Tremblay *et al*. 2005; Scopece *et al.* 2010), and several authors have suggested that deceitful species must be much less frequent than rewarding ones otherwise the evolution of lack of reward is difficult to explain (Darwin 1862; Smithson 2006). In fact we wonder if the cases in which orchids are being considered non-rewarding are not highly over-estimated.

General Discussion and Summaries

General Discussion

The present work brings together the results of systematic, phylogenetic and pollination studies of orchid species belonging to the genus *Specklinia*, with special emphasis on those with a Costa Rican distribution. It is organized in three distinct sections that contain manuscripts of similar topics for ease of the reader.

Contributions towards our systematic knowledge of *Specklinia*:—This section focuses on the systematics of *Specklinia* species by treating groups of closely related and badly understood species systematically.

The first group to be dealt with is that of the *Specklinia endotrachys* species complex. Traditionally considered a morphologically variable species, *S. endotrachys* is here treated as one of six distinct, albeit closely related, taxa. Four species were originally recognized in chapter 1, and two more are added in chapter 2. Of those species, *S. pfavii*, and *S. spectabilis* are described and illustrated from living material and removed from the synonymy of *S. endotrachys*, while *S. dunstervillei* and *S. remotiflora* are described as new to science.

The second group to be addressed is that of the *Specklinia glandulosa* species complex. Traditionally, and similar to the previous case, *S. glandulosa* has been considered a variable species along its broad range from Mexico to the Guiana Shield. In chapter 3 it is found to be one of at least six different, morphologically and ecologically similar, but well distinguished, species. Of those species, *S. pertenuis* and *S. vittariifolia*, are reconsidered and removed from the synonymy of *S. glandulosa*, while *S. alajuelensis* and *S. gersonii* are described as new to science. *Specklinia chontalensis* is described and illustrated from Costa Rican material.

In chapters 4 and 5 two single new species of *Specklinia* are described. In contrast with the species discussed in the previous chapters, these have not been confused with other similar species. In chapter 4 the very unique (even absurd) *Specklinia absurda* is described. It was discovered recently by the authors in the seldomly explored mountains of the Costa Rican south-Pacific. In chapter 5 the *Specklinia lugduno-batavae* is described. This tiny, creeping plant from the Caribbean lowlands of Costa Rica apparently had escaped previous collectors. The name honors Leiden University and the Hortus botanicus Leiden.

After the publication of these papers disentangling the mentioned species' complexes it became obvious that the species treated here are quite different. At the time we wondered why they were considered a variable, yet single, species for so long? The answer now seems to be straightforward, when similarities appear much larger than individual differences, we tend to overlook those differences. With little material at hand we find ourselves unable to adequately assess intra- and inter-specific variation. With more material for study it is possible to finally understand that even though our species' have certain similarities that set them aside from other species, they also have differences from each other. It is important to use all the evidence possible, linking morphology, ecology and molecular data, in order to avoid seeing things that are not there. It is important to point out that if we stare too long at copies of the same thing we will eventually find differences between them.

Phylogenetic reassessment of *Specklinia* and its allied genera:—After the publication of the first molecular phylogeny of the Pleurothallidinae many species were transferred to genus *Specklinia*. In the last decade different authors have assigned 40 to 420 species to the genus. In chapters 6 and 7 we re-circumscribe the genus in order not only to establish how many and which species belong to it, but also how they can be recognized both morphologically and ecologically.

We find that about 95 species belong to *Specklinia* in the broad sense, and that this includes the type species of the genera *Acostaea, Areldia, Cucumeria, Empusella, Gerardoa, Pseudoctomeria, Sarcinula, Sylphia, Tribulago* and *Tridelta*, which are therefore here considered synonyms of the first. Alternatively, *Specklinia* would be reduced to just a few species and quite a few additional generic names would require recognition. Taking into consideration that *Specklinia* in a broad sense is monophyletic with the removal of *Muscarella*, that it is manageable in size, and that it can also be easily recognized morphologically, it seems unnecessary to recognize those splinter generic concepts. *Specklinia* has a north-Andes to south-Central America speciation pattern. On one hand, even though the genus has an overall wide distribution, ranging from Mexico to Bolivia and Brazil, through Central America and the

Antilles, the highest species and clade diversity is found south-Central America. On the other hand, its sister taxa *Platystele*, *Teagueia* and *Scaphosepalum*, as well as their sister *Muscarella*, are most diverse in the north-Andes. The relatively young age of both geographical locations indicates that the diversification of the entire clade must have happened quite recently. Ongoing molecular clock studies are currently providing more insight in this.

Incaea, a monospecific genus that was previously unplaced, is here found embedded within *Dryadella* and synonymized. The also unplaced and monospecific *Rubellia* is found sister to *Platystele* and reduced as a synonym. *Teagueia*, which is morphologically similar to *Platystele* and from which it was segregated recently, was unexpectedly found sister to *Scaphosepalum* instead. The phylogenetic position of a group of species with "*Specklinia*-like habit and *Anathallis*-like flowers", and which have been going back and forth between both genera is finally resolved. They are segregated into the new generic concept proposed here, *Lankesteriana*. This name honors Lankester Botanical Garden and its scientific journal.

The superficial morphological dissimilarities among species of *Specklinia* led to a proliferation of generic concepts, proposing the segregation of several small species groups from the genus. Nevertheless, we have been able to show that *Specklinia* as here defined is monophyletic and can be recognized by several morphological characters that had not been considered before. Some of the taxonomic decisions presented in both chapters are likely not to be accepted unanimously, and indeed these are nothing but proposals based on the data available and our best interpretative capabilities. They are not sculptured in stone, and might eventually be trumped by newer techniques and evidence. In the meantime, without contradicting data a critique is nothing more than an opinion.

Assessing the pollination mechanisms of *Specklinia*:—The first documented observation of pollination in Pleurothallidinae was that of Augustus Endrés who noticed that the "viscid sepals" of *Specklinia endotrachys* were visited by a "small fly". Mark Chase would later identify the visiting flies as being of the genus *Drosophila*. In chapter 8 we document and describe how species of the *Specklinia endotrachys* complex are pollinated by different *Drosophila* species.

We found that *Specklinia endotrachys*, *S. pfavii*, *S. spectabilis* and *S. remotiflora* are pollinated by up to 13 different species of the *Drosophila*, especially of the *D. repleta* group. Several unrelated genera of Pleurothallidinae, including *Acianthera*, *Dracula*, *Masdevallia*, *Specklinia* and *Stelis* (in a broad sense), have species that share a similar system in which pollinia removal occurs when a fly is pushed against the column once it walks over the lip; whilst exiting in reverse, the pointed scutellum is smeared with a viscid substance found in the rostellum, and the pollinia are removed by touching their twisted base. In those genera the observed pollen removal is reported to occur mostly by flies of the families Chloropidae, Drosophilidae and/or Phoridae. It is thus essentially how the fly is guided to visit the column/lip cavity that differs between these different pleurothallid species' groups.

We showed that species of the *S. endotrachys* complex arrest the flies using aggregation pheromones, including ethyl tiglate, methyl tiglate and isopropyl tiglate. Pheromones are likely to play an important role in initially aggregating Diptera species on pleurothallid flowers. Here we have been able to confirm for the first time that aggregation pheromones are being released from the sepals of *Specklinia* species to attract pollinators. The pollinators showed frequent courtship behaviors even though they rarely copulated on the flowers. The use of pheromones, be it sexual or aggregation, might be generalized in Pleurothallidinae considering that a wide range of species have secretory structures. Scent is likely to play an important role in specific pollinator attraction thus mediating reproductive isolation, and nothing is more convincing than pheromones.

Visible nectar drops on the adaxial surface of sepals of these *Specklinia* species are secreted from nectar secreting stomata. These encourage the *Drosophila*, both males and females, to linger on the flowers for several hours at a time. Nectar guides are also commonly used by pleurothallids to guide the pollinators to the lip/column cavity. Many studies seem to report no "measurable" or "obvious" rewards, however evidence for nectar guides is frequently found in more detailed pollination studies in the pleurothallids. The appearance of nectary glands has been found to lead to an increase in reproduction success. Pollination efficiency was found to be significantly lower in food deceptive orchids as compared to rewarding species, and several authors have suggested that deceitful species must occur much less frequent than rewarding ones otherwise the evolution of lack of reward is difficult to explain. In fact we wonder if the cases in which orchids are being considered non-rewarding are not highly over-estimated, as absence of evidence is not evidence of absence.

A look into the future:—There are most likely more than 7000 species in subtribe Pleurothallidinae, several hundreds patiently awaiting discovery. The vastness of the subtribe allows for endless research opportunities, but it is also a tremendous challenge. Entire lineages were completely unknown until only a few years ago, and many are probably still unknown today. The alpha-taxonomy, the discipline of detecting, describing and classifying species, is still badly needed to set a strong base for evolutionary interpretations within the pleurothallids. Nevertheless, the future looks bright. The knowledge on species belonging to the group has had an exponential growth since the publication of the first modern monograph of Pleurothallidinae three decades ago. Luckily even though scientist are ephemeral, scientific knowledge lingers.

Floral morphology is immensely diverse in the subtribe suggesting a plethora of different pollination syndromes employing modifications of the deceit/reward system. Judging by the numerous cases of convergence in flower morphology it is highly likely that the employment of pheromones (both sexual and aggregation) arose several times independently in the subtribe. The adaptation to the same pollinator groups (such as Drosophilidae) by several unrelated groups of Pleurothallidinae strongly suggests cases of parallel evolution. In the future, with the pollinators of more pleurothallids being documented, we will be able to apply molecular dating techniques in order to establish wether these orchids and insects have radiated synchronically, or if, which is more likely considering their relatively young age, the pleurothallids exploited the preexisting fly diversity. Advances in the genome data will help unravel how aggregation and sex pheromones evolved in the Pleurothallidinae. Full genomes of orchids are nowadays being assembled with more frequency, for example the nuclear genome of the Costa Rican *Erycina pusilla* (L.) N.H. Williams & M.W. Chase, a deceptive orchid, is being assembled in a few labs around the world, including Leiden. Genome data will also allow to identify genes or groups of genes with accelerated evolutionary rates, which is key in understanding how so many Pleurothallidinae species could radiate in such a short evolutionary time.

The high diversity in the pleurothallids may also have potential practical uses. The flowers of these orchids are specialized in the attraction of a diversity of flies, many of which are serious agricultural plagues. As shown here, *Specklinia* species aggregate a diversity of *Drosophila* species employing aggregation pheromones. Some species of *Drosophila*, such as *D. suzukii*, are well-known greenhouse pests and it is likely that they too can be aggregated with certain pheromones. A pleurothallid-based pheromone dispenser would be an exciting and innovative alternative to the use of other chemical controls.

Samenvatting

De voorgaande pagina's beschrijven de resultaten van een reeks onderzoeken gericht op de systematiek, fylogenie en bestuiving van soorten van *Specklinia*, een genus van de Pleurothallidinae, de meest diverse groep van tropische orchideeën. *Specklinia* omvat bijna 100 soorten, verspreid over een gebied dat reikt van Mexico tot Bolivia en Brazilië, en dat eveneens Midden-Amerika en de Antillen omvat. In Costa Rica en Panama treffen we de grootste diversiteit van *Specklinia* soorten aan. De resultaten van de hier gepresenteerde onderzoeken richten zich dan ook voornamelijk op deze twee landen.

Dit proefschrift bevat acht hoofdstukken, verdeeld over drie delen, die hieronder worden samengevat.

Bijdragen aan de systematische kennis van *Specklinia*:—Het eerste deel bevat vijf systematische publicaties, waarin de kenmerkende eigenschappen van een reeks *Specklinia* soorten worden toegelicht. De eerste twee hoofdstukken richten zich op het soortcomplex *Specklinia endotrachys*, een naam die in gebruik is geweest voor tenminste zes soorten, die overduidelijk verschillend zijn, en als zodanig herkend en beschreven worden in dit proefschrift.

Het derde hoofdstuk behandelt een ander complex van soorten, in dit geval *Specklinia glandulosa*, een naam die evenzeer in gebruik is geweest voor verschillende soorten, tenminste vijf, die in dit hoofdstuk geïllustreerd en beschreven worden. Deze complexen worden hier geëvalueerd door middel van een combinatie van gegevens: de beschikbare documentatie over hun natuurlijke variatie, genetische identiteit, geografische verspreiding en ecologische voorkeuren. De combinatie van verschillende evidenties maakt de getrokken conclusies aanzienlijk robuuster.

Tegenwoordig lijkt het evident dat we met verschillend soorten te maken hebben en niet met één enkele, weliswaar zeer variabele soort. Desalniettemin is het opmerkelijk dat ze zolang als één enkele soort beschouwd werden. Een mogelijke verklaring hiervoor is dat als de gelijkenissen tussen soorten aanzienlijk significanter zijn dan de individuele verschillen, er een tendens bestaat om deze verschillen niet op waarde te schatten. Met andere woorden, deze soorten werden beschouwd als één enkele variabele soort, omdat ze erg op elkaar lijken, terwijl ze als groep zeer verschillend zijn van andere *Specklinia* soorten.

De laatste twee hoofdstukken betreffen twee nieuwe soorten, enerzijds *Specklinia absurda*, een soort met bloemen met unieke morfologie uit de bergbossen van Costa Rica en Panama; anderzijds *Specklinia lugduno-batavae*, opgedragen aan de Hortus botanicus Leiden en de Universiteit van Leiden uit de Caraïbische laaglanden van Nicaragua en Costa Rica. Beide soorten worden geïllustreerd en in detail beschreven.

Fylogenetische omschrijving van *Specklinia* en verwanten:—*Specklinia* werd beschouwd als synoniem van *Pleurothallis*, tot de identiteit van deze laatste werd erkend, op basis van de de fylogenie van Pleurothallidinae, gepubliceerd in 2001. Sindsdien hebben verschillende auteurs *Specklinia* op diverse wijzen omschreven, daarbij tussen 40 en 420 soorten erin onderbrengend. De fylogenetische onderzoeken die in de hoofdstukken zes en zeven gepresenteerd worden, evalueren op systematische en kritische wijze hoeveel en welke soorten in *Specklinia* moeten worden ondergebracht. Ons onderzoek geeft aan dat 95 soorten op dit moment kunnen worden beschouwd als onderdeel uitmakend van het genus, en dat Specklinia ook de genera *Acostaea, Areldia, Cucumeria, Empusella, Gerardoa, Pseudoctomeria, Sarcinula, Sylphia, Tribulago* en *Tridelta* omvat, die hier derhalve als synoniemen beschouwd worden. Het is bovendien duidelijk, dat het genus *Muscarella* verschillend is van *Specklinia*, en dat dit laatste een nauwe verwant is van *Platystele* en *Scaphosepalum*. Deze relatie is niet direct evident, maar in vegetatief opzicht zijn deze drie genera zeer gelijkend. Daarnaast werd de fylogenetische positie van een groep van 20 soorten geëvalueerd, die zowel in *Anathallis* als *Specklinia* geplaatst werden. Het werd duidelijk, dat ze tot geen van beide behoren. Als gevolg daarvan, en om de monofylie van beide genera te bewaren, werden ze apart geplaatst in een nieuwe genus naam *Lankesteriana*.

De oppervlakkige morfologische verschillen van de soorten die tot *Specklinia* behoren, veroorzaakten tal van voorstellen om verschillende soorten te groeperen en in andere genera onder te brengen. Desalniettemin hebben we hier kunnen aantonen dat er zowel morfologische eigenschappen zijn als geografische patronen, die eigen zijn voor alle soorten van *Specklinia*, en die derhalve gebruikt kunnen worden om de soorten van dit genus af te bakenen. Enkele taxonomische beslissingen hier genomen, zullen ongetwijfeld betwist worden, en zijn in feite niet meer dan voorstellen, gedaan op grond van de beschikbare gegevens. Het is mogelijk dat met toekomstige technieken en aanvullende aanwijzingen het voorgestelde beeld kan veranderen, maar zolang er geen gegevens zijn die onze conclusies weerspreken, is elke kritiek niet meer dan een -welkome- mening, die het wetenschappelijk proces versterkt.

Bestuiving van *Specklinia*:—Het laatste hoofdstuk van dit proefschrift behandelt de bestuiving van *Specklinia* en presenteert de gecombineerde resultaten van een reeks aan verschillende onderzoekstechnieken. We hebben kunnen vaststellen dat *Specklinia endotrachys, S. pfavii, S. spectabilis* en *S. remotiflora* bezocht en bestoven worden door tenminste 13 verschillende soorten bananenvliegjes uit het genus *Drosophila* (Diptera), in het bijzonder uit de groep *D. repleta* en verwanten. Deze orchideeën trekken zowel mannelijke als vrouwelijke vliegen aan door middel van aggregatieferomonen, die waarschijnlijk uitgestoten worden door klieren die aanwezig zijn op de onderzijde van de bloemen. Zodra de insecten landen op de bloemen beginnen ze zich te verplaatsen van de ene naar de andere kant, ondertussen kleine druppeltjes nectar opzuigend, die ook door deze orchideeën geproduceerd wordt. De nectar wordt uitgestoten door huidmondjes die zich op de bovenzijde van de bloemen bevinden. Tijdens het bewegen, lopen de vliegen bij tijd en wijle over de lip, een zeer kleine, centrale structuur die beweeglijk is. Als de vlieg zich hierop waagt in een bepaalde positie kantelt de lip en wordt het insect tegen het zuiltje gedrukt. Het kan tot meer dan 20 minuten duren alvorens de vlieg zich kan bevrijden door zich achterwaarts te bewegen. Hierbij wordt de vlieg deels bedekt met een kleverige stof afgescheiden door het viscidium en neemt daarmee pollinia mee die zich aan deze materie hechten. Dit proces moet dan herhaald worden in een andere bloem om bestuiving te bewerkstelligen. Gedurende lange tijd werd gedacht dat de Pleurothallidinae over het algemeen geen beloning aanboden aan hun bestuivers. De meerderheid van het hedendaagse onderzoek heeft echter een of andere vorm van beloning ontdekt. De bestuiving van deze groep van orchideeën lijkt dan ook niet gebaseerd te zijn op bedrog. Bij een derde van alle orchideeën wordt momenteel aangenomen dat de bestuivers bedrogen worden aangezien de beloning niet direct zichtbaar is. In het geval van *Specklinia* bleek de afwezigheid van direct bewijs echter geen bewijs voor afwezigheid van een beloning. Wellicht geldt dit voor meer orchideeën.

Resumen

Las páginas precedentes describen los resultados de una serie de investigaciones enfocadas en la sistemática, filogenética y biología reproductiva de especies del género *Specklinia*. Este género pertenece a la subtribu Pleurothallidinae, la más numerosa de las orquídeas del neotrópico. *Specklinia* incluye poco menos de 100 especies distribuidas desde México a Bolivia y Brasil, pasando por Centro América y las Antillas. La mayor diversidad de las especies del género se encuentra en Costa Rica y Panamá, y los resultados de los trabajos presentados aquí son también centrados en esos dos países.

Se presentan ocho capítulos divididos en tres secciones, que se describen a continuación.

Contribuciones al conocimiento sistemático de *Specklinia*:—La primera sección incluye cinco trabajos sistemáticos en los cuales se aclara la identidad de una serie de especies de *Specklinia*. En los primeros dos capítulos se trata el complejo *Specklinia endotrachys*, un nombre que había sido utilizado para por lo menos seis especies evidentemente diferentes, y que se reconocen y describen aquí. El tercer capítulo trata otro complejo de especies, en este caso el de *Specklinia glandulosa*, un nombre que había sido utilizado igualmente para por lo menos cinco especies distintas, todas debidamente ilustradas y descritas aquí. Estos complejos se han evaluado combinando varias técnicas de investigación: la documentación de la variación natural de cada una, la identidad genética, la distribución geográfica y las preferencias ecológicas. La combinación de estos tipos de evidencia hacen mucho más robustas las conclusiones que se derivan de ellas. Ahora parece evidente que estas especies son distintas y no una sola especie variable. Sin embargo es curioso que fueran consideradas como una sola especie por tanto tiempo. Una posible explicación es que cuando las similitudes entre las especies aparentan mucho más significativas que sus diferencias individuales, existe la tendencia a ignorar estas diferencias. En otras palabras, estas especies se consideraban una sola especie variable porque son muy similares entre si, mientras que son muy distintivas como grupo de otras *Specklinia*. Los últimos dos capítulos tratan dos especies nuevas, la *Specklinia absurda*, una especie con una morfología floral inusual, de los bosques de altura de Costa Rica y Panamá, y la *Specklinia lugduno-batavae*, dedicada al Hortus botanicus Leiden y la Universidad de Leiden, de las bajuras del Caribe de Nicaragua y Costa Rica. Ambas especies se ilustran y describen con detalle.

Revaluación filogenética de *Specklinia* y géneros afines:—*Specklinia* había sido considerado un sinónimo de *Pleurothallis* hasta que, con base en la filogenia de Pleurothallidinae publicada en el 2001, se restituyó el género. Desde entonces varios autores han tratado *Specklinia* de diversas maneras, incluyendo dentro del género desde 40 hasta 420 especies. Los trabajos filogenéticos presentados en los capítulos seis y siete evalúan de manera sistemática y crítica cuántas y cuáles especies deben ser incluidas en *Specklinia*. Nuestro estudio refleja que 95 especies pueden considerarse en este momento como parte del género, y evidencia que este incluye las especies tipo de los géneros *Acostaea, Areldia, Cucumeria, Empusella, Gerardoa, Pseudoctomeria, Sarcinula, Sylphia, Tribulago* y *Tridelta*, que por lo tanto son considerados sinónimos aquí. Queda claro también que el género *Muscarella* es distinto de *Specklinia*, y que este último es hermano de *Platystele* y *Scaphosepalum*. Esta relación no es evidente de inmediato, sin embargo vegetativamente las especies de estos tres géneros son muy similares. Adicionalmente se evaluó la posición filogenética de un grupo de unas 20 especies que habían sido colocadas tanto en *Anathallis* como *Specklinia*, y se constató que no pertenecen a ninguno de los dos. Debido a ello, y para mantener la monofilia de ambos géneros, fueron segregados en un concepto genérico nuevo bajo el nombre de *Lankesteriana*.

Las diferencias morfológicas superficiales de las especies que pertenecen a *Specklinia* provocaron una proliferación de propuestas de segregar a varios grupos de especies en otros géneros. Sin embargo aquí hemos podido demostrar que hay características morfológicas al igual que patrones geográficos que son propios de todas las especies de *Specklinia* y que por tanto se pueden utilizar para reconocer a las especies del género. Algunas decisiones taxonómicas aquí presentadas serán sin duda cuestionadas, y son en efecto sólo propuestas hechas con nuestras mejores capacidades, y basadas en los datos que tenemos disponibles. Es posible que con futuras técnicas y evidencia adicional el panorama propuesto pueda cambiar.

Evaluación de los mecanismos de polinización de *Specklinia*:—La última sección incluye únicamente un capítulo, y trata la polinización de *Specklinia*, presentando los resultados combinados de una serie de diversas técnicas de investigación.

Hemos logrado determinar que *Specklinia endotrachys, S. pfavii, S. spectabilis* y *S. remotiflora* son visitadas y polinizadas por al menos 13 especies distintas de *Drosophila* (Diptera), especialmente del grupo de *D. repleta*. Estas orquídeas atraen a las moscas, tanto machos como hembras, utilizando feromonas de agregación que probablemente son liberadas de tricomas hendidos presentes en el dorso de los sépalos de las flores. Una vez que los insectos aterrizan sobre los sépalos comienzan a deambular de uno a otro succionando el néctar que también es producido por estas orquídeas, esta vez secretados por estomas que se encuentran en la cara interna de los sépalos. Ocasionalmente al deambular de sépalo a sépalo, las moscas caminan sobre el labio, una estructura central muy reducida, que tiene movilidad. Al colocarse sobre este labio en una posición particular, este se vuelca presionando a la mosca contra la columna. Escapar de la columna le cuesta a la mosca hasta más de 20 minutos, y debe salir en retroceso. Al retroceder se embarra una sustancia viscosa que hay en el rostelo y remueve los polinios al pegarles esta sustancia. Este proceso se debe repetir en otra flor para lograr la polinización. Por mucho tiempo se pensó que los Pleurothallidinae por lo general no dan recompensas a sus polinizadores. Sin embargo, la mayoría de los estudios detallados modernos han encontrado algún tipo de recompensa floral y por tanto la polinización de este grupo parece no ser un caso de engaño.

En términos generales se considera que muchos grupos de orquídeas son polinizados por engaño porque las recompensas no son siempre visibles. Sin embargo hay que recordar que ausencia de evidencia no debe ser tomada como evidencia de ausencia.

References

References

Abele AD. 2007. Phylogeny of the genus *Masdevallia* Ruiz & Pav. (Orchidaceae) based on morphological and molecular data. disertación Phd. Hamburg University.

Ackerman JD. 1986. Mechanisms and evolution of food-deceptive pollination systems in orchids. *Lindleyana* 1: 108–113.

Ackerman JD. 2014. Orchids flora of the Greater Antilles. The New York Botanical Garden Press, 640 pp.

Adams PB, Lawson SD. 1993. Pollination in Australian orchids: a critical assessment of the literature 1882–1992. *Australian Journal of Botany* 41: 553–575.

Albores Ortiz O, Sosa V. 2006. Polinización de dos especies simpátricas de *Stelis* (Pleurothallidinae, Orchidaceae). *Acta Botanica Mexicana* 74: 155–168.

Ames O. 1923. New or noteworthy orchids. *Schedulae orchidianae* 6: 60–65.

Ames O, Schweinfurth C. 1925. New or noteworthy species of orchids from the American tropics. *Schedulae orchidianae* 8: 1–84.

Antoń S, Kamińska M, Stpiczyńska M. 2012. Comparative structure of the osmophores in the flower *Stanhopea graveolens* Lindley and *Cycnoches chlorochilon* Klotzsch (Orchidaceae). *Acta Agrobotanica* 65: 11–22.

Ascensãno L, Francisco A, Cotrim H, Pais MS. 2005. Comparative structure of the labellum in *Ophrys fusca* and *O. lutea* (Orchidaceae). *American Journal of Botany* 92: 1059–1067.

Ayasse M, Schiestl FP, Paulus HF, Ibarra F, Francke W. 2003. Pollinator attraction in a sexually deceptive orchid by means of unconventional chemicals. *Proceedings of the Royal Society of London Series B - Biological Sciences* 270: 517–522.

Barbosa AR, de Melo MC, Borba EL. 2009. Self-incompatibility and myophily in *Octomeria* (Orchidaceae, Pleurothallidinae) species. *Plant Systematics and Evolution* 283: 1–8.

Barros F. 2004. Taxonomic and nomenclatural notes on Brazilian Orchidaceae. pp. 7–22. In: Manilal KS, Satish Kumar C. (eds.) Orchid memories: a tribute to Gunnar Seidenfaden. Mentor Books, Kolkata.

Barros F. 2006. Seis novas combinacões para orquídeas brasileiras. *Bradea* 11: 29–32.

Barros F, Trettel Rodrigues V. 2009. Nomenclatural notes and new combinations on *Acianthera* Scheidw. and *Specklinia* Lindl. (Orchidaccae). *Bradea* 14: 21–26.

Bartelt RJ, Schaner AM, Jackson LL. 1985. cis-Vaccenyl acetate as an aggregation pheromone in *Drosophila melanogasler*. *Journal of Chemical Ecology* 11: 1747–1756.

Bartelt RJ, Schaner AM, Jackson LL. 1986. Aggregation pheromones in five taxa of the *Drosophila virilis* species group. *Physiological Entomology* 11: 367–376.

Bartelt RJ, Schaner AM, Jackson LL. 1988. Aggregation pheromones in *Drosophila borealis* and *Drosophila littoralis*. *Journal of Chemical Ecology* 14: 1319–1328.

Blanco MA, Barboza G. 2005. Pseudocopulatory pollination in *Lepanthes* (Pleurothallidinae, Orchidaceae) by fungus gnats. *Annals of Botany* 95: 763–772.

Boerhaave H. 1710. Index Plantarum Horti Lugduno Batavi. Cornelius Boutenstein, Leiden.

Bogarín D. 2007. A new *Lycaste* (Orchidaceae: Maxillarieae) from Costa Rica. *Lankesteriana* 7(3): 543–549.

Bogarín D, Pupulin F, Arrocha C, Warner J. 2013a. Orchids without borders: studying the hotspot of Costa Rica and Panama. *Lankesteriana* 13(1–2): 13–26.

Bogarín D, Karremans AP, Rincón R, Gravendeel B. 2013b. A new *Specklinia* (Orchidaceae: Pleurothallidinae) from Costa Rica and Panama. *Phytotaxa* 115(2): 31–41. http://dx.doi.org/10.11646/phytotaxa.115.2.1

Bogarín D, Serracín Z, Zamudio Z. 2014. Illustrations and studies in Neotropical Orchidaceae. The *Specklinia condylata* group (Orchidaceae) in Costa Rica and Panama. *Lankesteriana* 13(3): 185–206.

Bolaños R, Watson V, Tosi J. 2005. Mapa ecológico de Costa Rica (Zonas de Vida), según el sistema de clasificación de zonas de vida del mundo de L.R. Holdridge. Scale 1:750 000. Centro Científico Tropical, San José, Costa Rica.

Borba EL, Semir J. 2001. Pollinator specificity and convergence in fly-pollinated *Pleurothallis* (Orchidaceae) species: a multiple population approach. *Annals of Botany* 88: 75–88.

Brncic D. 1983. Ecology of flower breeding Drosophila. In: Ashburner MA, Carson HL, Thompson JN, eds. The Genetics and Biology of Drosophila 3b. London: Academic Press, 333–382.

Carson HL. 1974. Three flies and three islands: Parallel evolution in *Drosophila*. *Proceedings of the National Academy Sciences* 71: 3517–3521.

Chase MW. 1985. Pollination of *Pleurothallis endotrachys*. *American Orchid Society Bulletin* 54: 431–434.

Chase MW, Williams NH, de Faria AD, Neubig KM, Amaral MdCE, Whitten WM. 2009. Floral convergence in Oncidiinae (Cymbidieae; Orchidaceae): an expanded concept of *Gomesa* and a new genus *Nohawilliamsia*. *Annals of Botany* 104: 387–402. doi:10.1093/aob/mcp067.

Chiron GR, Guiard J, van den Berg C. 2012. Phylogenetic relationships in Brazilian *Pleurothallis sensu lato* (Pleurothallidinae, Orchidaceae): evidence from nuclear ITS rDNA sequences. *Phytotaxa* 46: 34–58.

Christensen DE. 1994. Fly pollination in the Orchidaceae. In: Arditti J, ed. Orchid Biology: Reviews and Perspectives, VI. USA: John Wiley & Sons, New York, 415-454.

Ciotek L, Giorgis P, Benitez-Vieyra S, Cocucci AA. 2006. First confirmed case of pseudocopulation in terrestrial orchids of South America: Pollination of *Geoblasta pennicillata* (Orchidaceae) by Campsomeris bistrimacula (Hymenoptera, Scoliidae). *Flora* 201: 365–369.

Cozzolino S, Widmer A. 2005. Orchid diversity: an evolutionary consequence of deception? *Trends in Ecology & Evolution* 20: 487–494.

Curry KJ, McDowell LM, Judd WS, Stern WL. 1991. Osmophores, floral features, and systematics of *Stanhopea* (Orchidaceae). *American Journal of Botany* 78: 610–623.

Darwin CR. 1862. On the various contrivances by which British and foreign orchids are fertilised by insects. London, UK: John Murray.

Davies KL, Turner MP. 2004. Pseudopollen in *Dendrobium unicum* Seidenf. (Orchidaceae): Reward or Deception? *Annals of Botany* 94: 129–132.

Davies KL, Stpiczyńska M, Rawski M. 2014. Comparative anatomy of floral elaiophores in *Vitekorchis* Romowicz & Szlach., *Cyrtochilum* Kunth and a florally dimorphic species of *Oncidium* Sw. (Orchidaceae: Oncidiinae). *Annals of Botany* 113(7): 1155–1173. doi:10.1093/aob/mcu045.

de Melo MC, Borba EL, Paiva EAS. 2010. Morphological and histological characterization of the osmophores and nectaries of four species of *Acianthera* (Orchidaceae: Pleurothallidinae). *Plant Systematics and Evolution* 286: 141–151. doi:10.1007/s00606-010-0294-1.

Den Otter CJ, Behan M, Maes FW. 1980. Single Cell Responses in Female Pieris-Brassicae (Lepidoptera, Pieridae) to Plant Volatiles and Conspecific Egg Odors. *Journal of Insect Physiology* 26: 465–472.

Dressler RL. 1993a. Field guide to the orchids of Costa Rica and Panama. Cornell University Press, Ithaca.

Dressler RL. 1993b. Phylogeny and classification of the Orchid family. Cambridge: Press Syndicate of the University of Cambridge.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 7: 214.

Dunsterville GCK, Garay LA. 1965. Venezuelan Orchids Illustrated 3. London: Andre Deutsch Ltd.

Duque O. 1993. Polinización en *Pleurothallis*. *Orquideología* 19: 55–69.

Duque-Buitrago CA, Alzate-Quintero NF, Otero JT. 2014. Nocturnal pollination by fungus gnats of the Colombian endemic species, *Pleurothallis marthae* (Orchidaceae: Pleurothallidinae). *Lankesteriana* 13: 407–417.

Edwards, S.V., Liu, L. & Pearl, D.K. 2007. High-resolution species trees without concatenation. *PNAS* 104(14): 5936–5941.

Endara L, Grimaldi DA, Roy BA. 2010. Lord of the flies: pollination of *Dracula* orchids. *Lankesteriana* 10: 1–11.

Endara L, Williams NH, Whitten WM. 2011. Filogenia molecular preliminar de *Scaphosepalum* (Orchidaceae: Pleurothallidinae). *Lankesteriana* 11(3): 245–252.

Fernández M, Bogarín D, Karremans AP, Jiménez D. 2014. New species and records of Orchidaceae from Costa Rica III. *Lankesteriana* 13(3): 259–282.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.

Garay LA. 1974. *Acostaea* y los géneros del complejo *Pleurothallis*. *Orquideología* 9: 103–124.

Garay LA, Sweet, HR. 1972. Notes on West Indian orchids, III. *Journal of the Arnold Arboretum* 53: 515–530.

Gravendeel B, Smithson A, Slik FJW, Schuiteman A. 2004. Epiphytism and pollinator specialization: drivers for orchid diversity? *Philosophical Transactions of the Royal Society of London B - Biological Sciences* 359: 1523–1535.

Greenspan RJ, Ferveur J-F. 2000. Courtship in *Drosophila*. *Annual Review of Genetics* 34: 205–232.

Grüber CE, Nakagawa S, Laws RJ, Jamieson IG. 2011. Multimodel inference in ecology and evolution: challenges and solutions. *Journal of Evolutionary Biology* 24: 699–711.

Hamer F. 1974. Las orquídeas de El Salvador. Ministerio de Educación. Dirección de Publicaciones, San Salvador, 374 pp.

Hamer F. 1981. Las orquídeas de El Salvador, III, suplemento. The Marie Selby Botanical Gardens, Sarasota.

Hamer F. 1984. *Pleurothallis endotrachys*. Sub pl. 1096. In: Hamer F. Orchids of Nicaragua, Icones plantarum tropicarum 11.

Harder LD, Johnson SD. 2009. Darwin's beautiful contrivances: evolutionary and functional evidence for floral adaptation. *New Phytologist* 183: 530–545.

Hebert PDN, Cywinska A, Ball SL. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B - Biological Sciences* 270: 313–321.

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. *Proceedings of the National Academy of Sciences of the United States of America* 101: 14812–14817.

Hedlund K, Bartelt RJ, Dicke M, Vet LEM. 1996. Aggregation pheromones of *Drosophila immigrans*, *D. phalerata*, and *D. suboscura*. *Journal of Chemical Ecology* 22: 1835–1844.

Heller AH, Hawkes AD. 1966. Nicaraguan orchid studies – 1. *Phytologia* 14: 1–35.

Heneghan C, Thompson M, Billingsley M, Cohen, D. 2011. Data from: Medical-device recalls in the UK and the device-regulation process: retrospective review of safety notices and alerts. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.585t4.

Holdridge LR. 1987. Ecología basada en zonas de vida. 3ª reimpresión. Instituto Interamericano de Cooperación para la Agricultura (IICA), San José, Costa Rica. 216 pp.

Hooker JD. 1890. *Pleurothallis platyrachis*. *Curtis' Botanical Magazine* 116: sub pl. 7129.

Humphreys AM, Linder P. 2009. Concept versus data in delimitation of plant genera. *Taxon* 58(4): 1054–1074.

Immonen E, Hoikkala A, Kazem AJN, Ritchie MG. 2009. When are vomiting males attractive? Sexual selection on condition-dependent nuptial feeding in Drosophila subobscura. Behavioral Ecology 20: 289–295.

Janzen D. 1967. Why mountain passes are higher in the tropics. *American Naturalist* 101: 233–249.

Janzen D. 1991. Historia Natural de Costa Rica. San José. Editorial de la Universidad de Costa Rica.

Jersáková J, Johnson SD, Kindlman P. 2006. Mechanisms and evolution of deceptive pollination in orchids. *Biological Reviews* 81: 219–235.

Karremans AP. 2010. Phylogenetics of *Stelis* (Orchidaceae: Pleurothallidinae) and closely related genera, based on molecular data, morphological characteristics and geographical distribution in the Central American and Andean Cordilleras. M.Sc. Thesis, Wageningen University. Available: http://edepot.wur.nl/146921. Accessed 22 October 2014.

Karremans AP. 2014. *Lankesteriana*, a new genus in the Pleurothallidinae (Orchidaceae). *Lankesteriana* 13(3): 319–332.

Karremans AP, Bogarín D, Fernández M, Smith CM, Blanco, MA. 2012. New species and records of Orchidaceae from Costa Rica. II. *Lankesteriana* 12(1): 19–51.

Karremans AP, Bogarín D. 2013. Costa Rica, land of endless orchids. *Orchids (West Palm Beach)* 82(7): 408–411.

Karremans AP, Bakker FT, Pupulin F, Solano-Gómez R, Smulders MJM (2013a). Phylogenetics of *Stelis* and closely related genera (Orchidaceae: Pleurothallidinae). *Plant Systematics and Evolution* 29(1): 69–86.

Karremans AP, Pupulin F, Gravendeel B. 2013b. Taxonomy, molecular phylogenetics, reproductive isolation, and niche differentiation of the *Specklinia endotrachys* species complex (Orchidaceae: Pleurothallidinae). *Lankesteriana* 13(1–2): 132–133.

Karremans AP, Bogarín D, Gravendeel B. 2015a. *Specklinia lugduno-batavae* (Pleurothallidinae: Orchidaceae), a new species in the *S. digitalis* group. *Blumea* 59: 180–184. http://dx.doi.org/10.3767/000651915X687859

Karremans AP, Pupulin F, Gravendeel B. 2015b. *Specklinia dunstervillei*, a New Species Long Confused with *Specklinia* *endotrachys* (Orchidaceae: Pleurothallidinae). *PLoS ONE* 10(7): e0131971. doi:10.1371/journal.pone.0131971

Karremans AP, Bogarin D, Pupulin F, Luer CA, Gravendeel B. 2015c. The glandulous *Specklinia*: morphological convergence versus phylogenetic divergence. *Phytotaxa* 218(2): 101–127.

Kowalkowska AK, Kozieradzka-Kiszkurno M, Turzynski S. 2014. Morphological, histological and ultrastructural features of osmophores and nectary of *Bulbophyllum wendlandianum* (Kraenzl.) Dammer (*B*. section *Cirrhopetalum* Lindl., Bulbophyllinae Schltr., Orchidaceae). *Plant Systematics and Evolution* DOI 10.1007/s00606-014-1100-2.

Kress JW, Wurdack KNJ, Zimmer EA, Weigt LA, Janzen DH. 2005. Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences of the United States of America* 102 (23): 8369–8374.

Kubatko, L.S. & Degnan, J.H. 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. *Systems Biology* 56(1): 17–24.

Lahaye R, van der Bank M, Bogarín D, Warner J, Pupulin F, et al. (2008) DNA barcoding the floras of biodiversity hotspots. *Proceedings of the National Academy of Sciences of the United States of America* 105: 2923–2928.

Leopardi C, Carnevali G, Romero-González GA. 2012. *Amoana* (Orchidaceae, Laeliinae), a new genus and a new species from Mexico. *Phytotaxa* 65: 23–35.

Lindley J. 1830. The genera and species of orchidaceous plants 8. Asher, London. xvii, 554 pp.

Lindley J. 1836. Notes upon some genera and species of American Orchidaceae. *Companion to the Botanical Magazine* 2: 353-357.

Lindley J. 1859. *Pleurothallis*. In: Lindley J. (Ed.). Folia Orchidacea. J. Matthews, London. pp. 1–46.

Luer CA. 1986. Systematics of *Pleurothallis* (Orchidaceae). Icones pleurothallidinarum III. *Monographs in Systematic Botany from the Missouri Botanical Garden* 20.

Luer CA. 1988. Icones Pleurothallidinarum V. Systematics of *Dresslerella* and *Scaphosepalum*. Addenda to *Porroglossum* (Orchidaceae). *Monographs in Systematic Botany from the Missouri Botanical Garden* 26.

Luer CA. 1990. Icones Pleurothallidinarum VII. Systematics of *Platystele*. *Monographs in Systematic Botany from the Missouri Botanical Garden* 38.

Luer CA. 1991. Icones Pleurothallidinarum VIII. Systematics of *Lepanthopsis*, *Octomeria* subgenus *Pleurothallopsis*, *Restrepiella*, *Restrepiopsis*, *Salpistele* and *Teagueia*. Addenda to *Platystele*, *Porroglossum* and *Scaphosepalum* (Orchidaceae). *Monographs in Systematic Botany from the Missouri Botanical Garden* 39.

Luer CA. 1997. Systematics of *Trichosalpinx*. *Monographs in Systematic Botany from the Missouri Botanical Garden* 64.

Luer CA. 1999. lcones Pleurothallidinarum XVIII. Systematics of *Pleurothallis* Subgen. *Pleurothallis* Sect. *Pleurothallis* Subsect. *Antenniferae*, Subsect. *Longiracemosae*, Subsect. *Macrophyllae-Racemosae*, Subsect. Perplexae, Subgen. *Pseudostelis*, Subgen. *Acuminatia*. *Monographs in Systematic Botany from the Missouri Botanical Garden* 76.

Luer CA. 2002. A systematic method of classification of the Pleurothallidinae versus a strictly phylogenetic method. *Selbyana* 23(1): 57–110.

Luer CA. 2004. *Pleurothallis* subgenus *Acianthera* and three allied subgenera. A second century of new species of *Stelis* of Ecuador. *Epibator*, *Ophidion*, *Zootrophion*. Icones Pleurothallidinarum XXVI. *Monographs in Systematic Botany from the Missouri Botanical Garden* 95.

Luer CA. 2005. *Dryadella* and *Acronia* section *Macrophyllae-Fasciculatae*: addenda to *Acianthera*, *Andinia*, *Dracula*, *Dresslerella*, *Lepanthes* and *Masdevallia* new taxa, validation of taxa, errata. Icones pleurothallidinarum XXVII. *Monographs in Systematic Botany from the Missouri Botanical Garden* 103.

Luer CA. 2006. A Reconsideration of *Masdevallia*. Systematics of *Specklinia* and vegetatively similar taxa (Orchidaceae). Icones Pleurothallidinarum XXVIII. *Monographs in Systematic Botany from the Missouri Botanical Garden* 105.

Luer CA. 2007. A third century of *Stelis* of Ecuador. Systematics of *Apoda-Prorepentia*. Systematics of miscellaneous small genera. Addenda: new genera, species and combinations (Orchidaceae). Icones pleurothallidinarum XXIX. *Monographs in Systematic Botany from the Missouri Botanical Garden* 112.

Luer CA. 2009. Icones Pleurothallidinarum XXX. *Lepanthes* of Jamaica and Systematics of *Stelis*, *Stelis* of Ecuador, part four and addenda: systematics of *Masdevallia*, new species of *Lepanthes* from Ecuador, and miscellaneous new combinations. *Monographs in Systematic Botany from the Missouri Botanical Garden* 115.

Luer CA. 2010. *Lepanthes* of Bolivia. Systematics of *Octomeria* species north and west of Brazil. Addenda: new species of *Brachionidium*, *Lepanthes*, *Masdevallia*, *Octomeria*, *Playstele*, *Pleurothallopsis* and *Porroglossum*. Corrigenda. Icones pleurothallidinarum 31. *Monographs in Systematic Botany from the Missouri Botanical Garden* 120.

Luer CA. 2011. Miscellaneous new species in the Pleurothallidinae excluding species from Brazil. *Harvard Papers in Botany* 16: 311–360.

Maddison WP, Maddison DR. 2007. Mesquite: a modular system for evolutionaryanalysis. Mesquite v. 2.72. Available: http://mesquiteproject.org. Accessed 13 January 2013.

Markow TA. 1988. Reproductive behavior of *Drosophila* in the laboratory and in the field. *Journal of Comparative Psychology* 102: 169–174.

Markow TA, O'Grady PM. 2005. Evolutionary genetics of reproductive behavior in *Drosophila*: Connecting the Dots. *Annual Review of Genetics* 39: 263–291.

Markow TA, O'Grady PM. 2008. Reproductive ecology of *Drosophila*. *Functional Ecology* 22: 747–759.

Meyer GE, Basquero L, Cameron KM. 2012. A new Ecuadorian species of *Dracula*: Pleurothallidinae (Orchidaceae). *OrchideenJournal* 19: 107–113.

Meyer CP, Paulay G. 2005. DNA Barcoding: Error Rates Based on Comprehensive Sampling. *PLoS Biology* 3(12): e422.

Mickeliunas L, Pansarin ER, Sazima M. 2006. Biologia floral, melitofilia e influência de besouros Curculionidae no sucesso reprodutivo de *Grobya amherstiae* Lindl. (Orchidaceae: Cyrtopodiinae). *Revista Brasileira de Botânica* 29: 251–258.

Miller, M.A., Pfeiffer, W. & Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8. New Orleans: Proceedings of the Gateway Computing Environments Workshop (GCE).

Misas Urreta G. 2006. Orquídeas de la Serranía del Baudó, Chocó, Colombia. Corporación Capitalina de Orquideología y Conconcreto. Medellín.

Moats RA, Bartelt RJ, Jackson LL. 1987. Ester and ketone components of the aggregation pheromone of *Drosophila hydei*. *Journal of Chemical Ecology* 13: 451–462.

Montes C, Cardona A, Jaramillo C, Pardo A, Sliva JC, Valencia V, Ayala C, Pérez-Angel LC, Rodriguez-Parra LA, Ramirez V, Niño H. 2015. Middle Miocene closure of the Central American Seaway. *Science* 348 (6231): 226-229.

Neyland R, Urbatsch LE, and Pridgeon, AM. 1995. A phylogenetic analysis of subtribe Pleurothallidinae (Orchidaceae). *Botanical Journal of the Linnean Society* 117: 13–28.

Oliveira DCSG, Almeida FC, O'Grady PM, Armella MA, DeSalle R, Etges WJ. 2012. Monophyly, divergence times, and evolution of host plant use inferred from a revised phylogeny of the *Drosophila* repleta species group. *Molecular Phylogenetics and Evolution* 64: 533–544.

Ortiz P, Uribe C. 2007. Galería de Orquídeas de Colombia (CD edition). Asociación Bogotana de Orquideología, Bogatá.

Ossenbach C. 2002. Breve historia de la orquideología en Costa Rica. Editorial de la Universidad de Costa Rica, 41 pp.

Pansarin ER, Amaral MCE. 2006. Biologia reprodutiva e polinização de duas espécies de *Polystachya* no sudeste do Brasil: evidência de pseudocleistogamia em Polystachyeae (Orchidaceae). *Revista Brasileira de Botânica* 29: 423–432.

Pansarin LM, Pansarin ER, Sazima M. 2008. Reproductive biology of *Cyrtopodium polyphyllum* (Orchidaceae): a Cyrtopodiinae pollinated by deceit. *Plant Biology (Stuttgart)* 10: 650–659.

Pansarin ER, Pansarin LM. 2011. Reproductive biology of *Trichocentrum pumilum*: an orchid pollinated by oil-collecting bees. *Plant Biology (Stuttgart)* 13: 576–581.

Pansarin ER, Aguiar JMRBV, Pansarin LM. 2013. Floral biology and histochemical analysis of *Vanilla edwallii* Hoehne (Orchidaceae: Vanilloideae): an orchid pollinated by *Epicharis* (Apidae: Centridini). *Plant Species Biology* 29(3): 242–252. doi: 10.1111/1442-1984.12014

Papadopulos AST, Powell MP, Pupulin F, Warner J, Hawkins JA, Salamin N, Chittka L, Williams NH, Whitten WM, Loader D, Valente LM, Chase MW, Savolainen V. 2013. Convergent evolution of floral signals underlies the success of Neotropical orchids. *Proceedings of the Royal Society of London Series B - Biological Sciences* 280: 20130960.

Peakall R, Ebert D, Poldy J, Barrow RA, Francke W, Bower CC, Schiestl FP. 2010. Pollinator specificity, floral odour chemistry and the phylogeny of Australian sexually deceptive *Chiloglottis* orchids: implications for pollinator-driven speciation. *New Phytologist* 188: 437–450.

Phillips RD, Faast R, Bower CC, Brown GR, Peakall R. 2009. Implications of pollination by food and sexual deception for pollinator specificity, fruit set, population genetics and conservation of *Caladenia* (Orchidaceae). *Australian Journal of Botany* 57: 287–306.

van der Pijl L, Dodson CH. 1966. Orchid flowers: their pollination and evolution. Coral Gables, FL: University of Miami Press.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

Pridgeon AM. 2005. Subtribe Pleurothallidinae. In: Prigeon AM, Cribb PJ, Chase MW, Rasmussen FN, editors, Genera Orchidacearum. Volume 4 Epidendroideae (Part One). Oxford: Oxford University Press. pp. 319–422.

Pridgeon AM, Chase MW. 2001. A phylogenetic reclassification of Pleurothallidinae (Orchidaceae). *Lindleyana* 16: 235–271.

Pridgeon AM, Solano R, Chase MW. 2001. Phylogenetic relationships in Pleurothallidinae (Orchidaceae): combined evidence from nuclear and plastid DNA sequences. *American Journal of Botany* 88: 2286–2308.

Pridgeon AM, Chase MW. 2002. Nomenclatural notes on Pleurothallidinae (Orchidaceae). *Lindleyana* 17: 98–101.

Pupulin F. 2010. Orchidaceae werckleanae: typification of Costa Rican orchid species described from collections by K. Werckle. *Botanical Journal of the Linnean Society* 163: 111–154.

Pupulin F, Ossenbach C, Jenny R, Vitek E. 2011. Typi Orchidacearum ab Augusto R. Endresio in Costa Rica lecti. *Annalen Naturhistorisches Museums Wien, Ser. B, Botanik. Zoologie* 112: 265–313.

Pupulin F, Karremans AP, Gravendeel B. 2012. A reconsideration of the empusellous species of *Specklinia* (Orchidaceae: Pleurothallidinae) in Costa Rica. *Phytotaxa* 63: 1–20.

Pupulin F, Karremans AP, Strigari S. 2013. Taxonomie in Aquarell: die *Specklinia-endotrachys*-Gruppe, Teil 1. Taxonomy in watercolor: the *Specklinia endotrachys* group, part 1. *Die Orchidee (Hamburg)* 64(5): 392–399.

Pupulin F, Karremans AP, Strigari S. 2013. Taxonomie in Aquarell: die *Specklinia-endotrachys*-Gruppe, Teil 2. Taxonomy in watercolor: the Specklinia endotrachys group, part 2. *Die Orchidee (Hamburg)* 64(6): 475–485.

Pupulin F, Ossenbach C, Jenny R. 2014. Mr. Auguste R. Endrés: not so mysterious, after all. *Orchids (West Palm Beach)* 83(10): 604–617.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org/

Rambaut A. 2009. FigTree v1.3.1. Available: http://tree.bio.ed.ac.uk/software/. Accessed 13 January 2013.

Ramírez SR, Eltz T, Fujiwara MK, Gerlach G, Goldman-Huertas B, Tsutsui ND, Pierce NE. 2011. Asynchronous diversification in a specialized plant-pollinator mutualism. *Science* 333: 1742–1746.

Ramos-Castro SE, Castañeda-Zárate M, Solano-Gómez R, Salazar GA (2012) *Stelis zootrophionoides* (Orchidaceae: Pleurothallidinae), a new species from Mexico. *PLoS One* 7(11): e48822. doi: 10.1371/journal.pone.0048822

Reichenbach HG. 1876. Orchidiographische Beiträge. *Linnaea* 41: 17–98.

Reichenbach HG. 1878. Zu Tafel 210. *Pleurothallis endotrachys* Rchb. *fil*. *Xenia Orchidacea* 3(1): 16–17.

Renner SS. 2005. Rewardless flowers in the angiosperms and the role of insect cognition in their evolution. In: Waser NM, Ollerton J, eds. Plant-Pollinator Interactions: from specialization to generalization. Chicago: University of Chicago Press, 123-144.

Reynolds ES. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* 17: 208–212.

Rolfe RA. 1888. New or noteworthy plants. *Gardeners' Chronicle* 4: 178–179 [18 August 1888].

Rolfe RA. 1890. The genus *Scaphosepalum* Pfitzer. *Journal of Botany, British and Foreign* 28: 135–137.

Schlechter FRR. 1910. Orchidaceae novae et criticae. Decas XIV-XV. *Repertorium Specierum Novovarum Regni Vegetabilis* 8: 561.

Scopece G, Cozzolino S, Johnson SD, Schiestl FP. 2010. Pollination efficiency and the evolution of specialized deceptive pollination systems. *The American Naturalist* 175: 98–105.

Singer RB. 2002. The pollination mechanism in *Trigonidium obtusum* Lindl. (Orchidaceae: Maxillariinae): sexual mimicry and trap-flowers. *Annals of Botany* 89: 157–163.

Singer RB, Flach A, Koehler S, Marsaioli AJ, Do Carmo E, Amaral M. 2004. Sexual mimicry in *Mormolyca ringens* (Lindl.) Schltr. (Orchidaceae: Maxillariinae). *Annals of Botany* 93: 755–762.

Silva MF, Silva JF. 1997. Novas ocorrências de orchidaceae para o Brasil. *Boletim do Museu Paraense emílio Goeldi, série Botanica* 13: 69–79.

Smets EF, Cresens E. 1988. Types of floral nectaries and the concepts “character” and “character-state” - a reconsideration. *Acta Botanica Neerlandica* 37: 121-128.

Smets EF, Ronse Decraene LP, Caris P, Rudall PJ. 2000. Floral nectaries in monocotyledons: distribution and evolution. In: Wilson KL, Morrison DA, eds. Monocots – Systematics and Evolution. Vol. 1 of Proceedings of the Second International Conference on the Comparative Biology of the Monocots, Sydney. CSIRO, Melbourne, 230-240.

Smith SD. 2010. Using phylogenetics to detect pollinator-mediated floral evolution. *New Phytologist* 188: 354–363.

Smithson A. 2006. Pollinator limitation and inbreeding depression in orchid species with and without nectar rewards. *New Phytologist* 169: 419–430.

Solano R, Soto M. 2008. *Specklinia endotrachys*. Sub pl. 1092. In: Hágsater, E. & Soto, M. (eds.). Orchids of Mexico, part 4. *Icones orchidacearum* 10: pl. 1001–1100.

Staden R, Judge DP, Bonfield JK. 2003. Analysing Sequences Using the Staden Package and EMBOSS. In: Krawetz SA, Womble DD. (eds.) Introduction to Bioinformatics. A Theoretical and Practical Approach. Human Press Inc., Totawa, NJ 07512. pp. 393–410.

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum Likelihood-based Phylogenetic Analyses with Thousands of Taxa and Mixed Models. *Bioinformatics* 22(21): 2688–2690.

Stamatakis, A. 2014. RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics* 10.1093/bioinformatics/btu033.

Steele RH. 1986. Courtship feeding in *Drosophila subobscura*. II. Courtship feeding by males influences female mate choice. *Animal Behaviour* 34: 1099–1108.

Stenzel, H. 2004. Systematics and evolution of the genus *Pleurothallis* R.Br. (Orchidaceae) in the Greater Antilles. Dissertation Thesis. Mathematisch-Naturwissenschaftlichen Fakultät I der Humboldt- Universität zu Berlin.

Stpiczyńska M. 1993. Anatomy and ultrastructure of osmophores of *Cymbidium tracyanum* Rolfe (Orchidaceae). *Acta Societatis Botanicorum Poloniae* 62: 5–9.

Stpiczyńska M, Matusiewicz J. 2001. Anatomy and ultrastructure of spur nectary of *Gymnadenia conopsea* (L.) Orchidaceae. *Acta Societatis Botanicorum Poloniae* 4: 267–272.

Stpiczyńska M, Davies KL. 2008. Elaiophore structure and oil secretion in flowers of *Oncidium trulliferum* Lindl. and Ornithophora radicans (Rchb.f.) Garay & Pabst (Oncidiinae: Orchidaceae). *Annals of Botany* 101: 375–384.

Sun Y, Skinner DZ, Liang GH, Hulbert H. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics* 89: 26-32.

Symonds MRE, Wertheim B. 2005. The mode of evolution of aggregation pheromones in *Drosophila* species. *Journal of Evolutionary Biology* 18: 1253–1263.

Szentesi Á. 2002. Insect-plant relationships – chance and necessity. *Acta Zoologica Academiae Scientiarum Hungaricae* 48: 55–71.

Teixeira SDP, Borba EL, Semir J. 2004. Lip anatomy and its implications for the pollination mechanisms of *Bulbophyllum* species (Orchidaceae). *Annals of Botany* 93: 499–505.

Tremblay RL, Ackerman JD, Zimmerman JK, Calvo RN. 2005. Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. *Biological Journal of the Linnean Society* 84: 1–54.

Villella A, Hall JC. 2008. Neurogenetics of courtship and mating in *Drosophila*. *Advances in Genetics* 62: 67–184.

Vogel S. 1990. The role of scent glands in pollination (transl. by Bhatti JS). Washington, DC: Smithsonian Institute.

Whitten WM, Blanco MA, Williams NH, Koehler S, Carnveli G, Singer RB, Endara L, Neubig KM. 2007. Molecular phylogenetics of *Maxillaria* and related genera (Orchidaceae: Cymbidieae) based on combined molecular data sets. *American Journal of Botany* 94: 1860–1889.

Wiens JJ. 2006. Missing data and the design of phylogenetic analyses. *Journal of Biomedical Informatics* 39: 34–42.

Williams NH. 1982. The biology of orchids and euglossine bees. In: Arditti J, ed. Orchid Biology: Reviews and Perspectives. II. Ithaca, NY: Cornell University Press, 119–171.

Acknowledgements

Acknowledgements

During this four-year period lots of people have contributed one way or another to the essence captured here, directly or indirectly they are all responsible for the end product. Writing the acknowledgements is both difficult, in the sense that one hopes to have credited everyone that deserves so, but also very fulfilling, in that one recognizes the role others have played during this journey.

For the supervision... I owe the words jotted down in this text basically to two persons. Franco Pupulin and Barbara Gravendeel have not only been the masterminds behind the study presented here, but they have also guided me throughout its completion. They have been there step by step, and it is certain that I would not have accomplished this without them. From Barbara I hope to have caught a bit of her precision and efficiency, as well as her capability of integrating different people and methods. I hope to follow Franco in his keen observation and writing skills, as well as in the development of such a magnificent detectivesque mind.

For their teachings... Marcel Eurlings helped me extensively throughout this research; he is thanked for “showing me ropes” in the DNA lab in Leiden, and putting up with my questions for four years. I owe Freek Bakker for the time spent with me working on phylogenetic interpretation and techniques. I owe Peter Roessingh for teaching me how to place electrodes on flies and doing Electroantennography. Bertie-Joan van Heuven taught me to prepare the LM samples and to do SEM work. Thank you all for the time spent and patience.

For access to and exchange of plant material... I am very grateful to Jorge Warner, director of Lankester Botanical Garden in Costa Rica, Paul Kessler, prefect of the Hortus botanicus Leiden, Christel Schollaardt, head of the collections of botany of Naturalis Biodiversity Center, and all their staff for their paramount effort in getting all the collecting and export permits, and cultivating the biological material. Karen Barquero, Maricruz Bonilla, Melania Fernández and Nicolien Sol have been especially helpful in preparing and sending plant material, thank you all.

For data... Alec Pridgeon and Mark Whitten were kind enough to aid in providing some of the DNA extractions here presented. Hagen Stenzel is thanked for letting me use some of the DNA data produced in his unpublished dissertation. I am thankful to Karsten Kaspers, Misli Kaya, André van Roon and Natasja Carol-Visser from the University of Applied Sciences Leiden, who produced the GC-MS analyses and chemical analyses. I have to thank Gregorio Fazzi for his excellent LM sections and photographs.

For the plant material... I am very grateful to Hermano Jorge de la Cruz, Wiel Driessen, Piet Dubbeldam, Jaco Kruizinga, Ton Sijm, Rogier van Vugt, Gerhard Vierling, Gerson Villalobos and Koos Wubben, for allowing me to access and use their personal orchid collections for the different stages of this research. Without their kindness and support this study would have been impossible.

For herbarium material... I am most grateful to Eric Hágsater and Rolando Jiménez at AMO, Gustavo Romero at AMES, Gordon McPherson and Michael Grayum at MO, Toscano de Brito at SEL. In general I would like to thank the personnel at CR, INB, JBL, L, and USJ for allowing me to access their collections. Karen Gil was kind enough to share the herbarium material prepared by Misas Urreta.

For the drawings and paintings... I am most thankful to Esmée Winkel and Lizbeth Oses, who kindly provided several of the ink illustrations presented here. I am also very thankful to Sylvia Stigari for allowing me to reproduce some of her paintings, including the one on the cover page.

For photographs... Kevin Beentjes, Guy Chiron, Wiel Driessen, Piet Dubbeldam, Carlos Ossenbach, Ton Sijm, Rogier van Vugt and Lubbert Westra have been kind enough to provide diverse photographic material.

For reviews and recommendations... Cassio van den Berg, Diego Bogarín, Toscano de Brito, Mark W. Chase, Miriam Contreras, Robert Dressler, Melania Fernández, Jan Karremans, Alec Pridgeon, Franco Pupulin and Norris H. Williams reviewed different chapters of this thesis and they are thanked for their time and the constructive criticism given. Without a doubt they improved them substantially.

For help in the field... Diego Bogarín, Melissa Díaz, József Geml, Daniel Jiménez, Jaco Kruizinga, Lizbeth Oses, Franco Pupulin, Christina M. Smith and Gerson Villalobos diversely joined the field trips and helped collecting the study material, thanks a lot.

For taking care of living orchids... Jaime Aguilar, Jorge "Koki" Brenes and Giovanni Meza cultivated and took care of the living plants at JBL. Rogier van Vugt and Jaco Kruizinga took special care of the plants at the Hortus botanicus in Leiden. I am very grateful for this, as without their help it would have been impossible to pursue this study.

For help in the DNA barcoding, climate rooms, NMR and anatomy labs... Kevin Beentjes has helped out in many stages of this research. Kevin, thanks for your patience and the excellent quality of everything you do. I need to thank Roland Butôt for the help in culturing flies and with several experiments; you have been of great help. Bobby Driessen and Martijn de Jong helped with diverse steps of DNA extraction, amplification and sequencing and are thanked. Young Hae Choi was kind enough to do an NMR analysis of floral compounds and is thanked. Rob Langelaan is thanked for the kindness in doing the TEM work presented here. Federico Albertazzi and his staff at CIBCM are thanked for allowing us to use their labs.

For permits... We are thankful to the CITES offices of the Netherlands and Costa Rica, the scientific services of Costa Rican Ministry of Environment and Energy (MINAE) and its National System of Conservation Areas (SINAC), and the Autoridad Nacional del Ambiente (ANAM) of Panama for issuing the Scientific Passports under which the wild species treated in this study were collected.

For financial support... The Alberta Mennega Foundation supported this research with grants at different stages and is thanked for playing a significant role in financing field trips and lab work. I wish to thank the Vice-Presidency of Research of the University of Costa Rica for providing support under the projects 814-A7-015, 814-BO-052, 814-B1-239, 814-B2-161 and 814-B3-075.

For "everyday" things... I have to thank, first and foremost, Nico Davin for helping me getting settled in Leiden (repeatedly). I owe you my bike, dozens of good meals and gallons of free coffee and beer. You have been a true brother. I owe thanks to Luigi Faino, Alice Pajoro, and Sebastian Sánchez for allowing me to stay at their place on several occasions, and I must thank João Luis Henriques for driving me around town and especially for the dance lessons; you have been like family. I have to thank Rachel Schwallier, my roommate at Leiden University, for the non-orchid conversations and overall kindness. I see all of you once a year and it always seems like we just saw each other yesterday, thanks for that. Andre, Andresito, Andreu, Diego, Eduard, Eduardo, Feli, Frederike, Helen, Inés, Ingrid, Jesús, Joel, Jose, Josema, József, Juanma, Luis, Nestor, Maarten, Manuel, Marco, Marcelita, Miriam, Nacho, Nelson, Patrick, Potter, Rebe, Renato, Rob, Robertico, Stef, Tico, with all of you I have shared more than just a beer, thanks for making the last years bearable.

For their unconditional support... I want to thank my wife Miriam for putting up with me. Thank you for reminding me that there are other thing besides orchids in life, and sharing your life with me. The truth is I did my PhD in Leiden to stay close to you. Please keep on being my travel partner on this earth. My mom, you are probably the reason I caught the orchid fever, an incurable disease that is sometimes a curse, but most of the time the only and most wonderful explanation for my existence. Thank you for your constant support and your unconditional love, I could not have done this without your guidance. My dad, thank you for supporting the orchid madness since I am a kid, for putting up with going to the field, finding and buying all sorts of old documents and instruments, and for reading many of the things I write (even-though it is not your field). You have stepped up even when it meant taking dangerous risks (remember the mine field?), thanks for that. Pedro, thanks for always being there, you have rescued me from troubles dozens of times. Thanks for the constructive criticism on both my work and life choices, it has meant a lot. Darius, Rosi and Tina, you have been wonderful and helped me out in your own manner along the way.

Curriculum Vitae

About the Author

Adam Philip Karremans was born on the 21st of December 1986 in Leiden, the Netherlands. At the age of four, after living for two years in Colombia, Adam moved with his parents, two graduates from the Anthropology Department of Leiden University, to the small town of Turrialba in Costa Rica. He spent his school years there, living at the Center of Agricultural Research and Education (CATIE) and attending the Jorge Debravo institute. Already by the age of twelve, Adam had started collecting orchids. However, it was more the curiosity of knowing how many and which different species he could find, rather than growing the orchids that really interested him. With 15 years of age, Adam was introduced to Franco Pupulin, who was looking for a very rare orchid known only from CATIE. Shortly after, and with Franco's orchid in hand, Adam arrived at Lankester Botanical Garden (JBL), where he was invited to do voluntary work. At the University of Costa Rica (UCR) in Turrialba, he started a BSc on Agronomy the following year, and during the four years of study he would be a student assistant at Lankester. During that period Adam started doing more serious research on orchids, publishing his first scientific publications. In 2008, he enrolled in the MSc program on Plant Breeding and Genetic Resources at Wageningen University in the Netherlands. His thesis, supervised by Rene Smulders, Freek Bakker and Franco Pupulin was on the phylogenetics of Stelis, a Neotropical genus of orchids. In 2010, Adam returned to Costa Rica to work as a researcher on orchids at JBL, UCR. Two years later he began looking for possibilities of doing a PhD in the Netherlands, and it was Barbara Gravendeel's name as supervisor on a vacancy for a PhD project that inspired him to apply. He had heard her name during his stay at the Herbarium Vadense in Wageningen. Barbara, Franco and Adam proposed a joint project between Naturalis Biodiversity Center, the Hortus botanicus of Leiden University and Lankester Botanical Garden, and studies on Specklinia, another Neotropical genus of orchids were started. Adam stood for the first time in front of the Sylvius building in Leiden in October 2012 where he completed his PhD project in October 2015. Currently, Adam is the head of research at JBL, where he manages four research projects on floristics, phylogenetics and pollination studies on orchids, supervises two MSc and two BSc students, is the Managing Editor of the journal Lankesteriana, and is a lecturer of the orchidology course at the Biology Department of UCR.

Publications

Pupulin F, **Karremans AP.** 2007. Prosthechea ionophlebia and P. chacaoensis two different species, but which is which? *Lindleyana in Orchids* 76(3): 202-208.

Bogarín D, **Karremans AP**, Pupulin F. 2008. New species and records of Orchidaceae from Costa Rica. *Lankesteriana* 8(2): 53-74.

Pupulin F, **Karremans AP.** 2008.The strange story of the two Hoffmann´s Epidendrums. *Lindleyana in Orchids* 77(6): 454-457.

Pupulin F, **Karremans AP.** 2008.The Orchid Pollinaria Collection at Lankester Botanical Garden. *Selbyana* 29(1): 69-86.

Pupulin F, **Karremans AP**, Merino G. 2008. *Trichocentrum loyolicum*, a new species in the genus *Trichocentrum s.s. Harvard Papers in Botany* 13(2): 219-222.

Karremans AP. 2009. *Prosthechea madrense*, a reconsideration of *Epidendrum madrense* Schltr.(Orchidaceae: Laeliinae). *Acta Botánica Mexicana* 88: 47-57.

Karremans AP, Hágsater E. 2010. Confusion in *Epidendrum brenesi* Schltr., and a new Costa Rican species: *Epidendrum sotoanum* (Orchidaceae). *Lankesteriana* 9(3): 403-409.

Bogarín D, **Karremans AP**. 2010. A new *Platystele* (Orchidaceae: Pleurothallidinae) from Central Costa Rica / Un nuevo *Platystele* (Orchidaceae: Pleurothallidinae) de la parte central de Costa Rica. *Orquideología* 27(2): 208-220.

Pupulin F, **Karremans AP**. 2010. Two new species and a new record of *Epidendrum* (Orchidaceae: Laeliinae) from Costa Rica. *Selbyana* 30(2): 196.

Pupulin F, **Karremans AP.** 2010. *Crossoglossa sotoana* (Orchidaceae: Malaxideae), a new species honoring the late Mexican botanist, Miguel Ángel Soto Arenas. *Lankesteriana* 9(3): 443-446.

Karremans AP. 2011. *Dracontia*, the little-known dragon orchids. *Orchids* 80(9): 560-566.

Karremans AP, Bogarín D. 2011. *Pleurothallis adventurae* (Orchidaceae: Pleurothallidinae), eine neue art aus einer unerforschten region in Costa Rica. *OrchideenJournal* 18(3): 111-114.

Karremans AP, Muñoz M. 2011. *Pleurothallis silvae-pacis* (Orchidaceae: Pleurothallidinae): a new species found at the Bosque de Paz Biological Reserve in Costa Rica. *Orchid Review* 121: 155-159.

Fernández M, **Karremans AP**, Pupulin F. 2011. Orchid Pollinaria up close and personal. *Orchids* 80: 362-363.

Karremans AP, Smith CM. 2012. A note on genus *Dracontia* (Orchidaceae: Pleurothallidinae), with a new species. *Harvard Papers in Botany* 17(1): 13-17.

Karremans AP. 2012. Illustrations and Studies in Neotropical Orchidaceae. 3. On the identity of *Dracontia pachyglossa* and *D. ramonensis*; with a new species, *D. lueriana* (Pleurothallidinae). *Ann. Naturhist. Mus. Wien, B*. 113: 119-132.

Karremans AP, Bogarín D, Fernández M, Smith CM, Blanco MA. 2012. New species and records of Orchidaceae from Costa Rica. II. *Lankesteriana* 12(1): 19—51.

Bogarín D, Pupulin F, **Karremans AP**. 2012. Three New *Lepanthes* (Orchidaceae: Pleurothallidinae) from South-East Costa Rica. *Lankesteriana* 12(2): 107—114.

Pupulin F, **Karremans AP**, Gravendeel B. 2012. A reconsideration of the Empusellous species of *Specklinia* (Orchidaceae: Pleurothallidinae) in Costa Rica. *Phytotaxa* 63: 1-20.

Karremans AP, Bakker FT, Pupulin F, Solano-Gomez R, Smulders MJM. 2013. Phylogenetics of *Stelis* and closely related genera (Orchidaceae: Pleurothallidinae). *Plant Systematics and Evolution* 29(1): 69-86.

Karremans AP, Bogarín D. 2013. Three New Species of *Dracontia* (Pleurothallidinae, Orchidaceae) from Costa Rica. *Systematic Botany* 38(2): 307-315.

Bogarín D, **Karremans AP**, Rincón R, Gravendeel B. 2013. A new *Specklinia* (Orchidaceae: Pleurothallidinae) from Costa Rica and Panama. *Phytotaxa* 115(2): 31-41.

Bogarín D, **Karremans AP**. 2013. *Trichocentrum pupulinianum* (Orchidaceae). *Lindleyana in Orchids (West Palm Beach)* 82(2): 106–110.

Pupulin F, **Karremans AP**, Strigari S. 2013. Taxonomie in Aquarell: die *Specklinia-endotrachys*-Gruppe, Teil 1. Taxonomy in watercolor: the *Specklinia endotrachys* group, part 1. *Die Orchidee (Hamburg)* 64(5): 392-399.

Pupulin F, **Karremans AP**, Strigari S. 2013. Taxonomie in Aquarell: die *Specklinia-endotrachys*-Gruppe, Teil 2. Taxonomy in watercolor: the *Specklinia endotrachys* group, part 2. *Die Orchidee (Hamburg)* 64(6): 475-485.

Karremans AP. 2014. *Lankesteriana*, a new genus in the Pleurothallidinae (Orchidaceae). *Lankesteriana* 13(3): 319-332.

Fernández M, Bogarín D, **Karremans AP**, Jiménez D. 2014. New species and records of Orchidaceae from Costa Rica III. *Lankesteriana* 13(3): 259-282.

Bogarín D, **Karremans AP**. 2015. *Prosthechea tinukiana* sp. nov. (Orchidaceae: Laeliinae): an update of the *Prosthechea prismatocarpa* complex. *Turkish Journal of Botany* 39: 499-505.

Dressler RL, **Karremans AP**. 2015. *Sobralia rogersiana* a marvelous monster. *Orquideología* 32(1): 31-37

Karremans AP. 2015. Nomenclatural notes in the Pleurothallidinae (Orchidaceae): *Stelis*. *Phytotaxa* 203 (3): 292-296.

Karremans AP. 2015. Visualizing Pleurothallids: *Lankesteriana*. *Lindleyana* in *Orchids (Bull. Amer. Orch. Soc.)* 84(5): 304-312.

Karremans AP, Bogarín D, Pupulin F, Luer CA, Gravendeel B. 2015. The glandulous *Specklinia*: morphological convergence versus phylogenetic divergence. *Phytotaxa* 218(2): 101--127.

Karremans AP, Bogarín D, Gravendeel B. 2015. *Specklinia lugduno-batavae* (Pleurothallidinae: Orchidaceae), a new species in the *S. digitalis* group. *Blumea* 59: 180-184.

Karremans AP, Pupulin F, Gravendeel B. 2015. *Specklinia dunstervillei*, a New Species Long Confused with *Specklinia endotrachys* (Orchidaceae: Pleurothallidinae). *PLoS ONE* 10(7): e0131971. doi:10.1371/journal.pone.0131971

Karremans AP, Pupulin F, Grimaldi D, Beentjes KK, Butôt R, Fazzi GE, Kaspers K, Kruizinga J, Roessingh P, Smets EF, Gravendeel B. 2015. Pollination of *Specklinia* by nectar-feeding *Drosophila*: the first reported case of a deceptive syndrome employing aggregation pheromones in Orchidaceae. *Annals of Botany* 2015: 1-19.

Rodríguez-Martínez L, Rincón-Useche C, **Karremans AP**. 2015. A New *Pleurothallis* from Colombia, with a Note on *Ancipitia* and *Colombiana* (Orchidaceae: Pleurothallidinae). *Systematic Botany* 40(1): 75-78.

PE&RC Training and Education Statement

The C.T. De Wit
Graduate School
PE&RC
PRODUCTION
ECOLOGY
& RESOURCE
CONSERVATION

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- Taxonomy molecular phylogenetics, reproductive isolation, and niche differentiation of the *Specklinia endotrchys* species complex (Orchidaceae: Pleurothallidinae)

Writing of project proposal (4.5 ECTS)

- Phylogenetics, reproductive biology and niche differentiation of the epiphytic orchid *Specklinia endotrachys* and close relatives in Pleurothallidinae (2012)

Post-graduate courses (9.5 ECTS)

- Current trends in phylogenetics; WUR (2012)
- Scanning Electron Microscopy (SEM) of floral part of *Specklinia* species; Leiden University (2012)
- Workshop VIBRANT tools for DNA taxonomists; Royal Belgian Institute of Natural Sciences, Brussels (2013)

Laboratory training and working visits (10 ECTS)

- Fossil calibration of the phylogeny of Pleurothallidinae; WUR (2015) Eletro-antenography (EAG) experiments with *Drosophila hydei*; UVA (2013)

Invited review of (unpublished) journal manuscripts (13 ECTS)

- Taxon (2012)
- Nordic Journal of Botany (2012)
- Plosone (2012)
- Acta Botanica Mexicana (2012)
- Blumea (2013)
- Blumea (2013)
- Nordic Journal of Botany (2013)
- Phytotaxa (2014)
- Phytotaxa (2014)
- Turkish Journal of Botany (2014)
- Phytotaxa (2015)
- Plant Systematics and Evolution (2015)
- Acta Botanica Galica (2015)
- Brittonia (2015)

Deficiency, refresh, brush-up courses (6 ECTS)

- Advanced biosystematics; Wageningen University and Research Centre (2009)

Competence strengthening / skills courses (0.6 ECTS)

- Scientific integrity; Leiden (2014)
- Effective communication; Leiden (2014)

PE&RC Annual meetings, seminars and the PE&RC weekend (0.7 ECTS)

- 6th Workshop Plant-insect; UVA (2011)
- Mini-symposium collections and spatial analysis-introduction of Naturalis Geoportal; Naturalis (2011)
- PhD Day; PE&RC (2011)

Discussion groups / local seminars / other scientific meetings (4.5 ECTS)

- Character evolution (2013-2015)
- Lankester seminars (2014-2015)

International symposia, workshops and conferences (9.6 ECTS)

- 4th Scientific Conference on Andean Orchids (IISCAO); poster presentation; Guayaquil, Ecuador (2012)
- 31st New Phytologist Symposium. Orchid symbioses: models for evolutionary ecology; poster presentation; Calabria, Italy (2013)
- V Monocots Symposium: Orchid relationships from species to subfamily; oral presentation; The Brox, New York (2013)
- Séptimo Congreso Colombiano de Botánica; oral presentation; Ibague, Colombia (2013)
- NERN Netherlands Annual Ecology Meeting; oral presentation; Lunteren, the Netherlands (2014)
- Primera Exposición Nacional de Orquídeas y Encuentro Académico: Bogotá Humana y florecida en el Jardín Botánico; oral presentation; Bogotá, Colombia (2014)

Lecturing / supervision of practical's / tutorials (8.1 ECTS)

- The most common orchids in Costa Rica; Costa Rican Ministry of Environment (2012)
- Orchidology; UCR (2012)
- Orchid agriculture; UCR (2012)
- Orchidology; UCR (2015)

Supervision of MSc students

- Molecular phylogeny of the Costa Rican species of Masdevallia Ruiz & Pav. (Orchidaceae: Pleurothallidinae)
- Reproductive biology of Costa Rican species of Masdevallia (Orchidaceae) sensu lato

LANKESTER
jardín botánico

Universiteit Leiden

Naturalis
Biodiversity
Center

UNIVERSIDAD DE
COSTA RICA