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Resinogalea humboldtensis gen. et sp. nov., a new resinicolous fungus from New Caledonia, placed in Bruceomycetaceae *fam. nova* (Ascomycota)

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A novel species of ascomycetes is described from resin of *Araucaria humboldtensis* on Mont Humboldt in New Caledonia. The fungus is placed in the new genus *Resinogalea* Rikkinen & A.R. Schmidt, with the species name *R. humboldtensis* Rikkinen & A.R. Schmidt. It has only been found growing on semi-hardened resin flows on branches of its endemic and endangered conifer host. The morphology and anatomy of the new fungus are compared with those of ecologically similar taxa, including *Bruceomyces castoris*. The new family Bruceomycetaceae Rikkinen & A.R. Schmidt is described to accommodate *Resinogalea* and *Bruceomyces*.

Introduction

During field studies of resin production in araucarian conifers in New Caledonia, a previously unknown fungus was found growing on semi-hardened resin of *Araucaria humboldtensis* (Araucariaceae) on the upper slope of Mont Humboldt in the Southern Province on Grande Terre, New Caledonia. The endemic and endangered host tree is only known from the upper slopes of four mountains in the southern part of the island: Mont Humboldt, Mont Kouakoué, Mont Mou, and Montagne des Sources, where it is restricted to ultramafic soils at elevations between 750 to 1600 m a.s.l. (Jaffré 1995, Gaudeul *et al*. 2012, Kranitz *et al*. 2014).

The fungus is characterized by unicellular non-septate ascospores produced in stalked ascomata that develop on solidified resin surfaces. It superficially resembles several other 'calicioid fungi', a polyphyletic assemblage of tiny Ascomycota, characterized by the production of a persistent spore mass (often a true mazaedium containing ascospores and sterile elements) on the tips of usually well-stalked ascomata (Tibell 1984, 1999, Rikkinen 2003a, Prieto *et al*. 2013, Selva 2014). Most previously known resinicolous calicioids belong to the Mycocaliciales

(Mycocaliciomycetidae, Eurotiomycetes), with numerous *Chaenothecopsis* species described from resin and resin-impregnated wood of boreal and temperate northern hemisphere conifers (Bonar 1971, Tibell & Titov 1995, Rikkinen 2003a, Tuovila *et al*. 2011b, 2013, Selva 2013). Recently we described as a new species *Chaenothecopsis neocaledonica* from resin of *Agathis ovata* (Araucariaceae) in New Caledonia (Rikkinen *et al*. 2014), this being the first resinicolous calicioid fungus from southern hemisphere conifers. Another lineage of resinicolous *Chaenothecopsis* species grows on angiosperm exudates and includes temperate, subtropical and tropical taxa (e.g. Tuovila *et al*. 2011a, 2014).

The new species from Mont Humboldt is in many respects similar to *Bruceomyces castoris*, an enigmatic, monotypic resinicolous fungus previously known from western North America. However, it is distinctive enough to merit a new genus. As several anatomical features suggest that it and *Bruceomyces* belong to the same lineage, we propose to include them in a new family.

Material and methods

Resinicolous fungi and other organisms were collected in 2011 from semi-solidified resin flows on branches of *Araucaria humboldtensis* (Araucariaceae) from Mont Humboldt in the Southern Province of Grande Terre, New Caledonia. Under closer inspection the resin samples were found to support twelve ascomata of what appeared to represent a previously unknown but highly distinctive resinicolous ascomycete.

Microscopic examination of the newly found fungus indicated that *Bruceomyces castoris* is its closest previously known analogue. The latter monotypic taxon is only known from three small collections from low elevation conifer forests in western North America (Rikkinen 2003c, Tuovila *et al*. 2012). As the original description of *Bruceomyces* did not include SEM images and/or photomicrographs of all relevant characters, the anatomy of that species was also revisited and illustrated in order to allow detailed comparison with the fungus from New Caledonia.

Ascomata on the dry-resin samples were imaged under a Carl Zeiss AxioScope A1 compound microscope using simultaneously transmitted and oblique incident lights. Hymenia and mycelia were imaged on a microscopic slide in water using 200× to 640× magnification, and ascospores were imaged at 1600× (oil immersion) magnification, using differential interference contrast (DIC) illumination. For scanning electron microscopy, one dried ascoma was placed on a carbon-covered SEM-mount, sputtered by gold/palladium and examined under a Carl Zeiss LEO 1530 Gemini field emission scanning-electron microscope as described by Beimforde *et al*. (2011). Energy-dispersive X-ray spectroscopy (EDX) was performed on the surface of the ascoma using an INCA-EDX system (Oxford Instruments) and an excitation voltage of 15 kV inside this electron microscope. The colour images presented in this paper are digitally-stacked photomicrographic composites obtained from several focal planes using the software package HeliconFocus 5.0 for enhanced illustration of the three-dimensional objects.

We made several careful attempts to isolate DNA from both the new fungus and *Bruceomyces castoris*, but without success. Further attempts were not possible due to the extreme scarcity of type material of both fungi. For the DNA isolation single ascomata of both species were crushed with a micro glass mortar. DNA isolation was then conducted by using QIAamp DNA micro kit (Quiagen) by following the provided protocol for tissue samples. Several attempts were performed to amplify nuclear-encoded ribosomal RNA gene regions of the large ribosomal subunit (LSU) and the internal transcribed spacer (ITS) region. Primers for amplification were ITS4 and ITS5 (White *et al*. 1990) or alternatively ITS4 and ITS1F (Gardes & Bruns 1993) for the ITS region and LR0R (Rehner & Samuels 1994), LR3R (Moncalvo *et al*. 2000), and LR5 and LR7R (Vilgalys & Hester 1990) for the large ribosomal subunit. PCR reactions were performed in a 25 μl volume containing final concentration of 0.5 μM of each primer, 0.5 μM of each dNTP, 1.25 unit of Gotaq hot start DNA polymerase (Promega), Green PCR buffer with a final concentration of 1.5 mM $MgCl_2$, and 2 μl template DNA. PCR cycles consisted of 2 min initial heating to activate DNA-polymerase and to ensure that template DNA

had denatured, 40 cycles at 94 °C for 45–60 sec of denaturation, at 50–56 °C for 45–60 sec of annealing, and at 72 °C for 45–60 sec of elongation and a final an elongation step at 72 °C for 10 minutes.

For the cultivation of the New Caledonian fungus, spores were detached from a single ascoma using a sterile needle, and transferred in 300 μl sterile 0.9% NaCl solution to obtain a spore solution. For germination of the spores the solution was inoculated on malt yeast agar (MYA, Ahmadjian 1961), malt extract peptone (MEA, Blakeslee 1915) and potato dextrose agar (PDA, Carl Roth), each supplemented with crumbs of the source resin of *Araucaria humboldtensis*. Unfortunately, all attempts to obtain cultures from spores of the new fungus were unsuccessful.

Taxonomy

Resinogalea Rikkinen & A.R. Schmidt, *gen. nov.*

MB817092. — TYPE SPECIES: *Resinogalea humboldtensis* Rikkinen & A.R. Schmidt.

ETYMOLOGY: The genus name (lat. *resina* = resin; *galea* = helmet) refers to the hat-like accumulation of resin-like substance on the young capitula of the ascomata.

Ascomata stipitate, capitate, developing on conifer resin. Stipe mainly consisting of periclinally arranged hyphae. Hymenium hyaline. Asci formed with croziers, broadly clavate and stalked, wall with two layers but not differentiated at apex, eight-spored and releasing mature spores into a powdery mass on top of capitulum. Paraphyses hyaline, filiform and non-branched, very long and septate. Ascospores non-septate, globose to broadly ellipsoidal, appearing smooth under light-microscope. Anamorph unknown.

Resinogalea humboldtensis Rikkinen & A.R. Schmidt *sp. nova* (Figs. 1–3)

MB817094. — TYPE: New Caledonia. Province Sud: Mont Humboldt Nature Reserve, close to Mont Humboldt refuge, along the foot path from shelter towards the mountain summit. On semi-hardened resin of *Araucaria humboldtensis*. Elevation 1320 m a.s.l. Coordinates 21°52′46.79′′S, 166°24′49.17′′E, 9 Nov. 2011 *Rikkinen JR010168a* (holotype P; isotype JR010168b H).

ETYMOLOGY: The species is named after the type locality, Mont Humboldt in New Caledonia, and the endemic conifer host, *Araucaria humboldtensis* that produces the resin substrate on which the fungus grows.

Apothecia on exudate of *Araucaria humboldtensis*, 650–1500 μm high. Stipe brownish black but covered by a granular yellowish-brown pruina, straight, sometimes flexuous, 80–140 μm wide. EDX analysis indicated that the pruina contains phosphorus and sulphur. Capitulum brownish black but usually covered by a crust of white or slightly yellowish matter, resembling a 'resin helmet', since its appearance is similar to dry *Araucaria humboldtensis* resin. Mature apothecia with well-developed reddish brown mazaedium protruding through central part of apothecial disk. Stipe consisting of two layers, hyphae of inner layer (2.5)3.0–4.5(5.4) μm wide, mostly parallel but slightly intertwined, with strongly agglutinated cell walls, outermost hyphae arching and partly horizontal, forming a loose network largely covered by amorphous matter and crystals. Excipulum brown, consisting of periclinally arranged or slightly intertwined hyphae, lower part with smooth surface layer, upper part thickly encrusted by amorphous matter, crystals and detached ascospores. EDX analysis indicates that the crystals are either potassium chloride or calcium chloride. Hymenium and hypothecium hyaline. Asci eight-spored, clavate, often with biseriately arranged spores, 14–28 × 6–9 μm, with 22–43 μm long stalks, with hyaline and relatively thick wall consisting of two layers but not differentiated at apex, formed with croziers. Ascospores non-septate, globose to broadly ellipsoidal, erythrocyte-like, pale brown, (3.0)3.6–4.7(5.8) × (2.7)3.3–4.3(4.6) μm, appearing smooth under light-microscope, but with very slight ornamentation of irregular polygonal areas visible in SEM. Paraphyses hyaline, filiform and non-branched, 2.0–4.2 μm wide and very long, septal intervals 12–25 μm.

HABITAT AND ECOLOGY: The type locality of *Resinogalea humboldtensis* supports open *Araucaria humboldtensis* stands intermixed with montane maquis, a shrubby vegetation type found above 1200 m on ultramafic soils in the southern part of the island of Grande Terre. The upper slopes of Mont Humboldt experience daily

Fig. 1. Light micrographs of *Resinogalea humboldtensis*. — **A**–**E**: Ascomata developing on solidifying resin of *Araucaria humboldtensis*. — **F**: Mature ascoma. Note dense network of endosubstratic hyphae visible through the clear resin surface below the ascoma. — **G**: Mature ascoma with partly cracked excipulum; ascospores from this apothecium were sampled for DNA extraction and culture experiments. — **H**: Detail of stipe surface. — **I**: Detail of fungal hyphae within the resin substrate. **A**–**D**, and **I** from *Rikkinen JR010168b* (isotype); **E** and **F** from *Rikkinen JR010168c*: GZG.BST.21892 (isotype); **G** and **H** from *Rikkinen JR010168a* (holotype). Scale bars = 10 μm.

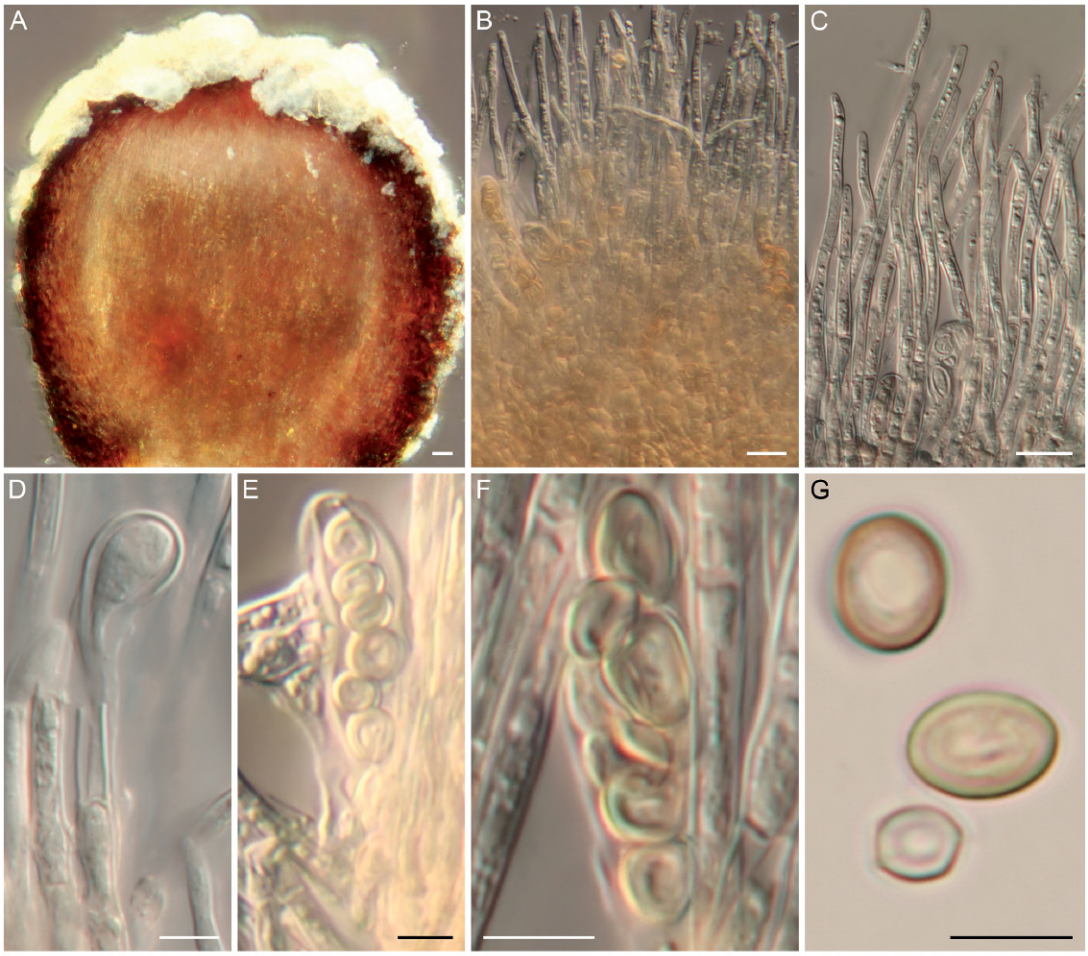

Fig. 2. Light micrographs of *Resinogalea humboldtensis* (isotype, *Rikkinen JR010168c*: GZG.BST.21892). — **A**: Longitudinal section of young capitulum. — **B**: Asci and paraphyses. — **C**: Paraphyses. — **D–F**: Asci. — **G**: Ascospores. Scale bars: **A–C** = 10 μm, **D–G** = 5 μm.

fog and rainfall which assure an almost constant high humidity year-round. A notable proportion of *Araucaria* trees at the type locality exhibit insect-induced resin production, sometimes associated with the death of entire branches. The ascomata of the fungus were seen growing solitarily or in small groups on resin surfaces, sometimes on the side walls of deep crevices between consecutive resin flows.

The ascomata of *Resinogalea humboldtensis* have a typical 'calicioid morphology' in having long and slender stalks and well-developed mazaedia that stain the finger if touched. The accumulation of mature ascospores into a mazaedium is consistent with a spore-saving strategy (Tibell 1994). Coupled with well-stalked ascomata the mazaedia may effectively promote animal dispersal, which is suggested to be important for the ecology of many calicioid fungi (Rikkinen 1995, 2003a, Tuovila *et al.* 2011a, Prieto *et al.* 2013). On Mont Humboldt resin flows caused by invertebrates provide the only available substrate for *Resinogalea*, indicating that the fungus may be dependent on wood-boring insects for both nutrition and dispersal.

TAXONOMIC NOTES: Calicioid fungi are known to represent a highly polyphyletic grouping of representatives from several different linages among the Ascomycota (e.g. Tibell 1984, 1999, Wedin & Tibell 1997, Wedin *et al.* 2000, Lumbsch & Huhndorf 2010, Prieto *et al.* 2013, Tuovila 2013). The widespread convergence among these fungi

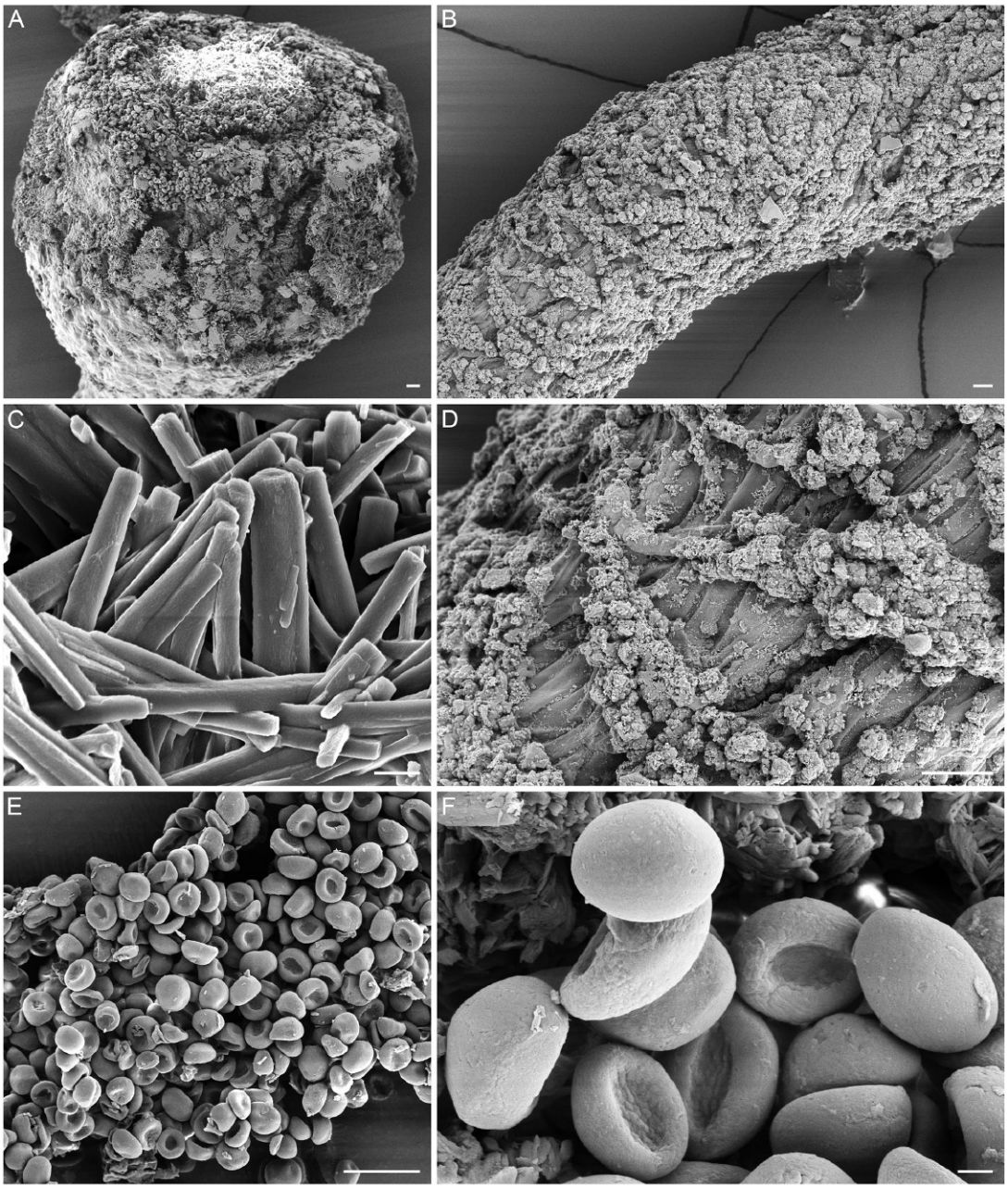

Fig. 3. Scanning electron micrographs of *Resinogalea humboldtensis* (isotype, *Rikkinen JR010168c*: GZG. BST.21892). — **A**: Capitulum. — **B**: Stipe. — **C**: Potassium chloride or calcium chloride crystals on excipulum surface. — **D**: Stipe surface. — **E** and **F**: Ascospores. Scale bars: **A**, **B**, **D** and **E** = 10 μm, **C** and **F** = 1 μm.

is probably linked to the adaptive value of producing stalked ascomata with spore-saving mazaedia in many of the microhabitats typically inhabited by calicioid fungi (Rikkinen 1995, 2003a). In fact, Paleogene amber fossils show that the morphology and ecology of three distantly related lineages of calicioid fungi have remained unchanged for at least tens of millions of years (Rikkinen & Poinar 2000, Rikkinen 2003b, Tuovila *et al*. 2013, Beimforde *et al*. 2014).

The ascus type and several other anatomical features of *Resinogalea humboldtensis* are clearly different to those of *Chaenothecopsis* species and they also rule out any close affinities with other genera of mycocalicioid fungi. In its overall habit *Resinogalea* more closely resembles *Roesleria*, *Roeslerina*, and also some species of *Sclerophora* and *Chaenotheca* (Table 1). All these fungi produce slender-stalked ascomata and have well-developed, often brown mazaedia. *Bruceomyces castoris* is an even closer analogue, both with respect to general morphology (Figs. 1 and 4) and the anatomy of asci and paraphyses (Figs. 2 and 4). Only the shape and ornamentation of ascospores and the anatomy of the stipe are different (Figs. 3 and 5).

Resinogalea and *Bruceomyces* both resemble *Sclerophora* and some *Chaenotheca* species (Coniocybales) in producing stalked asci singly with croziers. However, the asci of the two latter genera are cylindrical, thin-walled and dissolve at early stages. Furthermore, in contrast to *Resinogalea* and *Bruceomyces*, all presently known species of Coniocybales (Coniocybomycetes) are lichen-symbiotic and live in obligate association with green algae. *Resinogalea* and *Bruceomyces* have prominent paraphyses and broadly clavate, well-stalked and relatively thick-walled asci that persist until the spores are nearly mature. *Roesleria* and *Roeslerina* (Roesleriaceae, Helotiales) have similar paraphyses, but their cylindrical asci develop without croziers and their ascospores are subglobose to lentiform, sometimes appearing 1-septate from side view, and characteristically organized like a row of beads within the ascus. Furthermore, all presently known species of *Roesleria* and *Roeslerina* are parasitic on plant roots (Redhead 1984, Yao & Spooner 1999, Kirchmair *et al*. 2008, Neuhauser *et al*. 2011).

As *Resinogalea humboldtensis*, in our opinion, cannot be accommodated in *Bruceomyces*, it is here placed in a new genus. However, many shared anatomical features suggest that the two fungi are related and especially their asci and paraphyses are very similar. The relationship is also supported by a similar substrate and habitat ecology: both fungi produce ascomata on semi-solid conifer exudates and appear to be restricted to constantly humid temperate environments. However, the ascospore structure of the two fungi are distinctive enough to support their placement in different genera of the same family. We assume a larger potential diversity of both genera, but a review of all recent taxonomic literature of different groups of calicioid ascomycetes indicates that they cannot be accommodated in any of the currently recognized families. A new family is, therefore, proposed here for the two genera, without placing this family in any lineage at a higher taxonomical level (Ascomycota *incertae sedis*).

Bruceomycetaceae Rikkinen & A.R. Schmidt, *fam. nova*

MB817090. — Type genus: *Bruceomyces* Rikkinen.

Table 1. Distinguishing features of *Resinogalea* and *Bruceomyces* (Bruceomycetaceae, Ascomycota *incertae sedis*) and superficially similar calicioid ascomycetes. *Roesleria* and *Roeslerina* (Roesleriaceae, Helotiales) according to Redhead (1984), Yao and Spooner (1999), and Kirchmair *et al.* (2008), and *Chaenotheca* and *Sclerophora* (Coniocybaceae, Coniocybales) according to Tibell (1984, 1999).

Genus	Asci	Croziers	Ascospores	Ecology
Resinogalea	broadly clavate	yes	erythrocyte-like[3]	resinicolous
Bruceomyces	broadly clavate	yes	broadly ellipsoidal[4]	resinicolous
Roesleria	cylindrical[1]	no	lenticular/discoid[5]	root parasitic
Roeslerina	cylindrical[1]	no	lenticular/discoid	root parasitic
Chaenotheca	cylindrical[1,2]	yes	globose/ellipsoidal[6]	lichen-symbiotic
Sclerophora	cylindrical[1]	yes	globose	lichen-symbiotic

[1] The ascus wall dissolves at an early stage. [2] Catenulate in some species. [3] Globose to broadly ellipsoidal, appearing smooth under the light-microscope. [4] With distinctive ornamentation of longitudinally arranged, anastomosing wrinkles. [5] Septate across the broadest plane. [6] 1–3-septate in some species.

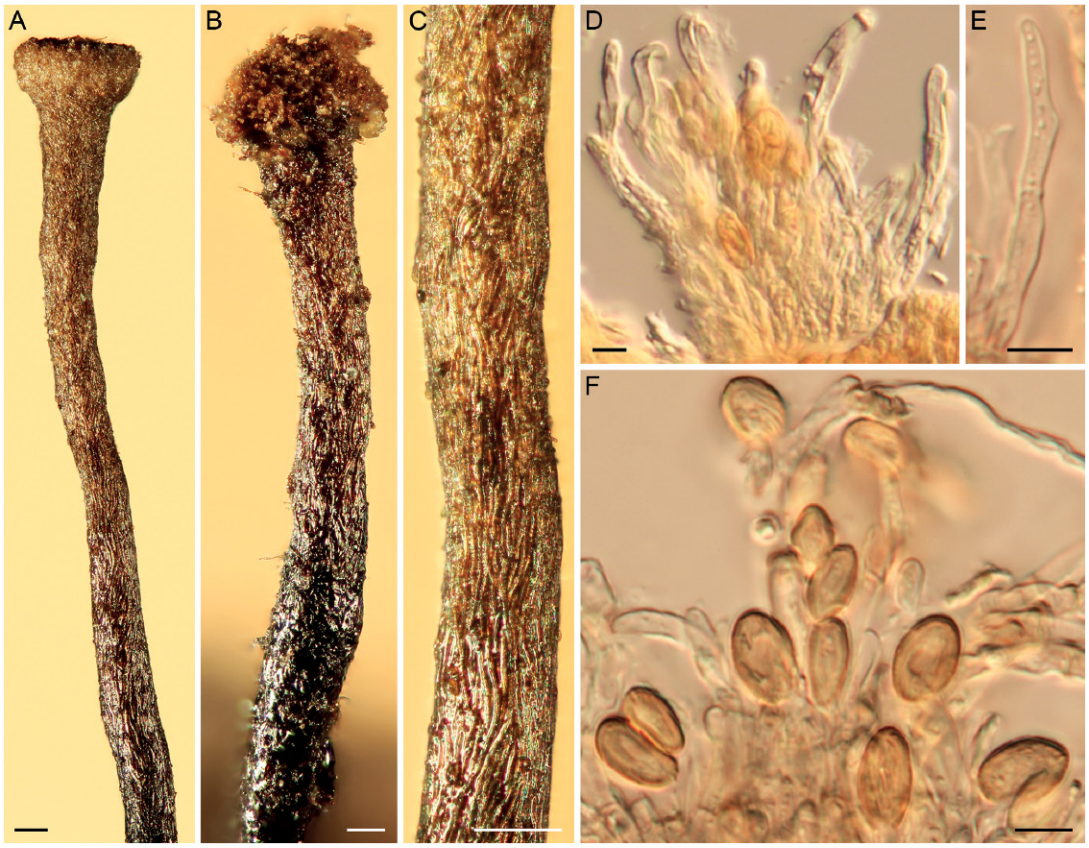

Fig. 4. Light micrographs of *Bruceomyces castoris* (*Rikkinen 98363*). — **A** and **B**: Ascomata. — **C**: Stipe surface. — **D**: Asci and paraphyses. — **E**: Paraphysis. — **F**: Ascospores. Scale bars: **A–C** = 50 *µ*m, **D–F** = 5 *µ*m.

Ascomata stipitate, capitate, developing on conifer resin or occasionally on resin impregnated lignum. Stipe slender, mainly consisting of periclinally arranged hyphae. Excipulum developed as extension of outer layers of stipe. Hymenium hyaline, loosely packed with asci and paraphyses. Asci formed with croziers, clavate, stalked, eight-spored, often with biseriately arranged spores; wall consisting of two-layers, not differentiated at apex, releasing mature spores into a powdery mass on top of capitulum. Paraphyses hyaline, wide and distinctly septate. Ascospores non-septate, globose or broadly ellipsoidal, hyaline to pale reddish brown, smooth or distinctly ornamented.

Two genera are currently recognized in Bruceomycetaceae, and ascospore structure is recognized as the primary character separating *Bruceomyces* from *Resinogalea*, with the former fungus having broadly ellipsoidal, distinctly ornamented spores, and the latter having erythrocyte-like, smooth spores. For convenience of reference, an emended description of *Bruceomyces* ascospores is given here. The ascospores of *Bruceomyces* are non-septate, broadly ellipsoidal with rounded ends, and have a surface ornamentation of longitudinally arranged wrinkles visible even under the light microscope (Fig. 4F). In SEM the outer layer of the spore is seen to have a distinctive ornamentation of interconnected, winding and branched ridges (Fig. 5G). The ridged outer layer of the spore wall eventually ruptures as it swells strongly after being mounted in water.

The inclusion of *Bruceomyces* and *Resinogalea* into the same family does not resolve the phylogenetic position of these enigmatic fungi. As they share several anatomical features with Coniocybomycetes they might have some affinity to that lineage of obligate lichen-symbionts.

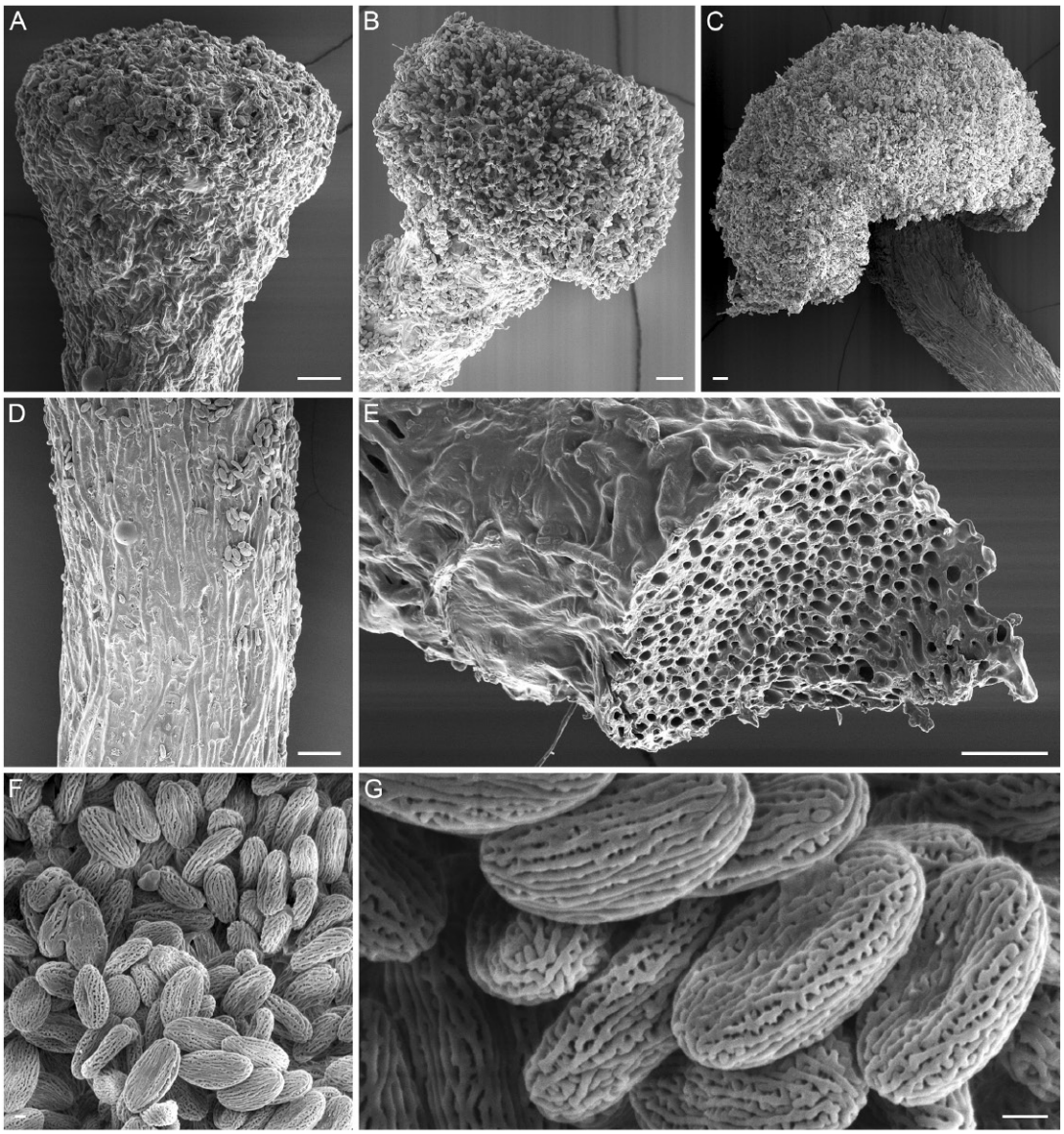

Fig. 5. Scanning electron micrographs of *Bruceomyces castoris* (*Rikkinen 98363*). — **A–C**: Capitulum development. — **D**: Stipe surface. — **E**: Cross-section of stipe. — **F** and **G**: Ascospores. Scale bars: **A–E** = 20 μm, **F** and **G** = 1 μm.

However, solving this matter will require more material and molecular evidence.

SPECIMENS EXAMINED: — *Resinogalea humboldtensis*. New Caledonia. Province Sud, Mont Humboldt Nature Reserve, close to Mont Humboldt refuge, along the foot path from shelter towards the mountain summit, on semi-hardened resin of *Araucaria humboldtensis*. Elevation 1320 m a.s.l., 21°52´46.79´´S, 166°24´49.17´´E, 9 Nov. 2011 *Rikkinen JR010168* (holotype JR010168a P; isotype JR010168b H; isotype JR010168c GZG.BST.21892 (Geoscientific Collections of the Georg August University Göttingen). The last mentioned isotype currently contains only the vegetative mycelium as both apothecia were used for SEM imaging and in attempts to extract DNA). — *Bruceomyces castoris*. USA. Oregon, Benton County, McDonald Research Forest, between Sulphur Springs trailhead and Forest Service Road 800, forest edge by beaver pond in moist gully, 44.0°38.47´N, 123°18.79´W. Elevation 185 m a.s.l., 44°38.5´N, 123°18.8´W, elev. 180 m a.s.l., on exudate and lignum in beaver scar at trunk base of living *Abies grandis*, 1998 (*Rikkinen 98363*, H).

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References

Ahmadjian V. 1961: Studies on lichenized fungi. — *Bryologist* 64: 168–179.

Beimforde C., Schäfer N., Dörfelt H., Nascimbene P.C., Singh H., Heinrichs J., Reitner J., Rana R.S. & Schmidt A.R. 2011: Ectomycorrhizas from a Lower Eocene angiosperm forest. — *New Phytologist* 192: 988–996.

Beimforde C., Feldberg K., Nylinder S., Rikkinen J., Tuovila H., Dörfelt H., Gube M., Jackson D., Reitner J., Seyfullah L.J. & Schmidt A.R. 2014: Estimating the Phanerozoic history of the Ascomycota lineages: combining fossil and molecular data. — *Molecular Phylogenetics and Evolution* 78: 386–398.

Blakeslee A.F. 1915: Lindner's roll tube method of separation cultures. — *Phytopathology* 5: 68–69.

Bonar L. 1971: A new *Mycocalicium* on scarred *Sequoia* in California. — *Madroñō* 21: 62–69.

Gardes M. & Bruns T.D. 1993: ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. — *Molecular Ecology* 2: 113–118.

Gaudeul M., Rouhan G., Gardner M.F. & Hollingsworth P.M. 2012: AFLP markers provide insights into the evolutionary relationships and diversification of New Caledonian *Araucaria* species (Araucariaceae). — *American Journal of Botany* 99: 68–81.

Jaffré T. 1995: Distribution and ecology of the conifers of New Caledonia. — In: Enright N.J. & Hill R.S. (eds.), *Ecology of the southern conifers*: 171–196. Melbourne University Press, Melbourne.

Kirchmai M. Neuhauser S., Buzina W. & Huber L. 2008: The taxonomic position of *Roesleria subterranea*. — *Mycological Research* 112: 1210–1219.

Kranitz M.L., Biffin E., Clark A., Hollingsworth M.L., Ruhsam M., Gardner M.F., Thomas P., Mill R.R., Ennos R.A., Gaudeul M., Lowe A.J. & Hollingsworth P.M. 2014: Evolutionary diversification of New Caledonian *Araucaria*. — *PLoS ONE* 10: e110308, doi:10.1371/journal.pone.0110308.

Lumbsch H.T. & Huhndorf S.M. 2010: Myconet, volume 14. Part One. Outline of Ascomycota. — *Fieldiana. Life and Earth Sciences* 1: 1–42.

Moncalvo J.M., Lutzoni F.M., Rehner S.A., Johnson J. & Vilgalys R. 2000: Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. — *Systematic Biology* 49: 278–305.

Neuhauser S., Huber L. & Kirchmair M. 2011: Is *Roesleria subterranea* a primary pathogen or a minor parasite of grapevines? Risk assessment and a diagnostic decision scheme. — *European Journal of Plant Pathology* 130: 503–510.

Prieto M., Balocha E., Tehler A. & Wedin M. 2013: Mazaedium evolution in the Ascomycota (Fungi) and the classification of mazaediate groups of formerly unclear relationship. — *Cladistics* 29: 296–308.

Redhead S.A. 1984: *Roeslerina* gen. nov. (Caliciales, Caliciaceae), an ally of *Roesleria* and *Coniocybe*. — *Canadian Journal of Botany* 62: 2514–2519.

Rehner S.A. & Samuels G.J. 1994: Taxonomy and phylogeny of *Gliocladium* analyzed by large subunit rDNA sequences. — *Mycological Research* 98: 625–634.

Rikkinen J. 1995: What's behind the pretty colours? A study on the photobiology of lichens. — *Bryobrothera* 4: 1–239.

Rikkinen J. 2003a: Calicioid lichens and fungi in the forests and woodlands of western Oregon. — *Acta Botanica Fennica* 175: 1–41.

Rikkinen J. 2003b: Calicioid lichens from European Tertiary amber. — *Mycologia* 95: 1032–1036.

Rikkinen J. 2003c: New resinicolous ascomycetes from beaver scars in western North America. — *Annales Botanici Fennici* 40: 443–450.

Rikkinen J. & Poinar G.O.Jr. 2000: A new species of resinicolous *Chaenothecopsis* (Mycocaliciaceae, Ascomycota) from 20 million year old Bitterfeld amber, with remarks on the biology of resinicolous fungi. — *Mycological Research* 104: 7–15.

Rikkinen J., Tuovila H., Beimforde C., Seyfullah L., Perrichot V. & Schmidt A.R. 2014: *Chaenothecopsis neocaledonica* sp. nov.: The first resinicolous mycocalicioid fungus from Araucariaceae (Pinales). — *Phytotaxa* 173: 49–60.

Selva S.B. 2013: The calicioid lichens and fungi of the Acadian Forest ecoregion of northeastern North America, I. New species and range extensions. — *Bryologist* 116: 597–714.

Selva S.B. 2014: The calicioid lichens and fungi of the Acadian Forest ecoregion of northeastern North America, II. The rest of the story. — *Bryologist* 117: 336–367.

Tibell L. 1984: A reappraisal of the taxonomy of Caliciales. — *Nova Hedwigia Beiheft* 79: 597–714.

Tibell L. 1994: Distribution patterns and dispersal strategies of Caliciales. — *Botanical Journal of the Linnean Society* 116: 159–202.

Tibell L. 1999: Calicioid lichens and fungi. — In: Ahti T., Jørgensen P.M., Kristinsson H., Moberg R., Søchting U. & Thor G. (eds.), *Nordic lichen flora*, vol. 1: *Introductory parts. Calicioid lichens and fungi*: 20–94. Nordic Lichen Society, Uddevalla.

Tibell L. & Titov A. 1995: Species of *Chaenothecopsis* and *Mycocalicium* (Caliciales) on exudate. — *Bryologist* 98: 550–560.

Tuovila H. 2013: Sticky business — diversity and evolution of Mycocaliciales (Ascomycota) on plant exudates. — *Publications from the Department of Botany, University of Helsinki* 44: 1–142.

Tuovila H., Cobbinah J.R. & Rikkinen J. 2011a: *Chaenothecopsis khayensis*, a new resinicolous calicioid fungus on African mahogany. — *Mycologia* 103: 610–615.

Tuovila H., Larsson P. & Rikkinen J. 2011b: Three resinicolous North American species of Mycocaliciales in Europe with a re-evaluation of *Chaenothecopsis oregana* Rikkinen. — *Karstenia* 51: 37–49.

Tuovila H., Huhtinen S. & Rikkinen J. 2012: Nomenclatural corrections in calicioid fungi. — *Karstenia* 52: 73–74.

Tuovila H., Davey M.L., Yan L.H., Huhtinen S. & Rikkinen J. 2014: New resinicolous mycocalicioid fungi from China. — *Mycologia* 106: 989–1003.

Tuovila H., Schmidt A.R., Beimforde C., Dörfelt H., Grabenhorst H. & Rikkinen J. 2013: Stuck in time — a new *Chaenothecopsis* species with proliferating ascomata from *Cunninghamia* resin and its fossil ancestors in European amber. — *Fungal Diversity* 58: 199–213.

Vilgalys R. & Hester M. 1990: Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. — *Journal of Bacteriology* 172: 4238–4246.

Wedin M. & Tibell L. 1997: Phylogeny and evolution of Caliciaceae, Mycocaliciaceae and Sphinctrinaceae (Ascomycota), with notes on the evolution of the prototunicate ascus. — *Canadian Journal of Botany* 75: 1236–1242.

Wedin M., Döring H., Nordin A. & Tibell L. 2000: Small subunit rDNA phylogeny shows the lichen families Caliciaceae and Physciaceae (Lecanorales, Ascomycotina) to form a monophyletic group. — *Canadian Journal of Botany* 78: 246–254.

White T.J., Bruns T.D., Lee S. & Taylor J.W. 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. — In: Innis M.A., Gelfand D.H., Sninsky J.J. & White T.J. (eds.), *PCR protocols: a guide to methods and applications*: 312–322. Academic Press, New York.

Yao Y.J. & Spooner B.M. 1999: Roesleriaceae, a new family of Ascomycota, and a new species of *Roeslerina*. — *Kew Bulletin* 54: 683–693.