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ABSTRACT

Ochnaceae s.str. (Malpighiales) are a pantropical family of about 500 species and 27 genera of almost exclusively woody plants. Infrafamilial classification and relationships have been controversial partially due to the lack of a robust phylogenetic framework. Including all genera except *Indosinia* and *Perissocarpa* and DNA sequence data for five DNA regions (ITS, *matK*, *ndhF*, *rbcL*, *trnL-F*), we provide for the first time a nearly complete molecular phylogenetic analysis of Ochnaceae s.l. resolving most of the phylogenetic backbone of the family. Based on this, we present a new classification of Ochnaceae s.l., with Medusagynoideae and Quiinoideae included as subfamilies and the former subfamilies Ochnoideae and Sauvagesioideae recognized at the rank of tribe. Our data support a monophyletic Ochneae, but Sauvagesieae in the traditional circumscription is paraphyletic because *Testulea* emerges as sister to the rest of Ochnoideae, and the next clade shows *Luxemburgia* + *Philacra* as sister group to the remaining Ochnoideae. To avoid paraphyly, we classify Luxemburgieae and Testuleeae as new tribes. The African genus *Lophira*, which has switched between subfamilies (here tribes) in past classifications, emerges as sister to all other Ochneae. Thus, endosperm-free seeds and ovules with partly to completely united integuments (resulting in an apparently single integument) are characters that unite all members of that tribe. The relationships within its largest clade, Ochnineae (former Ochneae), are poorly resolved, but former Ochninae (*Brackenridgea*, *Ochna*) are polyphyletic. Within Sauvagesieae, the genus *Sauvagesia* in its broad circumscription is polyphyletic as *Sauvagesia serrata* is sister to a clade of *Adenarake*, *Sauvagesia* spp., and three other genera. Within Quiinoideae, in contrast to former phylogenetic hypotheses, *Lacunaria* and *Touroulia* form a clade that is sister to *Quiina*. Bayesian ancestral state reconstructions showed that zygomorphic flowers with adaptations to buzz-pollination (poricidal anthers), a syncarpous gynoecium (a near-apocarpous gynoecium evolved independently in Quiinoideae and Ochninae), numerous ovules, septicidal capsules, and winged seeds with endosperm are the ancestral condition in Ochnoideae. Although in some lineages poricidal anthers were lost secondarily, the evolution of poricidal superstructures secured the maintenance of buzz-pollination in some of these genera, indicating a strong selective pressure on keeping that specialized pollination system.

1. Introduction

Malpighiales are one of the largest orders of flowering plants and are an important component of tropical rain forests worldwide (e.g., Gonmadje et al., 2011; Stropp et al., 2011). Its rapid

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diversification during the mid-Cretaceous made the order one of the phylogenetically most recalcitrant angiosperm groups (Davis et al., 2005; Wurdack and Davis, 2009). Additionally, the morphological foundation for new patterns of relationships based on recent molecular phylogenetics followed by a massive restructuring of the order has been poor (e.g., Savolainen et al., 2000; Davis and Chase, 2004; APG III, 2009; Endress et al., 2013). Only recently, with the advent of phylogenomics (Xi et al., 2012) and the availability of extensive floral morphological data (Endress et al., 2013), could major progress in our understanding of the phylogenetic backbone and familial relationships be achieved. Nonetheless, some relationships still remain unresolved and the Ochnaceae clade has been identified as being in strong need for comprehensive molecular phylogenetic studies (Matthews et al., 2012; Endress et al., 2013).

In higher-level molecular phylogenetic studies, Ochnaceae s.str. form a well-supported clade together with the monotypic Medusagynaceae – an endemic of the Seychelles – and the Neotropical Quiinaceae (Fay et al., 1997; Davis et al., 2005; Schneider et al., 2006; Korotkova et al., 2009; Bell et al., 2010; Soltis et al., 2011). In the most recent APG classification (APG III, 2009), all three were united to form an expanded Ochnaceae s.l. (if not specified, from here we refer to Ochnaceae s.str.). Nonetheless, some more recent studies keep all three as separate families because they form distinct clades that are also morphologically well-characterized (Wurdack and Davis, 2009; Matthews et al., 2012; Xi et al., 2012; Endress et al., 2013). The position of Ochnaceae s.l. within Malpighiales has been unclear until recently, when Xi et al. (2012) inferred that they are sister to the clusioid clade, though with low support (Bayesian posterior probability = 0.81). While higher-level relationships became clearer, molecular evidence for infrafamilial relationships of Ochnaceae was still wanting.

Ochnaceae in the traditional circumscription (Amaral, 1991; Amaral and Bittrich, 2014) are a largely woody pantropical family, comprising ~500 species and 27 genera. The highest diversity is found in the Neotropics with 15 genera and about 300–350 species; Africa has nine genera and about 150 species, and the lowest diversity is observed in Southeast Asia, which is home to ~20 species and eight genera (Kanis, 1968; Sastre, 2003; Verdcourt, 2005). Ochnaceae occur in rain forests, dry forests and savannas, and some taxa develop fire-adapted forms (Kanis, 1968). Most Ochnaceae are shrubs or small trees and only few are herbaceous (species of *Sauvagesia* L.) or large trees of the canopy strata (e.g., *Brackenridgea* A. Gray, *Lophira* Banks ex C.F. Gaertn. and *Testulea* Pellegr.). The African genus *Lophira*, and to a lesser extent also *Testulea*, provide valuable timber (azobé and izombé, respectively; Doumenge and Séné, 2012; Oduro, 2012). An important diagnostic character is the leaf venation with many Ochnaceae possessing rather densely spaced parallel secondary veins and the tertiary veins perpendicular to these. The number of sepals, petals, and stamens varies drastically across the family, but flowers are basically pentamerous. Potential synapomorphies of the family are poricidal anthers, stamen filaments with an abruptly narrow attachment zone of the anthers, and the crystal layer in the endostesta (Amaral, 1991; Matthews et al., 2012). The common poricidal anthers or the poricidal system (i.e., where the pore is formed by the staminodes enveloping the fertile anthers) are interpreted as adaptations to buzz-pollination (Kubitzki and Amaral, 1991). The best known feature, although only present in part of the family, are the fruits consisting of a swollen red receptacle bearing free black drupelets (resulting in the family's nick name, Mickey Mouse plants), but fruit type is widely variable in Ochnaceae s.str. (see, e.g., Dwyer, 1946).

Ochnaceae have traditionally been divided into the subfamilies Ochnoideae and Sauvagesioideae – corresponding to Engler's "Exalbuminosae" and "Albuminosae" (Fig. 1), respectively – on the basis of the absence (Ochnoideae) or presence (Sauvagesioideae) of endosperm in the seeds (Engler, 1874; Kanis, 1968; Amaral, 1991). In most classifications, both subfamilies have been subdivided into tribes and subtribes, but the concepts and circumscriptions have been controversial (Fig. 1). The majority of the genera of Sauvagesioideae are species-poor. The largest and morphologically most heterogeneous genus is *Sauvagesia* with about 35 species. By far the highest diversity is observed in tribe Ochneae which makes up more than two thirds of the species richness of the family, comprising the three most species-rich genera *Ouratea* Aubl. (~200 spp.; Sastre, 2003), *Ochna* L. (~80 spp.; Verdcourt, 2005) and *Campylospermum* Tiegh. (~50 spp.; Farron, 1985; Bissiengou et al., 2013) as well as the smaller *Brackenridgea* (9 spp.; Kanis, 1968; Callmander et al., 2010), *Rhabdophyllum* Tiegh. (8 spp.; Sosef, 2008), and *Idertia* Farron (1 spp.; Sosef, 2013).

Ochnaceae are noteworthy for their complex biogeographical history with radiations in the Old and New World. The occurrence of both forest and savanna species in several genera also makes Ochnaceae an excellent system for the study of forest-savanna transitions which have recently been used to address responses of rainforests to climate change and of climatic tipping points for tropical rainforests versus savannas (Hirota et al., 2011) or the reconstruction of the origin of these major tropical and subtropical biomes (Couvreur et al., 2008; Simon et al., 2009). Additionally, recent efforts to gain insights into the evolution of Ochnaceae using new floral morphological data (Matthews et al., 2012; Endress et al., 2013) further highlight the need for a sound molecular basis and an updated and stable classification of the family. The shifting circumscriptions and infrafamiliar classifications of Ochnaceae (s.str. and s.l.) hamper communication due to frequent changes and inconsistencies in floras and standard taxonomic and floristic databases worldwide.

Our study aims at providing for the first time a molecular phylogenetic framework for a modern classification of Ochnaceae based on a near-complete taxon sampling at the genus level and multiple DNA regions. Additionally, we examined evolutionary hypotheses for taxonomically important characters.

2. Materials and methods

2.1. Taxon sampling

Plant material was obtained during field excursions, from herbarium specimens, or from the DNA banks at Kew (K), the Missouri Botanical Garden (MO), and the National Herbarium of the Netherlands (Wageningen branch, WAG). Our final taxon sampling contains 93 accessions and 79 taxa of Ochnaceae s.l. (Table 1), comprising all genera of Ochnaceae s.str. – following the generic circumscriptions of Amaral (1991) –, except *Indosinia* J.E. Vidal and *Perissocarpa* Steyerm. & Maguire. In addition, the monotypic Medusagynaceae and all genera of Quiinaceae were included. Based on Xi et al. (2012), three representatives of the clusioid clade were chosen as outgroups. For the pantropical *Sauvagesia* and especially the larger genera of Ochneae (*Campylospermum*, *Ochna*, *Ouratea*, *Rhabdophyllum*), several species per genus were sampled.

2.2. Character sampling

For the taxa mentioned in the previous section, we produced sequence data for five loci, viz. the plastid loci *matK*, *ndhF*, *rbcL*, *trnL–trnF* (including the *trnL* intron, *trnL* 3′ exon and *trnL–trnF* intergenic spacer), and the nuclear ribosomal internal transcribed spacer (including ITS 1, 5.8 S, ITS 2).

For most taxa only herbarium specimens were available to obtain DNA. Amplification and sequencing of samples from

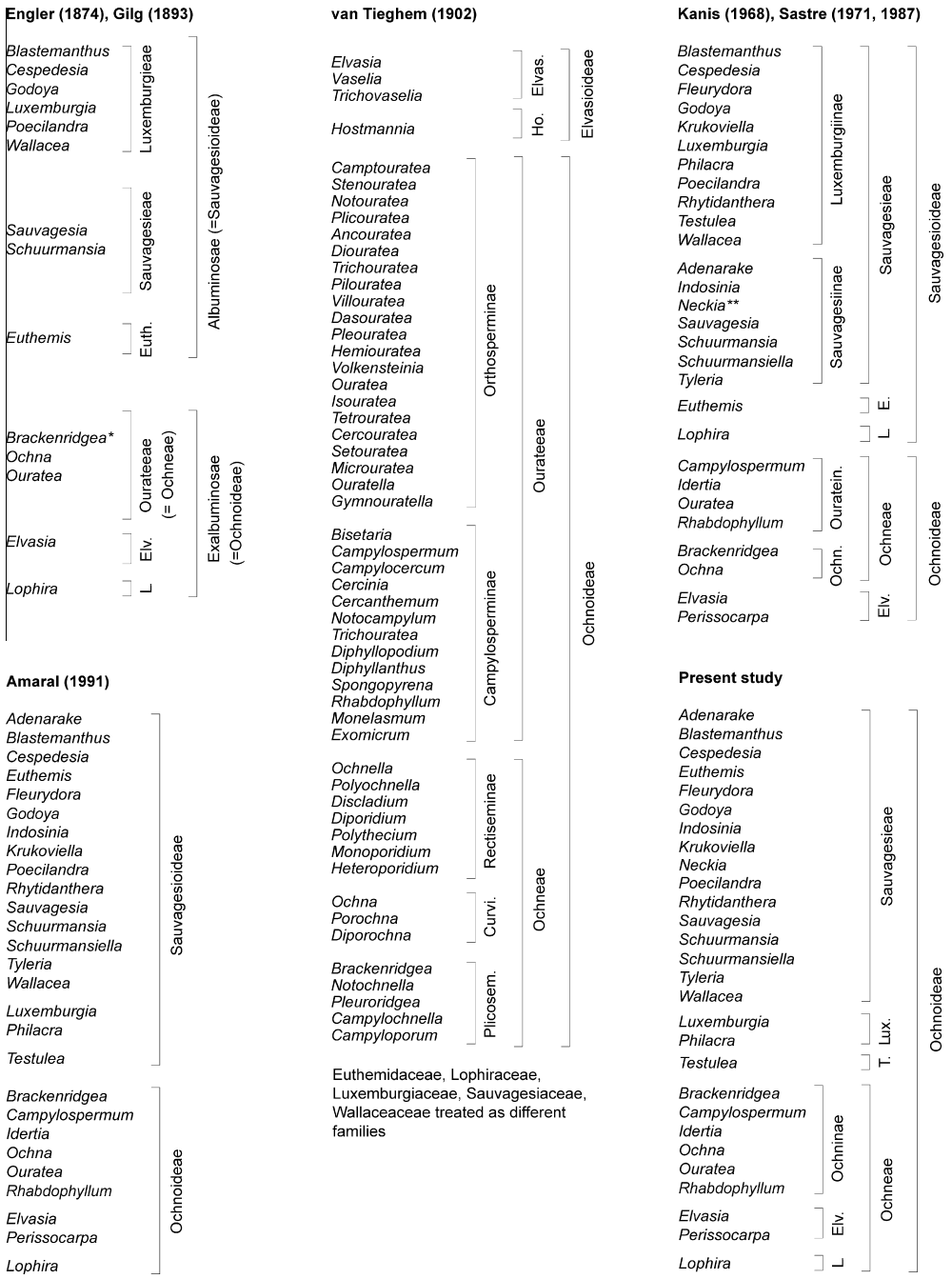

Fig. 1. Important classifications of Ochnaceae in a historical context, including the classification of the present study, showing the ranks of subfamily, tribe and subtribe. An asterisk indicates new additions or changes; ****Neckia** was placed in synonymy of *Sauvagesia* s.l. by Sastre (1971). [a]*Gomphia* is a synonym of *Ouratea*, but in Kanis (1968) it was used for the palaeotropical Ourateinae: *Campylospermum*, *Idertia* and *Rhabdophyllum*. Van Tieghem's (1902) classification, did not include "Sauvagesioideae". Curvi. = Curviseminae; Elv. = Elvasieae or Elvasiinae; E. or Euth. = Euthemideae; Ho. = Hostmannieae; L. = Lophireae or Lophirinae; Lux. = Luxemburgieae; T. = Testuleeae.

herbarium specimens often fails due to the highly degraded nature of the DNA (Särkinen et al., 2012). Thus, taxa represented only by such specimens may contain missing data. Excluding taxa, however, regrettably reducing taxon sampling, would negatively impact phylogenetic analyses as in general a reduced taxon sampling decreases phylogenetic accuracy, is more likely to introduce systematic error, and would lead to a deterioration in parameter estimation in model-based methods (e.g. Rannala et al., 1998; Heath et al., 2008). In particular, our data set represents only 20% of the species diversity in Ochnaceae, and pruning incompletely sampled terminals might give rise to systematic error known as long branch attraction (Felsenstein, 1978; Graybeal, 1998; Kück et al., 2012). On the other hand, it has been shown that the accuracy of phylogenetic analyses is robust to the inclusion of incomplete taxa – even those with large amounts of missing data (Cho et al., 2011; Wiens and Morrill, 2011). Therefore, taxa with incomplete sequence data were included in our data set. In a few cases, adequate representation of taxa of interest was achieved by the combination of data from different collections (Campbell and Lapointe, 2009), thus forming composite terminals (but only combining sequences of different individuals of the same species; not forming composite genera, with the exception of the outgroup genus *Hypericum*; see Table 1).

Table 1
Taxon list with information on herbarium vouchers (herbarium acronym), geographic location and GenBank accession numbers for five DNA regions. Data from additional accessions used in composite taxa are in parentheses; for cultivated material from Botanic Gardens (BG), the garden accession numbers are given.

Taxon	Voucher	Locality	ITS	Accession numbers			
				matK	*ndhF*	*rbcL*	*trnLF*
Outgroups							
Clusia rosea Jacq.				HQ331583	JX662746	HQ332043	AY144069/ AY144095
Garcinia mangostana L.			–	JX661944	JX662752	JX664049	GQ456077
Hypericum spp.			–	HM850929	JX662756	JX664053	KF267872
Quiinoideae							
Froesia diffusa Gereau & Vásquez	Villa & Alvia 256 (MA)	Ecuador: Río Tiputini	KF263222	KF263287	KF263336	KF263409	KF263479
Froesia venezuelensis Steyerm. & G.S.Bunting	Schneider 2 (FR)	Venezuela: Cerro La Chapa	KF263223	KF263288	KF263337	KF263410	KF263480
Lacunaria macrostachya (Tul.) A.C.Sm.	Zárate 16751 (HH)	Peru: Iquitos	KF263169	KF263232	KF263297	KF263346	KF263420
Lacunaria oppositifolia (Pires) Pires	Schneider 11 (FR)	Venezuela: La Esmeralda	KF263224	KF263289	KF263338	KF263411	KF263481
Quiina amazonica A.C.Sm.	Zárate 16753 (HH)	Peru: Iquitos	KF263170	KF263233	KF263298	KF263347	KF263421
Quiina pteridophylla (Radlk.) Pires	Schneider 22 (FR)	Venezuela: Pto. Ayacucho	KF263213	KF263291	KF263340	KF263412	KF263483
Quiina tinifolia Planch. & Triana	Schneider 7 (FR)	Venezuela: La Esmeralda	KF263226	KF263280	KF263328	KF263402	KF263471
Touroulia guianensis Aubl.	Prévost et al. 4595 (FR)	French Guiana: Montagnes Plomb	KF263214	KF263281	KF263329	KF263403	KF263472
Medusagynoideae							
Medusagyne oppositifolia Baker	RBGE 20030393 (ITS, *ndhF*); GenBank (*matK*, *rbcL*, *trnL-F*)	Seychelles	KF263188	JX661953	KF263309	JX664059	AY763244/ AY763259
Ochnoideae							
Adenarake muriculata Maguire & Wurdack	Maguire et al. 60447 (NY) (Kew DNA bank #3146)	Brazil: Serra da Neblina	–	KF263231	–	KF263345	KF263419
Blastemanthus sprucei Tiegh.	Amaral s.n (Kew DNA bank #2987)	Brazil	–	KF263229	KF263295	KF263343	KF263416
Brackenridgea palustris Bartell. (subsp. *kjellbergii* Kanis)	Aiyen Tjoa 25/S25 (L) (*rbcL*: Niyomdham 1102 [L])	Indonesia: S-Sulawesi (Thailand: Tak Bai)	KF263182	KF263240	KF263304	KF263376	KF263430
Brackenridgea zanguebarica Oliv.	Schultka K147 (FR) (*rbcL*: Reitsma 225 [WAG])	Kenya: Coast	KF263225	KF263290	KF263339	KF263389	KF263482
Campylospermum duparquetianum (Baill.) Tiegh.	Dauby 2135 (WAG)	Gabon: Ogooué-Maritime	KF263195	KF263252	KF263317	KF263370	KF263443
Campylospermum dybovskii Tiegh.	Wieringa 5455 (WAG)	Gabon: Cap Esterias	KF263196	KF263253	–	KF263371	KF263444
Campylospermum excavatum (Tiegh.) Farron	Bissiengou 1230 (WAG)	Cameroon: South Province	KF263205	KF263266	–	KF263387	KF263459
Campylospermum flavum Farron	RBGE 19697363A	RBG Edinburgh	KF263191	KF263247	KF263313	–	KF263438
Campylospermum gabonensis Biss.	Bissiengou 627 (WAG)	Gabon: Ngounié	–	KF263273	KF263325	KF263394	KF263464
Campylospermum glaucifolium Biss.	Bissiengou 1326 (WAG)	Congo (Brazzaville): Niari	KF263210	KF263271	–	KF263393	–
Campylospermum glaucum Farron	Bissiengou 1255 (WAG)	Cameroon: Bipindi	KF263207	KF263268	KF263322	KF263390	KF263461
Campylospermum glomeratum (Tiegh.) Biss.	Bissiengou 1008 (WAG)	Gabon: Lopé	KF263201	KF263261	KF263319	KF263382	KF263454
Campylospermum klainei (Tiegh.) Farron	Bissiengou 1299 (WAG)	Gabon: Libreville	KF263209	KF263270	KF263324	KF263392	KF263463
Campylospermum laeve Farron	Bissiengou 1067 (WAG)	Gabon: Ogooué-Ivindo	–	KF263263	KF263320	KF263384	KF263456
Campylospermum laxiflorum (De Wild. & T.Durand) Farron	Wieringa 6156 (WAG)	Gabon: Ogooué-Lolo	KF263197	KF263254	KF263318	KF263372	KF263445
Campylospermum louisii Biss. & Sosef	Bissiengou 1154 (WAG)	Gabon: Ogooué-Ivindo	KF263203	KF263264	KF263321	KF263385	KF263457
Campylospermum oliveranum Farron	Bissiengou 1239 (WAG)	Cameroon: South Province	KF263206	KF263267	–	KF263388	KF263460
Campylospermum plicatum Tiegh.	Sosef 2744 (WAG)	Gabon: Estuaire	–	KF263245	KF263310	KF263365	KF263435
Campylospermum schoenleinianum (Klotzsch) Farron	Jongkind et al. 8077 (WAG)	Guinea: Nimba Mountains	KF263200	KF263260	–	KF263381	KF263453
Campylospermum spec.	Bissiengou 1038 (WAG)	Gabon: Lopé	KF263202	KF263262	–	KF263383	KF263455
Campylospermum umbricola (Tiegh.) Farron	Bissiengou 1213 (WAG)	Cameroon: Elephant Mont	KF263204	KF263265	–	KF263386	KF263458
Cespedesia spathulata (Ruiz & Pav.) Planch.	Pérez et al. 1407 (MO) (*ndhF*: Panama-Exkursion 2012 no. 107 [FR])	Panamá: Sherman (Panamá: Bocas del Toro)	KF263171	KF263234	KF263316	KF263348	KF263422
Elvasia capixaba Fraga & M.M.Saavedra	Zamborlini 27 (MO)	Brazil: Espirito Santo	KF263172	–	KF263299	KF263349	KF263423
Elvasia calophyllea DC.	Amaral s.n. (Kew DNA bank #2986)	Brazil	–	KF263228	KF263294	KF263342	KF263415
Elvasia elvasioides (Planch.) Gilg	Hurtado 136 (MO)	Costa Rica: Corcovado	–	–	KF263300	KF263350	KF263424
Euthemis leucocarpa Jack	Djungai 028 (K) (Kew DNA bank #21769)	Brunei: Burkit Teraja	KF263167	KF263227	KF263293	KF263341	KF263414
Euthemis minor Jack	Beaman 8418 (L)	Malaysia: Sabah	–	KF263235	–	KF263351	–
Fleurydora felicis A. Chev.	Farron s.n. (WAG) (ITS: Adam 11940 [MO])	Guinea: Kindia	KF263173	–	–	KF263401	KF263470
Godoya obovata Ruiz & Pav.	Weigend et al. 5695 (MO)	Peru: Monobamba	KF263174	KF263236	KF263301	KF263352	KF263425
Idertia axillaris (Oliv.) Farron	Bissiengou 1291 (WAG)	Cameroon: Mafoko-Kindongi	KF263208	KF263269	KF263323	KF263391	KF263462

Idertia axillaris (Oliv.) Farron [formerly *Idertia morsonii* (Hutch. & Dalziel) Farron]	Jongkind et al. 6618 (WAG)	Liberia: Geeblo Town	KF263211	KF263276	–	KF263397	KF263466
Krukoviella disticha (Tiegh.) Dwyer	Neill et al. 15849 (MO)	Ecuador: Cordillera del Condor	KF263194	KF263250	KF263315	KF263368	KF263441
Lophira alata Banks ex C.F. Gaertn.	RBGE 20110701A (matK: Bissiengou 1409 [WAG])	Gabon: Ngounié	KF263190	KF263272	KF263312	KF263367	KF263437
Lophira lanceolata Tiegh. ex Keay	GenBank (ITS: Schmidt et al. 1902 [FR])	Ghana	KF263215	FJ670029	–	FJ670172	–
Luxemburgia ciliosa (Mart.) Planch.	Arbo et al. 4114 (Kew DNA bank #2326) (*matK*: Irwin et al. 20041 [L])	Brazil (Brazil: Serra do Cipó)	KF263216	KF263256	KF263330	KF263404	KF263473
Luxemburgia damazioana Beauverd	Feres et al. 98/37 (MO)	Brazil: Serra do Cipo	KF263175	KF263237	–	KF263353	KF263426
Luxemburgia schwackeana Taub.	Vita s.n. (NY) (Kew DNA bank #3212)	Brazil	KF263217	KF263282	KF263331	–	KF263474
Ochna afzelii R.Br. ex Oliv.	Mwangoka et al. 4849 (MO)	Tanzania: Ntakata	KF263176	KF263238	KF263302	KF263354	KF263427
Ochna integerrima (Lour.) Merr.	ITS: Svengsuksa et al. BT 225 (L); *rbcL* & *trnL-F*: Newman et al. LAO56 (L)	Laos: Khammouan	KF263177	–	–	KF263355	KF263449
Ochna macrantha Baker	Jongkind et al. 3479 (WAG)	Madagascar: Tsingy de Bemaraha	–	KF263279	–	KF263400	KF263469
Ochna membranacea Oliv.	Jongkind et al. 9584 (WAG)	Liberia: Nimba	KF263212	KF263278	KF263327	KF263399	KF263468
Ochna mossambicensis Klotzsch	Schultka K35 (FR)	Kenya	KF263218	KF263283	–	KF263405	–
Ochna multiflora DC.	Schneider 3077 (LZ)	BG Leipzig	–	KF263284	KF263332	KF263406	KF263475
Ochna natalitia (Meisn.) Walp.	RBGE 19490083B	RBG Edinburgh	KF263189	KF263246	KF263311	KF263366	KF263436
Ochna polycarpa Baker	Phillipson et al. 2869 (WAG)	Madagascar: Toliara	–	KF263277	–	KF263398	KF263467
Ochna serrulata Walp.	FHK46M (BG Utrecht University 0G 1984GR00402)	South Africa	–	KF263251	–	KF263369	KF263442
Ouratea erecta Sastre	Jansen-Jacobs et al. 6712 (L)	Suriname: Lely Mountains	–	KF263255	–	KF263374	KF263447
Ouratea lucens (Kunth.) Engl.	Stevens & Montiel 27915 (MO)	Nicaragua: Cuapa	KF263193	KF263249	KF263314	–	KF263440
Ouratea polyantha (Triana & Planch.) Engl.	Davidse 2773 (L)	Venezuela: Pto. Ayacucho	KF263198	–	–	KF263373	KF263446
Ouratea schomburgkii (Planch.) Engl.	Jansen-Jacobs et al. 6775 (L)	Suriname: Lely Mountains	–	–	–	KF263375	KF263448
Ouratea scottii Sastre	Chatrou 405 (WAG)	Bolivia: Riberalta	–	KF263257	–	KF263377	KF263450
Ouratea spec.	Lachenaud 1044 (BR)	French Guiana: Saut Takari Tante	KF263199	KF263259	–	KF263380	KF263452
Ouratea striata (Tiegh.) Urb.	Kuba-Exkursion 144 (FR)	Cuba	KF263219	–	KF263333	KF263407	KF263476
Ouratea vaccinioides Engl.	Seele 736 (LZ)	Brazil: Teresópolis	KF263220	KF263285	KF263334	–	KF263477
Philacra auriculata Dwyer	Amaral 9/96	Brazil	KF263221	KF263286	KF263335	KF263408	KF263478
Philacra auriculata Dwyer	Pipoly & Samuels 6867 (MO)	Brazil	KF263178	–	KF263303	KF263356	KF263428
Poecilandra retusa Tul.	Kelloff et al. 1049 (MO)	Guyana: Kaieteur National Park	KF263179	–	–	KF263357	–
Rhabdophyllum arnoldianum	Sosef 2239 (WAG)	Gabon: Ogooué-Ivindo	–	KF263258	–	KF263378	KF263451
Rhabdophyllum calophyllum (De Wild. & T.Durand) Tiegh.	Bissiengou 767 (WAG)	Gabon: Woleu-Ntem	–	KF263274	–	KF263395	–
Rhabdophyllum letestui Farron	Bissiengou 807 (WAG)	Gabon: Woleu-Ntem	–	KF263275	KF263326	KF263396	KF263465
Rhytidanthera splendida (Planch.) Tiegh.	Steyermark & Liesner 119012 (MO)	Venezuela: Cerro Las Minas	KF263180	–	–	–	–
Sauvagesia erecta L.	Nikolov 1846 (MO) (*rbcL*: Lachenaud 1018 [BR])	Madagascar: Analanjirofo (French Guaina: Savanes de Combi)	KF263192	KF263248	–	KF263379	KF263439
Sauvagesia fruticosa Mart. & Zucc.	Zarucchi & Balick 1780 (MO)	Colombia: Mitu	KF263181	KF263239	–	KF263358	KF263429
Sauvagesia serrata (Korth.) Sastre	Pater Agatho Elsener H205 (L)	Indonesia: West Borneo	KF263183	KF263241	–	KF263359	KF263431
Sauvagesia tafelbergensis Sastre	de Granville et al. 14953 (L)	French Guiana: Mont Bakra	KF263184	KF263242	–	KF263360	KF263432
Schuurmansia elegans Blume	Johns 9814 (K) (Kew DNA bank #18433) (*rbcL*: Yumte 229 [L])	Indonesia: Irian Yaya	KF263166	–	KF263292	KF263361	KF263413
Schuurmansiella angustifolia (Hook.f.) Hallier.f.	van Balgooy 7344 (L)	Malaysia: Sarawak	–	–	–	KF263362	–
Testulea gabonensis Pellegr.	Wieringa 6171 (WAG)	Gabon: Ogooué-Ivingo	KF263187	KF263244	KF263308	KF263364	KF263434
Tyleria silvana Maguire	Chase 3086 (Kew DNA bank #3086)	Brazil: Amazonia	KF263168	KF263230	KF263296	KF263344	KF263418
Wallacea insignis Spruce ex Benth. & Hook.f.	Berry et al. 5926 (MO) (*trnL-F*: Amaral s.n., Kew DNA bank #2994)	Venezuela: Laja Suiza (Brazil: Amazonas)	KF263185	KF263243	KF263306	KF263363	KF263417

2.3. DNA isolation, PCR and sequencing

Genomic DNA was isolated from leaves of herbarium specimens or silica gel-dried leaves using different methods: (1) the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's protocol; (2) the CTAB method (Doyle and Doyle, 1987) with a precipitation time of up to one week for herbarium specimens; (3) the AnaP method with PTB (*N*-phenacylthiazolium bromide) as an additive as described in Telle and Thines (2008). Tissue (~2–20 mg dry weight; if available, also up to 100 mg) was ground to a fine powder by a Mixer Mill MM200 (Retsch, Haan, Germany).

Amplifications were carried out in 25 µl volumes with 1.25 U of MyTaq DNA polymerase (Bioline, London, UK), 1x MyTaq reaction buffer (containing 3 mM $MgCl_2$, 1 mM of each dNTP, stabilizers and enhancers), 0.3 µM of each primer, and undetermined quantities of genomic DNA template in a Mastercycler S (Eppendorf, Hamburg, Germany). Alternatively, amplifications were performed using MangoTaq of the same manufacturer as described previously (Telle and Thines, 2008). The following primer combinations were used initially: ITS-F1 & -R1; ITS 1/.Leu & ITS 4; *matK*-400F & *trnK*-2R; *ndhF*-310F/331F & 2110R; *rbcL* aF & aR; *trnL-F* c & f (Table 2). If necessary, internal primers were used, and for some of the regions new primers were designed (Table 2). After initial denaturation (4:00 min at 94 °C), polymerase chain reaction (PCR) was performed for 35 cycles of denaturation (0:20 min at 94 °C), primer annealing (0:20 min at 54 °C [ITS], 52 °C [*matK*], 48 °C [*ndhF*, *trnLF*], 55 °C [*rbcL*]), and primer extension (0:30 min at 72 °C). The reactions ended with an elongation period of 7 min at 72 °C. PCR products were electrophoresed on 1% agarose gels in Tris–borate–EDTA buffer and stained with ethidium bromide. Afterwards, PCR bands were excised and purified with the Qiaquick gel extraction kit (Qiagen). Alternatively, unincorporated dNTPs and leftover PCR primers were directly removed in 20 µl reactions using 10 µl of the PCR product, 0.2 U Exonuclease I (New England Biolabs), 0.5 U Shrimp alkaline phosphatase (USB). This reaction mix was incubated for 20 min at 37 °C and deactivated for 20 min at 80 °C in a thermocycler. PCR products were mostly bidirectionally sequenced using the forward and reverse PCR primers (5 pmol) and the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) on an ABI 3730 xl capillary sequencer following the manufacturer's instructions. The edited DNA sequences were submitted to GenBank and accession numbers are listed in Table 1.

Table 2
List of PCR primers used in the present study.

Primer name	Primer sequence (5′-3′)	Reference
ITS		
ITS F1	GATCGCGGCGACTTGGGCGGTTC	Muellner et al. (2005)
ITS R1	GGTAGTCCCGCCTGACCTGGG	Muellner et al. (2005)
ITS.Leu	GTCCACTGAACCTTATCATTTAG	Vargas et al. (1998)
ITS 1	TCCGTAGGTGAACCTGCGG	White et al. (1990)
ITS 2	GCTGCGTTCTTCATCGATGC	White et al. (1990)
ITS 3b	GCATCGATGAAGAACGTAGC	White et al. (1990)
ITS 4	TCCTCCGCTTATTGATATGC	White et al. (1990)
matK		
400F	CCCTAATTTACGATCAATTCATTCAAT	Cameron et al. (2001)
472F	AAATTGGTTCAAACTCTTCGCTACTC	This study
825F	CATTATGTTAGATATCAAGGA	This study
1039F	GTACGGAGTCAAATGCTAG	This study
781R	TTAACA(M)AGACTTCTGCA	This study
887R	TTCATCAGAAGAGGCGTATCC	This study
1166R	TCGGATTACTAATGGGATG	This study
1184R	TCGGATTACTAATGGGATG	This study
1616R	TACTCGTATACTGCATGAGCA	This study
trnK2R	AACTAGTCGGATGGAGTAG	Johnson and Soltis (1995)
ndhF		
310F	GCCTTTTATATGTTTCGA	This study
331F	TATTTACTTACTTTTGAAGGG	This study
Och637F	CCTATACTTGTTTTAGTACTA	This study
Qui637F	CCTATACTTGTTTTAGCACTA	This study
838R	AATAAGCTATACTGACTGA	This study
2110R	CCCCCTAYATATTTGATACCTTCTCC	Olmstead and Sweere (1994)
rbcL		
aF	ATGTCACCACAAACAGAGACTAAAGC	CBOL Working Group (2009)
aR	GTAAAATCAAGTCCACCRCG	CBOL Working Group (2009)
trnL-F		
c	CGAAATCGGTAGACGCTACG	Taberlet et al. (1991)
d	GGGGATAGAGGGACTTGAAC	Taberlet et al. (1991)
e	GGTTCAAGTCCCTCTATCCC	Taberlet et al. (1991)
f	ATTTGAACTGGTGACACGAG	Taberlet et al. (1991)

2.4. Sequence alignment and phylogenetic analysis

Sequence alignment was done with the program mafft, version 7.015b (Katoh et al., 2002, 2009), using the L-INS-I algorithm with "maxiterate" set to 1000 (for ITS, the Q-INS-I algorithm); if necessary, the obtained alignment was subsequently adjusted manually. Alignment gaps (indels) were treated as missing data. The alignment was generally straightforward, except for the ITS region which contained several highly variable parts. Ambiguous or hypervariable alignment positions were excluded manually (for the *trnL-F* region) or, for the ITS region, using the software Gblocks 0.91b (Castresana, 2000; Talavera and Castresana, 2007) with default settings.

Because of the large proportion of unequal taxon sampling between the five regions, topology-based incongruence tests (Shimodaira–Hasegawa test; Shimodaira and Hasegawa, 1999) are either impossible or, as in the case of the incongruence length difference test (Farris et al., 1994), prohibitively time-consuming. Instead, we performed ML analyses for each region separately as outlined below and searched for well-supported significant incongruence (e.g., Couvreur et al., 2010). Due to the lack of such incongruence, the regions were combined following a supermatrix or total evidence approach (Eernisse and Kluge, 1993; de Queiroz and Gatesy, 2007). Data concatenation was done with the program SequenceMatrix, version 1.78 (Vaidya et al., 2011).

Tree searches were performed using maximum likelihood and Bayesian approaches. Prior to these analyses, the best-fit substitution model was determined using Modeltest 3.7 (Posada and Crandall, 1998). Based on the Akaike Information Criterion (AIC), different substitution models were suggested for each of the analyzed regions: TrN + I + G for ITS, GTR + G for *matK*, TVM + I + G for *ndhF*, GTR + G for *rbcL*, and TVM + G for *trnLF*. Maximum likelihood analyses were carried out using RAxML (Stamatakis, 2006) and the raxmlGui 1.3 (Silvestro and Michalak, 2012), setting partitions for each region, the model to GTRGAMMA and the bootstrap (BS) analysis to 1000 replicates.

For the Bayesian analysis of the concatenated data, the same partitions were defined as for the RAxML analysis. Analyses were performed with MrBayes, version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist et al., 2012), employing a Markov chain Monte Carlo (MCMC) procedure in order to simultaneously estimate an optimal phylogenetic tree and the posterior probabilities (PP) of interior branches (Rannala and Yang, 1996; Li et al., 2000). Four parallel Markov chains, incrementally heated by a temperature of 0.2, were run for 10 million generations, sampling every five hundredth generation. Convergence of runs was tested by inspecting whether the standard deviation of split frequencies of the runs was <0.01 and by using the effective sample sizes (ESS) as calculated with Tracer 1.4 (Rambaut and Drummond, 2007), considering ESS values >200 as good evidence. The first 2,500,000 generations

were discarded, and a consensus tree calculated from the remaining trees.

Analyses were partially run on the Cyberinfrastructure for Phylogenetic Research (Cipres Science Gateway; <http://www.phylo.org/index.php/portal/>; Miller et al., 2010). Phylogenetic trees were visualized in FigTree, version 1.4 (http://tree.bio.ed.ac.uk/software/figtree/).

2.5. Phylogenetic hypothesis testing

For a statistically based comparison between competing phylogenetic hypotheses and their associated classifications (i.e., the phylogenetic hypothesis based on the present study compared with those following previous classifications as outlined in Fig. 1), we used Bayesian inference. To test whether one phylogenetic hypothesis is strongly preferred over an alternative phylogenetic hypothesis, we estimated the marginal likelihoods of the different hypotheses, i.e., the models M_0, M_1, etc., using the stepping stone sampling approach (Xie et al., 2011) implemented in MrBayes, version 3.2.1 (Ronquist et al., 2012). For each model, we used an informed prior (i.e., monophyly constraints), following the "preferred" strategy of Bergsten et al. (2013). Thus, for each hypothesis we defined hard constraints for all major clades (see Fig. 2). For the stepping stone approach we set the number of generations to 7,500,000, the alpha-shape parameter to 0.3 – a value within the range Xie et al. (2011) found optimal – , the sampling fequency to 500, nsteps to 50, and the default for the burn-in (i.e., there are 147,000 generations per step). Convergence was assessed based on the standard deviation of split frequencies. According to Kass and Raftery (1995), there is strong support for a phylogenetic hypothesis if the 2ln Bayes factor ($2\ln B_{10}$) – resulting from the difference between the marginal likelihoods of both hypotheses – is >10.

2.6. Ancestral state reconstruction

The ancestral states of eight discrete morphological characters, which identify major groups of Ochnaceae in pre-molecular classifications, were inferred. Character states were scored for all taxa included in the molecular phylogeny following Amaral (1991) and Amaral and Bittrich (2014) for Ochnaceae s.str., Schneider and Zizka (in press) for Quiinaceae, and Dickison (1990) for *Medusagyne*. Two characters, carpel number and anther dehiscence, show considerable intrageneric variability. To reflect that polymorphism (which is not fully covered by the taxon sampling), all congeners were assigned a genus-level score which covers the range of character states. All characters are binary or multistate (for character states of terminals see Table S1 and Fig. 4).

Ancestral state reconstructions were performed using BayesTraits, version 2.0 (beta) (Pagel et al., 2004; Pagel and Meade, 2006). Pooled trees from independent runs of Bayesian analysis (see above) were loaded in Mesquite, version 2.75 (Maddison and Maddison, 2011) specifying a burn-in fraction of 0.25, as in the Bayesian analysis above. Trees were rooted with the outgroup taxa using the "Root tree with selected taxa as outgroup" option of the Alter/Transform module. Subsequently, the outgroups were pruned and a subsample of 1000 post-burnin trees produced with the option "sample trees from separate NEXUS file". Based on the Bayesian consensus tree, well-supported crown nodes of the major taxonomic lineages were selected for ancestral state reconstruction. The command lines for the specified nodes as required by the addMRCA command in BayesTraits were generated with the programme BayesTrees, version 1.3 (Meade and Pagel, 2011). Ancestral state reconstructions were performed for each character separately using the module MultiState as implemented in BayesTraits (Pagel et al., 2004; Pagel and Meade, 2006) and the sample of 1000 post-burnin trees. Initially, a maximum likelihood analysis was run to derive an empirical prior. Preliminary Bayesian analyses were run with a reversible jump Markov chain Monte Carlo approach, an exponential hyperprior with the mean seeded from a uniform distribution of 0–20, 10×10^6 generations, sampling every 1000th generation, discarding the first 2,500,000 iterations as burnin, and varying RateDev parameters until acceptance rates reached values of 20–40% to ensure adequate mixing. Finally, ancestral states were reconstructed for the selected nodes with the adjusted RateDev parameter and the same settings as before but running the analysis for 50×10^6 generations and sampling every 10,000th generation. Three independent runs were performed to check for stationarity of the harmonic means. If probability values for a given state were <0.70, alternative states were tested using the fossil command in BayesTraits and calculating the Bayes factor (BF) as described in the programme's manual. A BF of two to five indicates positive, and a BF of greater than five indicates strong support.

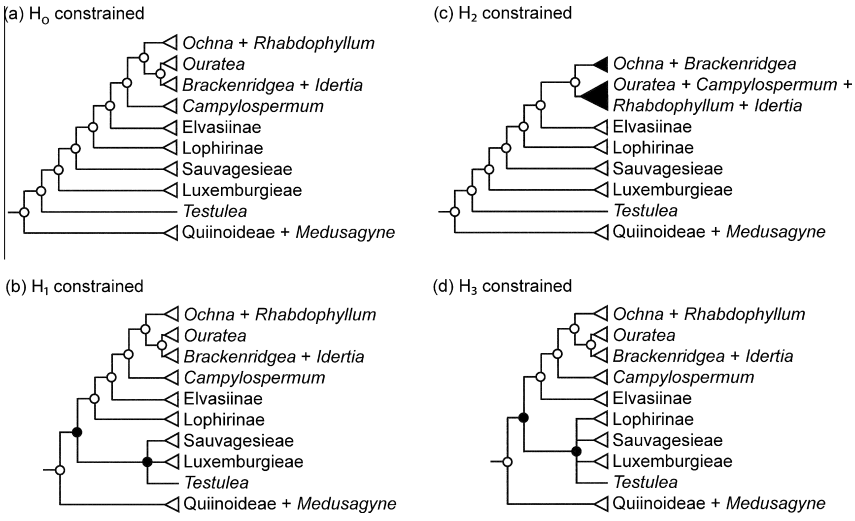

Fig. 2. Phylogenetic hypotheses used for Bayesian hypothesis testing. The null hypothesis H_0, which corresponds to the phylogeny obtained in the present study, is compared to three alternative phylogenetic hypotheses (H_1–H_3) that represent assumptions about phylogenetic relationships from previous classifications of Ochnaceae. Monophyly constraints are defined for all major clades (open circles and triangles). Solid circles and triangles indicate different constraints compared to H_0.

3. Results

3.1. Sequences/matrices

Our concatenated data set contained 3932 nucleotide sites after the exclusion of ambiguous characters. All 63 ITS and 69 *matK*, 49 *ndhF*, 75 *rbcL*, and 72 *trnL-F* sequences were newly obtained for this study. For 33 taxa all five gene regions were successfully sequenced, for 28 taxa four regions, for 15 taxa three regions, for four taxa two regions, and for two taxa only one region could be obtained (in some cases only partially). Important characteristics of the sequences and the alignment are summarized in Table 3.

3.2. Phylogenetics of Ochnaceae s.l

Maximum likelihood and Bayesian analyses of the combined data produced largely congruent trees and contained no well-supported incongruences. The names of suprageneric taxa used in this section follow the new classification presented here (see Section 4). Ochnaceae were maximally supported as monophyletic (100% BS, 1.0 PP), and Ochnoideae were inferred as sister to a clade of Medusagynoideae and Quiinoideae, though the latter is weakly supported only (Fig. 3). *Testulea* emerged as sister to the rest of Ochnoideae (100% BS, 1.0 PP). Within Quiinoideae all nodes received maximum support: *Froesia* Pires is sister to the rest of Quiinoideae, and *Quiina* Aubl. is sister to a clade of *Touroulia* Aubl. and *Lacunaria* Ducke. The clade of *Luxemburgia*/*Philacra* (99% BS, 1.0 PP) formed the sister clade to the remaining genera of Ochnoideae (100% BS, 1.0 PP). The clades of the tribes Sauvagesieae (96%, 1.0 PP) and Ochneae (100% BS, 1.0 PP) – the former Sauvagesioideae *pro parte* and Ochnoideae – are monophyletic and sister to each other (96% BS, 1.0 PP). Within Sauvagesieae, *Blastemanthus* is sister to the the rest of this tribe (with low support), and a clade uniting *Cespedesia* Goudot, *Godoya* Ruiz & Pav., *Krukoviella* A.C.Sm. and *Rhytidanthera* Tiegh. is sister to the remaining genera, although with weak support. *Wallacea* Spruce ex Benth. & Hook.f./*Poecilandra* Tul. are sister to a clade comprising *Adenarake* Maguire & Wurdack, *Euthemis* Jack, *Sauvagesia*, *Schuurmansia* Blume, *Schuurmansiella* Hallier f., and *Tyleria* Gleason (100% BS, 1.0 PP). *Sauvagesia* is polyphyletic when including the Old World species *Sauvagesia serrata* (Korth.) Sastre, as this species is sister to a clade consisting of *Adenarake*, the New World species of *Sauvagesia*, and the clade of *Euthemis*, *Schuurmansia*, *Schuurmansiella*, and *Tyleria*. The clade of *Euthemis* and *Schuurmansiella* is sister to *Schuurmansia*, but their relationships are poorly supported. Within Ochneae, *Lophira* and *Elvasia* DC. are strongly supported as subsequent sister lineages to the clade composed of the remaining genera (100% BS, 1.0 PP) as is *Elvasia* to the remaining genera of the tribe (96% BS, 1.0 PP). The relationships within Ochninae are less clear due to low support values, although the analyses infer each of the genera as monophyletic.

Maximum likelihood analyses of each of the five regions separately (topologies not shown) did not show significant differences compared to the analysis of the combined data with the exception that *Idertia* changed the position with *Elvasia* as sister to the rest of Ochneae (without *Lophira*; 100% BS) in the *trnL-F* tree.

3.3. Hypothesis testing

A comparison of the marginal likelihoods obtained from the stepping stone approach revealed that the null hypothesis (H_0; see Fig. 2), which corresponds to the tree obtained from Bayesian inference, is strongly preferred over each of the alternative phylogenetic hypotheses H_1–H_3. The mean log likelihood of H_0 was −20436.85, whereas H_1 (enforcing a clade of Sauvagesieae, *Testulea*, *Luxemburgia* and *Philacra*) received a log likelihood of −20532.21. The hypothesis H_2, forcing Ochninae (*Brackenridgea*, *Ochna*) and Ourateinae (*Campylospermum*, *Idertia*, *Ouratea*, *Rhabdophyllum*) as circumscribed in previous classifications to be monophyletic, received a log likelihood of −20452.31. The log likelihood for the hypothesis (H_3) forcing *Lophira* to be included in a clade of Sauvagesieae, *Testulea*, *Luxemburgia* and *Philacra* was −20557.48. Thus, the Bayes factors ($2\ln B_{10}$) for the comparisons between H_0 and each of the alternative hypotheses are >10.

3.4. Ancestral state reconstruction

Character state reconstructions showed that zygomorphic flowers are ancestral and actinomorphic flowers derived in Ochnoideae. The presence of zygomorphy already at early stages of flower development has a higher probability than zygomorphy that develops only at anthesis (Fig. 4, node 3; PP 0.57 [BF 3.0] versus 0.33; see also Table S2). A reversal to actinomorphic flowers arose independently in Ochneae (node 9, PP 0.97) and the clade uniting *Sauvagesia* s.l. and related genera (node 7, PP 0.98). The lack of endosperm is probably ancestral in Quiinoideae (node 2, PP 0.86), but derived in Ochnoideae (node 3, PP 0.8). A syncarpous gynoecium is ancestral in Ochnaceae (node 1, PP 0.99), and it was most likely present in the most recent common ancestors (MRCA) of Quiinoideae (node 2, PP 0.66, BF 5.8) and Ochnoideae (node 3, PP 0.98) too. A near-apocarpous gynoecium evolved twice, once in Quiinoideae (*Froesia*) and once in the entire Ochnineae. Ovule numbers of 4–50 are probably the ancestral condition in Ochnoideae (node 3, PP 0.71), whereas two ovules per carpel is the ancestral condition for Ochnaceae (node 1, PP 0.85). A single ovule per carpel is a derived state that emerged early in the evolution of Ochneae, along the branch subtending Elvasiinae + Ochninae (nodes 10). The evolution of carpel number along the backbone of Ochnaceae is unclear due to the lack of strong support for any of the states. However, either two or five carpels is inferred as the ancestral condition in Ochnoideae (e.g. nodes 3, 5, 8), whereas three carpels is ancestral in *Sauvagesia* s.l. and related taxa (node 7, PP 0.95). For Quiinoideae (node 2), the ancestral number of carpels remains undetermined. Anther dehiscence was poricidal in the MRCA of Ochnoideae (node 3, PP 0.91) and in all other major clades of that subfamily. Only in *Sauvagesia* s.l. and related taxa (node 7), the ancestral state is unclear. Therefore, dehiscence by longitudinal slits, either over most of the length of the anther or confined to the apical zone, is a derived state in Ochnoideae. Septicidal capsules are ancestral in Ochnaceae (node 1, PP 0.67, BF 1.54) and Ochnoideae (node 3, PP 0.84). Nut-like to samaroid fruits are the ancestral condition in Ochneae (node 9, PP 0.80). Seeds with wings is probably ancestral in Ochnoideae (node 3, PP 0.84). In contrast, the loss of wings in the MRCA of Ochneae is well supported (node 9, PP 0.92). In Sauvagesieae the loss of wings is also derived.

4. Discussion

4.1. Relationships and circumscription of Ochnaceae

Earlier molecular phylogenetic studies revealed that Medusagynaceae, Ochnaceae, and Quiinaceae form a well-supported clade within Malpighiales, but relationships between all three have long been unclear due to the lack of support in earlier molecular phylogenetic studies (Fay et al., 1997; Savolainen et al., 2000; Davis and Chase, 2004). This phylogenetic uncertainty together with the opportunity to eliminate a monogeneric family Medusagynaceae led APG III (APG, 2009) to unite the three families, forming a broadly defined Ochnaceae. In contrast, in some more recent studies all three families are kept separate because

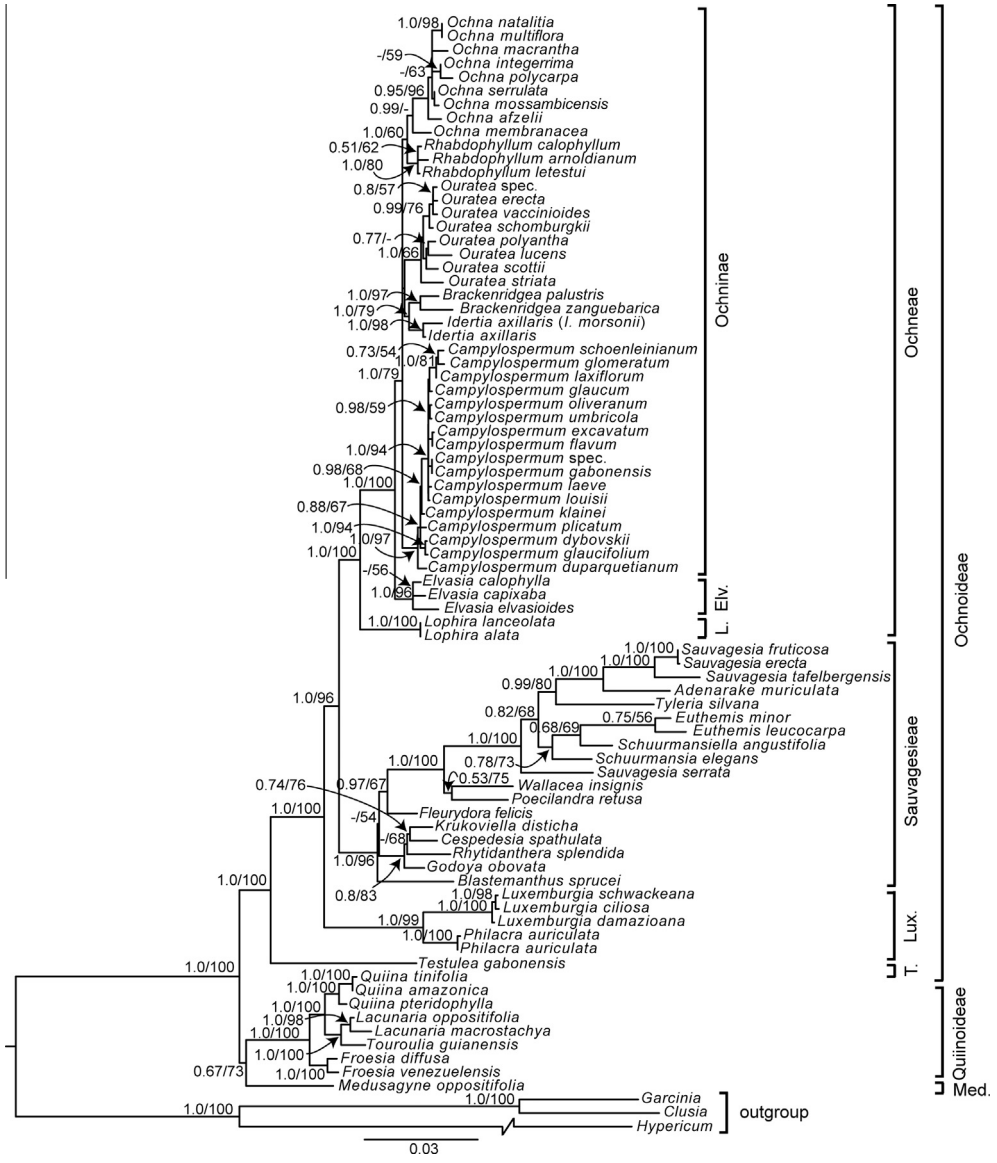

Fig. 3. Maximum likelihood tree of Ochnaceae s.l. based on the combined DNA regions with posterior probabilities obtained from Bayesian Inference and bootstrap support from RAxML analysis (only values ⩾ 0.5 [PP] or 50% [BS] are shown). The ranks and suprageneric names are according to the new classification presented here; Ochnaceae, Medusagynaceae and Quiinaceae are accepted at the rank of subfamily, in accordance with APG III (2009). Elv. = Elvasiinae; L. = Lophirinae; Lux. = Luxemburgieae; Med. = Medusagynoideae; T. = Testuleeae.

they form distinct clades that are also morphologically well characterized (Wurdack and Davis, 2009; Matthews et al., 2012; Xi et al., 2012; Endress et al., 2013). As inferred from a phylogenomic study, Ochnaceae are sister to a clade of Medusagynaceae and Quiinaceae (Xi et al., 2012). In the present study, which is based on the most complete taxon sampling of Ochnaceae to date, the relationships between the three families remain, unfortunately, poorly supported, but Ochnaceae and Quiinaceae form a well-supported clades.

There are several potential synapomorphies in support of a broadly defined Ochnaceae including, for example, nectarless flowers, sepals often being of different size within flowers and with more than three vascular traces, petals reflexed over the sepals and directed toward the pedicel, anthers basifixed or almost basifixed, and the presence of a short gynophore (Matthews et al., 2012; Endress et al., 2013). However, the families in the narrow sense are also morphologically well characterized by a set of potential apomorphies. Ochnaceae s.str. share adaptations to buzz-pollination with poricidal anthers, the stamen filaments with an abruptly narrow attachment zone of the anthers, and a crystal layer in the endotesta (Amaral, 1991; Matthews et al., 2012). The alternate phyllotaxis and the often fimbriate or intrapetiolarly fused stipules also distinguish them from Medusagynaceae and Quiinaceae, in which phyllotaxis is opposite and whorled, respectively, and stipules are interpetiolar (Quiinaceae) or absent (Medusagynaceae). The formation of morphologically and/or functionally unisexual flowers characterizes Medusagynaceae and Quiinaceae (only lacking in *Froesia*), whereas in Ochnaceae this is restricted to *Euthemis*, *Schuurmansia*, and *Schuurmansiella* in Sauvagesioideae (Kanis, 1968; Amaral, 1991). Potential apomorphies of Quiinaceae are a floral cup, a conspicuously ribbed ovary at anthesis with the ribs being not transversally subdivided, but vascularized, and in each locule there are two superimposed (collateral in *Froesia*) ovules that face in the same direction (Matthews et al., 2012), whereas in Medusagynaceae there are also two superimposed ovules in each locule but the micropyles of the ovules are directed towards each other, while the ovary ribs are transversally subdivided and not vascularized.

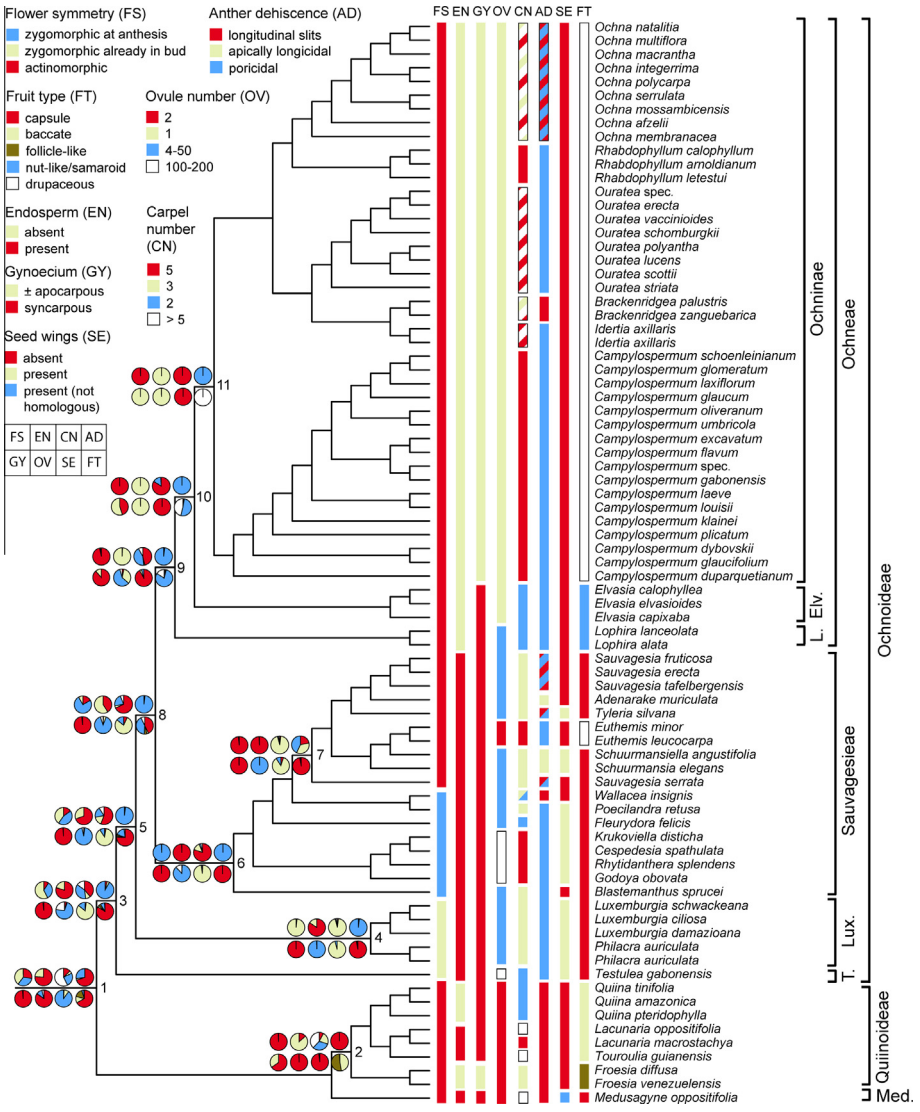

Fig. 4. Ancestral state reconstructions for eight taxonomically important morphological characters of well-supported major clades of Ochnaceae. The proportions of the posterior probabilities (see also Table S2) are displayed as pie charts (for arrangement of characters see the rectangular scheme). Node numbers refer to nodes analyzed in BayesTraits. Character scores are color-coded. To represent intrageneric variability in carpel number and anther dehiscence, all congeners were assigned a genus-level score which covers the range of the character states (see bars with mixed colors; including states not covered by the taxon sampling of the phylogeny). For the classification, see Fig. 3 (outgroups not shown).

Table 3
Alignment characteristics according to region.

	ITS	*matK*	*ndhF*	*rbcL*	*trnL-F*
Alignment length	626	1315	858	552	1048
# Characters excluded	273	21	–	–	173
# Variable characters	135	517	250	80	333
# Constant characters	218	777	608	472	542

Leaf venation is an important character that unites Medusagynaceae, Ochnaceae and Quiinaceae (e.g., Hickey and Wolfe, 1975), but it also distinguishes Quiinaceae from the other two. In Quiinaceae and Ochnaceae there is a tendency for parallel and densely spaced secondary and/or tertiary veins. However, the extremely dense and parallel tertiary veins of Quiinaceae that are perpendicular to the secondary veins are unique and also a potential apomorphy (Schneider et al., 2002, 2006). Shared features of a clade uniting Medusagynaceae and Quiinaceae are, for example, extreme polystemony, a massive thecal septum that persists after anther dehiscence, styles radiating outward from the ovary, two lateral superimposed ovules per carpel, and conspicuous lateral ribs on the ovary wall at anthesis (Matthews et al., 2012). Vestured pits, which have been supposed to contain a strong phylogenetic signal (Jansen et al., 2001), may be a synapomorphy of Ochnaceae s.l.: they characterize Ochnaceae (except *Sauvagesia*) and Medusagynaceae (Jansen et al., 2001), and Gottwald and Parameswaran (1967) also reported them for Quiinaceae, but Jansen et al. (2001) could not confirm their presence in that family.

In summary, there are good arguments from anatomy, morphology and molecular phylogenetics for both a narrow and a broad circumscription of Ochnaceae. Which one to choose is therefore a rather subjective decision. However, there are some additional points to consider. First, because stability of classifications is desirable (Godfray and Knapp, 2004) and because APG III (2009), with a broadly defined Ochnaceae, is now widely accepted and adapted in recent flora works, classifications and databases (e.g., Fiaschi et al., 2010; Stevens, 2001 onwards; Tropicos, 2013), keeping Ochnaceae s.l. is preferable. Second, elimination of

monogeneric families is also a desirable goal of modern classifications (e.g., APG III, 2009). Here, the question is whether the monogeneric Medusagynaceae are sufficiently distinct from the other two families to merit recognition at family rank comparable to, for example, Platanaceae (see APG III, 2009). Compared to Platanaceae, distinction of Medusagynaceae is, however, not strong. Thus, given the basic similarity of the three families with numerous potential synapomorphies for the Ochnaceae s.l. clade and the arguments outlined above, we agree with the classification of APG III (2009) in merging all three families.

4.2. Infrafamilial relationships and classification

Accepting a broadly defined Ochnaceae has immediate consequences for infrafamilial classification. Because Medusagynaceae and Quiinaceae are recognized at the rank of subfamily, infrafamilial names of previous classifications of Ochnaceae have to be accommodated accordingly. For example, subfamily Ochnoideae sensu Amaral (1991) will here be recognized at the rank of tribe. Thus, Ochneae and Sauvagesieae correspond to the Sauvagesioideae and Ochnoideae of pre-molecular classifications and to Engler's (1874) "Exalbuminosae" and "Albuminosae."

The separation into infrafamilial groups based on the absence (=Ochneae; Exalbuminosae) or presence of endosperm (=Sauvagesieae; Albuminosae) as suggested by Engler (1874) finds partial support in the monophyletic Ochneae in the present analysis. However, Sauvagesieae in their traditional circumscription (including *Testulea*, *Luxemburgia*, and *Philacra*) are paraphyletic because *Testulea* is the sister group of the rest of Ochnoideae and a clade with *Luxemburgia* and *Philacra* is the next sister group to the rest of the subfamily (Fig. 3). Therefore, it is necessary to elevate *Testulea* and *Luxemburgia* + *Philacra* to tribal rank. There are morphological characters (probably apomorphic) supporting tribes Luxemburgieae and Testuleeae: *Testulea* differs from the rest of Ochnoideae in having tetramerous flowers with only one bracteole, a single fertile stamen and the staminodes fused into a column up to two thirds of their length, besides leaves with brochidodromous venation; *Luxemburgia* and *Philacra* differ in flowers that are obliquely zygomorphic already in bud with the stamens surrounding the ovary only adaxially and filaments that are basally or completely fused. Thus, in our new classification Ochnoideae (former Ochnaceae s.str.) contain tribes Ochneae, Sauvagesieae, Luxemburgieae and Testuleeae.

The fact that Sauvagesioideae sensu Amaral (1991; including *Testulea*, *Luxemburgia* and *Philacra*) is not monophyletic is unexpected and it would hardly be inferred by the analysis of morphological characters alone, as both the former subfamilies Ochnoideae (Ochneae in the new classification) and Sauvagesioideae were apparently supported by synapomorphies (Amaral, 1991). The synapomorphies for Ochnoideae (now Ochneae) are the indehiscent fruits, ovules with united integuments for most of their length or completely so (see Matthews et al., 2012), and seeds with a strongly simplified testa and without endosperm. On the other hand, the supposed synapomorphies for the former Sauvagesioideae were zygomorphic flowers (with reference to the androecium at least), seeds with wings formed by the exotesta, and the presence of a crystal layer in the endotesta. An explanation for the unexpected result of a paraphyletic Sauvagesioideae may be found by re-evaluating the synapomorphies.

When dehiscent, the fruits of *Medusagyne* and the clusioid clade as circumscribed in Xi et al. (2012) open along the septal radius (Stevens, 2001 onwards). The presence of septicidal capsules that occur in Testuleeae, Luxemburgieae and Sauvasegieae (with the exception of *Euthemis*) is therefore probably a symplesiomorphic character. On the other hand, the winged seeds and the presence of a crystal layer in the endotesta might be apomorphic characters by comparison with the outgroups (but see below). The seeds of Quiinoideae are wingless, and the wings of the seeds of *Medusagyne* are probably not homologous to those of Testuleeae, Luxemburgieae and Sauvagesieae, because they include a chalazal vascular bundle and have several cell layers (Dickison, 1990; Amaral, pers. obs.).

The presence of winged seeds and the crystalliferous endotesta is correlated with the capsular fruits that occur in the current tribes Testuleeae, Luxemburgieae and Sauvagesieae. Only in *Wallacea* and *Blastemanthus* both the wings and the crystalliferous layer are reduced and their relatively large seeds are water-dispersed in riverine environments in the Amazon region. In other genera of Sauvagesieae with wingless seeds (e.g., *Indosinia*, *Adenarake*, *Sauvagesia* s.l.) the crystalliferous layer is present, even in the genus *Euthemis* with its indehiscent, drupaceous fruits. Indehiscent fruits occur in all genera belonging to tribe Ochneae and this, together with the more or less complete fusion of the integuments, apparently led to a simplification of the seed coat including the reduction of the seed wings and crystal layer. Another character that, according to Amaral (1991), could be synapomorphic for former Sauvagesioideae is the presence of zygomorphic flowers. Floral zygomorphy, however, is a non-homologous characters as it arises in different ways: the flowers of *Testulea* and *Luxemburgia* + *Philacra* are already zygomorphic in the floral bud as the stamen and staminodes (in *Testulea*) or the stamens (in *Luxemburgia* + *Philacra*) are situated at the adaxial side of the flower (in *Luxemburgia* the stamen primordia are already asymmetrically arranged at the early stages of the flower development; Amaral and Bittrich, 1998). In the early-branching genera of Sauvagesieae the development of the zygomorphy occurs only during the anthesis with the stamina shifting to the adaxial and the gynoecium to the abaxial region of the flower. If zygomorphic flowers are ancestral in Ochnoideae as now recognized, actinomorphic flowers arose twice in the subfamily: in subtribe Ochninae and in *Sauvagesia* and related genera of Sauvagesieae, many of them with petaloid staminodes around or enveloping the fertile stamens and gynoecium. Additionally, the flowers becoming zygomorphic by shifting stamens and gynoecium only during the anthesis are also derived.

Thus, most of the supposed synapomorphies for the former subfamily Sauvagesioideae, uniting *Testulea*, *Luxemburgia* + *Philacra* and the genera that now belong to Sauvagesieae, are probably synapomorphies for the whole subfamily Ochnoideae (as now recognized) as is largely confirmed by the ancestral state reconstructions.

Quiinoideae (former Quiinaceae) are not subdivided further (Schneider and Zizka, in press). They form a well supported monophyletic group as in previous studies (Schneider et al., 2002, 2006). *Froesia* is sister to the rest of Quiinaceae, as observed in former studies (Schneider et al., 2002, 2006), but the clade of *Lacunaria* and *Touroulia*, sister to *Quiina*, has not been retrieved previously in analyses including all four genera. *Lacunaria* and *Touroulia* share a similar leaf venation pattern, petals with three vascular traces, a polymerous gynoecium with (4–)5–16 carpels (3 in *Froesia*, 2[–5] in *Quiina*), a deep ovary roof, a distinctive epidermis with radially elongate, potentially secretory cells in the furrows between the ovary ribs, and a synascidiate placental zone (Zizka and Schneider, 1999; Schneider et al., 2002, 2006; Matthews et al., 2012; Schneider and Zizka, 2012), most of them being potential synapomorphies.

4.3. Relationships below the rank of tribe

Ochneae (former Ochnoideae) are a rather homogeneous group and the circumscription has been clear for a long time with the exception of the odd genus *Lophira*, which was included in Ochnaceae for the first time by Gilg (1893a,b). This genus shares the non-distichous leaves and a two-carpelled, one-celled,

many-ovuled ovary with Sauvagesieae (former Sauvagesioideae), whereas the lack of endosperm and unitegmic ovules agree with Ochneae. Here, the molecular data strongly support its inclusion in Ochneae, which in turn also corroborate the taxonomic importance of the endosperm character. Furthermore, the arguments used by Kanis (1968) for its inclusion in Sauvagesioideae (here Sauvagesieae) are weak because a two-carpelled ovary is not an exclusive feature of Sauvagesioideae/Sauvagesieae (see below) and the phyllotaxis is taxonomically problematic (Amaral, 1991). In the morphology-based cladistic analysis of Amaral (1991), *Lophira* formed a clade with *Elvasia* and *Perissocarpa*. However, such a relationship is most likely an artifact caused by homoplastic characters because after downweighting these characters (using the "weighting procedure" suggested by Farris, 1969) *Lophira* emerged as sister to the rest of the subfamily (Amaral, 1991). Although its inclusion in Ochneae is well-supported, the odd gynoecium as described above and the fruit with the outer two sepals being extremely accrescent, wing-forming (the outermost sepal increasing by a factor 10–12, attaining a length of up to 13 cm) clearly separate *Lophira* from the rest of the tribe. Therefore, it seems appropriate to recognize subtribe Lophirinae, following Gilg (1893a), who was the first to suggest its formal separation from the remaining genera.

Besides former Ochneae (here Ochninae) and Lophireae (here Lophirinae), many of those who studied the family also recognized a third tribe, Elvasieae, within Ochnoideae (Engler, 1874; Gilg, 1893a; Dwyer, 1943; Kanis, 1968; Sastre and Lescure, 1978). Elvasieae were monotypic until Steyermark and Maguire (Steyermark, 1984) segregated the new genus *Perissocarpa*. Both are morphologically similar (Wallnöfer, 1998) and differ from the rest of the family in a two(–seven)-celled ovary with a single ovule per cell and a globose, thin-walled, usually one-seeded indehiscent fruit that in *Elvasia* often possesses aerenchyma. In the present study, *Elvasia* (*Perissocarpa* was not included) is sister to the rest of Ochneae (without *Lophira*). Thus, combining morphological and molecular evidence and applying our criteria for recognizing subtribe Lophirinae, *Elvasia* and *Perissocarpa* are segregated as subtribe Elvasiinae.

The rest of Ochneae, namely Ochninae (=Ourateeae sensu Engler, 1874; Farron, 1963), are monophyletic in the present study and differ from the remaining tribes by a five-parted perianth, gynobasic styles, almost free carpels that are postgenitally fused, and an accrescent and usually red receptacle that bears one to several black drupelets. Several authors proposed a further subdivision of former Ochneae – which correspond to our Ochninae – into subtribes Ochninae and Ourateinae (Farron, 1963, 1985; Kanis, 1968; Sastre, 1988; Sosef, 2008), the first comprising *Ochna* and *Brackenridgea*, the second *Campylospermum*, *Idertia*, *Ouratea* and *Rhabdophyllum*. *Ouratea* is clearly distinct from the rest in having free stipules, straight cotyledons, and generally caducous sepals (Sastre, 1988; Sosef, 2008). Additionally, it is the only Neotropical genus. The African genera of Ourateinae sensu Kanis (1968) all have stipules that are intrapetiolarly fused at the base, persistent sepals and curved cotyledons; *Idertia* is an exception in having straight cotyledons (Sosef, 2013). All four genera of Ourateinae have 10 sessile to shortly stalked stamens with poricidal anthers. In contrast, the two genera of Ochninae generally have more than 10 stamens, and the anthers open by longitudinal slits or pores, but with the filaments well developed, attaining a length of at least one third the length of the anther. Sosef (2008), based on Farron's (1963) and his own observations, suggested that *Ouratea* and *Idertia* are the "basal" genera of Ourateinae, whereas *Rhabdophyllum* is nested in an paraphyletic *Campylospermum*. Although there are good morphological characters supporting the relationships outlined above, our molecular data do not support such relationships or classification. Here, *Campylospermum* is sister to the rest of Ochneae, *Brackenridgea* forms a clade with *Idertia*, and *Ochna* with *Rhabdophyllum*. This also contradicts the assumption that *Brackenridgea* is closely related to or even congeneric with *Gomphia* (syn. *Ouratea*, incl. *Campylospermum*) (van Tieghem, 1902; Ridley, 1922). However, several of the intergeneric relationships of Ochninae as circumscribed here require corroboration by additional data because the branch support, especially in the Maximum Likelihood analysis, is rather low. Additionally, the short branches suggest a rather recent diversification of the whole clade. For these reasons we decided not to recognize formal groups within subtribe Ochninae.

Sauvagesioideae in their traditional circumscription were generally subdivided into tribes Sauvagesieae (=Luxemburgieae sensu Gilg, 1893a) and the monogeneric Euthemideae (Kanis, 1968; Sastre, 1987). However, a segregation of *Euthemis* as a subtribe in our classification lacks support from morphological (Amaral, 1991) or molecular data (this study), because *Euthemis* is embedded within Sauvagesieae. In the present study, *Euthemis* groups with *Schuurmansiella*, *Schuurmansia*, *Tyleria*, *Adenarake* and *Sauvagesia*. Thus, the five-carpelled, five-locular ovary and the baccate fruit with five usually one-seeded pyrenes that distinguish *Euthemis* from the rest are derived (autapomorphic) characters and there is no reason to infer a sister group relationship to the rest of Sauvagesieae. The grouping of *Euthemis* with *Schuurmansiella* and *Schuurmansia*, the only strictly SE Asian genera of Sauvagesieae, points to a common origin of that clade in this region. The pantropical *Sauvagesia* is the only other member of this subfamily with SE-Asian representatives, but it is polyphyletic according to our molecular data because the Asian *S. serrata* is sister to a clade composed by *Adenarake*, the rest of *Sauvagesia* spp., and three other genera. *Sauvagesia serrata* has been considered to represent a separate monotypic genus – *Neckia* – by Kanis (1968) and predecessors. Sastre (1970, 1971) included *Neckia*, the Neotropical genera *Lavradia* Vell. ex Vand., *Leitgebia* Eichler, *Pentaspatella* Gleason, and *Roraimanthus* Gleason and the African *Vausagesia* Baill. in *Sauvagesia*, considering it – i.e., *S. serrata* – the species with the highest number of putative ancestral characters. Subsequent classifications (Amaral, 1991, 2006) agreed with that of Sastre and also put two other monotypic E- to SE-Asian genera in synonymy of *Sauvagesia* (*Indovethia*, *Sinia*; not included in our molecular study). To resolve polyphyly of *Sauvagesia*, the genus *Neckia* needs to be re-established for *N. serrata* Korth. *Sauvagesia* s.l. (but not including *Adenarake*) is in many characters polymorphic, but has persistent stipules (deciduous in *S. nudicaulis* Maguire & Wurdack) with long marginal cilia, globose or ovoid seeds without wings, and the testa has a conspicuous pattern of flattened hexagonal cells. Now, with the phylogenetic relationships at hand, a critical revision of the broadly circumscribed *Sauvagesia* as established by Sastre (1971) and Amaral (1991, 2006), and a phylogenetic analysis of the genus based on a wider sample of species, seems to be highly desirable.

A clade uniting *Cespedesia*, *Godoya*, *Krukoviella* and *Rhytidanthera* was also found in the morphological cladistic analyis of Amaral (1991; as sister group of *Testulea*). All of them are Neotropical and characterized by a scalariform tertiary leaf venation, long stipules that leave an elongate scar, five carpels, high numbers of ovules, sessile stigmas, a capsular fruit that dehisces from the base upwards, and long-winged seeds. *Godoya* and *Rhytidanthera* additionally share the unique club-shaped trichomes at the sepals. *Cespedesia* and *Krukoviella* both have comparatively short sepals, which from an early stage of flower development do not envelop the flower bud. All four genera were part of tribe Luxemburgiinae of Kanis (1968) and Sastre (1987), but in the present study Luxemburgieae form a different clade, only comprising the genera *Luxemburgia* and *Philacra*.

Our finding of a clade uniting *Poecilandra* and *Wallacea* confirms former phylogenetic hypotheses by Engler (1874) and Amaral (1991). Both genera share retuse to emarginate leaves with closely

parallel secondary veins, many leaf traces, and anthers covered with wax crystals. These are potential synapomorphies although they occur in some other genera as well (Amaral 1991).

Some names of tribes and subtribes have yet to be validly published. Therefore, new names and diagnoses are provided below.

Elvasiinae Schneider, subtrib. nov., based on Elvasieae Engl. in Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 37: 20. 1875. – Type: *Elvasia* DC.

Lophirinae Schneider, subtrib. nov., based on Lophireae Baill., Hist. Pl. 4: 210, 218. 1872. – Type: *Lophira* Banks ex C.F. Gaertn.

Ochninae Kanis ex Schneider, subtrib. nov. – Type: *Ochna* L.

Shrubs or trees; gynoecium almost apocarpous; style gynobasic; receptacle accrescent in fruit, red, bearing usually black drupelets.

Testuleeae Schneider, trib. nov. – Type: *Testulea* Pellegr.

Tree; perianth four-parted; one fertile stamen, staminodes fused into a column; endosperm present.

4.4. Character evolution

Polysymmetric, i.e. actinomorphic, flowers have been supposed to be the ancestral condition in angiosperms from which monosymmetry (=zygomorphy) originated several times independently, and reversals from monosymmetric to polysymmetric flowers have been considered rare (Stebbins, 1974; Cronquist, 1988; Takhtajan, 1991). More recent evidence from molecular phylogenetic analyses revealed that shifts between both states occurred in both directions in different lineages and that changes to polysymmetry are much more frequent than assumed earlier. Genetic and developmental factors rather favor transitions to polysymmetry (Donoghue et al., 1998). In asterids, for example, actinomorphic flowers were ancestral and zygomorphy arose several times independently. However, reversals to actinomorphic flowers were also frequent (Donoghue et al., 1998). This corresponds with the ancestral state reconstruction in Ochnaceae. Although the ancestral condition of the family is ambiguous, zygomorphic flowers are ancestral in Ochnoideae and actinomorphic flowers evolved secondarily in Sauvagesieae and Ochneae. These reversals are partially associated with a reduction in flower size (Amaral, 1991), a phenomenon known also from other angiosperm groups (Donoghue et al., 1998). In Ochnoideae two types of monosymmetry are distinguished, one that is present early in flower development and one that arises just at anthesis, a difference that may be due to a different evolutionary age and differential depth of rooting in the genetic system (Endress, 2011). Here, the presence of monosymmetry already early in bud is reconstructed as the state originating earlier in the evolution of Ochnoideae.

Floral monosymmetry is a means of precisely positioning the pollinators on the flower and generally leads to more efficient pollination and pollinator specificity (Sargent, 2004). Thus, zygomorphy might have a selective advantage. Most Ochnoideae, including all monosymmetric taxa except *Wallacea*, possess a specialized pollination system because of their poricidal anthers or poricidal system (see below). The pollinators are pollen-collecting bees capable of vibrational, i.e. buzz, foraging (Kubitzki and Amaral, 1991). However, this adaptation to buzz-pollination is independent of flower symmetry because it is present in actinomorphic and zygomorphic flowers. Nonetheless, the ancestral condition of Ochnoideae was a combination of monosymmetry and specialized pollination system that probably was advantageous for the nectarless plants (see De Luca and Vallejo-Marín, 2013).

Poricidal anthers are ancestral in Ochnoideae and are widely distributed across the subfamily. In Ochneae, only *Ochna* (partial) and *Brackenridgea* show dehiscence by longitudinal slits. In Sauvagesieae, several genera exhibit dehiscence by longitudinal slits that either extend along the anther or are confined to the apical part. Especially interesting is the case of *Sauvagesia*, in which several species have anthers with longitudinal slits (e.g. former *Lavradia*), but the androecium is completely enveloped by petaloid staminodes that leave only a small apical pore for pollen transmission. In a similar way, the poricidal system is established in *Tyleria* and *Adenarake*: in the first, the staminodes are only basally fused but are stabilized by ventral keels, whereas in the latter, they are free but still constitute a poricidal superstructure (Kubitzki and Amaral, 1991). Thus, although anther opening reversed to longitudinal dehiscence in these taxa, buzz-pollination was maintained through the poricidal system, suggesting that there was a strong selective pressure on keeping this pollination type. Once buzz-pollination is established, plants will hardly be able to escape from this rigid pollination type (Kubitzki and Amaral, 1991).

The great majority of the core eudicots are syncarpous and secondary apocarpy arose only in few clades of rosids (e.g. Rosaceae, Sapindales, Malvales) and asterids (Apocynaceae) (Endress, 2011). Syncarpy is considered a key innovation and its evolutionary success is supposed to be linked with the novel intragynoecial compitum which allows an increased offspring quality and quantity (Armbruster et al., 2002). Despite the overall benefits of syncarpy, Ochnaceae reveal two independent reversals to a near-apocarpous gynoecium (see Matthews et al., 2012), one in the MRCA of *Froesia* (Quiinoideae) and one in Ochninae. *Froesia* had long been considered apocarpous (e.g. Schneider and Zizka, 1997) and only recently it was detected that the carpels are united over a short zone at the base (Matthews et al., 2012). Ochninae had long been considered apocarpous too (e.g. Gilg, 1893a; but see Baum, 1951), but are syncarpous at the base of the ovary. In contrast to *Froesia*, in which the free parts remain free, these parts are united postgenitally in Ochninae (Matthews et al., 2012). Both groups also differ strongly in their gynoecium structure. *Froesia* has three carpels that in fruit resemble a follicle with the fibrous pericarp characteristic of Quiinoideae (Schneider and Zizka, in press); it is doubtful whether a compitum is present (Matthews et al., 2012). Ochninae have a short compitum, bulging ovaries, a variable carpel number (three to 15) and carpels that develop into indehiscent drupelets. Despite their structural differences, the independent reversal to apocarpy in both lineages might have been facilitated by a predisposition to variable and higher carpel numbers in the family (in Ochnoideae and Quiinoideae; see Fig. 4). According to Endress (2006), it is architecturally more difficult to allow an efficient compitum with more than five carpels. Hence, there is less constraint for carpel position in such multicarpellate gynoecia.

The presence versus absence of endosperm has long been used to subdivide Ochnaceae into subfamilial clades (e.g. Engler, 1874; Amaral, 1991). However, we reconstruct the presence of endosperm as plesiomorphic in Ochnaceae and thus it is not suitable for characterizing taxa in Ochnoideae. In contrast, the early absorption of the endosperm is a derived state in Ochneae and confirms Engler's recognition of "Exalbuminosae". For Quiinoideae, on the other hand, the early absorption of endosperm was inferred as the ancestral state. Thus, the reversal to the presence of endosperm unites *Touroulia* and *Lacunaria*. The number of ovules varies between one and up to 200. High ovule numbers were reconstructed as the ancestral condition in Ochnoideae, whereas a single ovule is derived and unites Ochninae and Elvasiinae.

Seeds with wings and, thus, wind-dispersal are ancestral in Ochnoideae and Ochnaceae (however, it is unclear if the wings of *Medusagyne* are homologous to those of Ochnoideae). Winged seeds are therefore not suited for grouping the early branching taxa. Within Sauvagesieae, the loss of wings occurred independently in *Blastemanthus*, *Wallacea*, *Euthemis*, and in the clade of *Adenarake* and *Sauvagesia*. The loss of wings in *Blastemanthus* and *Wallacea* is probably related to their occurrence in inundated forests with the fruits being adapted to hydrochory. In *Euthemis*, the

loss is coupled with the evolution of indehiscent fruits and the different dispersal mode. The same is true for unwinged seeds of Ochneae.

5. Conclusion

Our molecular data provide for the first time a robust phylogenetic framework for the evaluation of previous phylogenetic assumptions and a modern classification of Ochnaceae s.l. Ochnaceae comprise the subfamilies Ochnoideae Burnett, Quiinoideae Luerss., and Medusagynoideae Reveal. The long-standing subdivision of Ochnoideae into Ochneae and Sauvagesieae (=Ochnoideae and Sauvagesioideae in traditional classifications) was basically confirmed, except that the latter is paraphyletic in its traditional circumscription due to the position of *Testulea* as sister to the rest of Ochnaceae. To avoid paraphyly, we segregate the tribes Luxemburgieae (*Luxemburgia*, *Philacra*) and Testuleeae (*Testulea*) and redefine the circumscription of Sauvagesieae. Within the Ochneae, we corroborate the inclusion of *Lophira*, thus recognizing the subtribes Ochninae (*Brackenridgea*, *Campylospermum*, *Idertia*, *Ochna*, *Ouratea*, *Rhabdophyllum*), Elvasiinae (*Elvasia*, *Perissocarpa*), and Lophirinae (*Lophira*). Ochninae comprise all six genera which had formerly been partitioned between Ochninae and Ourateinae, a concept that was not confirmed in the present study. In contrast to previous classifications, Sauvagesieae (*Adenarake*, *Blastemanthus*, *Cespedesia*, *Euthemis*, *Fleurydora*, *Godoya*, *Indosinia*, *Krukoviella*, *Neckia*, *Poecilandra*, *Rhytidanthera*, *Sauvagesia*, *Schuurmansia*, *Schuurmansiella*, *Tyleria*, *Wallacea*) are not further subdivided here. Polyphyly of *Sauvagesia* s.l. is here resolved by re-establishing the monotypic genus *Neckia*, to which *Sauvagesia serrata* is transferred. Especially the circumscription of *Sauvagesia* and the unclear subdivision of and relationships within Ochninae require further investigation using additional molecular data and/or a more comprehensive taxon sampling. Ancestral state reconstructions confirmed the taxonomic value of most characters used in previous classifications for defining major clades. However, some states previously considered synapomorphic turned out to be plesiomorphic (e.g. zygomorphic flowers for Sauvagesieae). It was shown that zygomorphic flowers, poricidal anthers, a syncarpous gynoecium, numerous ovules, septicidal capsules, and winged seeds with endosperm are the ancestral condition in Ochnoideae. It is noteworthy that although poricidal anthers reversed to longicidal in several lineages, buzz-pollination was maintained by the evolution of poricidal superstructures, indicating a strong selective pressure on keeping that specialized pollination system. Now that a molecular framework is available, we are looking forward to resolving pending issues such as the timing of the diversification process and the reconstruction of the complex biogeographic history of Ochnaceae.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.05.018.

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