Rommelaarsia flavovirens gen. et sp. nov. (*Helotiales*), a new discomycete on *Equisetum* with a peculiar asexual state

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Ascomycete.org, 7 (6) : 321-330.
Novembre 2015
Mise en ligne le 30/11/2015

Summary: *Rommelaarsia flavovirens* gen. et sp. nov. is proposed. This inoperculate discomycete is known only from two collections in Western Europe, fruiting in spring on dead stems of *Equisetum arvensis*. An affiliation with the family *Hyaloscyphaceae* is suggested by the presence of short hairs on the superficially growing apothecium. The species is associated with an unusual, likewise unknown asexual state with large multiguttulate, holoblastic phragmoconidia formed in sporodochia. Phylogenetic analyses of the internal transcribed spacer (ITS) and large subunit (LSU) ribosomal DNA show that *Rommelaarsia* has an uncertain position within the *Helotiales*. Our analyses consistently show a close relationship to *Cistella* and *Psilachnum*, but there is no support for this placement. Bayesian analysis moderately supports the *Cistella* + *Psilachnum* + *Rommelaarsia* clade, which hints at the placement of *Rommelaarsia* within *Hyaloscyphaceae*. This agrees with the morphological data.
Keywords: Ascomycota, *Helotiales*, ITS rDNA, LSU rDNA, *Psilachnum*, taxonomy.

Introduction

In spring 2012 Luciën Rommelaars found a small hairy, greenish olive-yellow discomycete on dead stems of *Equisetum arvense* in the far north of the Netherlands. After many attempts to identify it he still had no clue as to its generic placement. In 2013 he presented this collection with a Dutch description under the heading Unknown beauty as "olijfgeelgroen heermoesschijfje" (= olive-yellow-green horsetail cup, Rommelaars, 2013). His description includes the following characters: sessile, olive-green-yellow apothecia, a rather strong yellow pigmentation of the whole tissue, which does not change in KOH and is particularly found on the encrusted cortical hyphae, the latter covering an ectal excipulum of *textura prismatica* and ending at the margin in short, cylindrical, thin-walled, smooth, yellowish-brownish, somewhat flexuous hairs.

Three years later, in May 2015, Patrice Tanchaud reported the same species in western France on the same host plant. A portion of each collection was kindly sent in the fresh state to the first author. A third collection on this host from the south of the Netherlands came to our notice shortly before the manuscript was finished and was seen by us from macrophotos only.

Sporodochia with large, guttulate phragmoconidia occurred in the two collections examined and seemed to belong in the life cycle of this species. After screening our personal database on helotialean species and their substrates and the host lists of Sydow (1898), Oudemans (1919-1924) and Farr *et al.* (1989), no taxon could be detected that matches either of these two morphs. No appropriate genus appears to exist for this peculiar fungus.

In this paper, we provide detailed descriptions of both the sexual (teleomorph) and asexual (anamorph) states of the fungus. In addition, the phylogenetic position of the discomycete was examined through phylogenetic analyses of internal transcribed spacer (ITS) and large subunit (LSU) ribosomal DNA (rDNA) sequences.

Material and methods

Morphological studies

Microscope slides were examined with a Zeiss Standard 14 compound microscope. Macro- and microphotographs were taken with a Nikon Coolpix E4500. Sections and squash mounts were studied in tap water. The iodine reaction was tested with Lugol's solution (IKI) without KOH-pretreatment, with a concentration of iodine around 0.5–1%. KOH (5–10%) was used to test the solubility of the greenish-yellow pigment. CRB (aqueous Cresyl Blue) was applied to stain gel on cell walls. The description is mainly based on study of fresh, living specimens. Measurements for living (*) and dead (†) elements are given separately. Further abbreviations are: LBs = lipid bodies (lipid content: 0 = without lipid bodies (LBs), 5 = maximum possible lipid content in relation to ascospore volume), VBs = vacuolar bodies. Numbers in curled brackets {} indicate the number of collections from which the given data were gained. Voucher specimens are preserved in the herbarium of the Botanische Staatssammlung München (M), the Farlow Herbarium at Harvard University in Cambridge, MA (FH), and the private herbaria of L. Rommelaars (L.R.), P. Tanchaud (P.T.) and H.-O. Baral (H.B.).

Cultural studies were not undertaken.

DNA extraction, PCR and sequencing

DNA was extracted from dry apothecia from both collections and sporodochia from Tanchaud's collection from France, using the Qiagen DNeasy Plant Mini Kit and the Sigma-Aldrich Extract-N-Amp Plant PCR Kit. Undiluted DNA or a 1/10 and 1/100 dilution was used for PCR amplification of ITS and LSU rDNA regions. The ITS was amplified using the primers ITS1f (Gardes & Bruns, 1993) and ITS4 (White *et al.*, 1990). The LSU region was amplified using primers LR0R and LR5 (Moncalvo *et al.*, 2000), and LR3R (Hopple & Vilgalys, 1999). All PCR reactions were done in Eppendorf Mastercycler ep gradient thermocyclers and used TaKaRa Ex Taq or Sigma-Aldrich REDTaq DNA Polymerase.

PCR conditions were as follows: an initial denaturation step at 93 °C for 5 min, then 40 cycles of 93 °C for 30 sec, 53 °C for 2 min and 72 °C for 2 min, and a final extension step at 72 °C for 10 min (ITS); or an initial denaturation step at 94 °C for 2 min, then 40 cycles of 94 °C for 1 min, 52 °C for 1 min and 72 °C for 1:30 min, and a final extension step at 72 °C for 5 min (LSU); or, in the case of the Extract-N-Amp Plant PCR Kit, according to the suggested protocol in the manufacturer's instructions.

Products that showed clear bands on agarose gel were cleaned with Qiagen Qiaquick PCR Purification Kit and subsequently sequenced. We prepared 10 µl sequencing reactions with the same primer pairs and 3 µl of purified PCR product. The sequencing reactions were performed using the Big Dye® Terminator v3.1 Cycle Sequencing Kit.

Sequences were trimmed, edited and assembled in Sequencher v. 4.10.1. We performed BLAST searches on sequences at http://ncbi.nlm.nih.gov/blast/Blast.cgi for similar sequences.

Sequence alignment and phylogenetic analyses

A combined ITS+LSU rDNA data matrix containing 47 isolates representing 43 species was constructed (Table 1 for GenBank accession numbers). Taxonomical sampling covered seven families in the order *Helotiales* (*Arachnopezizaceae*, *Calloriaceae*, *Cenangiaceae*, *Hyaloscyphaceae*, *Pezizellaceae*, *Rutstroemiaceae*, *Sclerotiniaceae*), the *Stamnaria* + *Roseodiscus* clade, and some species without clear affinities. *Geoglossum nigritum* (*Geoglossales*, *Geoglossaceae*) served as outgroup taxon. Alignment of the DNA sequences was done using Se–Al v. 2.0a11 (Rambaut, 2002). The concatenated dataset in-

Table 1. Strains included in phylogenetic analyses. GenBank (and NBRC) accession numbers are given for the ITS and LSU rDNA regions

Genus	Species	Isolate	ITS rDNA	LSU rDNA
Geoglossum	*nigritum*	AFTOL-ID 56	DQ491490	AY544650
Hyphodiscus	*hymeniophilus*	MUCL 40275	DQ227258	DQ227258
Cistella	*spicicola*	CBS 731.97	GU727553	GU727553
Venturiocistella	*japonica*	TNS-F18030	JN033447	JN033447
Cistella	*acuum*	CCF 3970	FR667211	FR667860
Psilachnum	*chrysostigma*	14793	JF908572	–
Psilachnum	*ellisii*	KUS-F52663	JN033428	JN086731
Hamatocanthoscypha	*laricionis*	TNS-F24336	JN033455	JN086755
Cistella	*acuum*	CBS 605.77	GU727552	GU727552
Hamatocanthoscypha	*laricionis*	TNS-F13530	JN033441	JN086742
Rodwayella	*sessilis*	H.B. 9913	KT876974	KT876974
Arachnopeziza	*aurata*	TNS-F11212	JN033436	JN033436
Arachnopeziza	*obtusipila*	TNS-F12769	JN033446	JN086747
Calycina	*citrina*	Andy 9/27/03	AY789386	AY789385
Calycina	*herbarum*	KUS-F51458	JN033390	JN086693
Calycina	*populina*	CBS 247.62	JN033382	JN086685
Roseodiscus	*formosus*	SBRH 686	KT972711	KT972712
Chlorencoelia	*torta*	KUS-F52256	JN033400	JN086703
Heyderia	*abietis*	HMAS71954	AY789297	AY789296
Heyderia	*abietis*	OSC60392	AY789290	AY789289
Rutstroemia	*firma*	G.M. 01-Dec-2014	KT876987	KT876987
Sclerotinia	*sclerotiorum*	KR1121_1	KC311494	KC311494
Ciboria	*amentacea*	A.U. 2760	KT970066	KT970066
Monilinia	*laxa*	SK278	LN714571	LN714571
Roseodiscus	*lapponicus ad int.*	KH.10.18	KT972716	KT972716
Roseodiscus	*rhodoleucus*	H.B. 8488	KT972704	KT972705
Stamnaria	*austriaca*	Gruber 151/225	KT972708	KT972709
Stamnaria	*americana*	H.B. 7261	KT972707	–
Stamnaria	*laetissima*	TNS-F39244	NBRC108774	NBRC108774/AB773854
Leohumicola	*minima*	DAOM 232587	AY706329	–
Leohumicola	sp.	E3-24a	JX912155	JX912155
Rodwayella	*citrinula*	KUS-F52443	JN033414	JN086717
Hyaloscypha	*albohyalina*	TNS-F17137	JN033431	JN086734
Hyaloscypha	*aureliella*	M235	JN943610	EU940153
Hyaloscypha	*vitreola*	M236	JN943615	EU940156
Rhizoscyphus	*ericae*	pkc29	AY394907	AY394907
Urceolella	*carestiana*	TNS-F18014	JN033443	JN086744
Calloria	*urticae*	G.M. 12-Apr-2015	KT185667	KT185667
Laetinaevia	*carneoflavida*	G.M. 25-Jul-2014	KT185666	KT185666
Roseodiscus	*subcarneus*	J.S. 01-Jul-2013	KT972714	KT972715
Rommelaarsia	*flavovirens*	H.B. 9684 (sexual)	KT958772	KT958769
Rommelaarsia	*flavovirens*	H.B. 9951 (asexual)	KT958774	KT958771
Rommelaarsia	*flavovirens*	H.B. 9951 (sexual)	KT958773	KT958770
Cistella	*albidolutea*	KUS-F52678	JN033429	JN086732
Cistella	sp.	KUS-F52527	JN033419	JN086722
Cistella	*grevillei*	JHH 1602	U57089	–
Psilachnum	sp.	KUS-F52448	JN033415	JN086718
Psilachnum	*staphyleae*	KUS-F52105	JN033396	JN086699

cluded 47 taxa and 1489 characters, 946 of which were constant and 364 were parsimony informative.

Phylogenetic analysis was performed using PAUP* 4.0b10 (Swofford, 2002). Maximum parsimony (MP) analysis with heuristic searches consisted of 500 stepwise-addition trees obtained using random sequence addition replicates followed by tree bisection-reconnection (TBR) branch swapping, MulTrees in effect, and saving all equally most parsimonious trees (MPTs). Robustness of individual branches was estimated by maximum parsimony bootstrap proportions (BP), using 200 bootstrap replicates, with TBR branch swapping, a rearrangement limit of 1000, and MaxTrees set at 100. Maximum likelihood (ML) was estimated under a GTR + I + G model of nucleotide substitution, rapid bootstrapping was implemented with 1000 replicates.

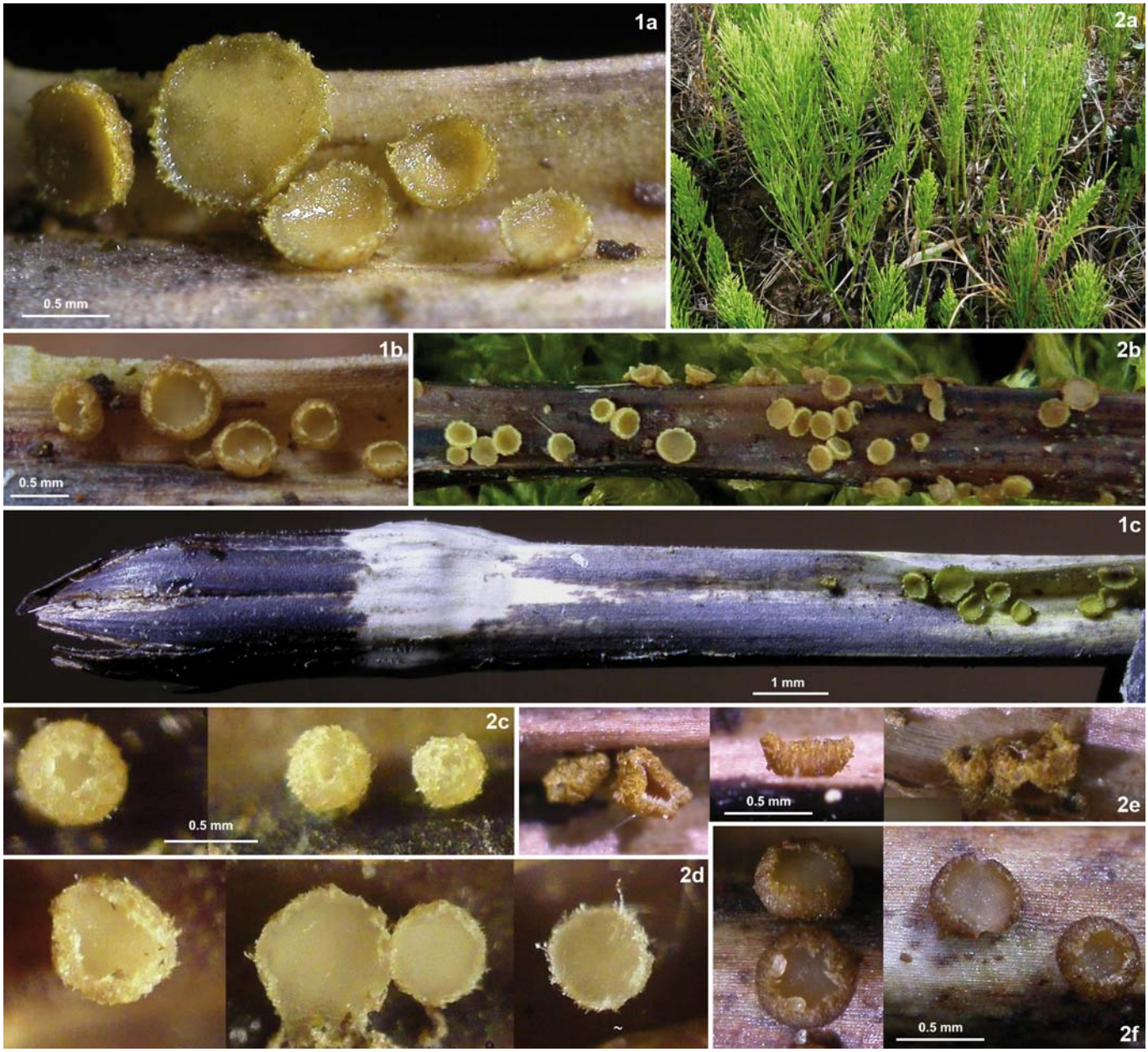

Plate 1. Figs. 1–2. *Rommelaarsia flavovirens* (sexual state), 1a–c: H.B. 9684 (The Netherlands, Groningen), 2a–f: H.B. 9951 (France, Rochefort). – 2a: Collection site with *Equisetum arvense* at La Grande Vergne. 1a–c, 2b–d: Apothecia in fresh state. 2e: Apothecia in dry state. 2f: Apothecia after rehydration. Pictures: 1b: L. Rommelaars, 2a–d: P. Tanchaud.

Bayesian analyses were done with a Markov chain Monte Carlo (MCMC) coalescent approach implemented in BEAST 1.8.2 (Drummond & Rambaut, 2007), with an uncorrelated lognormal relaxed molecular clock allowing for rate variation across the tree. A Bayesian skyride coalescent tree prior was used in all simulations with the general time-reversible model of nucleotide substitution + gamma + invariant sites; the starting tree was randomly generated. Four independent runs were undertaken. Chain length was 10 million generations, with a sampling frequency of 1000. TRACER 1.5 (Drummond & Rambaut, 2007) was used to check the effective sample size (ESS), and burnin values were adjusted to achieve an overall ESS of ≥ 200. Consensus trees with 0% burnin value were generated using TREEANNOTATOR 1.8.2. and visualized in FIGTREE 1.4.2.

Results

Rommelaarsia Baral & Haelew., *gen. nov.* – MB 814656

Diagnosis: The sexual state resembles the genus *Psilachnum*, but deviates by a greenish-yellow exudate and by the absence of refractive vacuoles in paraphyses and hairs. The exudate forms warts on the cortical excipular hyphae, which terminate at the margin in yellowish, smooth, flexuous hairs. The asexual state forms sporodochia with large, multiguttulate, holoblastic phragmoconidia; in species of *Psilachnum* no asexual state is known.

Type species: *Rommelaarsia flavovirens* Baral, Tanchaud & Rommelaars

Etymology: named after Luciën Rommelaars, the first collector of the type species of the genus.

Rommelaarsia flavovirens Baral, Tanchaud & Rommelaars, *sp. nov.* – MB 814657 – Plates 1–3: Sexual state. Plates 4–5: Asexual state.

Etymology: referring to the greenish-yellow pigment in the apothecia and conidiomata.

Description

Sexual state: Apothecia moist 0.4–0.8(–1.1) mm diam., 0.22 mm thick (receptacle 0.12–0.13 mm), non-translucent, round, non-gelatinous, scattered to sub-gregarious; disc pale cream to yellowish-

olivaceous, slightly concave to flat, margin distinct, somewhat hairy-toothed, 30–40 µm protruding (including hairs), pale yellowish-olivaceous, exterior concolourous; subsessile to shortly stipitate, stipe obconical, 0.05–0.08 × 0.15–0.3 mm, superficial, loosely attached to the substrate; dry with ± closed disc, externally light brown, with yellowish ridges and whitish-hairy margin. **Asci** *58–75 × 7.7–9 µm {2}, †50–65 × 6–7.7 µm {1}, 8-spored, spores *obliquely biseriate, *pars sporifera* *24–30(–37) µm long, living mature asci protruding ~0–10 µm beyond paraphyses; apex (†) stongly conical, apical ring †0.7 × 1–1.3 µm, IKI strongly blue, euamyloid, *Calycina*-type; base with short to long, broad stalk arising from croziers {2}. **Ascospores** *(8.5–)9.5–12(–14) × (2–)2.5–3.5(–4) µm {2}, †8–10 × 2.4–2.7 µm {1}, subcylindrical to fusoid or often clavate-cuneate, apex obtuse, rarely subacute, base often attenuated, straight to slightly inequilateral, containing 1–3 minute LBs near each end {2}, lipid content 0.5–1, uninucleate, without sheath; overmature spores not observed. **Paraphyses** apically cylindrical or mostly narrowly lanceolate, smooth, without exudate, terminal cell *21–43 × (2.7–)3.5–4.5 µm {2}, without VBs, contents IKI pale to light redbrown (granular), lower cells *9–17(–23) × 2–3.5 µm {1}; unbranched at upper septum. **Medullary excipulum** hyaline, 40 µm thick, of dense, irregular texture, rather sharply delimited from ectal excipulum. **Ectal excipulum** light greenish yellow, of thin-walled, horizontally oriented *textura prismatica* from base to margin, 30 µm thick at lower flanks, cells *17–35 × 7.5–12 µm {1}; whole exterior covered by a network of cortical hyphae 5–8 µm wide, incrusted by a rough, bright greenish yellow exudate; cortical hyphae ending up at the margin in thin-walled, smooth, sparsely septate, somewhat flexuous, pale to bright yellowish-ochraceous hairs *~30–50 × 3–4 µm {2}. **Anchoring hyphae** sparse, *2.5–3.5 µm wide, light yellow, walls 0.2–0.4 µm thick {1}.

Asexual state (observed on the natural substrate in vicinity of apothecia {2}): **Sporodochia** 0.15–0.27 mm diam., pulvinate, sessile, whitish to light yellow, surface ragged by the projecting conidia. **Conidiophores** ~22–35 µm long, ± cylindrical, straight to ± flexuous, branched below, hyaline, partly with bright sulphur-yellow exudate, conidiogenesis solitary holoblastic, conidiogenous cells *(12–)17–22 × 2.5–4 µm. **Conidia** *(83–)87–105(–118) × (14–)14.5–16(–18.5) µm {2}, †13–15 µm wide, cigar-shaped to fusiform, ± strongly tapered at the ends, hyaline, thin-walled, base sometimes with yellow exudate, (7–)8–11(–12)-septate, slightly constricted at septa, inequilateral or slightly to rather strongly curved, often somewhat flexuous, often ± bent below, multiguttulate (high lipid content), larger LBs 2–4.5(–5.5) µm diam., also each cell with one large central LB (by confluence); young conidia eguttulate, even when septa are formed; wall surface unstained in CRB.

Crystals and pigment: Entire tissue without crystals. Yellow exudate and cell contents not changing colour in KOH, pigment not extruding in medium (non-ionomidotic); dead cells of all elements with yellow cytoplasm.

Habitat: 0–26 m alt., on previous year's stems of *Equisetum arvense* {2} lying on the moist ground (without running or standing water). **Associations:** none observed. **Drought tolerance:** only conidia and ascospores were found to be viable when examined one week after drying of the stems. **Phenology:** May.

Studied collections: France: Poitou-Charentes, department Charente-Maritime, 20 km S of Rochefort, 3 km WSW of Sainte-Gemme, W-border of village La Grande Vergne, 45°45'45" N, 0°55'37" W, 26 m a.s.l., previous year's stems of *Equisetum arvense*, 5. & 16.V.2015, *leg*. P. Tanchaud (M-0276613, ex H.B. 9951, **holotype**; P.T. 20150506, isotype; FH 00304335, isotype). – The Netherlands: province of Groningen, 30 km NW of Groningen, 2.8 km S of Lauwersoog, Ballastplaat, 53°22'48" N, 6°12'45" E, -1 m a.s.l., previous year's fertile stems of *Equisetum arvense*, 11.V.2012, *leg*. L. Rommelaars (ex H.B. 9684, paratype; FH 00304334, paratype, L.R. 12-058, paratype).

Not studied collection: The Netherlands: province of Noord-Brabant, 16 km ESE of Tilburg, 1.1 km NNW of Oirschot, crossing of Peperstraat and Ekerschotweg, 51°30'49" N, 5°18'14" W, 12 m a.s.l., previous year's stem of *Equisetum arvense*, 20.V.2015, *leg*. H. van Hooff, *vid*. L. Rommelaars (L.R. 15-054).

Phylogenetic analyses

The LSU rDNA nucleotide sequences generated for the two sexual state collections H.B. 9648 and H.B. 9951 were identical. Their ITS sequences differed in a single nucleotide (on a total of 446 nucleotides). For H.B. 9951 we were able to generate SSU and LSU sequences of the asexual state, confirming with certainty the connection between the sexual and asexual state of this fungus (the sequences of the sexual state are identical to those of the asexual state).

The best scoring ML tree (-lnL = 12708.70821) is shown in Plate 6. Maximum parsimony, maximum likelihood, and Bayesian phylogenetic analyses of rDNA sequence data support the placement of *Rommelaarsia* in the *Helotiales*. However, the phylogenetic position of the genus within this order remains unclear. In all three analyses, *Rommelaarsia* groups with species in the genera *Cistella* Quél. and *Psilachnum* Höhn. In the MP analysis the relationship is as follows: (*Psilachnum*, (*Cistella*, *Rommelaarsia*)). In the ML analysis: (*Rommelaarsia*, (*Cistella*, *Psilachnum*)). After Bayesian inference: (*Cistella*, (*Psilachnum*, *Rommelaarsia*)). Although there is no bootstrap support for these placements, the posterior probability of the *Cistella* + *Psilachnum* + *Rommelaarsia* clade is 0.72. This tentatively hints at the placement of *Rommelaarsia* in the *Hyaloscyphaceae*. However, this family appears to be phylogenetically heterogeneous, as are the genera *Cistella* and *Psilachnum*.

Discussion

Remarks on the sexual state

One of the very few taxa resembling ours is *Psilachnum equisetinum* (Quél.) Svrček, which has a long nomenclatural history. The collections assigned to this species by different authors vary from one another in several details, although they apparently grew on the same host species, *Equisetum arvense*. In all of them the spore length does not exceed 8 µm, which appears to separate *P. equisetinum* from *R. flavovirens*. Also pigmentation of the excipulum was partly absent in *P. equisetinum*, and the apothecial stipe was longer than wide.

Psilachnum equisetinum was described as *Peziza equisetina* (Quélet, 1879), and a year later reassigned to the genus *Helotium* Pers. (Quélet, 1881: 673). It was said to grow on *Equisetum limosum* in Alsace (France) and to have smooth, yellow-white apothecia, 0.5–0.8 mm diameter (in Quélet, 1879: as 0.05–0.8 mm), a sulphur-yellow disc, a thin yellowish stipe, 0.05 mm long, cylindrical asci, and rod-shaped spores, 5–6 µm long. The new combinations in *Phialea* (Pers.) Gillet and *Calycina* Nees ex Gray by Rehm (1893: 739) and Kuntze (1898: 448), respectively, were done without studying the actual specimen. Velenovský (1934) referred to *Helotium equisetinum* collections from near Prague (Czech Republic), on *Equisetum limosum*, with apothecia 0.5–1 mm in diameter, totally lemon-coloured, smooth, with a stipe length equal to the disc diameter, and cuneate spores 5–8 µm long. Kirschstein (1935: 228), who combined the species in *Pezizella* Fuckel, studied a collection on *E.* ?*limosum* from Vidzeme (Latvia), with subsessile, externally whitish apothecia of about 0.5 mm in diameter, with a yellowish hymenium and hyaline prosenchymatic excipulum, cylindric-clavate, 8-spored asci, 32–40 × 4–5 µm, cylindrical spores 6–8 × 2 µm, and filiform, 2 µm wide paraphyses. Svrček (1985) considered the species to be closely related to *Psilachnum inquilinum* (P. Karst.) Dennis and, therefore, he made the currently accepted combination *Psilachnum equisetinum* (Quél.) Svrček. Svrček (*op. cit.*) redescribed one of Velenovský's collections, with apothecia with rather long and thin stipes 0.5–0.8 × 0.14 mm, a pale yellow-brown excipulum of up to 15 × 10 µm large, thin-walled cells, filiform, smooth, 0–1-septate hairs 30–35 × 1.5–2.5 µm, oblong-clavate asci 45–50 × 5–6 µm with amyloid apex, nar-

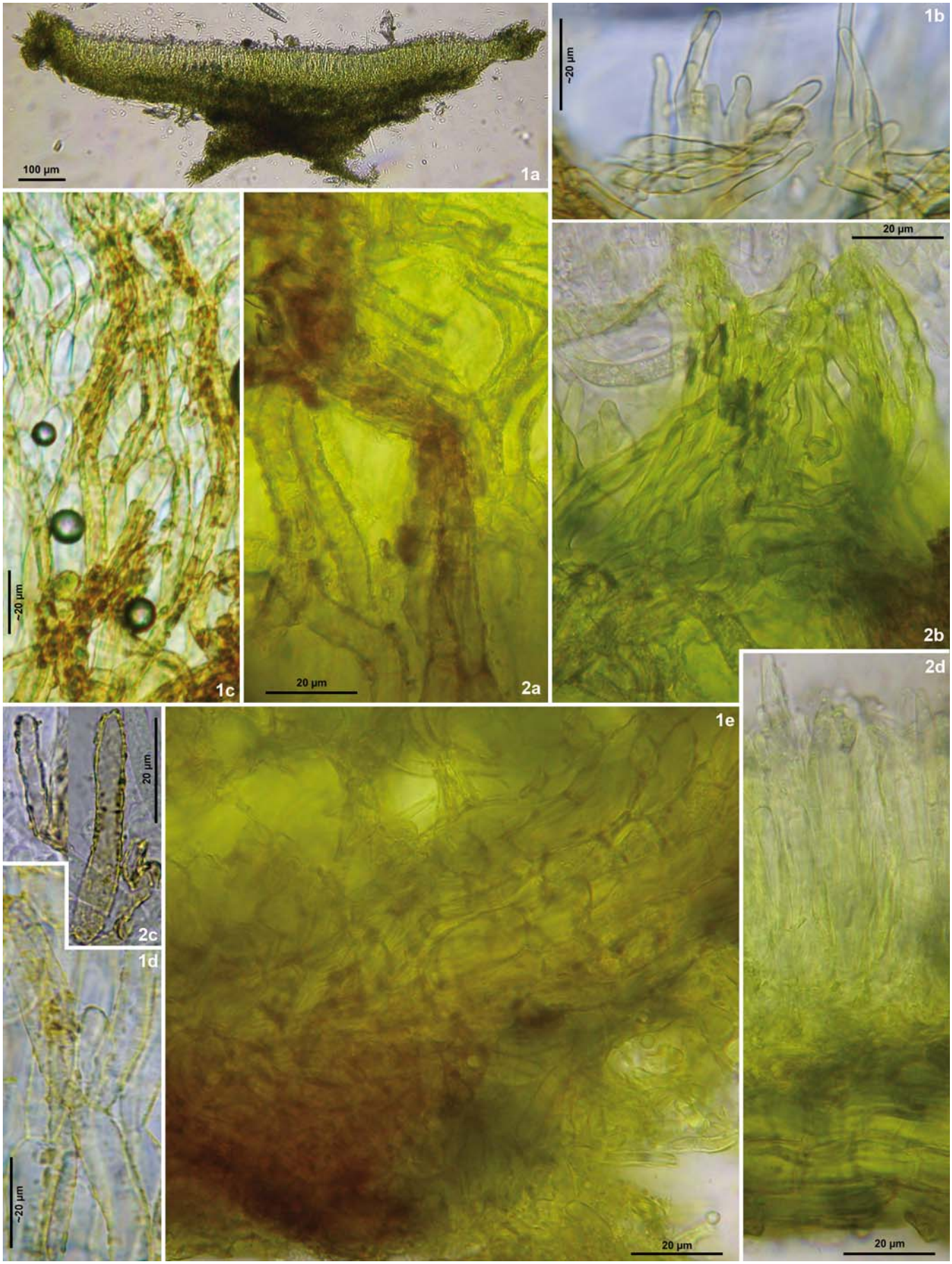

Plate 2. Figs. 1–2. *Rommelaarsia flavovirens* (sexual state), 1b–d: H.B. 9684, 2a–d: H.B. 9951. – 1a: Apothecium in median section. 1b, 2b: Smooth marginal hairs. 1c, 2a, 2c: Encrusted cortical hyphae on ectal excipulum. 1d: Detail of (1a) near base showing thin-walled ectal excipulum of *textura prismatica*. 2d: Median section of excipulum and hymenium at lower flanks. All in living state (in water). Pictures: 1b–d: L. Rommelaars, 2c: P. Tanchaud.

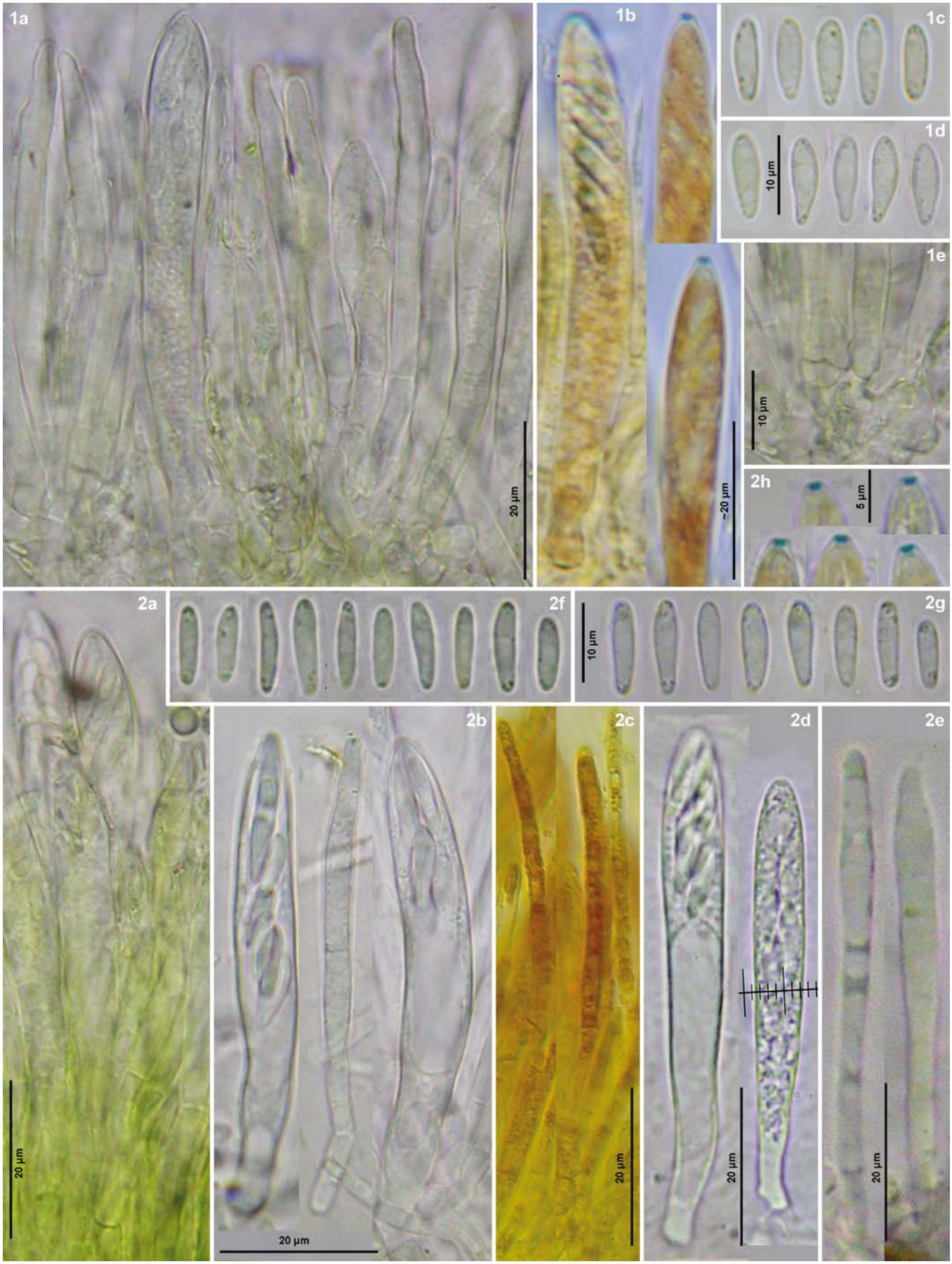

Plate 3. Figs. 1–2. *Rommelaarsia flavovirens* (sexual state), 1a–e: H.B. 9684, 2a–h: H.B. 9951. – Asci and paraphyses, ascospores. All in living state (in water, 2c in IKI), except for 2d: right ascus (plasmalemma damaged). 2h: Ascus apices (in IKI). Pictures: 1b–c: L. Rommelaars, 2d–f and h: P. Tanchaud.

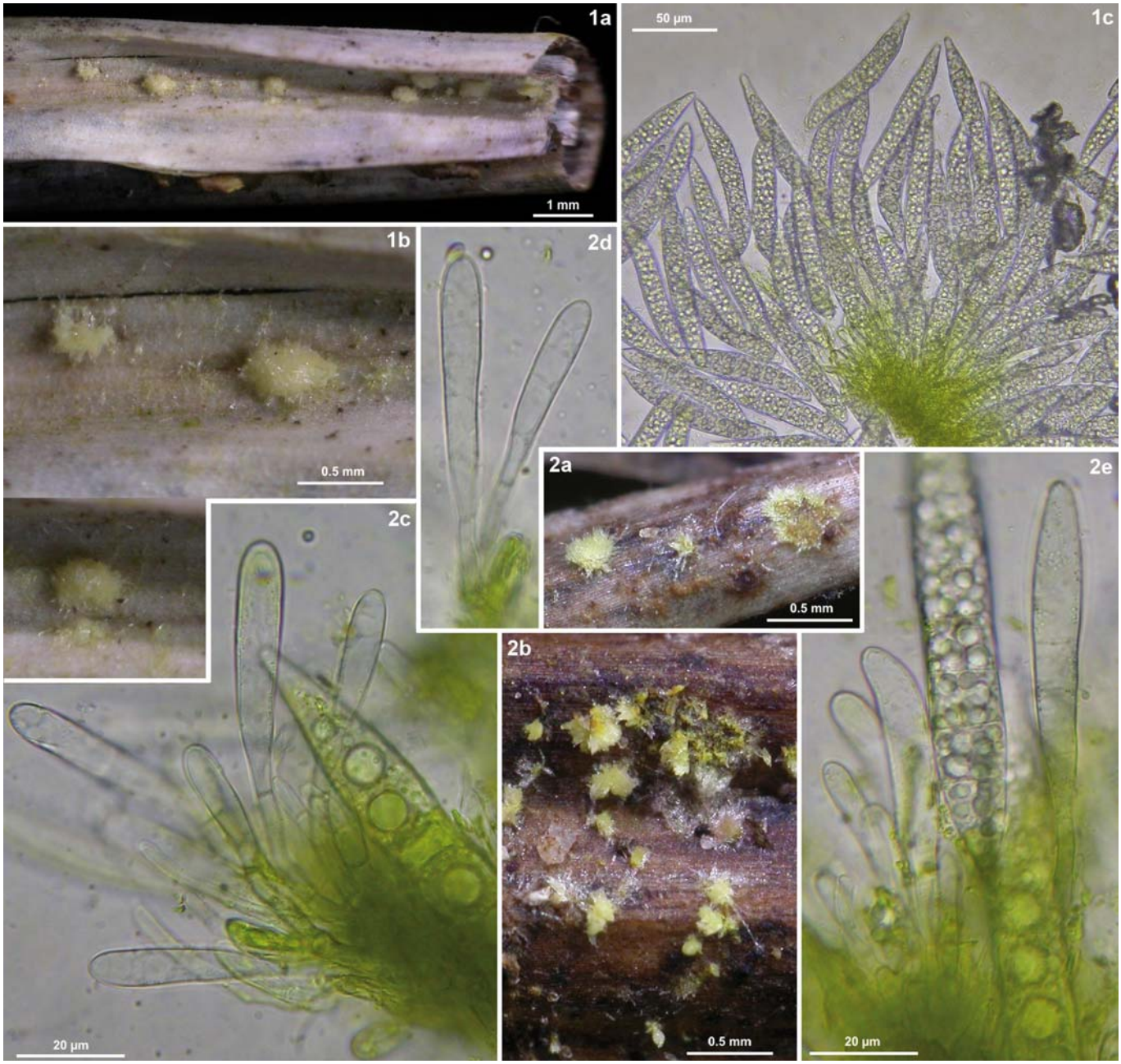

Plate 4. Figs. 1–2. *Rommelaarsia flavovirens* (asexual state), 1a–c: H.B. 9684, 2a–e: H.B. 9951. – 1a–b: Conidiomata (sporodochia) on natural substrate (remotely associated with apothecia on the same stems), fresh state. 1c: Squash mount of conidioma with mature conidia. 2c–e: Conidiophores with young, eguttulate conidia. All in living state.

rowly cuneate, eguttulate spores 6–8 × 1.5(–2) µm, and narrowly lanceolate, protruding paraphyses. Especially the long apothecial stipe and the shorter and narrower spores exclude a relationship with *R. flavovirens*.

Another equiseticolous species, *Pseudohelotium elaphoides* Sacc. (≡ *Mollisia elaphines* Quél.), was described from dry stems of Equisetum in the French Jura (Quélet, 1880; Saccardo, 1889). It macroscopically resembles the present fungus based on its apparently sessile apothecia, brownish exterior, yellowish, later darker disc, and pruinose-pubescent margin. However, the ellipsoid-oblong spores are said to be 4 µm long (width not stated). Although the apothecia are said to be finally expanded and undulating, their size is given as 0.1 mm in diameter. Mainly the small spores exclude conspecificity with *R. flavovirens*.

Remarks on the asexual state

The sporodochia of *R. flavovirens* are reminiscent of members of the genus *Bactridium* Kunze (K. Seifert, pers. comm.). However, the type species *B. flavum* Kunze and the similar *B. subglandis* Tubaki differ in having much larger conidia with a very large central cell and finely granular, yellowish-orange cytoplasm (e.g. Tubaki & Okuda, 1981), which is very different compared to *R. flavovirens*. Most of the *Bactridium* species recognized in Index Fungorum are lignicolous, and none of them fits the present species. A single sequence of *Bactridium subglandis* is available in the Japanese NBRC database (No. NBRC31322), comprising ITS and LSU rDNA. A BLAST search in GenBank results in *Trichophaea abundans* (P. Karst.) Boud. (93% similarity over 67% query cover), *Pseudaleuria quinaultiana* Lusk (92% over 50%), *Scutellinia* sp. (95% over 45%), and *Ramsbottomia crechqueraultii* (P. Crouan & H. Crouan) Benkert & T. Schumach. (94% over 47%) as top hits. All these belong to the family *Pyronemataceae*, so it can be concluded that *Bactridium* perhaps belongs in the *Pezizales*.

Ecological remarks

Although the two collection sites are 990 km distant from each other, *Rommelaarsia flavovirens* does not show significant morphological differences between the holo- and paratype, also the mole-

cular data are almost identical. Both sites are very close to the sea, either the North Sea or the Bay of Biscay. The temperate-oceanic "Ballastplaat" sandbank is a broad and flat open area at the border of the Lauwersmeer, a large man-made freshwater lake that was separated from the Wadden Sea in 1969 by a dike, which changed its flora and fauna (Nijborg, 2014). The sandbank area is composed of arid grasslands, with creeping willow and young deciduous bushes on calcareous soil, forming a damp thicket of maximum 1,5 m height, with an undergrowth of *Rhytidiadelphus squarrosus* and *Equisetum arvense*. The collection site at "La Grande Vergne" is submediterranean-oceanic. It is very close to the Lac de Cadeuil, a system of small lakes, which is located about 17 km off the coast of the Bay of Biscay. *Equisetum arvense* occurs here on sandy soil in an open semi-wetland. The shrubs include *Calluna vulgaris*, *Erica scoparia*, *E. cinerea*, *Ulex europaeus*, *Sarothamnus scoparius*, and the herbs *Rumex acetosella*, *Pteridium aquilinum*, *Tuberaria guttata*, and *Arenaria montana*. Trees occur at a ca. 30 m distance and include *Salix*, *Betula pubescens*, *Quercus pubescens*, *Q. pyrenaica*, *Q. ilex*, and *Pinus pinaster*.

Phylogenetic placement

Our phylogenetic analyses place *Rommelaarsia flavovirens* in the *Hyaloscyphaceae*. However, this family is clustered in three different clades (Plate 6), suggesting a polyphyletic origin. Also some of the recognized genera appear to be polyphyletic (*Cistella*, *Psilachnum*, *Roseodiscus* Baral). However, from a morphological point of view a

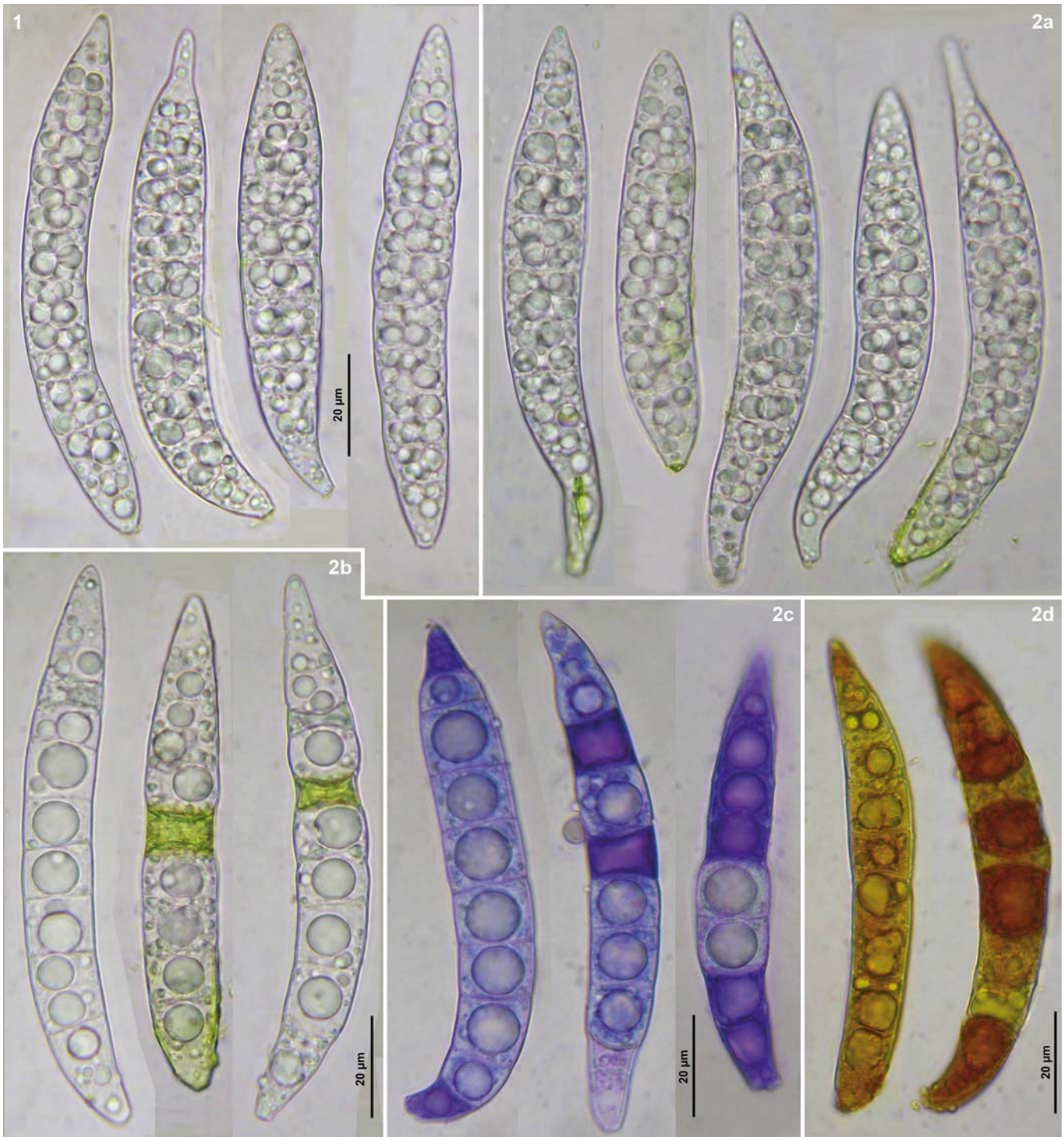

Plate 5. Figs. 1–2. *Rommelaarsia flavovirens* (asexual state), 1: H.B. 9684, 2a–d: H.B. 9951. – 1, 2a: Mature conidia in living state (in water). 2b–c: Mature conidia, partly with dead cells (in water or CRB), living cells containing large, confluent LBs due to de- and rehydration. 2d: Two mature conidia in dead state (in IKI).

polyphyletic origin of the *Hyaloscyphaceae* is hard to accept: (1) each of the three *Hyaloscyphaceae* clades comprises morphologically heterogeneous taxa, while (2) between clades some representatives show high morphological similarities. We hope to further elucidate the family *Hyaloscyphaceae* by including more molecular markers and more taxa.

Within the clade that contains *Rommelaarsia*, the macroconidial asexual state and the greenish-yellow warted exudate appear to be unique. Among the species of this clade, *Psilachnum staphyleae* J.G. Han, M.J. Park & H.D. Shin matches typical members of *Psilachnum*, such as *P. lateritioalbum* (P. Karst.) Höhn. and *P. acutum* (Velen.) Raitv. in its long and straight, tapering hairs, and lanceolate, strongly protruding paraphyses. Regrettably, no sequences are available for the two latter *Psilachnum* species. Two other species in the genus *Psilachnum*, *P. chrysostigma* (Fr.) Raitv. and *P. ellisii* (Dennis) E. Weber & Baral, form a separate clade (Plate 6). They differ from the above in more or less cylindrical hairs and cylindrical, not protruding paraphyses. Contrary to *Rommelaarsia*, *Psilachnum* species generally contain refractive vacuoles (VBs) in their paraphyses and hairs.

Morphologically, *Cistella albidolutea* (Feltgen) Baral and *C. grevillei* (Berk.) Raitv. are extremely similar to *C. acuum* (Alb. & Schwein.) Svrček and *C. spicicola* Huhtinen & Söderh. when comparing descriptions of the types or other collections. Therefore, the heterogeneity of the genus *Cistella* as observed in our phylogenetic tree is surprizing, given that the strains were correctly identified and the sequences not confused. A possible confusion concerns the ITS+LSU rDNA sequence of CBS 605.77 (*C. acuum*), which we think is a chimeric sequence (see Nilsson *et al*., 2012, for discussion about chimeras). Its ITS is not much different from *Hamatocanthoscypha laricionis*

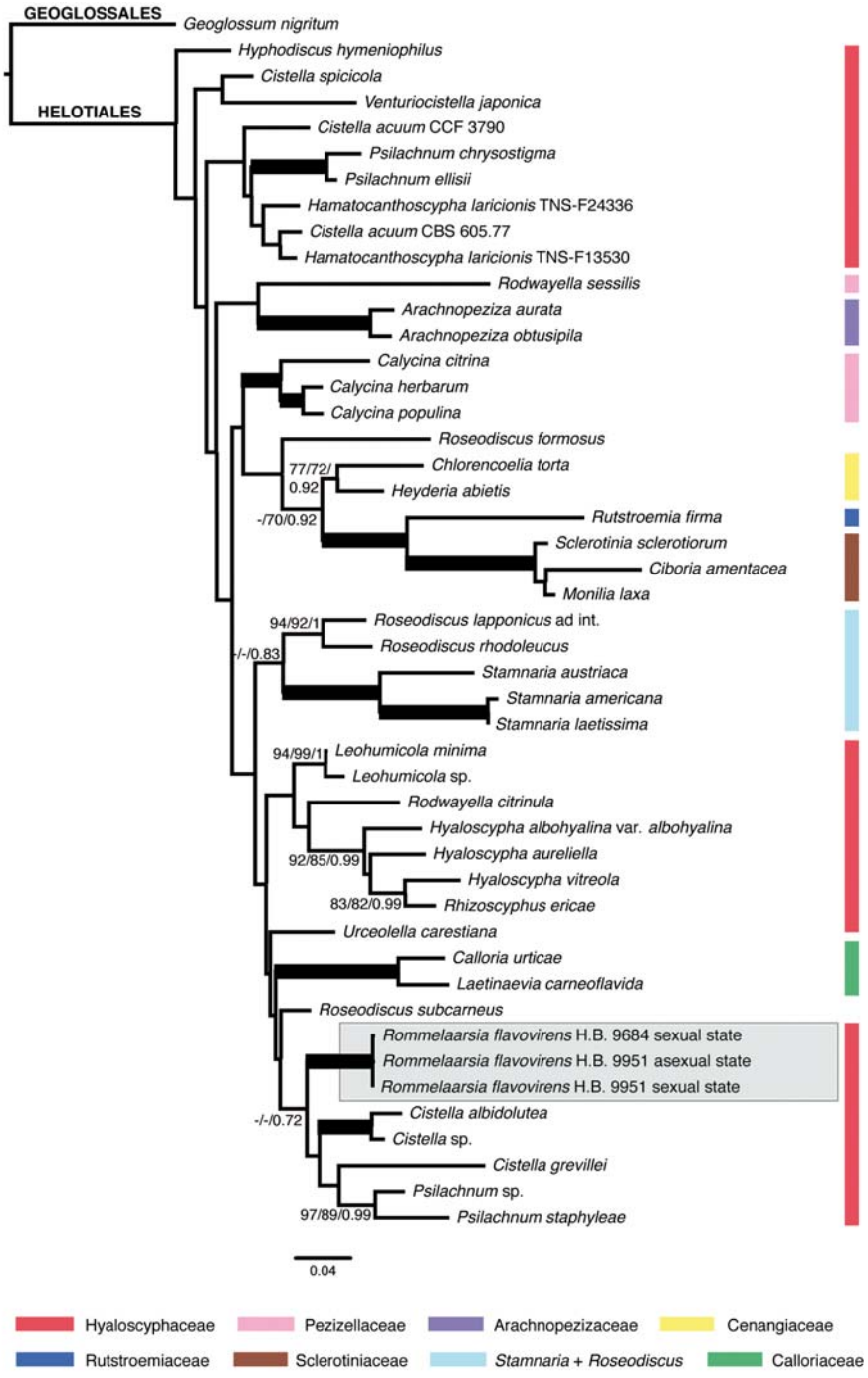

Plate 6. Phylogeny of *Helotiales* produced from ML analysis of the combined ITS + LSU rDNA dataset. An isolate of *Geoglossum nigritum* was used to root the tree. Thick branches received high support (MP/ML bootstrap ≥ 95 % and Bayesian posterior probabilities ≥ 0.95). Other branches received indication of MP/ML bootstrap support values if ≥ 70 and posterior probabilities if ≥ 0.7.

(Velen.) Svrček, while the LSU suggests a relationship to *Cistella spicicola*.

The genus *Psilachnum* differs from *Rommelaarsia* in various morphological characters. Typical are the refractive vacuoles in both paraphyses and hairs. The living mature asci often include a large globose refractive drop below the spores, which is not seen in *Rommelaarsia*. Unlike *Rommelaarsia*, several species of *Psilachnum* turn yellowish or reddish when bruised or when senescent, due to a colour change of the VBs, apparently by oxidation, but a yellow or greenish exudate was not observed in that genus.

The adaptation to an ancient linage of spore-forming vascular plants might indicate that *Rommelaarsia* is of ancient origin too. The present phylogenetic analysis does not support this view. Instead, moss, fern, and horsetail inhabiting taxa [*Psilachnum chrysostigma*, *Rommelaarsia flavovirens*, *Roseodiscus* spp., *Stamnaria* spp.] occur scattered throughout the tree, and only the *Roseodiscus-Stamnaria* clade includes exclusively equiseticolous taxa.

In Han *et al.*'s (2014) multigene phylogenetic analyses of the family *Hyaloscyphaceae*, the genus *Psilachnum* is represented by *P. staphyleae* and *Psilachnum* sp. in a clade with *Urceolella* spp. and *Cistella albidolutea*, and *Psilachnum ellisii* [as *Microscypha ellisii*] in a clade with *Hamatocanthoscypha laricionis*. These analyses do not provide support for the close morphological similarity between the genera *Hyaloscypha* Boud. and *Cistella*. The observed molecular heterogeneity of different genera calls for further research to include unsequenced species. We, and other groups, are currently undertaking such studies.

Acknowledgements

Thanks are due to Luciën Rommelaars (Tilburg, The Netherlands) and Patrice Tanchaud (Soulignonne, France) for sending their collections and photographs, also to Jaap Kloosterhuis and Jasper Schut (Staatsbosbeheer, The Netherlands) for providing ecological information about the Lauwersmeer area. Donald H. Pfister (Cambridge, MA, USA) is thanked for reviewing the manuscript.

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