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TESTUDINACEAE, A NEW FAMILY OF ASCOMYCETES

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(With two Plates)

The Testudinaceae, a new family of the Pseudosphaeriales, is characterized by astomatous ascomata with a dark peridium which is often made up of plates, by bitunicate asci, and by dark, 2-celled ascospores, about 10 μ long. Keyed out are the genera *Testudina*, *Neotestudina*, *Lepidosphaeria*, *Argynna* and *Pseudophaeotrichum*.

In Zygomycetes, Ascomycetes, and Basidiomycetes phylogenetic relationships are known to exist between epigeous and hypogeous fungi. Epigeous fructifications which were gymnocarpous or hemiangiocarpous originally, became angiocarpous and spherical or tuberiform when the development took place subterraneously. At the same time the special spore dispersal mechanisms, associated with open sporocarps tend to disappear. The sporocarps remained closed for an extended period and spore dispersal was brought about by soil inhabiting animals and other agents.

Often such soil inhabiting fungi are closely related to fungi with epigeous fructifications. For example a number of hypogeous, gasteromycete-like Basidiomycetes are related to well-defined genera of Agaricales, Boletales, or Russulales.

Well known in this respect is the phylogentic relationship between the astrogastraceous series, comprising *Russula*, *Lactarius* and a number of genera with hypogeous or semihypogeous species (Heim, *1948*; Singer & Smith, *1960*).

In Ascomycetes the relationship between some operculate Discomycetes (Pezizales) and most of the genera of the Tuberales is often recognized as a classical model with all possible intermediates. Also well known is the relationship between many genera of Sphaeriales with ostiolate ascomata and genera with cleistothecia, hitherto mostly arranged in Plectascales or Eurotiales (compare Müller & von Arx, *1972*, in press). For example the genus *Heleococcum* is the angiocarpous relative of *Nectria*, *Chaetomidium* that of *Chaetomium*, *Zopfiella* that of *Podospora*, and *Microthecium* that of *Melanospora*. The two last-mentioned genera often are united, because most of the species of the one have related species in the other. Moreover, in pure culture the ascomata of a single strain may be ostiolate or astomatous, depending on environmental conditions. This phenomenon has been observed also for other genera of the Sordariaceae and the Melanosporaceae (including Chaetomiaceae and Microascaceae).

In the bitunicate Pyrenomycetes (Loculoascomycetidae) only two cases of relationship between ostiolate and astomatous genera are known. One concerns *Trichodelitschia*, which is ostiolate, and *Phaeotrichum*, which is astomatous (Cain, *1956*).

The other is the phylogenetic group comprising the genera *Sporormia*, *Preussia*, and *Westerdykella* (von Arx & Storm, *1967*). In *Sporormia* the ascomata are ostiolate, in the

two other genera astomatous. In *Sporormia* and in some species of *Preussia* the asci are cylindrical-clavate and bitunicate. In other species of *Preussia* and in *Westerdykella* the asci are obovate or nearly spherical and the two membranes of the ascus wall are no longer distinguishable. In *Sporormia* the asci are arranged in a hymenial layer and extend to the ostiolum, in *Westerdykella* the asci are arranged irregularly at different levels, and *Preussia* again is intermediate.

Remarkable is the species *Sporormia aemulans* (Rehm) v. Arx & Storm, where in one and the same strain the ascomata may be provided with a conical ostiolum or may be astomatous. In the latter instance, the upper part of the ascomata is flat or slightly umbilicate and the peridial wall is thinner than on the sides and the base. The formation of an ostiolum in the *Sporormia—Preussia* series can be influenced by the choice of medium and humidity when the ascomata are in an early state of development. In species considered to belong to *Westerdykella* the ascomata in every case are astomatous and spherical to tuberous.

A study of recently collected material showed that *Testudina terrestris* Bizz., hitherto placed in the Eurotiales, in fact has bitunicate asci and also is an astomatous member of the Pseudosphaeriales. The genus is related to some other astomatous Ascomycetes, described as type species of the genera *Neotestudina*, *Pseudophaeotrichum*, *Lepidosphaeria* and *Argynna*. Common characters are the dark cleistothecia, 2-celled, pigmented ascospores without germ pores or germ slits and the 'parenchymatic' initials. Related genera with ostiolate ascomata and elongated asci might be *Didymosphaeria* and *Herpotrichia* of the Pleosporaceae, but no intermediates are known. *Testudina* and the related genera can hardly be assigned to the Pleosporaceae, therefore the description of a new family of the Pseudosphaeriales becomes necessary:

Testudinaceae von Arx, *nov. fam.*

Ascomata e cellulis plerumque intercalaribus, inflatis, undique divisis oriuntur; cleistothecia tuberosa vel globosa, pariete in bracteas e cellulis crassitunicatis obscuris radiantibus compositas diviso; asci irregulares vel fasciculati, clavati, obovati vel fere globosi, pariete inspissato duplici vel tenui; ascosporae bicellulares, brunneae, plerumque crassitunicatae; paraphyses filamentosae, saepe absunt.

GENUS TYPICUM *Testudina* Bizz.

Colonies spreading, aerial mycelium abundant or scarce; composed of brown, thick-walled hyphae; initials of ascomata consisting mostly of intercalary swollen cells dividing in all directions; ascomata tuberiform or spherical, without ostiolum; peridial wall usually divided into plates, composed of elongated, radially arranged, thick-walled, dark cells; asci irregularly arranged or in fascicles borne on ascogenous hyphae, clavate, obovate or nearly spherical, with a thickened double or a thin single membrane; ascospores 2-celled, brown, glabrous or ornamented, mostly thick-walled; paraphyses absent or scarce, filamentous, often septate and ramose.

TYPE GENUS.—*Testudina* Bizz.

KEY TO THE GENERA

1. Ascomata with continuous peridium composed of flattened cells; asci obovate or spherical, with a thin membrane . . . . . *Pseudophaeotrichum*
1. Ascomata with peridium composed of plates of radiating cells; asci clavate or obovate, bitunicate 2
2. Ascospores ornamented 3
2. Ascospores glabrous 4
3. Asci obovate or broadly clavate; ascospores reticulate. . . . . *Testudina*
3. Asci clavate, with a long stalk; ascospores finely echinulate, darker near the septum *Lepidosphaeria*
4. Asci clavate, stalked; ascospores darker near the septum . . . . . *Argynna*
4. Asci obovate or nearly spherical; ascospores not darker near the septum. *Neotestudina*

1. TESTUDINA Bizz.

Testudina Bizz. *in* Atti Ist. veneto Sci. (Lett. Arti) VI **3**: 303. 1885.
Marchaliella Wint. *apud* Bommer & Rouss. *in* Bull. Soc. r. Bot. Belg. **29**: 243. 1891.

TYPE SPECIES.—*Testudina terrestris* Bizz.

The ascomata develop in a dark mycelial mat; they are spherical or tuberous, dark, and 400–600 μ in diameter. The 'plates' of the peridium are composed of thick-walled, brown cells, 4–7 μ long, 2–4 μ wide and arranged in radiating rows. The asci, borne on branched, hyaline ascogenous hyphae, are obovate, broadly clavate or nearly spherical, with a short stalk, 15–20 × 12–16 μ in size. The outer membrane is thin, the inner one is up to 2–3 μ thick at the apex. The reticulate, brown ascospores are composed of 2 rounded cells, and measure 9–10.5 × 4.5–5.5 μ (without ornamentation 8–9 × 4–5 μ). Plate 12.

The description is based on a freshly collected specimen, received from Dr. Roswitha Schneider (Berlin) and on some herbarium collections. In most of them the fungus had developed on twigs of conifers. Type specimens of *T. terrestris* and *M. zopfielloides* were not available.

In the collections studied the fungus develops in association with other fungi, e.g. *Fusarium* sp., *Acremonium* sp., and *Phialophora* sp., probably as a fungal parasite. Isolation in pure culture did not succeed, and only cultures of the last-mentioned genera developed on the agar plates.

2. NEOTESTUDINA Segretain & Destombes

Neotestudina Segretain & Destombes *in* C.r. hebd. Séanc. Acad. Sci. Paris **253**: 2577. 1961.

TYPE SPECIES.—*Neotestudina rosatii* Segretain & Destombes.

In the type strain (CBS 427.62), ascomata were observed only in cultures developed on oatmeal agar from revived lyophilized ascospores. The ascomata are black, 300–450 μ in diameter, and the peridial wall is divided into plates. The cells of the wall, arranged in rows, are brown, thick-walled, and measure 8–12 × 4–6 μ. The asci develop laterally on wide ascogenous hyphae; they are bitunicate, with a thick inner membrane, at the apex up to 2–3 μ, and measure 16–24 × 10–16 μ. The ascospores are irregular in shape, often broadly truncate, often rounded or attenuated at the ends, provided with a rather thick, smooth wall and measure 9–12 × 5–8 μ.

3. LEPIDOSPHAERIA Parguey-Leduc

Lepidosphaeria Parguey-Leduc *in* C.r. hebd. Séanc. Acad. Sci. Paris **270**: 2784. 1970.

TYPE SPECIES.—*Lepidosphaeria nicotiae* Parguey-Leduc.

The development of this fungus has been fully described by Parguey-Leduc (*1970*). In cultures of the type strain (CBS 559.71) on malt- and oatmeal-agar, only a few cleistothecia were observed on the dark, dense mycelial mat; these have a diameter of 230–500 μ; the peridial wall is subdivided into plates. The bitunicate asci are arranged in dense fascicles in a hymenium-like layer around the center of the ascoma on ascogenous hyphae; they are clavate, long stalked and measure (without stalk) 27–37 × 9–13 μ. The ascospores consist of 2 rounded cells; they measure 8–10 × 5–6.5 μ; the rather thick wall is finely echinulate, brownish, darker at maturity and nearly opaque along the septum.

4. ARGYNNA Morgan

Argynna Morgan *in* J. Cincinn. Soc. nat. Hist. **18**: 41. 1895.

TYPE SPECIES.—*Argynna polyhedron* (Schw.) Morgan

No specimen of this fungus could be studied, but it is fully described and illustrated by Martin (*1941*).

Its relationship with *Neotestudina* has also been recognized by D. Malloch (personal communication). The fungus seems to be closely related to *Lepidosphaeria nicotiae*, but differs in the ascospores which are smooth, asymmetrical, and attenuated at both ends.

5. PSEUDOPHAEOTRICHUM Aue & *al.*

Pseudophaeotrichum Aue & *al. in* Nova Hedwigia **17**: 84. 1969.

TYPE SPECIES.—*Pseudophaeotrichum sudanense* Aue & *al.*

Studied was the type strain (CBS 512.69). The fungus was described and illustrated fully by Aue & al. (1969). In culture on oatmeal agar the ascomata attain a diameter of 300–600 μ. The asci have a thin membrane and measure 17–24 × 15–20 μ; the ascospores are smooth, brown, attenuated at both ends, and measure 10–12 × 6–7.5 μ. Plate 13.

Another cleistocarpous genus with two-celled ascospores is *Zopfia* Rabenh. (see Müller & von Arx, *1962*). *Zopfia rhizophila* Rabenh. and other species develop on roots. All species are easily recognized because of the very large ascospores. Whether this genus is a further angiocarpous member of the Pseudosphaeriales is still uncertain.

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EXPLANATION OF PLATES 12 AND 13

PLATE 12

Testudina terrestris, young and mature asci and ascospores. — 1800 ×.

PLATE 13

Pseudophaeotrichum sudanense, ascogenous hyphae with young asci and a mature ascus. — 1800 ×.