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ORIGINAL ARTICLE

Morphology and molecules: the Sebacinales, a case study

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Abstract Morphological and molecular discrepancies in the biodiversity of monophyletic groups are challenging. The intention of this study was to find out whether the high molecular diversity in Sebacinales can be verified by micromorphological characteristics. Therefore, we carried out molecular and morphological studies on all generic type species of Sebacinales and additional representative taxa. Our results encouraged us to disentangle some phylogenetic and taxonomic discrepancies and to improve sebacinalean classifications. This comprises generic circumscriptions and affiliations, as well as higher taxon groupings. At the family level, we redefined the Sebacinaceae, formerly the Sebacinales group A, and set it apart from the Sebacinales group B. For taxonomical purposes, it seems appropriate to refer *Paulisebacina, Craterocolla, Chaetospermum, Globulisebacina, Tremelloscypha,* and *Sebacina* to the Sebacinaceae and *Piriformospora,* and *Serendipita* to the Sebacinales group B. At the lower taxonomic level, we propose within the Sebacinaceae (1) to introduce *Paulisebacina* for *Sebacina allantoidea*, (2) to transfer *Efibulobasidium rolleyi* into a new monotypic genus, *Globulisebacina*, (3) to include *Tremellostereum* in *Tremelloscypha*, (4) to transfer *Sebacina amesii* into *Tremelloscypha*, (5) to combine *S. helvelloides* and *S. concrescens* in their own genus, *Helvellosebacina*, (6) to transfer *Tremellodendron* spp. into *Sebacina*, (7) to define *S. epigaea* s.str. without cystidia and flagelliform dikaryophyses, but with star-shaped resting spores, and (8) to separate *S. cystidiata* with simultaneously irregular germinating spores and inconspicuous cystidia, and *S. flagelliformis* with flagelliform dikaryophyses from *S. epigaea* s.str. Additional clades in *Sebacina*, based on molecular differences, cannot be distinguished morphologically at present.

Introduction

Based on longitudinally septate meiosporangia in their mature stage, sebacinoid fungi were originally grouped together with tremelloid and exidioid taxa. Sebacinales in the present circumscription were reviewed in detail recently (Oberwinkler et al. 2013). We refer to this publication for traditional classification of genera and interpretation of some species. Here, we summarize data that accumulated within several years of intensive sampling, from morphological and molecular studies of all species of Sebacinales group A that were accessible for this combined approach. At the same time, calculations about divergence times in Sebacinales have been carried out (Garnica et al. 2014). In the latter work, the same specimens as in the present study were used for molecular analyses, and the phylogenetic framework is identical in both surveys. For that reason, we focus here on morphology and compare our results with phylogenetic hypotheses of both trials.

Materials and methods

Taxon sampling

Sebacinales collections including representative genera and species were used in this study. Most of the specimens were collected by us during the last four years from various sites in Austria and Germany. Our sampling was complemented by specimens from various countries, provided by several

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herbaria (see Supplementary Files 1–3). In addition, with the aim to incorporate as much genetic information as possible from a wide geographical range for Sebacinales, sequences spanning the D1/D2 regions of the LSU rDNA were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/) and UNITE (http://unite.ut.ee/) databases.

Light microscopy analyses

Samples were mounted in tap water and studied with a Zeiss Standard microscope. Then the preparations were treated with 10 % KOH, phloxine, and glycerine. Drawings were made at a scale of 10 μm=3 cm or 6 cm, respectively. Measurements of cellular structures were done approximately 20 times each. All drawings are originals of FO.

Molecular techniques, phylogenetic and genetic variation analyses

DNA isolation, polymerase chain reaction (PCR) conditions, cloning, sequencing, and phylogenetic analyses are described in detail in Garnica et al (2014). Briefly, an aligned dataset (556 bp lengh) comprising D1/D2 regions of the LSU rDNA sequences of badiomata and strains of Sebacinales was used to circumscribe clade boundaries. All clusters with support values over 84 % are considered as clades (except for *S. dimitca*, 72 % bootstrap).

For new species, the intraspecific genetic variation in the ITS and the D1/D2 regions of nuclear rDNA was analysed from sequences aligned using MAFFT v.5 with the option E-INS-i (Katoh et al. 2005). Multiple sequence alignments were used to calculate sequence divergences in Mesquite v.2.75 (Maddison and Maddison 2011) from uncorrected *p*-distance matrixes.

Results and discussion

Overview of phylogenetic diversification in Sebacinales

The purpose of this study was to compare morphological and molecular features of a representative number of species in Sebacinales. This approach provides some convincing evidences for substantially interpreting evolutionary trends in the Sebacinaceae s.str. As discussed in the preceding parts and summarized in phylogenetic trees (Fig. 1 and Supplementary File 4), saprobic species seem to be restricted to basal clades.

In *Paulisebacina* (see below), only a few generative hyphae, basidia, and basidiospores constitute the complete cellular construction (Fig. 3, a-c). Macroscopically distinct, globose basidiocarps are realized in *Chaetospermum* and *Globulisebacina* (Fig 4, d, e, i), and in very young developmental stages of *Craterocolla*, cup-shaped basidiomata occur (Fig. 3, i).

Fig. 1 Simplified cartoon phylogenetic tree of Sebacinaceae s.str. (Sebacinales group A), based on nucleotide sequences of the D1/D2 regions of large subunit (28S) rDNA. Best ML tree topology calculated from 1,000 searches of all available sequences from basidiomata and strains under the GTRCAT model of DNA substitution. Only bootstrap values ≥ 70 are given. For the complete tree see Supplementary File 4 and Garnica et al (2014). Results and discussions in this study are arranged according to clade numbers. Saprobic species cluster in basal positions, mycorrhizal ones appear to be derived (marked with arrows). As explained in the text, we propose three new genera to accommodate an adequate taxonomy. The genus *Sebacina* is emended and considered to include *Tremellodendron* species, as outlined in the text. New taxa are bold marked ▶

In species of these genera, hymenia do not only contain basidia, but also dikaryophyses. These sterile terminal hyphae seem to have a protective function as layers above the meiosporangia. They are present in all species of subsequent clades.

Tremelloscypha species switched to mycorrhizal nutrional modes, apparently a singular event in Sebacinaceae s.str. with a clear dominance for ECM mycosymbionts, present in all following clades (Fig. 1).

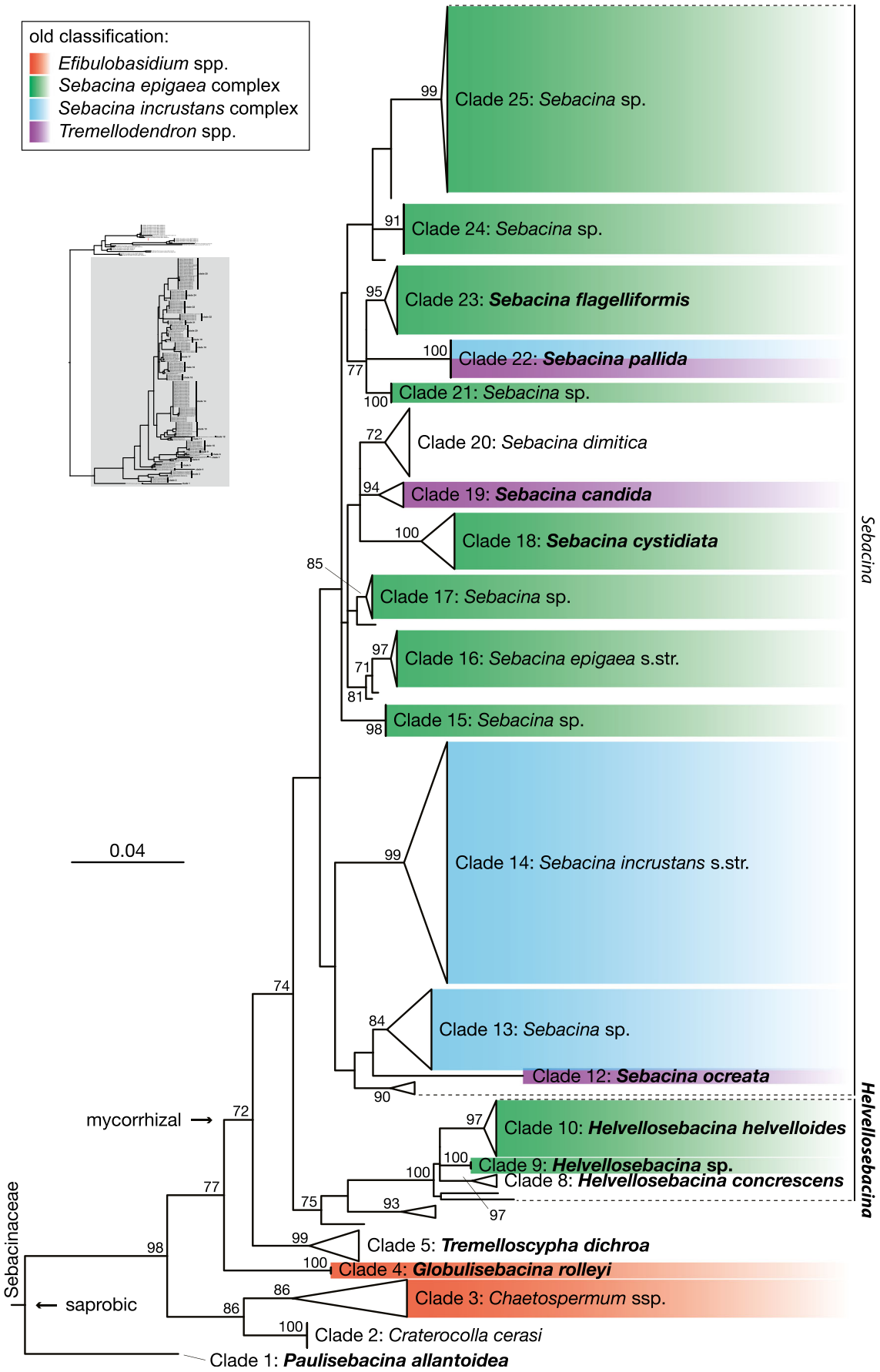
The proposed new genus *Helvellosebacina* (see below) comprises species of clades 7–10 in a cluster that is supported by a 100 % bootstrap value. As a micromorphological equivalent we consider rather simple-structured dikaryophyses in species of the genus.

Clades 11–25 are considered as *Sebacina* s.str., with clade 14 containing the generic type, *S. incrustans* s.str. Unfortunately, this cluster is not supported molecularly by an adequate bootstrap value. Micromorphological characteristics share rather uniform features with minor variations, such as hyphal context, ramification of dikaryophyses, and, if present, resting spore morphology. Upright growth is considered as an evolutionary trend, convergently developed several times and approaching clavarioid fructifications, so far named *Tremellodendron*.

As explained above, Sebacinales of clades 26–32 (Supplementary File 4) totally lack any kind of macroscopic fruiting structures. Also, the micromorphology of the few teleomorphic species known at present (Fig. 21), and of the majority of the anamorphic ones, is so scant that comparative interpretations are impossible. In addition, host plant associations are difficult to assess in most cases. Therefore, phylogenetic hypotheses are based exclusively on sequence data.

Descriptive characteristics of Sebacinales clades

In this section, we present morphological data of representative species of Sebacinaceae. To facilitate an


old classification:
Efibulobasidium spp.
Sebacina epigaea complex
Sebacina incrustans complex
Tremellodendron spp.
0.04
Clade 25: Sebacina sp.
Clade 24: Sebacina sp.
Clade 23: Sebacina flagelliformis
Clade 22: Sebacina pallida
Clade 21: Sebacina sp.
Clade 20: Sebacina dimitica
Clade 19: Sebacina candida
Clade 18: Sebacina cystidiata
Clade 17: Sebacina sp.
Clade 16: Sebacina epigaea s.str.
Clade 15: Sebacina sp.
Clade 14: Sebacina incrustans s.str.
Clade 13: Sebacina sp.
Clade 12: Sebacina ocreata
Clade 10: Helvellosebacina helvelloides
Clade 9: Helvellosebacina sp.
Clade 8: Helvellosebacina concrescens
Clade 5: Tremelloscypha dichroa
Clade 4: Globulisebacina rolleyi
Clade 3: Chaetospermum ssp.
Clade 2: Craterocolla cerasi
Clade 1: Paulisebacina allantoidea
Sebacina
Helvellosebacina
mycorrhizal
saprobic
Sebacinaceae

understanding of the ontogenetic phases in hymenial structures and spores, such developmental stages are illustrated and explained separately in Fig. 2a. Basidial ontogeny is essentially the same in all taxa treated here. Resting spores were found in members of clades 15, 16, 18, 21, 23, and 24 (Fig. 2b, c).

The grouping and arrangement of the clades follows the molecularly-based phylogenetic hypothesis (Fig. 1, Supplementary File 4). This phylogeny is treated in detail and underlined by divergence time estimations in a separate publication (Garnica et al. 2014). Both papers are well-matched to each other and should be conceived as a concerted approach.

Clade 1: *Sebacina allantoidea*, Fig. 3, a-c

The basal clade of the Sebacinaceae s.str. contains only *Sebacina allantoidea* (Weiß and Oberwinkler 2001; Kirschner and Oberwinkler 2002), a species with inconspicuous basidiocarps that produce few thin-walled and hyaline substrate hyphae without clamps, and lacks a subhymenium (Fig. 3, a-c). The hymenium consists only of scattered, globose basidia, ca. 10 μm in diam., sometimes in clusters, and dikaryophyses and cystidia are lacking. The thin- and smooth-walled, hyaline, and allantoid spores germinate by repetition. *Sebacina allantoidea* was collected on basal parts of the stem of a dead ornamental *Spiraea* sp. in winter; its substrate

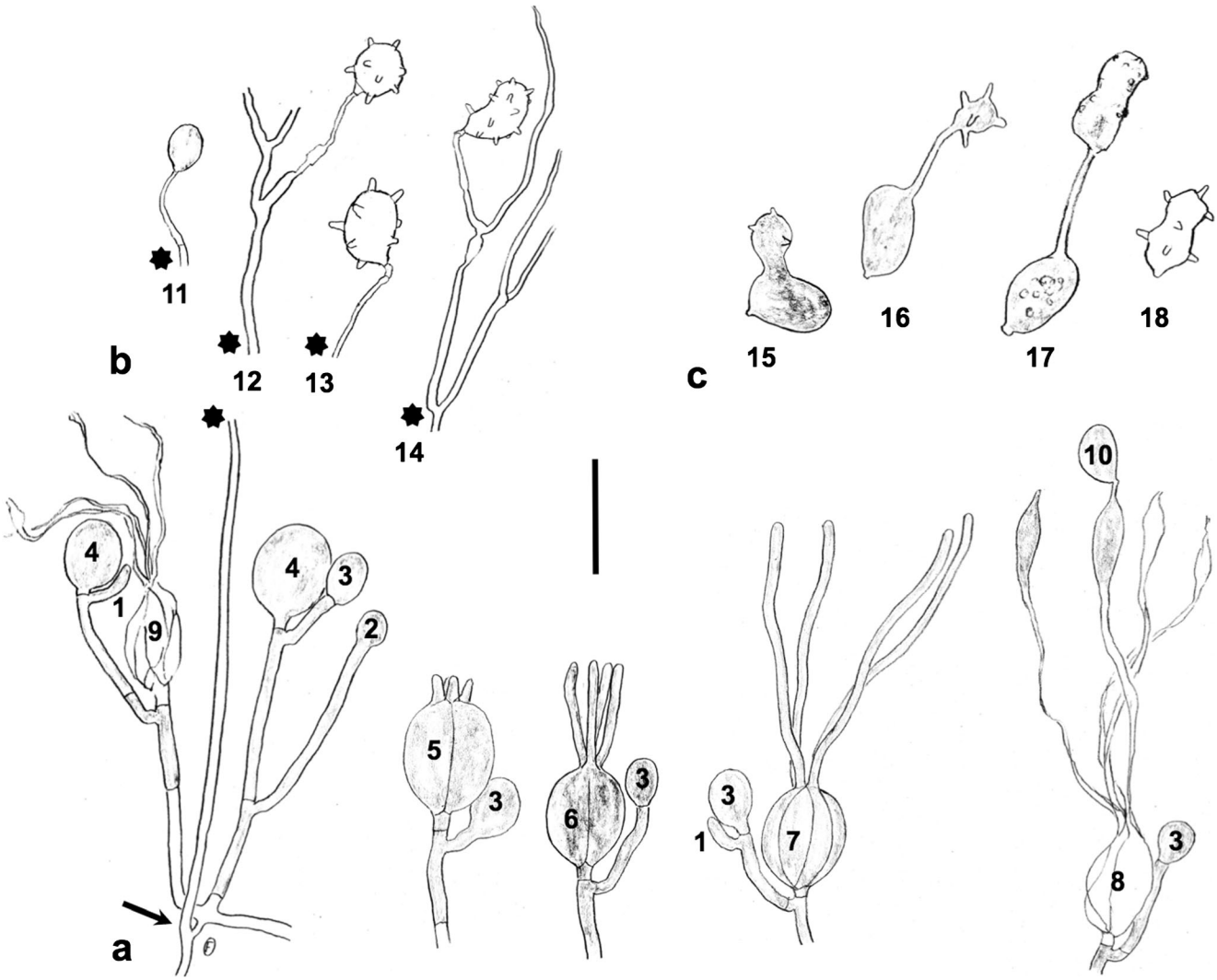


Fig. 2 Ontogenetic stages of basidia, basidiospores, and resting spores of *Sebacina* sp.; a, b, stars mark disconnected dikaryophyses (TUB 019655, clade 23)**: a** basidial development: **1** lateral hyphal branches; **2** first probasidial hyphal swellings; **3** young probasidia with basal septa, approximate stage of karyogamy; **4** probasidia in the stage of meiosis; **5, 6** postmeiotic longitudinal septation of basidia and beginning of sterigma formation; **7** basidium with well-developed sterigmata; **8** basidial cytoplasm moved to swollen tips of sterigmata and spores; three spores ejected, one on top of sterigma (10); **9** empty and collapsed basidium; **10** basidiospore formation; **11**–**14** resting spore formation on top of terminal cells of dikaryophyses; arrow: hyphal fusion of dikaryophysis and generative hypha; **c, 15**–**17** resting spore formation by germination of primary basidiospores; **18** resting spore. Bar for all figures 20 μm

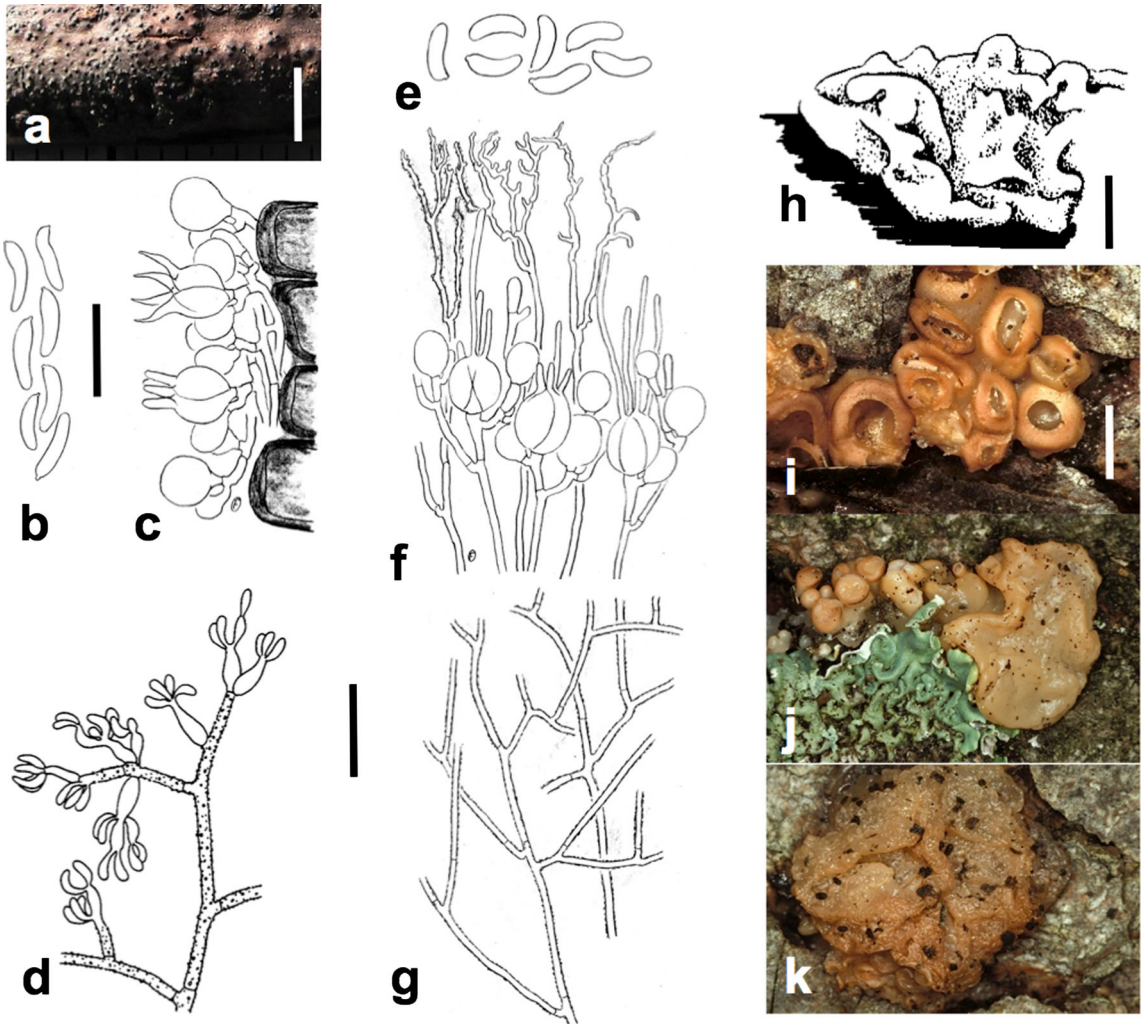


Fig. 3 Species of clades 1 and 2: **a-c** *Sebacina allantoidea* from holotypus; **a** dead stem of *Spiraea* sp. with stromatic ascocarps overgrown by *S. allantoidea*; note the snip from where the material for microscopy was taken; **b** basidiospores; **c** section of basidiocarp growing on a pseudoparenchymatous pyrenomycete; basidia in different developmental stages; note the extreme thin fructification. **d-k** *Craterocolla cerasi*: **d** anamorph, *Ditangium cerasi*, after Oberwinkler et al. (2013); **e-g** F. Oberwinkler 42814; **e** basidiospores; **f** part of the hymenium with basidia in different developmental stages, prominent and heavily encrusted dikaryophyses, the encrustations illustrated on the left side; **g** part of the trama with widely separated, heavily gelatinized, and dot-like encrusted hyphae; **h** habit sketch of a mature basidiocarp, v. Höhnel 1903; **i-k** basidiocarps on fallen *Picea abies*, F. Oberwinkler 44666; **i** young, crater-like fructifications; **j** well-developed, and **k** old basidiocarps. Bars: **a** 5 mm; **b-g** 20 μm; **h** 1 mm; **i-k** 1 cm

dependency remained unclear. It is tempting to assume that fructifications of such a simple construction could represent basal phylogenetic types in the order. The available molecular data support this view (Fig. 1; Weiß and Oberwinkler 2001). As mentioned already by Kirschner and Oberwinkler (2002), sebacinalean fungi without basidiocarps can only be found by chance and may be much more common than presently estimated.

Clade 2: *Craterocolla cerasi*, Fig. 3, d-k

Craterocolla cerasi (Fig. 3, d-k), the type species of the genus (Brefeld 1888), is composed of soft, gelatinous, finely-encrusted hyphae in a thick subhymenial and trama layer. Young fructifications are crater-like (Fig. 3, i), followed by the expansion of the hymenium with densely packed basidia and a conspicuous layer of strongly encrusted dikaryophyses, resulting in a hirneoloid surface (Fig. 3, h, j, k). Often, an anamorph stage, formerly named *Ditangium cerasi* (Fig. 3, d), is associated with basidiocarps. This set of morphological characters is unique within the Sebacinales.

Craterocolla cerasi, as indicated by its name, is believed to prefer *Prunus avium* as a main substrate. However, even conifers like *Picea abies* (Fig. 3, f-g) can be colonized by huge populations in comparatively early wood decay stages.

The taxonomic status of additional species included in *Craterocolla*, like *C. insignis*, *C. minuta*, and *C. rubella* var. *minor*, remains dubious.

Craterocolla can easily be recognized as a distinct monophyletic taxon. The apparent basal position in the Sebacinales may be interpreted as an expression of its saprobic life style (Fig. 1).

Clade 3: *Efibulobasidium albescens* and *Chaetospermum* spp., Fig. 4, a-f

The genus *Efibulobasidium* was proposed by Wells (1975), including two species, *E. albescens*, the type species, and *E. rolleyi*. Both have tiny globose, softly gelatinous fructifications that may fuse in older developmental stages. The hyphal construction with thin-walled, heavily gelatinizing cell-walls, and apically ramified dikaryophyses is essentially the same in both taxa. Also, basidial development and spore morphology does not show any remarkable difference. Nevertheless, we would like to draw attention to an overlooked feature of *E. albescens* with a marked restriction of basidia to the core of basidiomata, requiring rather long sterigmata for reaching the surface of the fructification, and resulting in a halo-like peripheral zone (Fig. 4, a, e). We are not aware of similar differentiations in other basidiomycetous fungi. In addition, a *Chaetospermum* anamorph (Fig. 4, f) developed when *E. albescens* was cultivated (Wells and Bandoni 2001; Kirschner and Oberwinkler 2009). Because of its appendages, these conidia are ecologically adapted for water dispersal that certainly plays a considerable role in the preferred wet habitats where *E. albescens* occurs, favorably on dead herbaceous substrates.

Saccardo (1892) proposed a new genus, *Chaetospermum*, for *Tubercularia chaetospora*, a grass decaying fungus described by Patouillard (1888). A detailed morphological

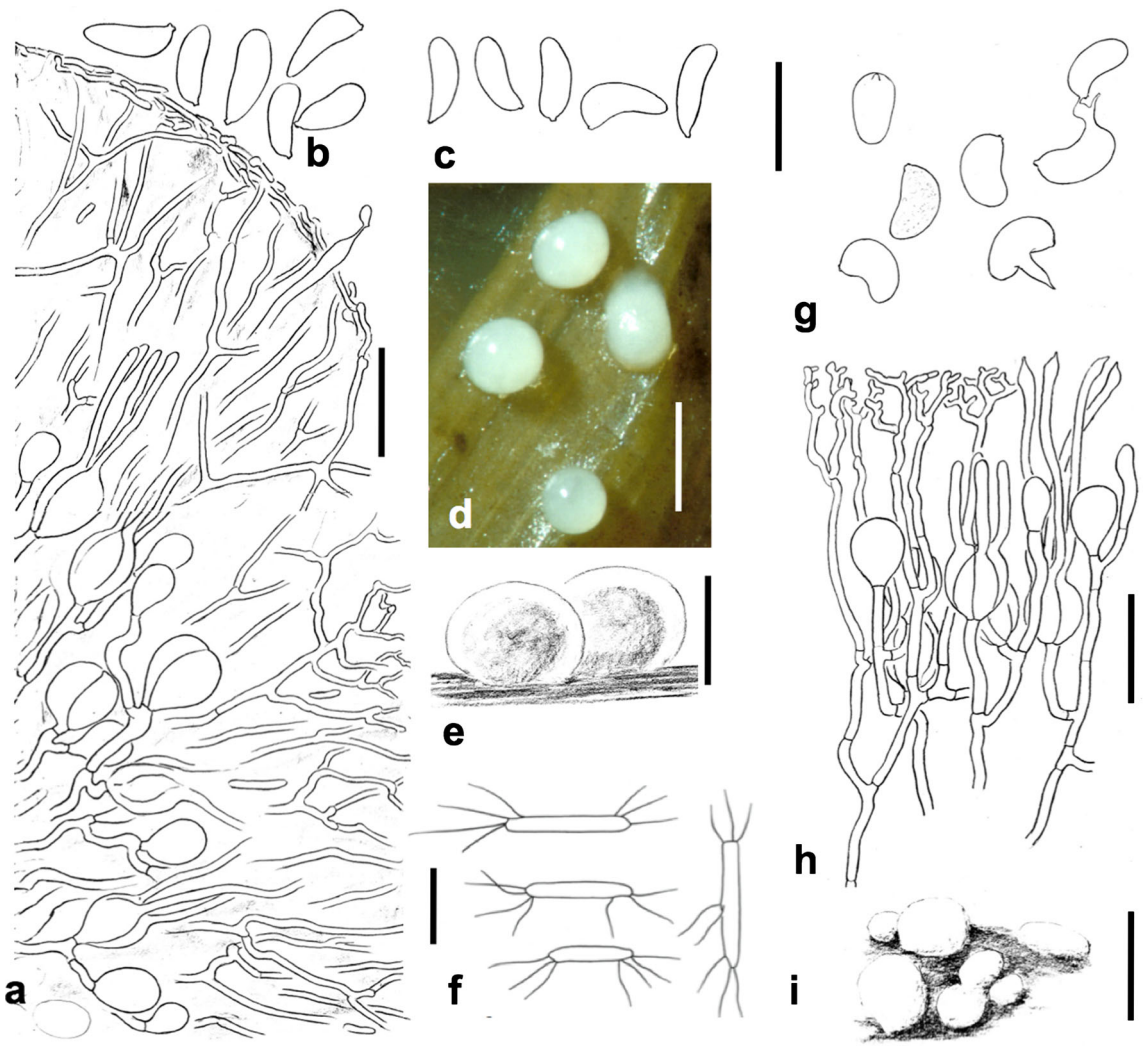


Fig. 4 *Efibulobasidium* spp., clades 3, 4: **a-f** *E. albescens*; **a, b** section of basidiocarp with basidia in different developmental stages and basidiospores (Parmasto 15852); **c** basidiospores of *Epidochium albescens* (holotypus), **d, e** basidiocarps of *Efibulobasidium albescens* (F. Oberwinkler 34705), note halo-like peripheral zone in **e**; **f** *Chaetospermum gossypinum* from Kirschner and Oberwinkler (2009); **g-i** *E. rolleyi,* **g, i** basidiospores, two germinating with secondary spores, and basidiocarps (R.J. Bandoni 6647); **h** hymenium with basidia in different developmental stages and dikaryophyses (Olive 1956, holotypus). Bars: **a, b, c, f, g, h** 20 μm; **d** 1 mm; **e, i** 0.5 mm

analysis has been carried out by De Fonseka (1960). In his treatment of the genus *Chaetospermum*, Nag Raj (1993) accepted four species. An additional species with conidiomatal setae, *Ch. setosum*, was proposed by Rajeshkumar et al. (2010).

In molecular analyses, Weiß et al. (2004) found *E. albescens* in a sister clade of *Craterocolla cerasi,* and *E. rolleyi* in a separated relationship. Rungjindamai et al. (2008) found *Chaetospermum* spp. clustering within the Sebacinales. Also, Weiß et al. (2011) included *Ch. artocarpi* and *Ch. camelliae* in their comprehensive analyses in which these species grouped with *E. albescens*. These findings have been confirmed in our study (Fig. 1, Supplementary File 4).

Clade 4: *Efibulobasidium rolleyi*, Fig. 4, g-i

In contrast to *E. albescens*, conidial anamorphs have not been found to be associated with *E. rolleyi*, neither by culture nor by molecular evidence. *Efibulobasidium rolleyi* (Fig. 4, g-i) apparently is also a saprobic fungus, but grows on decaying wood.

The taxonomic status of two additional species, *E. dimorphobasidii* and *E. patiliense*, remains unclear.

As discussed already for previous clades, we suggest that the rather basal position of *Efibulobasidium* spp. may be due to their saprobic nutrition modes. That the species are distinctly separated in two clades by molecular evidences was unexpected. However, if the *Chaetospermum* anamorphs (Fig. 4, f) occur only in the *E. albescens* clade, a genetic distance to *E. rolleyi* could be indicated that justifies a generic separation.

Clade 5: *Tremelloscypha*, *Tremellostereum* and *Sebacina amesii*, Fig. 5

The genus *Tremelloscypha* was erected by Reid (1979) for the single species *T. australiensis* (Fig. 5, a-e). Wells and Oberwinkler (1982) found that *Tremella gelatinosa* (Fig. 5, f) also shares essential features with this genus. They attempted

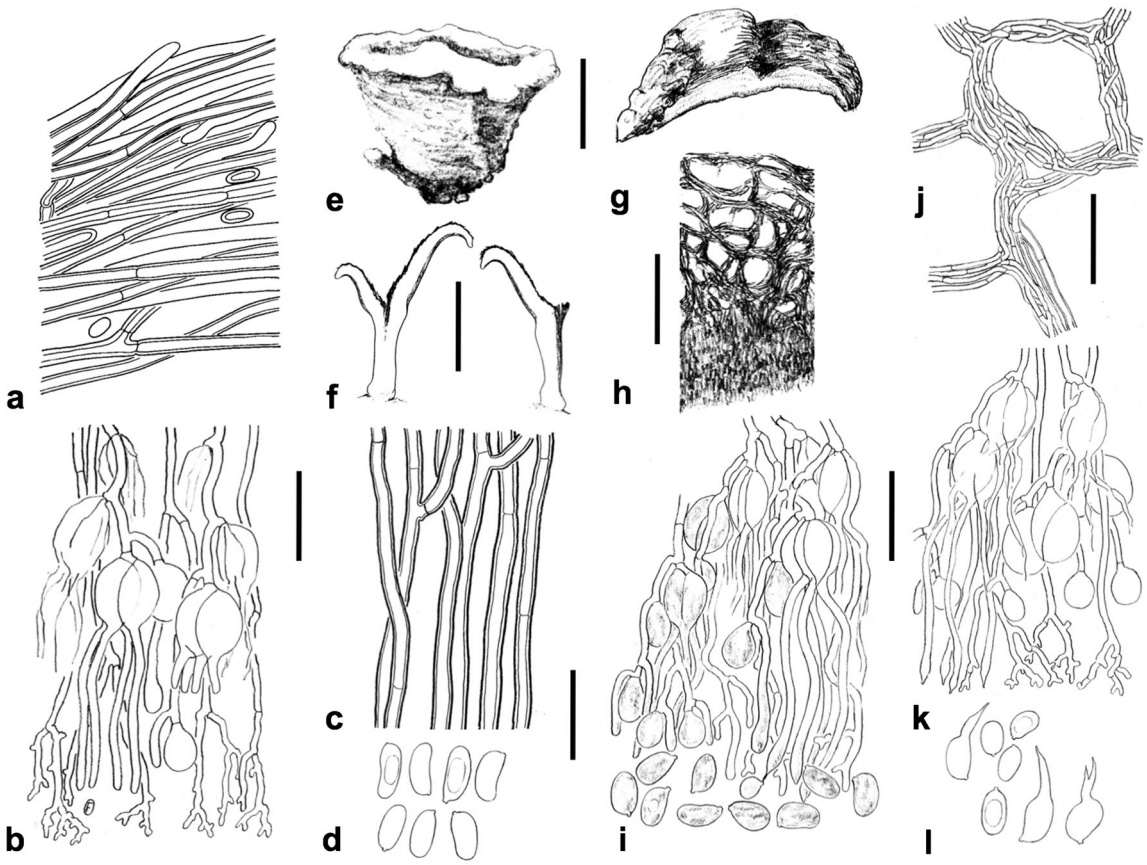


Fig. 5 Species of clade 5: **a-e** *Tremelloscypha australiensis*, holotypus; **a** sterile upper surface of basidiocarp with mainly thick-walled and non-gelatinized hyphae; **b** part of hymenium with basidia in different developmental stages and dikaryophyses; **c** hyphal arrangement of stype; **d** basidiospores; **e, f** longitudinal sections of two stipitate and pileate basidiocarps with the hymenia restricted to the underside of the caps; **f** *Tremelloscypha gelatinosa* (Bandala VB 4210) basidiocarp with hymenium on the lower surface; **g-i** *Tremellostereum dichroum* (Gomez 58/78); **g** longitudinal section of basidiocarp; **h** loose hyphal context in basidiocarp trama; subhymenium and hymenium in the lower parts; **i** part of hymenium with basidia in different developmental stages, dikaryophyses and basidiospores; **j-l** *Sebacina amesii*, isotypus; **j** loose hyphal context in basidiocarp trama; **k** part of hymenium with basidia in different developmental stages and dikaryophyses; **l** basidiospores. Bars: **a-d, i, k, l** 20 μm; **e-g** 1 cm; **h** 100 μm; **j** 50 μm

to evaluate the relationship between these fungi and concluded by incorporating *Sebacina* s.str., *Tremellodendron*, and *Tremelloscypha* in a newly proposed family, Sebacinaceae. Tentatively, they also included *Efibulobasidium* in this family. Using molecular data, Weiß and Oberwinkler (2001) confirmed this phylogenetic reinterpreation.

Ryvarden (1986) proposed the genus *Tremellostereum* for *Stereum dichroum* (Fig. 5, g-i). A re-examination of this species revealed identical micromorphological features, thus indicating a very close relationship with *Tremelloscypha* that was conclusively supported by molecular data in this study (Fig. 1).

Habit structures and micromorphological details of *Sebacina amesii* (Lloyd 1916), studied by the first author at Kew in 1971, indicate a close relationship of this species with *Tremelloscypha* (Fig. 5, j-l). However, so far, molecular data are not available for *S. amesii*.

The common structural features of *Tremelloscypha* spp. include a conspicuous basidiocarp morphology (Lloyd 1916), and dry and loose hyphal textures. Ectomycorrhizal host dependencies (ECM) appear to occur in *T. gelatinosa*, growing in tropical *Gymnopodium floribundum* (Polygonaceae) forests in southern Mexico (Bandala et al. 2011).

Sebacina amesii was collected by F.H. Ames "in rather thin, but moist woods" (Lloyd 1916), indicating the high probability of being an ECM taxon.

In the evolution of Sebacinales (Fig. 1), *Tremelloscypha* marks the switch from saprobic to mycorrhizal nutrition modes, the latter one being most likely maintained in all further evolved taxa.

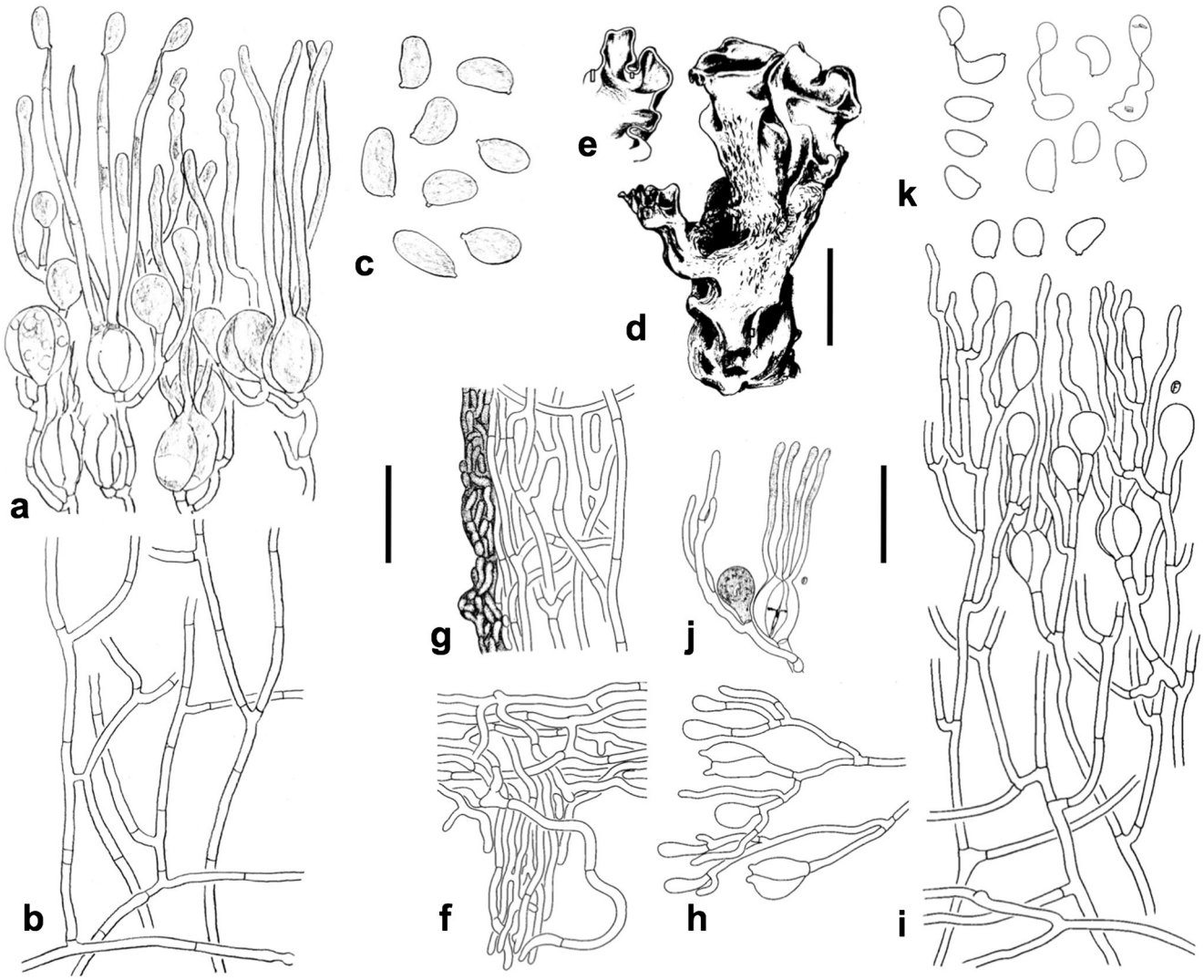


Fig. 6 *Sebacina* sp., clades 8, 9: **a-c** *Sebacina* sp. TUB 020028; **a** hymenium with basidia in different developmental stages and dikaryophyses; **b** subhymenial hyphae in a loose and strongly gelatinized context; **c** basidiospores. **d-k** *Sebacina concrescens* (W.B. Cooke 1960): **d** basidiocarp; **e** section of basidiocarp; **f, g** sterile surfaces of basidiocarp; **h, i, k** details of hymenium with basidia in different developmental stages and dikaryophyses; **c, k** basidiospores, three germinating with secondary spores. Bars: **a-c, f-k** 20 μm; **d, e** 1 cm

Clades 7–10: *Sebacina concrescens* and *S. helvelloides* s.l., Figs. 6, 7

In clades 8–10, we found *Sebacina* species with an apparently similar micromorphology, at least in essential parts of hyphae, hymenia, dikaryophyses, basidia, and basidiospores, and in contrast, a considerable macromorphological diversity. For comparison, we have selected the resupinate *Sebacina* sp., TUB 020028 (Fig. 6, a-c) and the stipitate-merulioid *S. concrescens* (Fig. 6, d-k). Both taxa share basidiocarps with widely separate hyphae, producing a gelatinous matrix (Fig. 6, b, i). It is obvious that the upright growing *S. concrescens* requires structural stability, provided by strongly agglutinating peripheral hyphae (Fig. 6, f). Other parts of sterile surfaces of this species are structured with hyphal pegs (Fig. 6, g).

Species of clades 7–10 grow in ECM communities. Therefore, they have to be considered as mycosymbionts. We were unable to discriminate the four clades, indicated by molecular data, using micromorphological features. Well-developed *S. concrescens* may display a characteristic macromorphology (Fig. 6, d).

We identified collection TUB 019681 (Fig. 7) as *Sebacina helvelloides* s.l., based on detailed descriptions by Burt (1915), referring to gross morphology and hymenial construction with simple to sparingly ramified dikaryophyses (Fig. 7, a). In contrast to an Estonian sample (Tedersoo et al. 2006) that clusters in clade 7 (see Supplementary File 4), our material groups within clade 10 (Fig. 1). However, the micromorphological conformities of members of clades 7–10 are striking, and are strongly supported by a 100 % bootstrap value (Fig. 1), indicating a very close relationship.

Clades 12 and 13: *Tremellodendron ocreatum* and *Sebacina* sp., Fig. 8

We selected four taxa for sequence comparison, *Tremellodendron ocreatum* (clade 12), and *Sebacina* sp. (clade 13), and the latter three also for comparative micromorphological analyses (Fig. 8).

Already, Corner (1968) recognized *Thelephora ocreata* (Berkley 1856) as a *Tremellodendron* species, and Roberts (in Henkel et al. 2004) formally transferred it into the sebacinalean genus, including a description taken from the holotypus collection and Berkeley's original account. Roberts remarked that hyphae were thick-walled and hyphidia (dikaryophyses) were not seen. Unfortunately, his scanty illustration does not provide valuable information for a substantial micromorphological interpretation. In contrast, except for *Sebacina allantoidea* (Fig. 3, a-c), we are not aware of any sebacinalean species of Sebacinaeae being devoid of dikaryophyses. Thick-walled trama hyphae are typical for *Tremellodendron* species in the traditional sense (compare Figs. 14, 17). Therefore, it is not surprising that these skeletal elements are also present in *T. ocreatum*. However, members of the related clade 13 have clearly monomitic hyphal systems (Fig. 8). Fasciculate basal hyphae (Fig. 8, m) may serve to support upright growth. Their function might be supported by thick-walled hyphae.

Tremellodendron in the common circumscription (Bodman 1942), comprising 8–15 species, does not represent a monophylum in molecularly-based phylogenetic trees. In our cladogram, *Tremellodendron* species cluster with

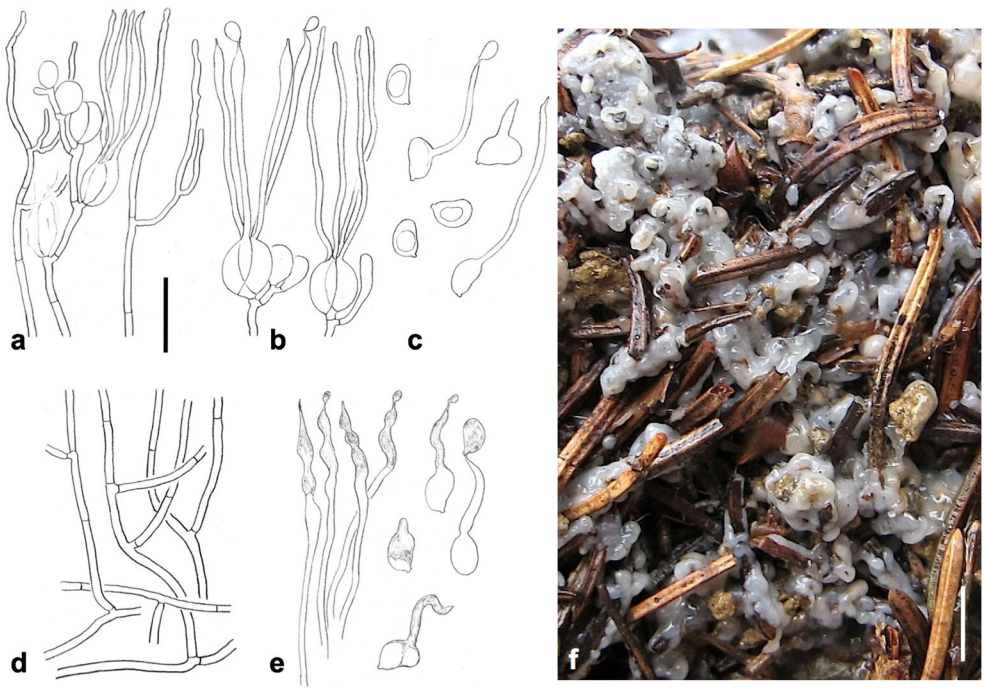


Fig. 7 *Sebacina helvelloides* s.l., clade 10: TUB 019681, **a** hymenium with basidia in different developmental stages and dikaryophyses; **b** mature basidia; **c** basidiospores, three germinating by repetition; **d** subhymenial hyphae in a loose and strongly gelatinized context. **e** torulose apices of sterigmata and germinating basidiospores; **f** fresh basidiocarps on litter of *Picea abies* needles. Bars: **a-e** 20 μm; **f** 1 cm

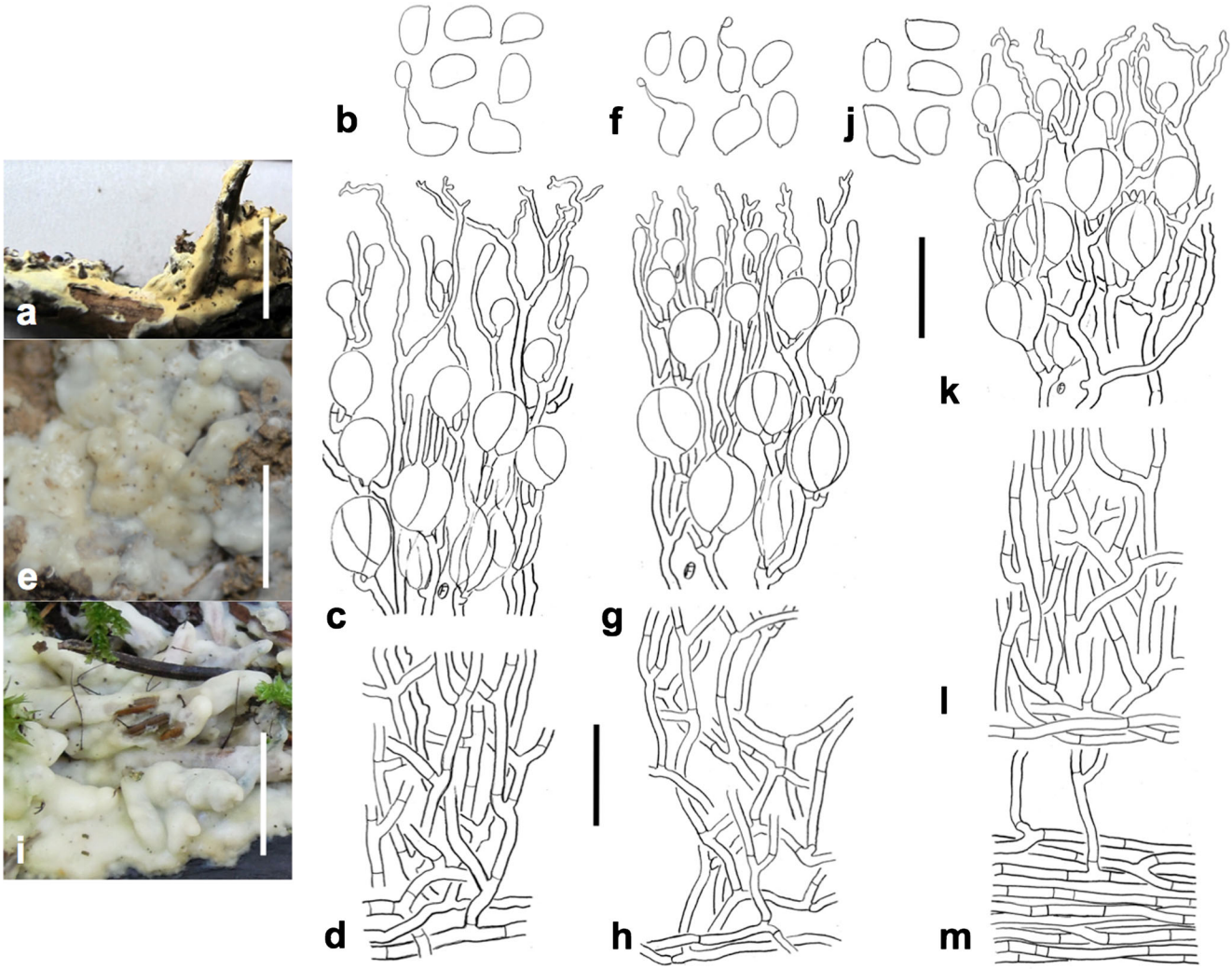


Fig. 8 *Sebacina* sp., clade 13: **a-d** TUB 019635, **e-h** TUB 019641, **f-m** TUB 020011. **a** dry basidiocarp, **e, i** fresh basidiocarps; **b, f, j** basidiospores, several germinating by repetition; **c, g, k** hymenia with basidia in different developmental stages and dikaryophyses; **d, h, l** subhymenial hyphae in a loose, not gelatinized context; **m** fasciculate basal hyphae. Bars: b-d, f-h, j-m 20 μm; a, e, i 1 cm

Sebacina species s.l. in three separate clades (12, 19, and 22). Our attempts to find morphological grounds for that fact were strongly hampered by insufficient sampling. On the other hand, we are aware of the tendency of certain *Sebacina* species fruiting on soil to form irregular upright outgrowths under optimal developmental conditions. With the help of thick-walled hyphae, such protuberances can eventually reach clavarioid structures, thus approaching the habit of a *Tremellodendron*.

Walker and Parrent (2004) found convincing evidence that *Tremellodendron* species are ECM mycobionts.

Clade 14: *Sebacina incrustans* s.str., Fig. 9

Clade 14 includes the type species of *Sebacina, S. incrustans*, described by Tulasne and Tulasne (1872), who revealed that *Corticium incrustans* (Persoon 1796) is phragmobasidiate. This species is easily recognized in the field because it tends to encrust all kinds of substrates, including living ones. Also, dry basidiocarps (Fig. 9, f) are rather characteristic in their growth habit and ochraceous color. Microscopically, *S. incrustans* has a clearly distinctive hyphal construction with slightly thick-walled and loosely interwoven trama hyphae (Fig. 9, b, h), and thin-walled, densely arranged subhymenial and hymenial hyphae (Fig. 9, a, g), all non-gelatinized. We found identical structural details in a previous study (Fig. 7 in Riess et al. 2013).

The molecular variation in *S. incrustans* samples of clade 14 certainly reflects genetic diversity in populations. As explained above, a *Sebacina* sp. TUB 019641 (Fig. 8), also named *S. incrustans* (lineage 2) in our previous study (Riess et al. 2013), does not share the above description and certainly belongs in the monophyletic group of clade 13.

Sebacina incrustans has been identified several times as a mycobiont of ECMs (Urban et al. 2003; Weiß et al. 2004;

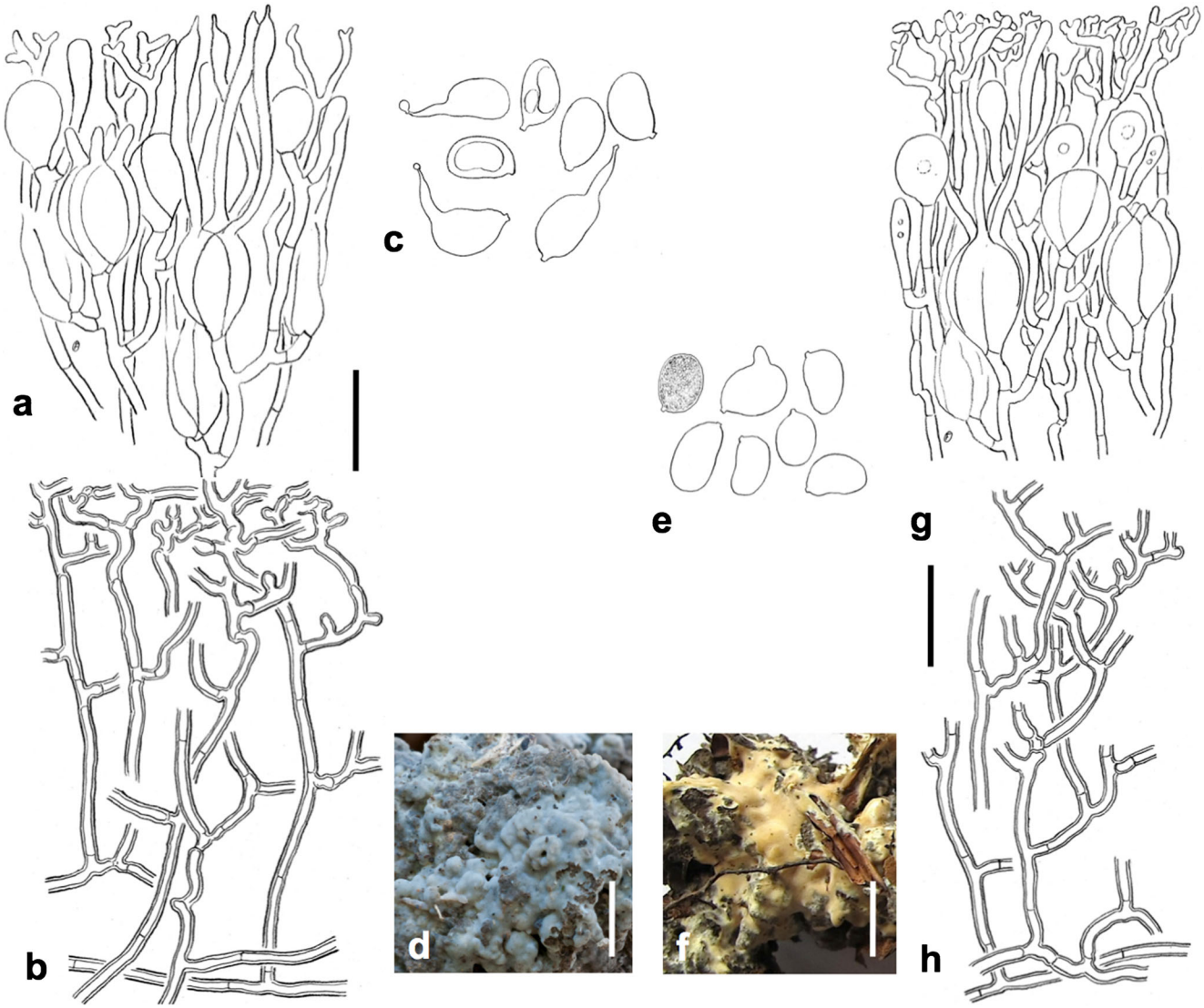


Fig. 9 *Sebacina incrustans*, clade 14 : **a-d** TUB 019623, **e-h** TUB 019629. **a, b** Hymenia with basidia in different developmental stages and dikaryophyses. **b, e** thick-walled subhymenial hyphae. **c, d** basidiospores, three germinating with secondary spores. **d** fresh basidiocarp; **f** dried basidiocarp. Bars: **a-c, e, g, h** 20 μm; **d, f** 5 mm

Tedersoo et al. 2006; Mühlmann and Peintner 2008; Henkel et al. 2011; Garnica et al. 2013).

Clade 15: *Sebacina* sp., Fig. 10

Six collections of *Sebacina epigaea* s.l. cluster in the highly supported clade 15 (Fig. 1). The samples show strictly resupinate growth and are composed of thin-walled, strongly gelatinized hyphae in a dense context, basally parallel to the substrate and regularly vertically oriented in the subhymenium. As in all *Sebacina* species, there is a mixture of sterile dikaryophyses and generative hyphae, gradually producing a thickening hymenium. The dikaryophyses form a covering layer above the basidia, inducing them to produce rather long sterigmata to reach the fructification surface for sporulation (Fig. 10, a, i). As characteristic for *Sebacina* species, primary basidiospores are smooth and thin-walled. In addition, thick-walled spores with irregular spore walls are commonly present. However, the general morphology of these resting spores is quite distinct from those of typical *S. epigaea* (Fig. 11, d, g). We could not clarify their ontogeny. Whether the differences in resting spore morphology are statistically significant cannot be estimated at present.

Clade 16: *Sebacina epigaea* s.str., Fig. 11

Berkley and Broome (1848) noticed longitudinally septate, tremelloid basidia in a soil-inhabiting basidiomycete, collected in the Leigh Wood near Bristol in August 1848, and assigned it to *Tremella* as *T. epigaea*. Unfortunately, their illustration of the hymenial micromorphology omits

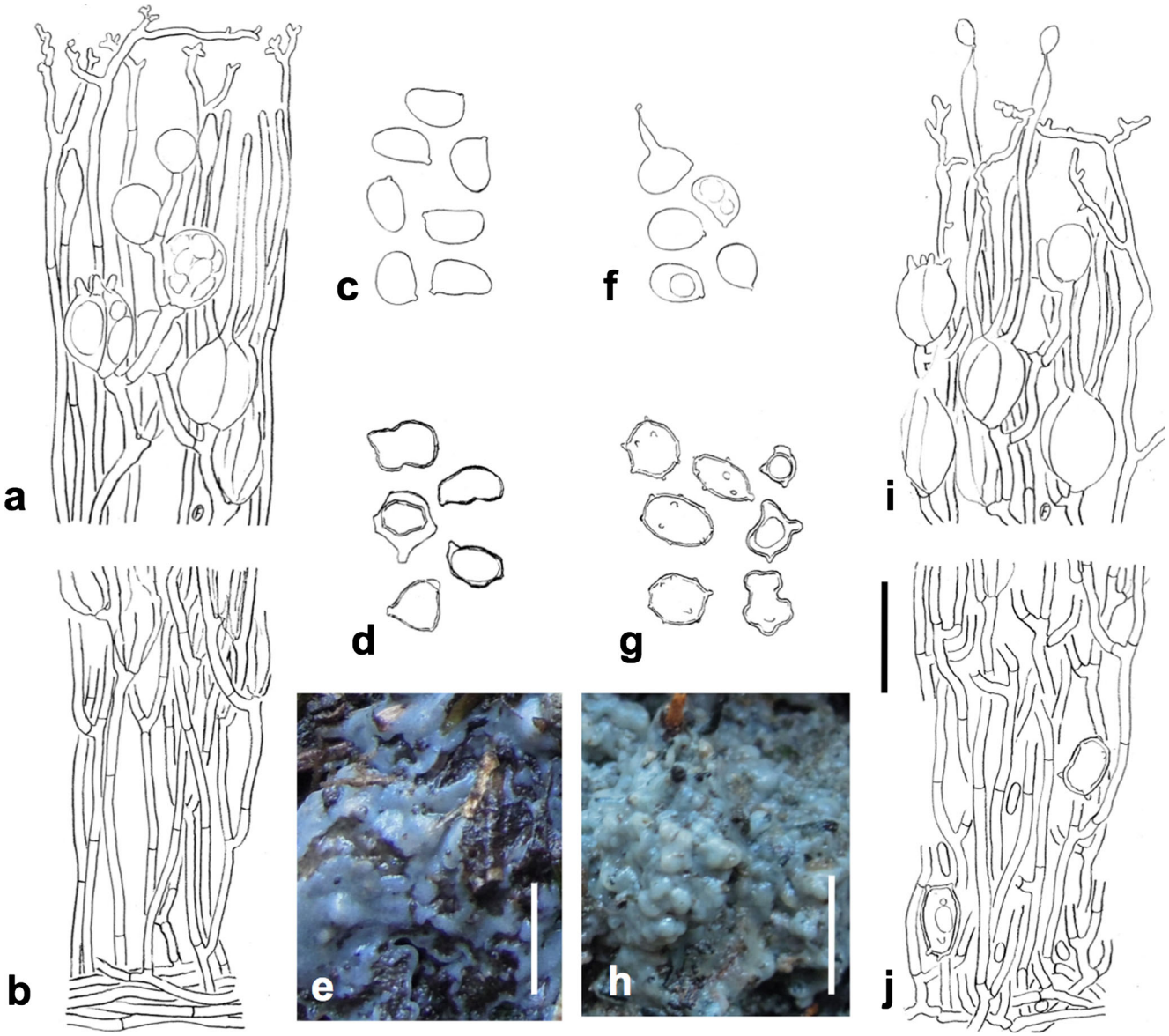


Fig. 10 Species of clade 15: *Sebacina* sp. (a-e TUB 019999; f-j TUB 020017). **a, i** parts of hymenia with basidia in different developmental stages and dikaryophyses; **b, j** thin-walled basal and subhymenial hyphae, the upright ones widely separated through a gelatinized matrix; **c, f** basidiospores; **d, g** irregular resting spores; **e, h** fresh basidiocarps. Bars: a-d, f, g, i, j 20 μm; e, h 5 mm

basidiospores. Later, Bourdot and Galzin (1927) transferred the species into *Sebacina*, recognizing and illustrating star-like resting spores that were since then considered as a distinguishing feature of the species (Fig. 11, d, g), i.a. by Oberwinkler (1963): Abb. 21 and Riess et al. (2013): Fig. 6. However, we are not aware that the holotypus has been restudied to confirm this characteristic spore morphology. We found star-like resting spores developing from secondary spores (Fig. 11, g), but we do not exclude that also primary basidiospores can be transformed into resting spores (Fig. 11, d; Fig. 7 in Riess et al. 2013).

Other common features of *S. epigaea* s.str. are thin-walled and strongly gelatinizing hyphae, slightly coralloid dikaryophyses, and predominant fruiting on soil in ECM tree communities. The species has often been identified from ECM roots (Glen et al. 2002; Weiß et al. 2004; Tedersoo et al. 2006; Palmer et al. 2008; Rineau 2008; Rineau and Garbaye 2009; Garnica et al. 2013; Oberwinkler et al. 2013; Riess et al. 2013).

Clade 17: *Sebacina* sp., Fig. 12

Two *Sebacina* samples (TUB 019686, TUB 020023) of clade 17 were taken to document their monomitic hyphal systems and their apically strongly and tinily ramified dikaryophyses. Both features are not unique in the *Sebacina* clades, nevertheless, they appear to characterize *Sebacina* species of clade 17 adequately.

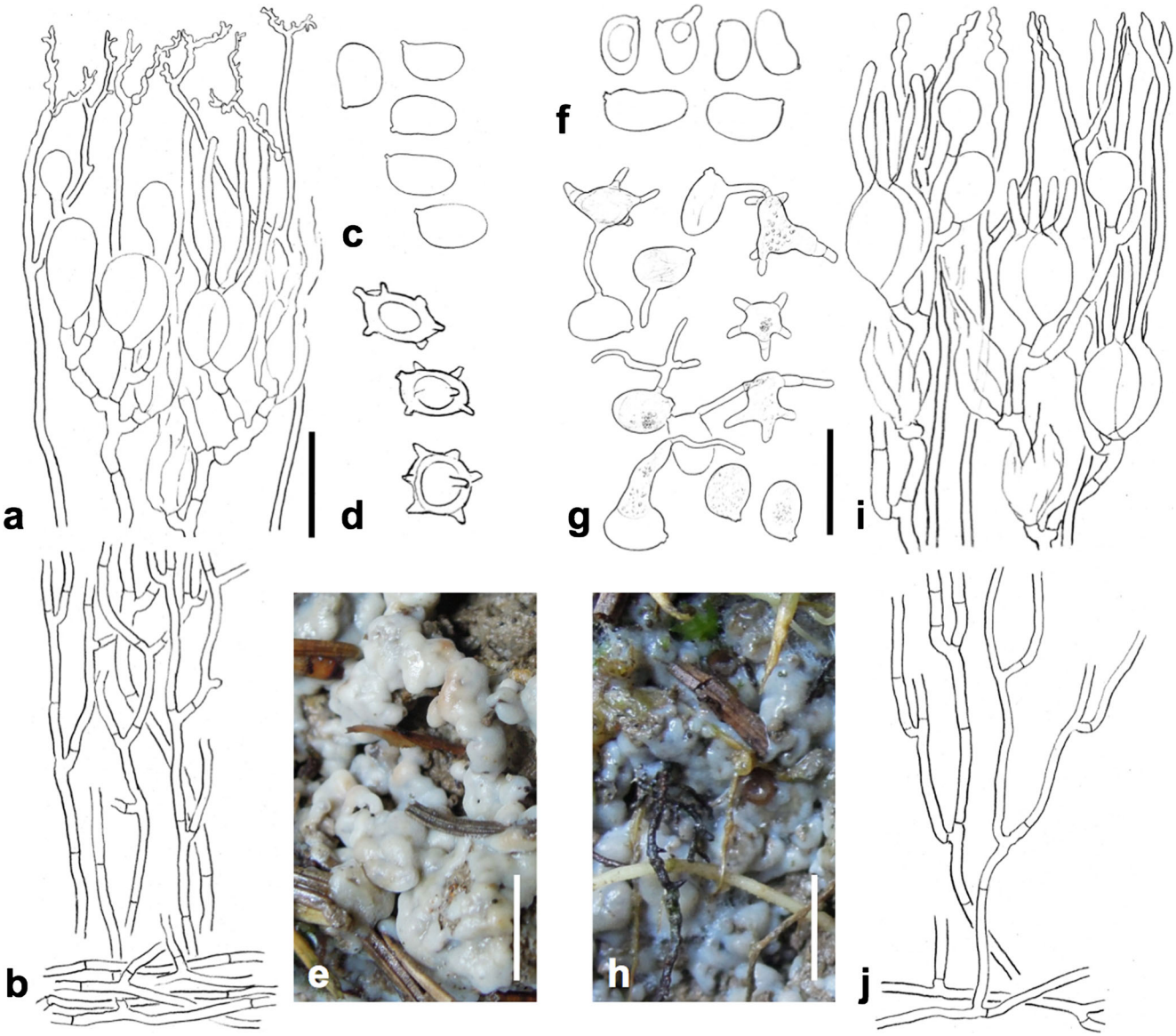


Fig. 11 *Sebacina epigaea,* clade 16: **a-e** TUB 019979; **f, h-j** TUB 020003; **g** FO 31755). **a, i** parts of hymenia with basidia in different developmental stages and dikaryophyses; **b, j** thin-walled basal and subhymenial hyphae, the upright ones widely separated through a gelatinized matrix; **c, f** basidiospores; **d** star-like resting spores; **e, h** fresh basidiocarps; **g** basidiospore germination with star-like secondary spores. Bars: **a-d, f, g, i, j** 20 μm; **e, h** 5 mm

According to their collecting sites in ECM forests, the fungi of this clade are most likely symbionts.

Clade 18: *Sebacina* sp., Fig. 13

The micromorphology of two *Sebacina* collections (TUB 020024, TUB 020025), clustering in clade 18, shares peculiar features. Unique for *Sebacina* sp. are cystidia-like elements, i.e., sterile hyphae originating in the subhymenium and ending in the hymenium, or protruding from it, at least partly. These hyphae can also be categorized as simple, non-ramified dikaryophyses. Sometimes, these hyphae are difficult to see, as in Fig. 13a, b. In both collections, basal hyphae are thick-walled (Fig. 13, b, e, l), and thin-walled ones are originating from them (Fig. 13, l). Another common feature is a probably unique spore germination (Fig. 13, d, k) with multipolar germtube formation that reflects the morphology of resting spores. In general, spore germination in *Sebacina* species is commonly documented when secondary ballistospores are observed. Other germination types are rarely mentioned and not illustrated. When basidiocarps develop luxuriantly, hollow protuberances can be produced (Fig. 13, m). In total, clade 18 appears to represent a monophyletic group that can also be characterized with morphological features. We are not aware of any other *Sebacina* species with cystidia or cystidia-like hyphae. Samples of clade 18 were collected in ECM forest communities, thus indicating that these *Sebacina* species are symbionts.

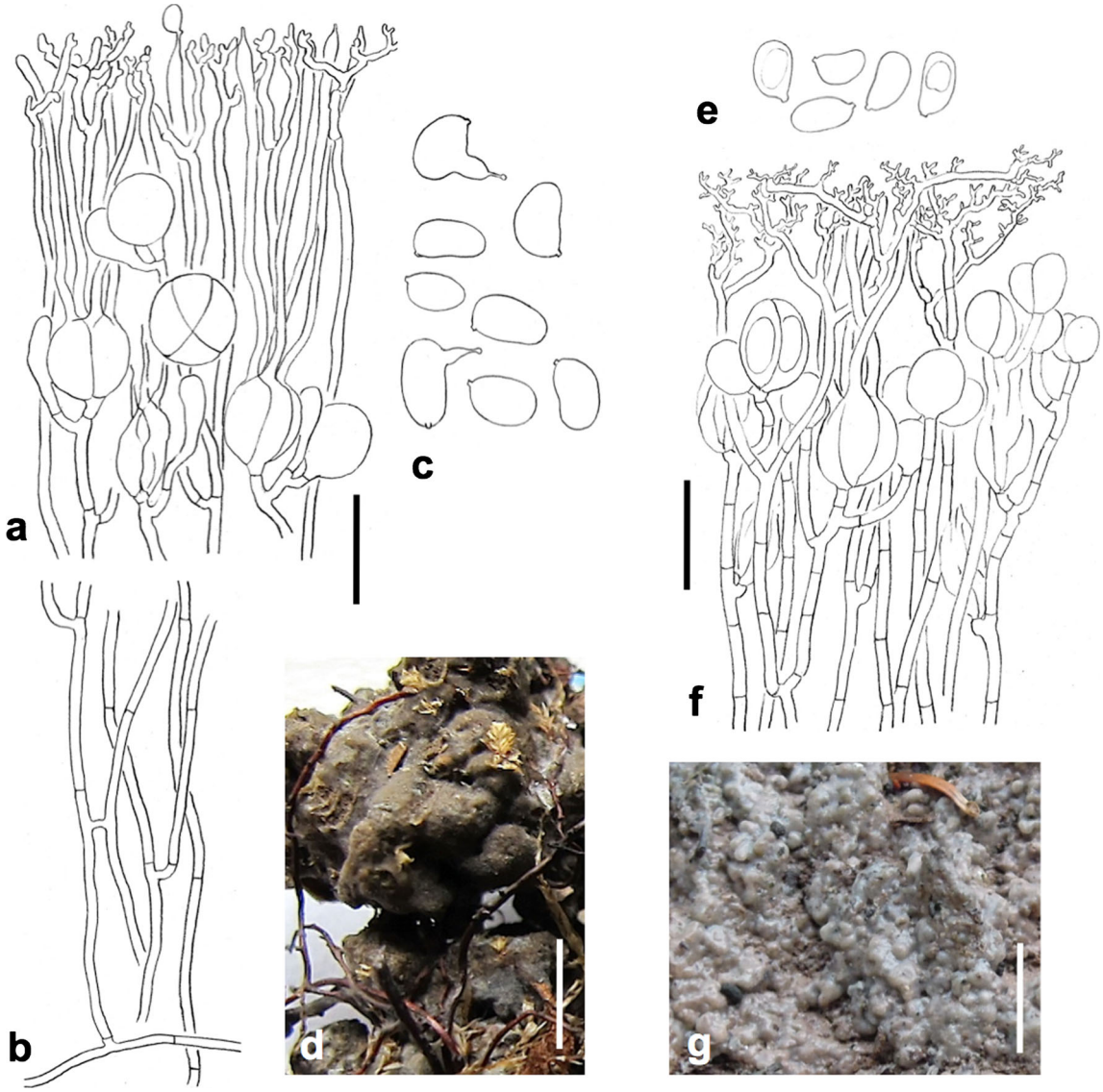


Fig. 12 *Sebacina* sp., clade 17: **a-d** TUB 019686, **e-g** TUB 020023. **a, f** parts of hymenia and subhymenia with basidia in different developmental stages and apically, strongly ramified dikaryophyses. **b, f** (**f** lower part) thin-walled subhymenial hyphae embedded in a gelatinized matrix; **c, e** basidiospores, two germinating by repetition (**c**). **d** dry basidiocarp on soil and plant litter; **g** fresh basidiocarp on soil. Bars: **a-c, e, f** 20 μm; **d, g** 5 mm

Clade 19: *Tremellodendron candidum, Sebacina* s.l. sp., Fig. 14

As mentioned above under clades 12 and 13, species traditionally assembled in *Tremellodendron* appear to be paraphyletic in molecularly-based phylograms. Clade 19 of our phylogenetic tree comprises the generic type, *T. candidum*, and four additional collections of *Tremellodendron* spp. from various locations. Unfortunately, several of them (TUB 020330, TUB 020331) were not fertile, and therefore not usable for micromorphological comparisons. The two species illustrated in Figs. 14 and 17, tentatively identified as *T. candidum* and *T. schweinitzii*, cluster in clades 19 and 22, respectively. Both species differ considerably in gross morphology (Figs. 14, e; 17, b, c, f), but share common structural features in micromorphology. As discussed above (clade 12), apparently all *Tremellodendron* species have dimitic hyphal systems, the thick-walled hyphae in central parts of the fructifications for the functional purpose of stabilizing the upright growth of basidiocarps. Subhymenial and hymenial hyphal arrangements are rather similar. There appear to be differences in ramification types of dikaryophyses (compare Figs. 14, a, b, f; 17, a, d); those in *T. candidum* are drawn in bold to facilitate tracing them from their origin to the hymenial surface. However, these singular analyses cannot be used for a conclusive distinction. As all *Tremellodendron* species, these fungi grow in ECM forest communities.

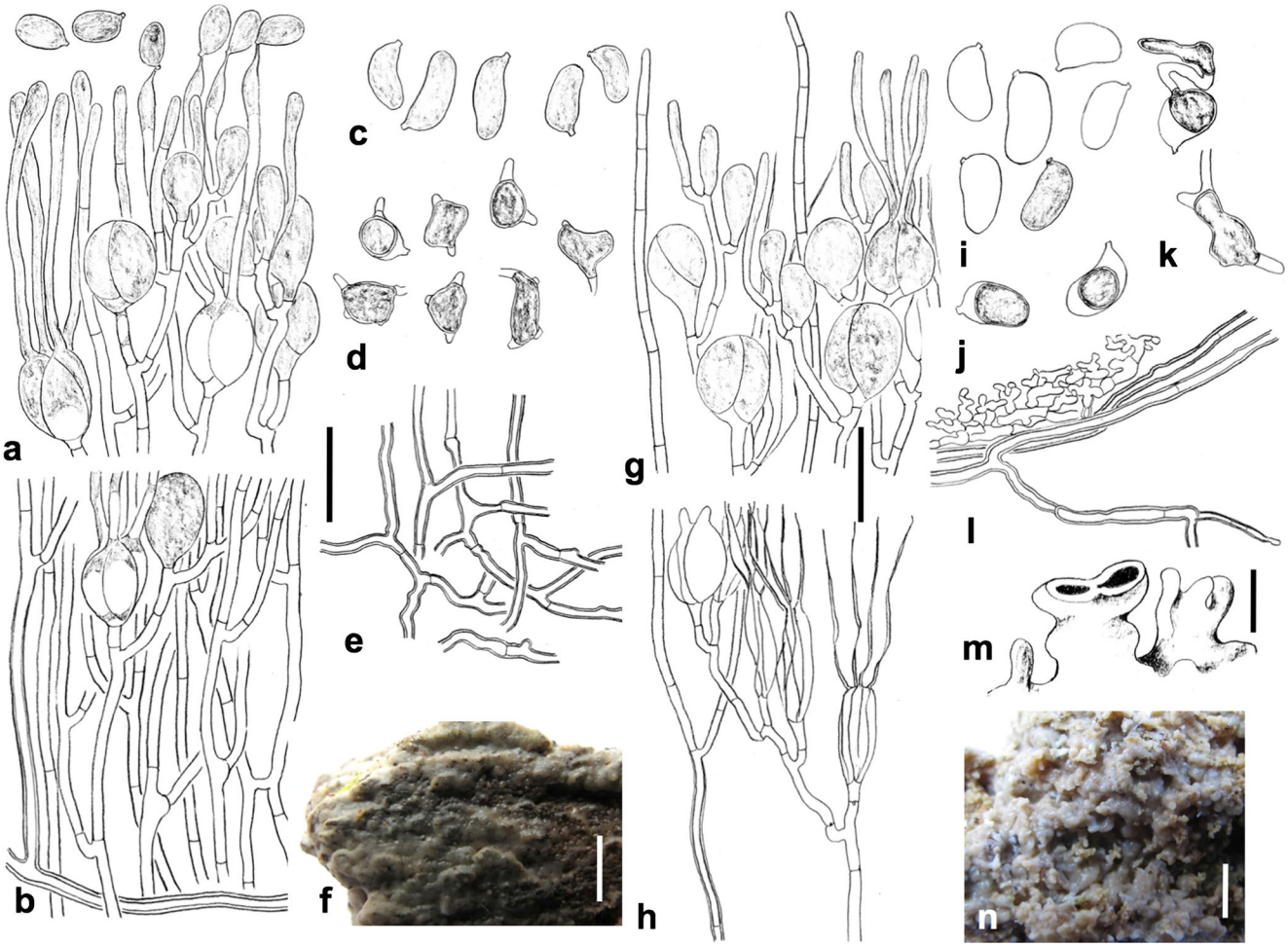


Fig. 13 *Sebacina* sp., clade 18: **a-f** TUB 020024, **g-n** TUB 020025. **a, b, g, h** parts of hymenia with basidia in different developmental stages and simple cystidial hyphae, easily visible in g and h; **b, e, l** basal hyphae thick-walled; **c, i, j** basidiospores. **d, k** germinating basidiospores; **f, n** photographs of dry basidiocarps; **m** detail of hymenial protuberance, partly longitudinally cut off and showing the internal cavities, drawn in black. Bars: **a-e, g-l** 20 μm, **f, n** 5 mm, **m** 1 mm

Clade 20: *Sebacina dimitica*, Fig. 15

Though thick-walled trama hyphae typically occur in *Sebacina* species belonging to the *S. incrustans* group, *S. dimitica* is easily distinguishable by extremely thickened walls of many subhymenial and basal hyphae. While *S. incrustans* fungi have a rather dry to waxy hyphal context, *S. dimitica* is always distinctly gelatinous under well-developed and fresh conditions. It is interesting to note that the phylogenetic position of *S. dimitica* is close to other distinctly dimitic sebacinalean fungi, like *Tremellodendron* spp. It seems logical to assume that dimitic hyphal systems had to be evolved to facilitate upright growing basidiocarps. Interestingly, there are parallels in the Dacrymycetales and in *Typhula*, but most of homobasidiomycetous clavarioid fungi lack such structural prerequisites.

Commonly, ECM fungi develop fructifications on soil. In contrast, *S. dimitica* preferably produces basidiocarps on wood. However, similar ecological specializations occur also in other relationships, e.g., in *Tomentella* of the Thelephorales. ECM associations with *S. dimitica* have been reported several times (Glen et al. 2002; Tedersoo et al. 2006). An orchid mycosymbiont of *Neottia nidus-avis* has been considered to match best with *S. dimitica* (McKendrick et al. 2002).

Clades 21–25: *Sebacina* sp. and *Tremellodendron schweinitzii*, Figs. 16, 17, 18, 19, 20

Extensive molecular and morphological analyses of *Sebacina* sp. in clades 21–25 revealed rather divergent results. All terminal clades are well-supported by high bootstrap values (91-100 %), but they lack deep resolutions (Fig. 1). There is a high congruence of micromorphological traits within the clusters, however, besides predominant resupinate growth forms in all clades, an evolutionary trend for tremellodendroid basidiocarps exists in clade 22. All collecting sites are in ectomycorrhizal forests, indicating that these sebacinoid taxa might be ECM symbionts.

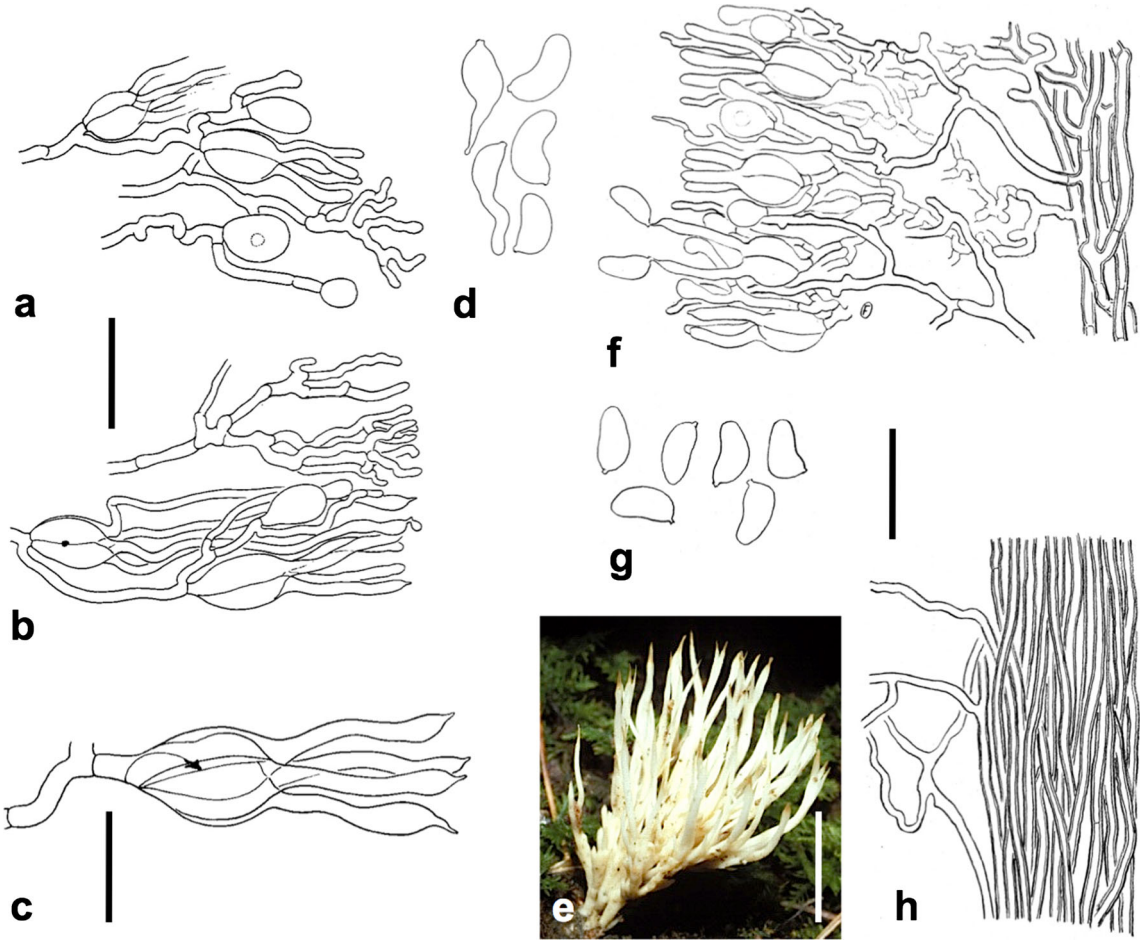


Fig. 14 *Tremellodendron candidum*, clade 19: **a-d** DAOM 73070, **f-h** FO 36700; **a, b, f** parts of hymenia with basidia in different developmental stages, dikaryophyses and subhymenia. **c** basidium with mature sterigmata; **d, g** basidiospores. **h** hyphal context in central part of fructification; **e** living basidiocarp in natural habitat. Bars: **a, b, d, f-h** 20 μm; **c** 10 μm; **e** 2 cm

However, more detailed substrate dependencies cannot be deduced from our data, and there is no sufficient proof that these species, fruiting on decaying wood, are also mycorrhizal symbionts.

Clade 21: *Sebacina* sp., Fig. 16

Sebacina sp. of clade 21 shares essential morphological features in basidiocarp construction, hyphal context, morphology of dikaryophyses, basidia, and primary basidiospores with *S. epigaea* s.str. (clade 16). However, star-shaped resting spores, typical for *S. epigaea* s.str. (Fig. 11, d, g), could not be detected in these collections. In one sample, thick-walled resting spores of irregular shapes were present (Fig. 16, g). All together, morphological features do not allow a concise, specific circumscription of these fungi as a separate taxon. This is in contrast to a 100 % bootstrap support of clade 21. Here, as in the clades 24 and 25, morphological features are so uniform that they cannot be used for further taxon separation. We are also not aware of ecological traits that could explain the genetic differentiation in diverse clades.

Clade 22: *Sebacina* sp. and *Tremellodendron schweinitzii*, Fig. 17

Tremellodendron schweinitzii (=*T. pallidum*) shares essential morphological characteristics with other *Tremellodendron* species treated in this study (clades 12, 19), such as spathulate to clavarioid basidiocarps, thick-walled central hyphae, thickening hymenia, and branched dikaryophyses, as well as mycosymbiontic interactions in ECM forest communities.

In our analysis, *T. schweinitzii* clusters with six *Sebacina* samples, one of which (TUB 019648), named *S. incrustans* s.l. by Riess et al. (2013), is illustrated here (Fig. 17, i-l) for comparison. It shares most micromorphological features with *T. schweinitzii*, especially dry hyphal context, partly fasciculate hyphae, and only sparsely ramified dikaryophyses.

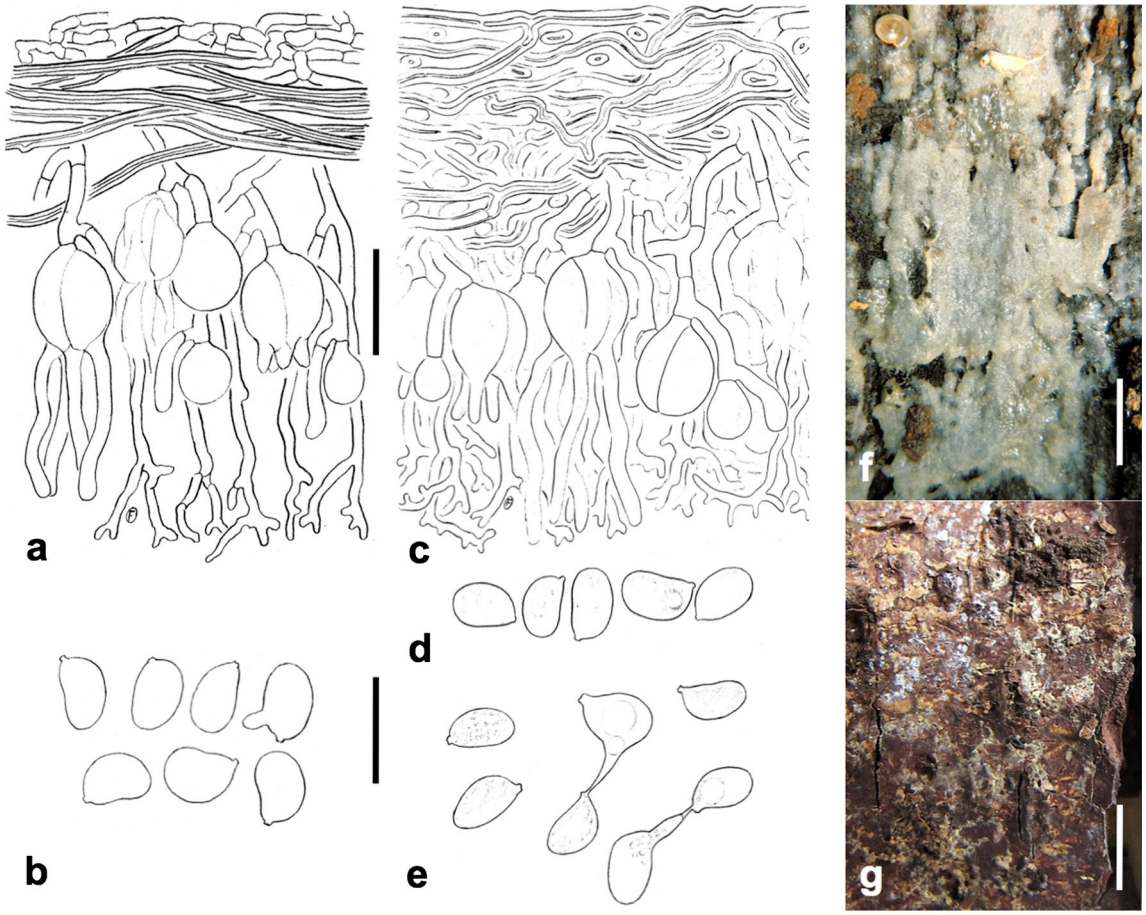


Fig. 15 *Sebacina dimitica*, clade 20: **a, b**, **g** FO 2982; **c, d** FO 3964a holotypus; **e** FO 31795; **f** FO 36305). **a, c** longitudinal sections through whole basidiocarps showing dimitic hyphal systems, hymenia with basidia in different developmental stages, and dikaryophyses. **b, d, e** basidiospores, two germinating with secondary spores. **f, g** basidiocarps growing on wood, **f** well-developed, fresh fructification, **g** dry basidiocarp. Bars: **a-e** 20 μm; **f, g** 1 cm

However, subhymenial and basal hyphae are only slightly thick-walled (Fig. 17, l).

Here again, a 100 % bootstrap value supports a clade, in this one not only containing resupinate but also clavarioid fungi. As discussed already above, the evolutionary development of upright growth was convergently realized in several sebacinoid relationships.

Clade 23: *Sebacina* sp., Figs. 1, 18

Our collections assembled in clade 23 are supported by a 95 % bootstrap value. Basidiocarps, growing on decaying wood, are strictly resupinate and strongly gelatinous when fresh, drying out to inconspicuous, filmy, and horny patches (Fig. 18, j). We found very specific hyphal differentiations, including strongly thick-walled and fasciculate basal hyphae, dikaryophyes originating from them, narrow and thick-walled, in lower parts ramified at large distances, thinning out in the hymenial top layer to fine, thin-walled, flagellate-like terminal cells (Fig. 18, a, h). Occasionally, the upper, thin-walled parts of dikaryophyses are broadened, strongly and shortly ramified, appearing as pseudoparenchymatic cell agglomerations (Fig. 18, h top right) or terminating with resting spores (Figs. 2, b; 18, d, g). The ontogeny of basidia is illustrated in Fig. 2, a. Primary basidiospores are comparatively big, thin-walled, and hyaline (Fig. 18, c, f), and can germinate by repetition or produce resting spores. These (Figs. 2, c; 18, d, g) are normally smaller, irregularly shaped, thick-walled, mostly warty, and faintly pigmented.

Clade 24: *Sebacina* sp., Fig. 19

All collections clustering in clade 24 have strictly resupinate basidiocarps (Fig. 19, d, e), growing on soil mixed with plant litter. Basal hyphae are mostly strongly fasciculate and thick-walled (Fig. 19, i), subhymenial hyphae are thin-walled, embedded in a gelatinous matrix, and predominantly vertically

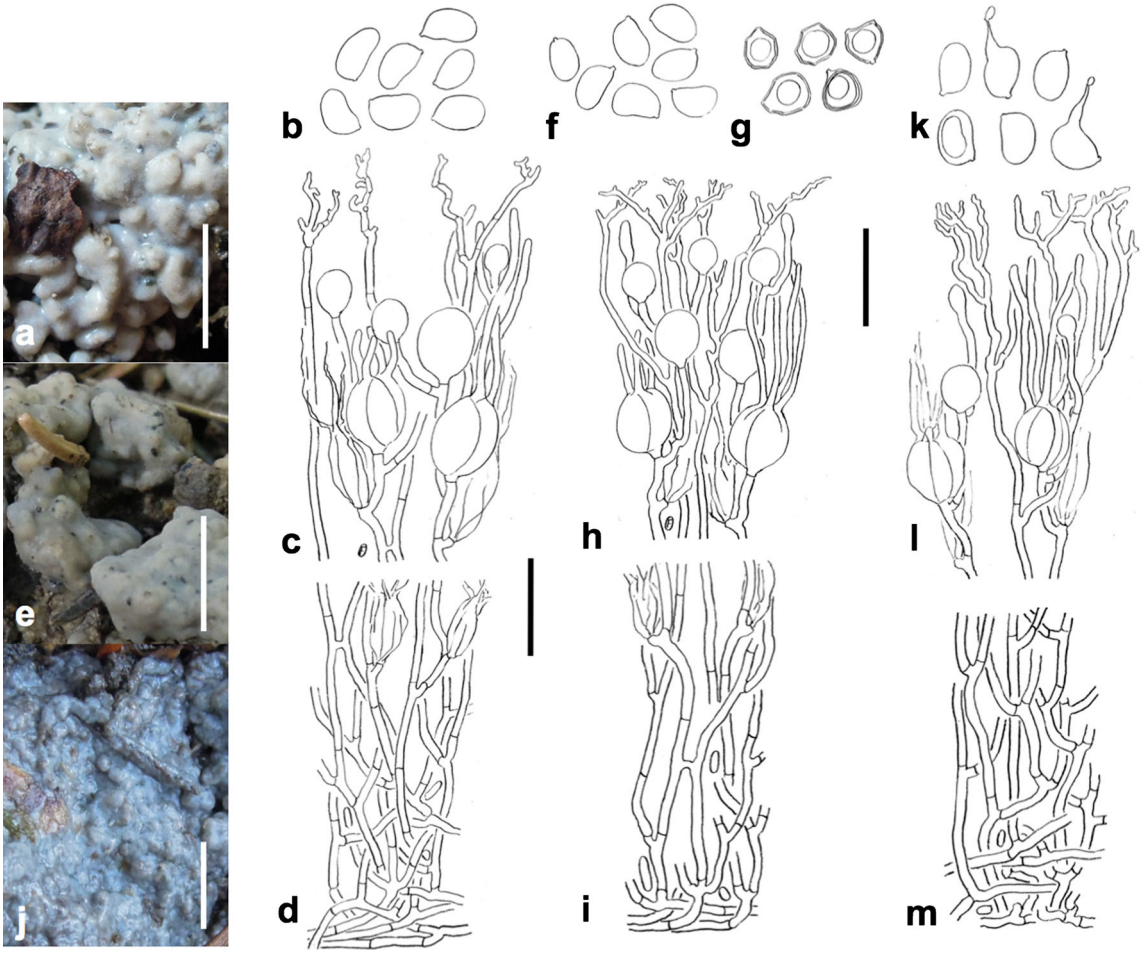


Fig. 16 *Sebacina* sp., clade 21: **a-d** TUB 019985; **e-i** TUB 020007; **j-m** TUB 020000). **a, e, j** fresh basidiocarps; **b, f, k** basidiospores, two germinating with secondary ballistospores; **g** irregularly shaped resting spores; **c, h, l** parts of hymenia with basidia in different developmental stages and dikaryophyses; **d, i, m** thin-walled basal and subhymenial hyphae, the upright ones separated through gelatinized matrices. Bars: **b-d, f-i , k-m** 20 μm; **a, e, j** 5 mm

arranged. Dikaryophyses are loosely branched in upper parts. Basidiospores potentially germinate by repetition. Irregularly-shaped resting spores are rarely observed (Fig. 19, f below to the right). This micromorphological set of characters does not allow a discriminating circumscription of the clade that is supported by a 91 % bootstrap support (Fig. 1, see also Supplementary File 4).

Clade 25: *Sebacina* sp., Fig. 20

Sebacina collections of clade 25 share essential ecological and morphological characteristics. Basidiocarps were exclusively collected on bare soil or on humus layers mixed with soil in ectomycorrhizal forests (Fig. 20, a, e, i). Hyphal systems are always monomitic, hyphae share strongly gelatinizing walls, similar to *Sebacina* species of clades 8, 9, 10, 15, 16, 17, 18, 20, 21, 23, and 24. Basidial ontogeny and spore morphology are of the same structural types and vary only in minor proportions of size. Star-like resting spores of the *Sebacina epigaea* s.str. type (clades 15, 16, 21, 23) have never been observed. However, in all collections of these clades, conspicuous and strongly ramified dikaryophyses occur (Fig. 13, c, g, j).

We hypothesize that the genetic diversity in these morphologically uniform fungi expresses population dynamics rather than fixed evolutionary lines.

Clades 26–32: Supplementary File 4

Many species of the Sebacinaceae (clades 1–25) develop macroscopically visible basidiocarps. Such fructifications are not known in clades 26–32. The few samples with sporulating teleomorphs, collected by chance and finally detected in preparations for light microscopy (Oberwinkler 1964; Roberts 1993), are complemented by isolations from orchid roots that developed in sterile cultures to fruiting structures (Warcup and Talbot 1967; Warcup 1971, 1981, 1988).

Surprising examples for culturable species are *Piriformospora indica* (Verma et al. 1998), *P. williamsii* (Basiewicz et al. 2012), and *Serendipita herbamans* (Riess et al. 2014).

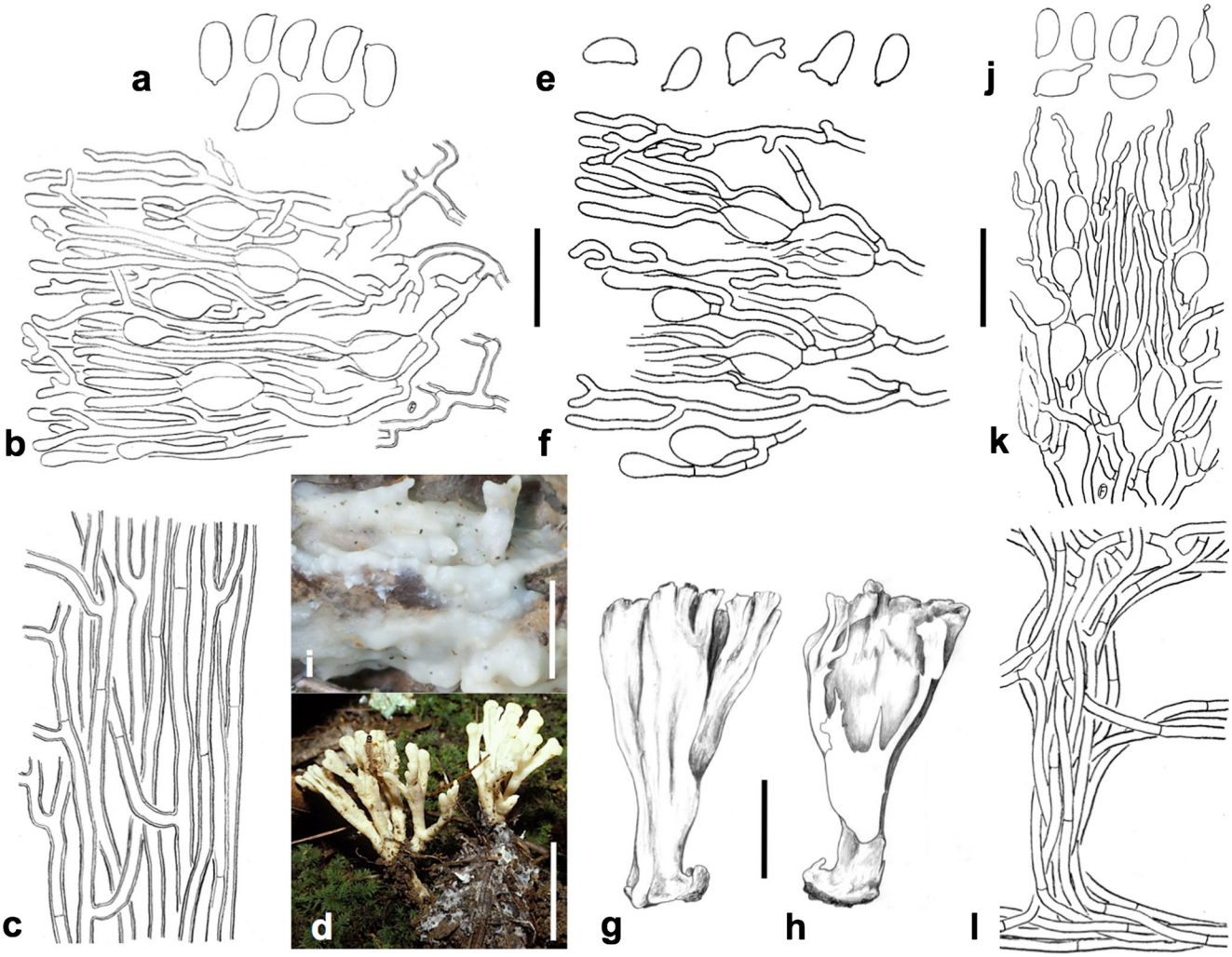


Fig. 17 *Tremellodendron schweinitzii* (**a-h**) and *Sebacina* sp. (**i-l**), clade 22: a-d FO 36701, **e-h** DAOM 73074, **j-l** TUB 019648; **a, e, j** basidiospores, one germinating by repetition; **b, f, k** parts of hymenia with basidia in different developmental stages, dikaryophyses and subhymenia; **c** hyphal context in central part of fructification; **d, i** living basidiocarps in their natural habitats; **g, h** dried basidiocarps, **h** basidiocarp longitudinally cut; **l** subhymenial and basal hyphal context. Bars: **a-c, e, f, j-l** 20 μm; **d** 2 cm; **i** 1 cm; **g, h** 5 mm

The overwhelming majority of sebacinalean fungi belonging to clades 26–32 are not known as individual fungi, but were detected with molecular techniques as plant associates. These cryptic fungi cannot be treated here morphologically.

Clade 29: *Serendipita vermifera*, Fig. 21

When Oberwinkler (1964) described *Sebacina vermifera*, the generic scope of *Sebacina* matched the traditional concept of resupinate basidiomycetous fungi with longitudinally septate basidia and clampless hyphae, including very scanty basidiocarps with scattered hyphae and separated basidia or small clusters of them, but not forming a hymenium (Fig. 21). Unique features of *S. vermifera* are very small hyphae, meiosporangia with subbasidial clamps, and vermiform, nematode-like basidiospores. Later, Roberts (1993) proposed the genus *Serendipita* for *Sebacina vermifera* and added five more species, a treatment that requires both morphological and molecular confirmation.

Taxonomic implications

To accommodate higher systematic groupings within the Sebacinales we propose to restrict the Sebacinaceae to clades 1–25 (Fig. 1, Supplementary File 4), formerly called Sebacinales group A. For clades 26–32 currently assigned to Sebacinales group B (Urban et al. 2003; Weiß et al. 2004, 2011; Setaro et al. 2006; Selosse et al. 2007, 2009; Oberwinkler et al. 2013), a family description will be introduced in a future work.

Sebacinaceae Oberw. & K. Wells, emend. Oberw., Garnica, R. Bauer & K. Riess

These clades differ from members of Sebacinales group B by the possession of the rRNA 5.8S gene sequence signature GTATGCYYGT and the 28S gene sequence

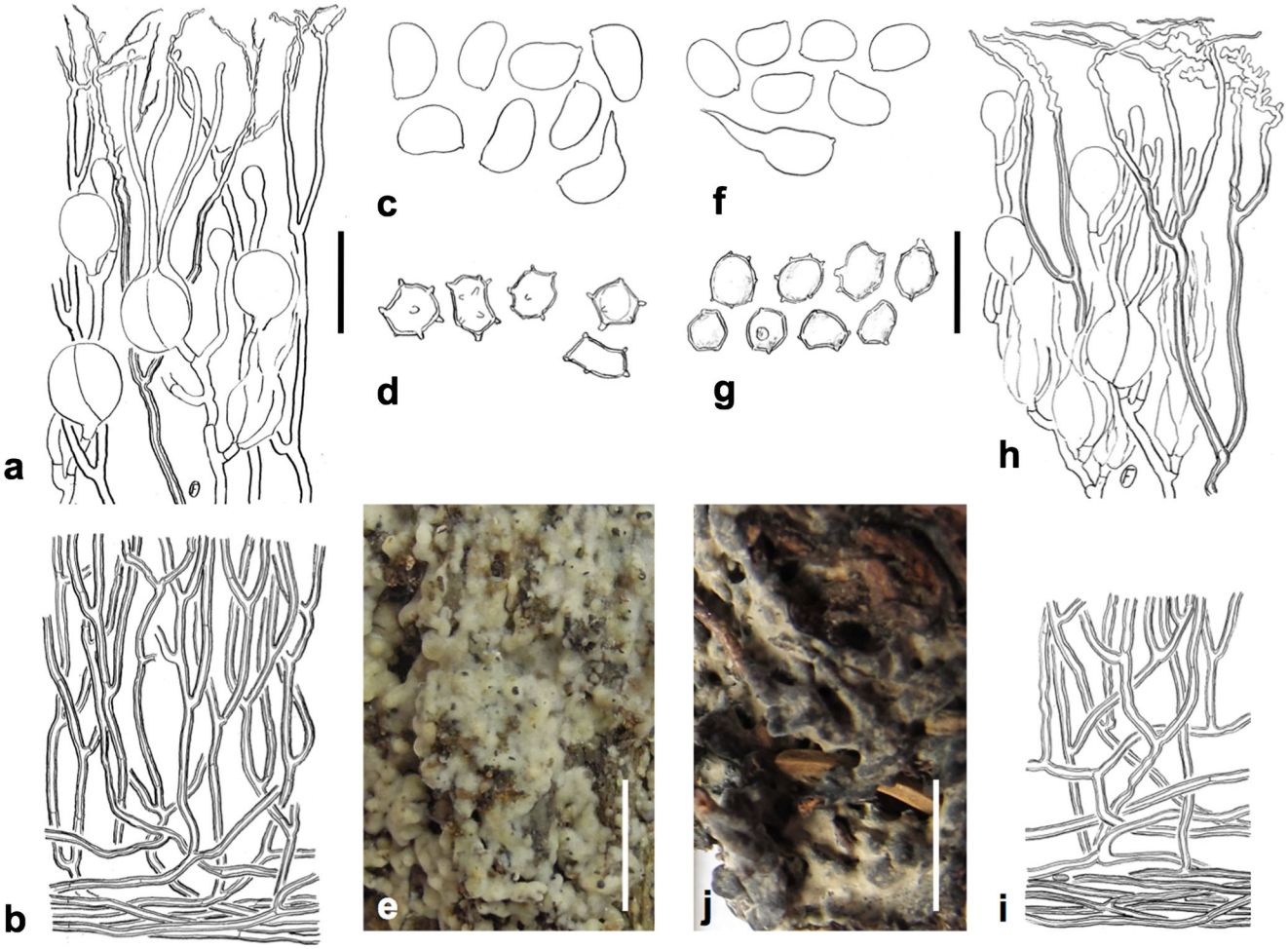


Fig. 18 *Sebacina* sp., clade 23: **a-e** TUB 020036, **f-j** TUB 019662; **a, h** parts of hymenia with basidia in different developmental stages, dikaryophyses and subhymenia; **b, i** subhymenial and basal hyphal context; **c-f** basidiospores, two germinating by repetition; **d, g** resting spores; **e** fresh basidiocarp; **j** dried basidiocarp. Bars: **a-d, f-i** 20 μm; **e, j** 5 mm

signatures CTTGRCCTCAAMTCGRGTA and AAACRCTT, with the underlined nucleotides being specific for the taxon.

This family includes the genera *Chaetospermum*, *Craterocolla*, *Globulisebacina*, *Helvellosebacina*, *Paulisebacina*, *Sebacina*, and *Tremelloscypha*, which are explained in detail below. The type of the family was designated by *Sebacina* Tul., J. Linn. Soc. Bot. 13:35, 1873 (Oberwinkler and Wells in Wells and Oberwinkler 1982, p. 329).

Remarks: This taxonomic rearrangement requires to introduce a new family, Serendipitaceae, for Sebacinales group B that was already announced informally in posters at IMC IX in Edinburgh (Weiß et al. 2010) and at a meeting of the German Phytopathological Society in Hohenheim (Weiß et al. 2012). It was also used without citations in recent papers (Tedersoo et al. 2011, 2013; Tedersoo and Smith 2013). Members of the Sebacinales group B sensu Weiß et al. (2004) differ from species belonging to Sebacinaceae by the possession of the rRNA 5.8S gene sequence signature GTACRCCCGT, and the LSU rDNA sequence signatures TTYGACCTCARATCGRGYG, and AAGCRTTT, with the underlined nucleotides being specific for the taxon. The Sebacinales group B comprises the genera *Piriformospora* (Verma et al. 1998) and *Serendipita* (Roberts 1993), as well as various records of cultures and environmental sequences, named "*Sebacina vermifera*", but of uncertain taxonomic status, not representing members of *Sebacina* as circumscribed in this study.

The clade 1 containing *Sebacina allantoidea* collections strongly deviates from all other sebacinalean taxa analyzed in this study and known to the authors. Therefore, we propose a new genus, and *Sebacina allantoidea* is newly combined and placed within it.

Paulisebacina Oberw., Garnica & K. Riess, gen. nov., [MycoBank MB 808187].

Remarks: The proposed monospecific genus is characterized by its extremely thin basidiocarps lacking dikaryophyses (Fig. 3, a-c).

Type: *Sebacina allantoidea* R. Kirschner & Oberw. (2002)

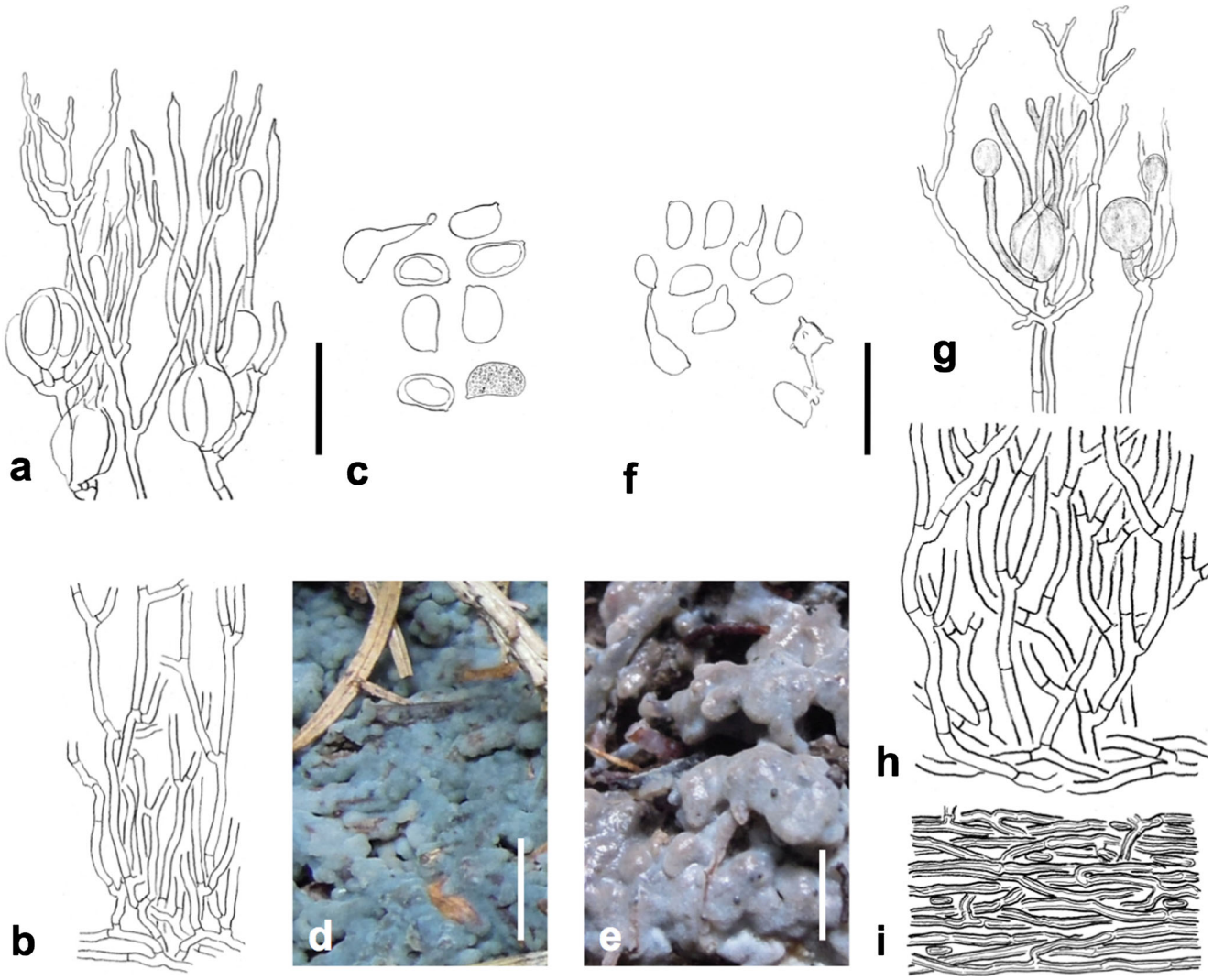


Fig. 19 *Sebacina* sp., clade 24: **a-d** TUB 019992, **e-i** TUB 019994; **a, g** parts of hymenia with basidia in different developmental stages, dikaryophyses and subhymenia; **d, e** fresh basidiocarps; **b, h** basal and subhymenial hyphae, all embedded in a gelatinized matrix; **c, f** basidiospores, several germinating by repetition; **d, e** fresh basidiocarps; **i** thick-walled basal hyphae. Bars: **a-c, f-i** 20 μm; **d, e** 1 cm

Paulisebacina allantoidea (R. Kirschner & Oberw.) Oberw., Garnica, K. Riess & R. Kirschner, comb. nov., [MycoBank MB 808201].

Basionym: *Sebacina allantoidea* R. Kirschner & Oberw., Cryptog. Mycol. 23(2): 130 (2002).

Remarks: *Renatobasidium notabile* Hauerslev (1993) lacks clamps, indicating that it may belong to the Sebacinales. Ramified dikaryophyses occur in the hymenium and basidia are partly repetitive. Therefore, this species clearly deviates from *P. allantoidea*.

A sequence including ITS, 5.8S and D1/D2 regions of the nuclear rDNA from the holotype of *P. allantoidea* is available at GenBank (accession no. KF06126).

Members of the genera *Efibulobasidium* and *Chaetospermum* cluster in clades 3 and 4. When introducing *Efibulobasidium*, Wells (1975) typified the new genus by *E. albescens* (syn. *Epidochium albescens*). Donk (1958) has chosen *Agyrium atrovirens* as type species for *Epidochium*. It is considered to be a synonym of *Tremella exigua*, and therefore, is not applicable as a new generic name (Wells 1975). The type species of *Chaetospermum, Ch. chaetosporum,* and *Efibulobasidium, E. albescens*, are strikingly similar. Kirschner and Oberwinkler (2009) reported basidiospores and conidia of *Ch. gossypinum* on a single Taiwanese collection of *E. albescens*. Based on these data, we conclude that *Chaetospermum* and *Efibulobasidium* are congeneric, and the latter should be proposed as a synonym of *Chaetospermum*. A nomenclatorial rearrangement of species seems premature because specific connections of anamorph and teleomorph stages are not yet clarified. Because of strikingly similar teleomorph characteristics, Wells (1975) also included *Exidia rolleyi* in *Efibulobasidium*. In our phylogenetic analysis, the two *Efibulobasidium* species cluster in separate

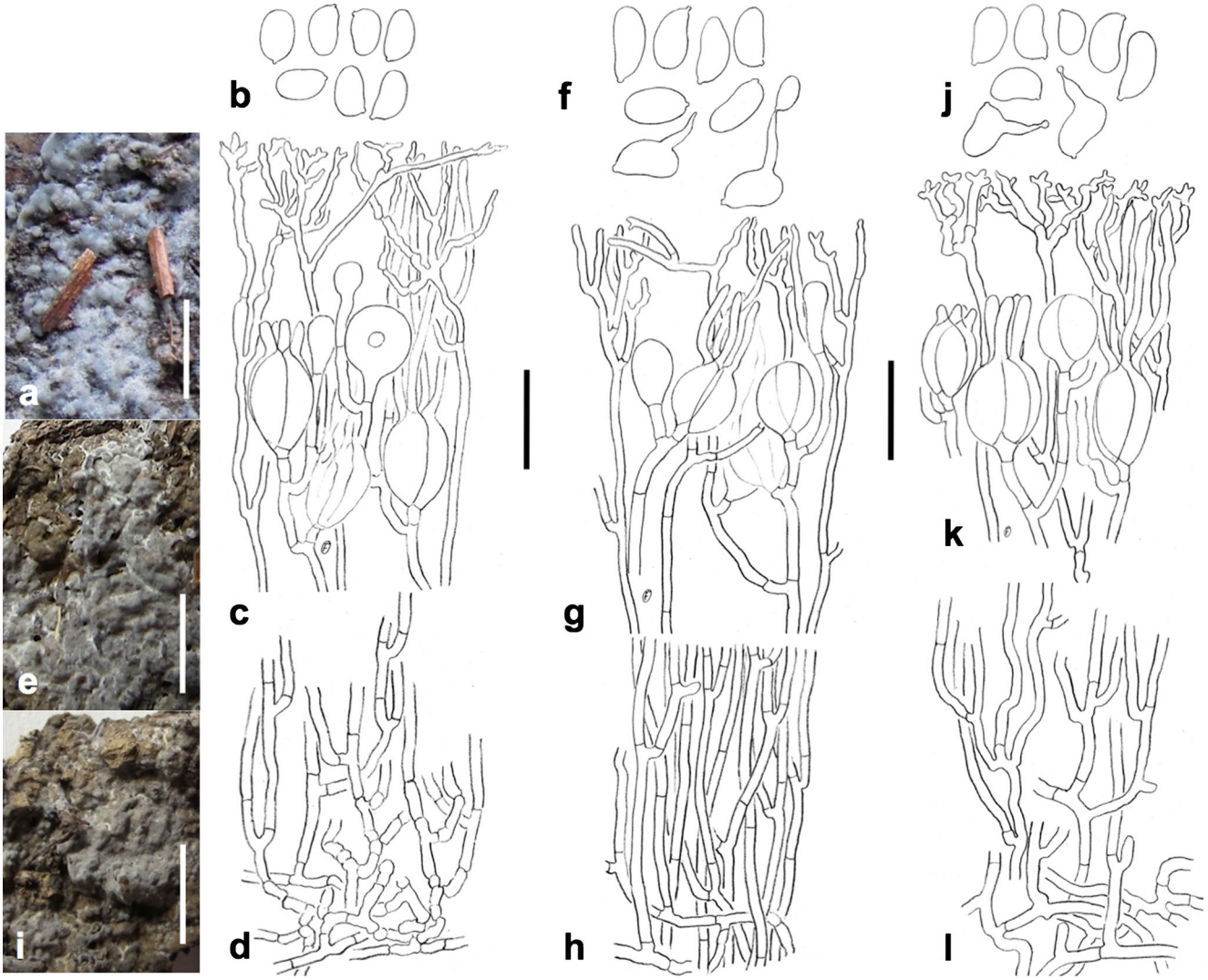


Fig. 20 *Sebacina* sp., clade 25: **a-d** TUB 019996; **e-h** TUB 019654; **i-l** TUB 019696 (=TUB 019844). **a** fresh basidiocarp; **e, i** dry basidiocarps; **b, f, j** basidiospores, germination by repetition in **f** and **j**, not observed in **b. c, g, k** parts of hymenia with basidia forming slightly thickening layers, and apically, strongly ramified dikaryophyses. **d, h, l** basal and subhymenial hyphae, all embedded in a gelatinized matrix. Bars: **a, e, i** 1 cm; for all other figs 20 μm

clades. We consider the differences in hymenial construction and the presence of a specific anamorph in *E. albescens* as convincing indicators to separate both taxa generically.

Globulisebacina Oberw., Garnica & K. Riess, gen. nov., [MycoBank MB 808188].

Remarks: Genus of the Sebacinaceae with tiny globular basidiocarps lacking *Chaetospermum* anamorphs (Fig. 4, g-i).

Type: *Exidia rolleyi* L.S. Olive (1958).

Globulisebacina rolleyi (L.S. Olive) Oberw., Garnica & K. Riess, comb. nov., [MycoBank MB 808191].

Basionym: *Exidia rolleyi* L.S. Olive, Bull. Torrey bot. Club 85: 95 (1958), syn. *Efibulobasidium rolleyi* (L.S. Olive) K. Wells (1975).

Our comparative analyses have shown that essential morphological (Fig. 5, a-i) and DNA sequence features (clade 5, Fig. 1, Supplementary File 4) of *Tremelloscypha* and *Tremellostereum* are identical. Therefore, we transfer *Tremellostereum dichroum* into *Tremelloscypha*.

Tremelloscypha dichroa (Lloyd) Oberw., Garnica & K. Riess, comb. nov., [MycoBank MB 808189].

Basionym: *Stereum dichroum* Lloyd, Mycol. Writ. 7 (Letter 67): 1158 (1922), syn. *Tremellostereum dichroum* (Lloyd) Ryvarden (1986).

When describing *Sebacina amesii*, Lloyd (1916) discussed the justification to erect a new genus for the species, but he refrained from doing so. We found that *S. amesii* matches best with *Tremelloscypha* spp. (Fig. 5). Even when molecular data are lacking, a taxonomic improvement is justified.

Tremelloscypha amesii (Lloyd) Oberw., Garnica & K. Riess, comb. nov., [MycoBank MB 808190].

Basionym: *Sebacina amesii* Lloyd, Mycol. Writ. 5 (Letter 42): 576 (1916).

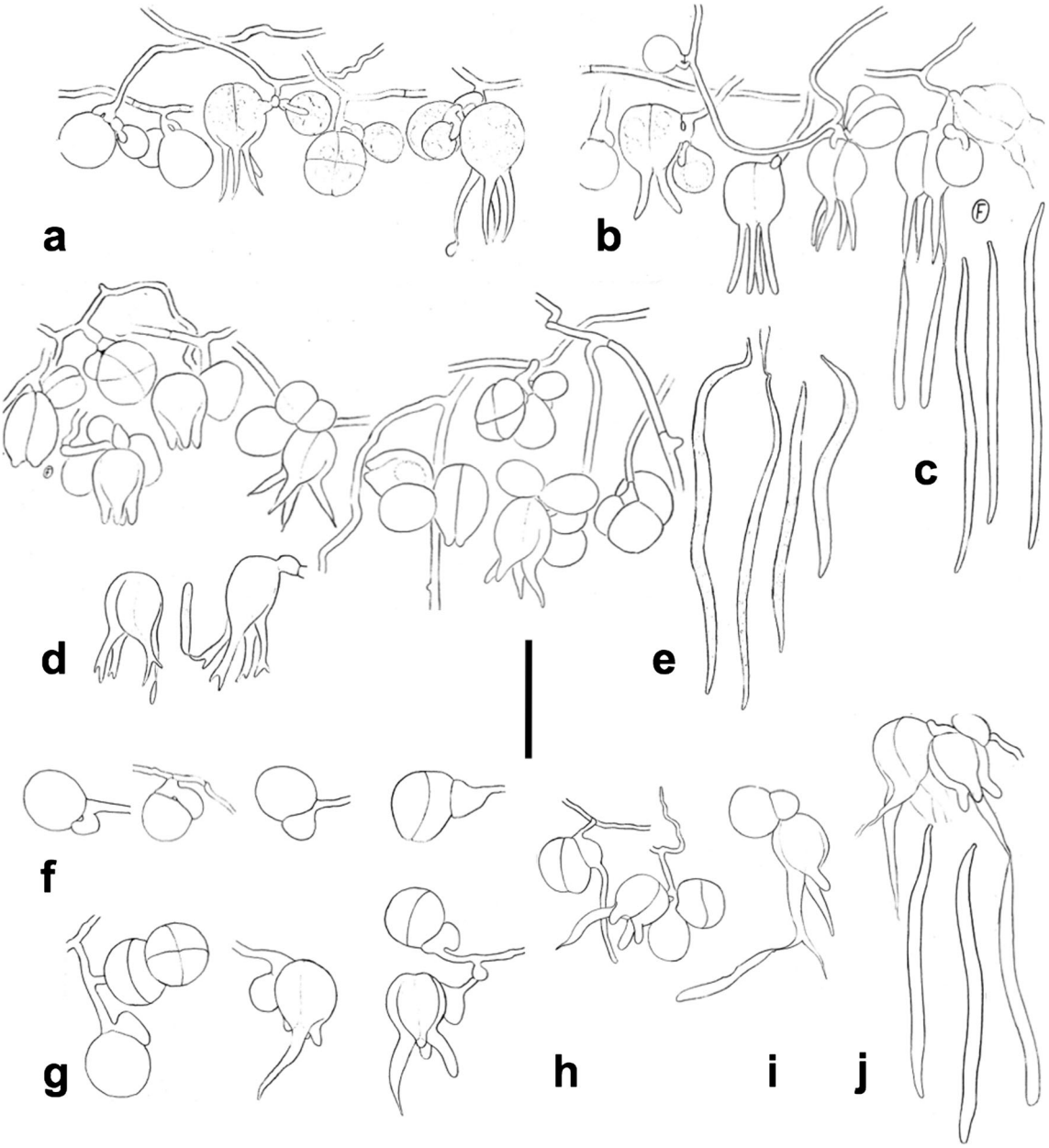


Fig. 21 *Serendipita vermifera*, clade 29: **a-c** FO 11851a; **d-e** FO 6475 holotypus of *S. vermifera*, modified after Oberwinkler (1964); **f-j** FO 13734b. Substrate and generative hyphae, basidia in different developmental stages, and basidiospores. Note that hymenia, dikaryophyses, and subhymenia are lacking. Bar 20 μm

Members of clades 7–10 appear to be closely related by micromorphological features and a well-supported clustering (Fig. 1). Therefore, it appears justified to propose a separate genus for these clades that are clearly distinguished from *Sebacina* s.str.

Helvellosebacina Oberw., Garnica & K. Riess, gen. nov., [MycoBank MB 808192].

Genus of the ectomycorrhizal Sebacinaceae with resupinate to upright merulioid-helvelloid basidiocarps, and hymenia with simple to sparingly ramified dikaryophyses.

Type: *Sebacina helvelloides* (Schwein.) Burt (1915).

The following two species are proposed to be included in *Helvellosebacina*:

Helvellosebacina concrescens (Schwein.) Oberw., Garnica & K. Riess, comb. nov., [MycoBank MB 808193].

Basionym: *Peziza concrescens* Schwein., Schr. Naturf. Ges. Leipzig 1: 118 (1822).

Helvellosebacina helvelloides (Schwein.) Oberw., Garnica & K. Riess, comb. nov., [MycoBank MB 808194].

Basionym: *Thelephora helvelloides* Schwein., Schr. Naturf. Ges. Leipzig 1: 108 (1822).

As explained under clades 12, 19, and 22, *Tremellodendron* species, including the type of the genus, *T. candidum*, so far

studied morphologically and molecularly, cluster within *Sebacina* s.str. (Fig. 1). Consequently, *Tremellodendron* has to be put under synonymy of *Sebacina*. Therefore, we propose the following new combinations:

Sebacina candida (Schwein.) Oberw., Garnica & K. Riess, comb. nov., [MycoBank MB 808195].

Basionym: *Merisma candidum* Schwein., Schr. Naturf. Ges. Leipzig 1: 110 (1822).

Sebacina ocreata (Berk.) Oberw., Garnica & K. Riess, comb. nov., [MycoBank MB 808196].

Basionym: *Thelephora ocreata* Berk., Hooker's J. Bot. Kew Gard. Misc. 8: 239 (1856).

Sebacina pallida (Schwein.) Oberw., Garnica & K. Riess, comb. nov., [MycoBank MB 808197].

Basionym: *Thelephora pallida* Schwein., Trans. Am. Phil. Soc., Ser. 2, 4 (2): 186 (1832), syn. *Thelephora schweinitzii* Peck (1876), *Tremellodendron pallidum* Burt (1915).

The clades 18 and 23 contain *Sebacina* collections with peculiar microscopical features that are, to our knowledge, not known in other *Sebacina* species.

Sebacina cystidiata Oberw., Garnica & K. Riess, sp. nov., [MycoBank MB 808198].

Fresh basidiocarps gelatinous, resupinate to merulioid, dry forming horny crusts. Basal hyphae, 2–4 μm in diameter, thick-walled, subhymenial hyphae, inclusive generative ones, thin-walled, 2–4 μm in diameter. Cystidia simple, unbranched, thin-walled, 3–4 μm in diameter, often septate, protruding the hymenium. Mature basidia globose to oviform, 15–22 μm in diameter, longitudinally septate, four-celled, with tubular sterigmata, 2–3 × 30–70 μm. Basidiospores thin-walled and hyaline, 7–9 × 10-16-(20) μm, with multipolar germtube formation, possibly also functioning as resting spores.

Habitat: Basidiomata growing on dead wood, under coniferous (*Abies alba*) and deciduous (*Corylus avellana*, *Fagus sylvatica*, *Quercus robur* and *Q. rubra*) trees, holotype TUB 020024, Germany.

Remarks: Collections of the new species *S. cystidiata* cluster in clade 18 (Fig. 13), with 100 % bootstrap value (Fig. 1), and these share simple cystidia or cystidia-like elements (Fig. 13, a, g). Sequence divergences between collections of *S. cystidiata* ranged from 0 % to 2.04 % in the ITS region and from 0 % to 1.14 % in D1/D2 regions of the LSU rDNA, respectively.

A sequence including the ITS, 5.8S, and D1/D2 regions of the nuclear rDNA from the holotype of *S. cystidiata* is available at GenBank (accession no. KF000452).

Sebacina flagelliformis Oberw., Garnica & K. Riess, sp. nov., [MycoBank MB 808199]

Fresh basidiocarps gelatinous, resupinate, drying to filmy crusts on rotten wood. Basal hyphae, 2–3 μm in diameter, strongly thick-walled, subhymenial hyphae 2–4 μm in diameter, moderately thick-walled, generative hyphae thin-walled 2–4 μm in diameter. Mature basidia globose to oviform, 15–20 μm in diameter, longitudinally septate, four-celled, with tubular sterigmata, 2–3 × 20–50 μm, ultimately protruding the layer of dikaryophyses. Primary basidiospores thin-walled and hyaline, 9–12 × 12–16 μm, capable of germinating by repetition. Resting spores irregular in outline, mostly warty, 8–11 × 10–12 μm, thick-walled, pigmented, originating from terminal cells of dikaryophyses or by germination of primary basidiospores.

Habitat: Basidiomata growing on dead wood, under coniferous (*Abies alba*) and deciduous (*Fagus sylvatica*) trees, holotype TUB 020036, Germany.

Remarks: According to the most striking micromorphological feature, the flagellate dikaryophyses (Fig. 18, a, h), we propose S. *flagelliformis* as a new species. Sequence divergences between collections of *S. flagelliformis* ranged from 0 % to 0.37 % in the ITS region and from 0 % to 0.18 % in the D1/D2 regions of the LSU rDNA, respectively.

A sequence including ITS, 5.8S, and D1/D2 regions of the nuclear rDNA from the holotype of *S. flagelliformis* is available at GenBank (accession no. KF000464).

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Author Contributions FO, KR, and SG collected fungal samples, FO performed the microscopic work and illustrations, and wrote the paper. KR and SG conceived and designed the molecular studies, and constructed the phylogenetic tree. RB added critical comments.

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