



## ***Chaenothecopsis neocaledonica* sp. nov.: The first resinicolous mycocalicioid fungus from an araucarian conifer**

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### **Abstract**

A new resinicolous species of the genus *Chaenothecopsis* (Ascomycota, Mycocaliciales) is described from resin of *Agathis ovata* in New Caledonia. *Chaenothecopsis neocaledonica* sp. nov. is the first mycocalicioid fungus to be described from the exudates of Southern Hemisphere conifers of the family Araucariaceae. It is easily distinguished from related Northern Hemisphere species by its larger ascospores and unique capitulum structure.

**Key words:** Araucariaceae, exudate, resinicolous fungi

### **Introduction**

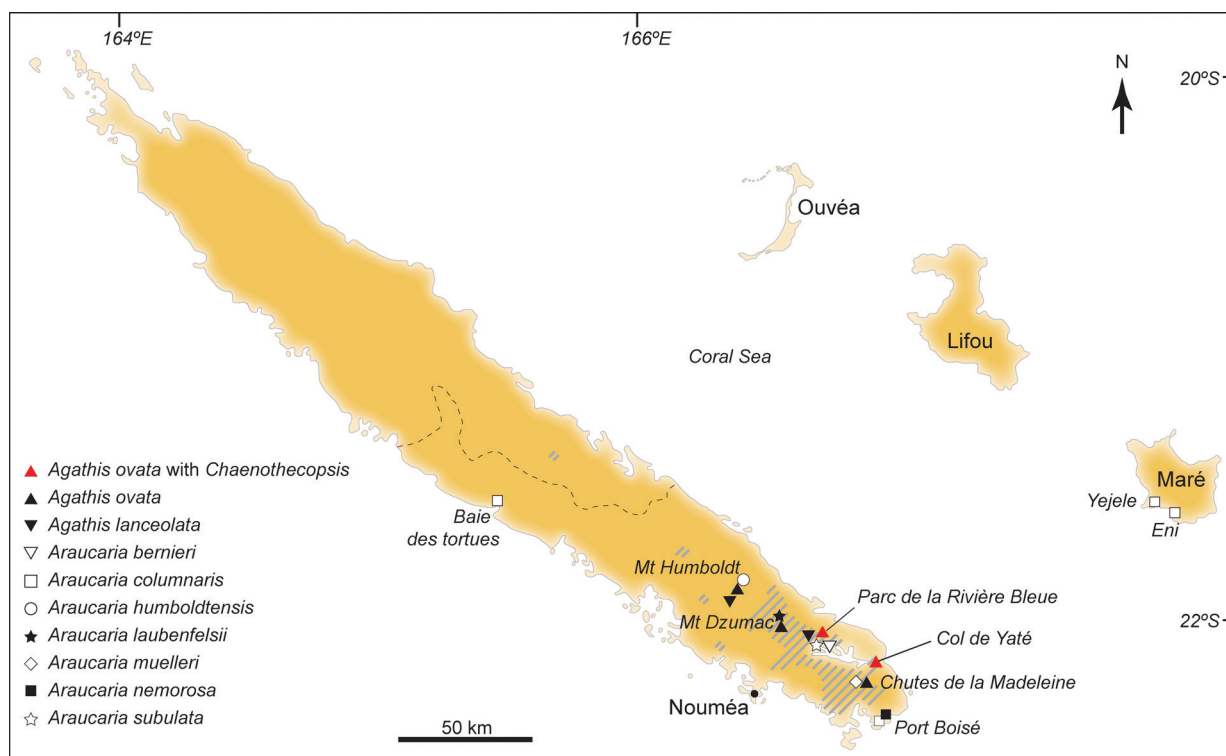
*Chaenothecopsis* Vainio (1927: 70) is a genus of ascomycetes in the order Mycocaliciales (Mycocaliciomycetidae). Species in the genus are either saprotrophic on wood (lignicolous) or resin (resiniculous), or associate with atmophytic green algae (algicolous) or green algal symbionts of lichens (lichenicolous) (e.g. Tibell 1999, Titov 2006, Tuovila 2013). Recent molecular studies have shown that *Chaenothecopsis* is polyphyletic, but taxonomic changes await wider taxon sampling that is needed to resolve the generic delimitations within the family and order (Tibell & Vinuesa 2005, Tuovila *et al.* 2013).

The majority of previously known resinicolous mycocalicioid species grow on resins of Northern Hemisphere conifers, especially different species of Pinaceae (Titov & Tibell 1993, Tibell & Titov 1995, Rikkinen 2003a, Tuovila *et al.* 2011b). Species on conifer resin have been described from both Eurasian and North-American boreal and temperate forests. Several other *Chaenothecopsis* species live on angiosperm exudates and these include temperate, subtropical and tropical taxa (e.g. Funk & Kujt 1982, Samuels & Buchanan 1983, Tuovila *et al.* 2011a).

Here, we describe a new resinicolous *Chaenothecopsis* species growing on resin of *Agathis ovata* (C. Moore ex Vieillard) Warburg (1900: 186) in New Caledonia, a geologically old island in the southwest Pacific Ocean (Figure 1). The new species is the first resinicolous mycocalicioid fungus from the conifer family Araucariaceae, the natural range of these conifers is mainly restricted to the Southern Hemisphere.

### **Material and methods**

Resiniculous fungi were collected in 2011 from semi-solidified resin flows on trunks of *Agathis ovata* (Araucariaceae) from two locations in the Southern Province of New Caledonia (Figures 1 and 2). The first site (type locality) is along the road RP 3 about 5 km west of Yaté (Figure 2B). The second site is along road GR NC1 in Blue River Provincial Park near the Refuge des Scientifiques (Figure 2A). The vegetation at both sites is semi-open maquis scrubland, typical of ultramafic rocks in south-eastern New Caledonia.



**FIGURE 1.** Field localities with resinous Araucariaceae trees visited in November 2011. *Agathis ovata* (Yaté, Rivière Bleue, Mont Dzumac road, and Mont Humboldt trail), *A. lanceolata* (Rivière Bleue and Mont Humboldt trail), *Araucaria bernieri* (Rivière Bleue), *A. columnaris* (Baie des tortues, Port Boisé, and two sites on Maré), *A. humboldtensis* (Mont Humboldt summit), *A. laubenfelsii* (Mont Dzumac), *A. muelleri* (Chutes de la Madeleine), *A. nemorosa* (Port Boisé), and *A. subulata* (Rivière Bleue). The shaded area shows the total distribution of *Agathis ovata*.

Morphological and chemical characters for the fungal specimens were determined using the methodology described in Tuovila *et al.* (2013). Morphological features of the extant fungal specimens were observed and measured in water under a light microscope (Leica DMLS) with a 100× oil-immersion objective. Potassium-hydroxide (KOH), Lugol's reagent (IKI), Melzer's reagent (MLZ), Congo Red (CR) were used when observing some diagnostic structures, like paraphyses and stipe hyphae. Ascospores from dry *Agathis* resin were imaged under a Carl Zeiss AxioScope A1 compound microscope using simultaneously incident and transmitted lights. Ascospores were imaged on a microscope slide in water using 1600× (oil immersion) magnification and differential interference contrast (DIC) illumination. For scanning electron microscopy, several dried ascospores were removed from the substrate, 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). The images of Figure 3A–F are digitally-stacked photomicrographic composites obtained from several focal planes using the software package HeliconFocus 5.0 for a better illustration of the three-dimensional objects.

Genomic DNA was extracted and the ITS and LSU regions of rDNA were amplified and sequenced as described in Tuovila *et al.* (2013). Bayesian analyses employing four Markov chain Monte Carlo (MCMC) chains were carried out with MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003). The four chains were run simultaneously for 1 million generation for the partitioned datasets with trees sampled every 100th generation. Average standard deviations of split frequency (ASDSF) values lower than 0.01 were taken as an indication that convergence had been achieved. A burn-in sample of 25% of the trees was discarded for the run and the remaining trees were used to estimate branch lengths and posterior probabilities. Additionally, maximum likelihood support values were estimated using the same model parameters in Garli 2.0 (Zwickl 2006) with 1,000 bootstrap searchreps. The models of evolution selected by jModelTest 2.0 (Darriba *et al.* 2012) for ITS1, 5.8SR, ITS2, and partial LSU were: TPM2uf+G, TPM2+I, TPM2uf+G, TIM2+I+G, respectively.

Voucher information and GenBank accession numbers of all fungal specimens used in this study are listed in Table 1.

**TABLE 1.** Voucher information and NCBI GenBank accession numbers for the fungal ITS and LSU sequences used in this study.

Species	GenBank Accession numbers ITS/LSU	Reference ITS/LSU, if not same
<i>Pyrgillus javanicus</i> (Montagne & Bosch) Nylander (1858:334)	DQ826741/DQ823103	James <i>et al.</i> 2006
<i>Caliciopsis</i> sp. Peck (1880:32)	GQ259981/GQ259980	Pratibha <i>et al.</i> 2011
<i>Chaenothecopsis</i> sp. 1	X119110/JX119119	Tuovila <i>et al.</i> 2013
<i>Chaenothecopsis</i> sp. 2	KC590480/KC590485	Tuovila <i>et al.</i> 2014
<i>Chaenothecopsis consociata</i> (Nádvorník) A.F.W.Schmidt (1970:148)	AY795851/DQ008999	Tibell & Vinuesa 2005
<i>Chaenothecopsis diabolica</i> Rikkinen & Tuovila (2011:40)	JX119109/JX119114	Tuovila <i>et al.</i> 2011
<i>Chaenothecopsis dolichocephala</i> Titov (1995:551)	AY795854/AY795993	Tibell & Vinuesa 2005
<i>Chaenothecopsis fennica</i> (Laurila) Tibell (1940:10)	AY795857/AY795995	Tibell & Vinuesa 2005
<i>Chaenothecopsis golubkova</i> Tibell & Titov (1993:320)	AY795859/AY795996	Tibell & Vinuesa 2005
<i>Chaenothecopsis khayensis</i> Rikkinen & Tuovila (2011:611)	JX122785/HQ172895	Tuovila <i>et al.</i> 2013/Tuovila <i>et al.</i> 2011a
<i>Chaenothecopsis montana</i> Rikkinen (2003c:446)	JX119105/JX119114	Tuovila <i>et al.</i> 2013
<i>Chaenothecopsis neocaledonica</i> Rikkinen, A.R.Schmidt & Tuovila	KF815196/ KF815197	this study
<i>Chaenothecopsis nigripunctata</i> Rikkinen (2003:99)	JX119103/JX119112	Tuovila <i>et al.</i> 2013
<i>Chaenothecopsis pallida</i> Rikkinen & Tuovila	JX122779/JX122781	Tuovila <i>et al.</i> 2014
<i>Chaenothecopsis pusiola</i> (Acharius) Vainio (1817:231)	JX119106/JX119115	Tuovila <i>et al.</i> 2013
<i>Chaenothecopsis quintralis</i> Messuti, Amico, Lorenzo & Vidal-Russell (2012:1224)	-/ JQ267741	Messuti <i>et al.</i> 2012
<i>Chaenothecopsis resinophila</i> Rikkinen & Tuovila	JX122780/JX122782	Tuovila <i>et al.</i> 2014
<i>Chaenothecopsis sitchensis</i> Rikkinen (1999:366)	JX119102/JX119111	Tuovila <i>et al.</i> 2013
<i>Chaenothecopsis tsugae</i> Rikkinen (1999:367)	JX119104/JX119113	Tuovila <i>et al.</i> 2013
<i>Mycocalicium</i> sp.	KC590482/KC590487	Tuovila <i>et al.</i> 2014
<i>Mycocalicium sequoiae</i> Bonar (1971:64)	-/AY796002	Tibell & Vinuesa 2005
<i>Mycocalicium subtile</i> (Persoon) Szatala (1797:60)	AF225445/AY796003	Vinuesa <i>et al.</i> 2001/Tibell & Vinuesa 2005
<i>Phaeocalicium</i> sp. A. F. W. Schmidt (1970:128)	KC590483/KC590484	Tuovila <i>et al.</i> 2014
<i>Phaeocalicium populneum</i> (Brond ex Duby) A.F.W. Schmidt (1830:638)	AY795874/AY796009	Tibell & Vinuesa 2005
<i>Sphinctrina leucopoda</i> Nylander (1860:144)	AY795875/AY796006	Tibell & Vinuesa 2005
<i>Sphinctrina turbinata</i> (Persoon ex Fries) de Notaris (1801:95)	AY795877/DQ009001	Tibell & Vinuesa 2005
<i>Stenocybe pullatula</i> (Acharius) Stein (1816:121)	AY795878/AY796008	Tibell & Vinuesa 2005

## Results

### Taxonomy

*Chaenothecopsis neocaledonica* J. Rikkinen, H. Tuovila & A.R. Schmidt, *sp. nov.* (Figures 2C and 3–5)

**Type:**—NEW CALEDONIA, Province Sud: Yaté. Along road RP 3 about 5 km west of Yaté. On resin, resin-soaked bark and lignum of *Agathis ovata*, 411 m, 22°10'03.63"S, 166°54'10.15"E, 5 November 2011, Rikkinen 010179 (holotype P, isotypes H, GenBank KF815196/KF815197, MycoBank MB 806059).

**Diagnosis:**—*Chaenothecopsis neocaledonica* differs from related *Chaenothecopsis* species in having very robust ascomata with wide and flat capitula, wide and thick-walled hyphae in the stipe, and noticeably large one-septate ascospores.

**Description:**—*Apothecia* on exudate of *Agathis ovata*, 1400–2400 µm high. *Stipe* shining black or sometimes olivaceous, straight, sometimes flexuous, occasionally branched, 180–260 µm wide. *Capitulum* black, in young apothecia usually lenticular to subspheric, later lobate and plano-concave, 150–600 µm wide. *Asci* cylindrical, 96–115 × 5.5–6.5 µm (n = 10), apex variously thickened, thickening gradually disappearing, mature asci usually without a thickening, apices often covered with hyaline, amorphous material IKI+ weakly blue, dissolving in KOH; formed with croziers. *Ascospores* uniseriate, usually obliquely to periclinally oriented in asci, septate, septa as thick or slightly thinner than spore wall, brown, ellipsoid to cylindrical, smooth, sometimes thickened at the ends; (12.3–) 13.4–17.6 (–18.6) × (4.4–) 4.9–6.3 (–6.7) µm (n = 100, from 7 ascocarps, 2 populations), mean 15.1 × 5.7 µm, Q = (2.2–) 2.3–3.1 (–3.8), mean Q = 2.7. *Paraphyses* hyaline, filiform, 1.0–1.5 µm wide (n = 10), with several branches, very long, reaching and curving above asci; septate, septal intervals 10–25 µm. *Stipe* hyphae covered with hard glass-like layer dissolving in KOH, 10–16 µm wide, walls two layered: outer wall hyaline, 2–5 µm wide, inner wall yellowish green to yellowish brown to dark brown, 1.5 µm wide; hyphae intertwined and outer cell walls fused (*textura intricata-oblita*); IKI+ slowly violet red; hyphae swell in KOH and brown pigment leaks into the medium; hyphae in inner part of stipe hyaline, slightly intertwined, walls up to 8 µm wide. *Excipulum* hyphae yellowish brown to greenish brown, 5–8 µm wide, wall 1–1.5 µm, periclinally arranged or slightly intertwined, outer cell walls fused (*textura prismatica-oblita*); IKI+ slowly violet red, hyphae swell in KOH, some brown pigment leaks into the medium. *Hymenium* IKI+ blue, slowly disappearing; full of colorless, CR– crystals, only partly dissolving in KOH. *Hypothecium* hyaline, hyphae swell in KOH. *Epithecium* yellowish green or almost hyaline, composed of elements from excipulum and paraphyses. The thick-walled hyphae of excipulum reach to cover the asci, intertwine and form a tight net, hard to break, with small holes, on average 3–5 × 5–8 µm in size. Paraphyses curve at the level of ascus tips to cover the asci, branch repeatedly just beneath the net of excipular hyphae, forming an inner layer of the epithecium; excipular part of epithecium IKI+ faint violet red. All parts MLZ –.

**Etymology:**—In reference to New Caledonia, France, where the species was first discovered.

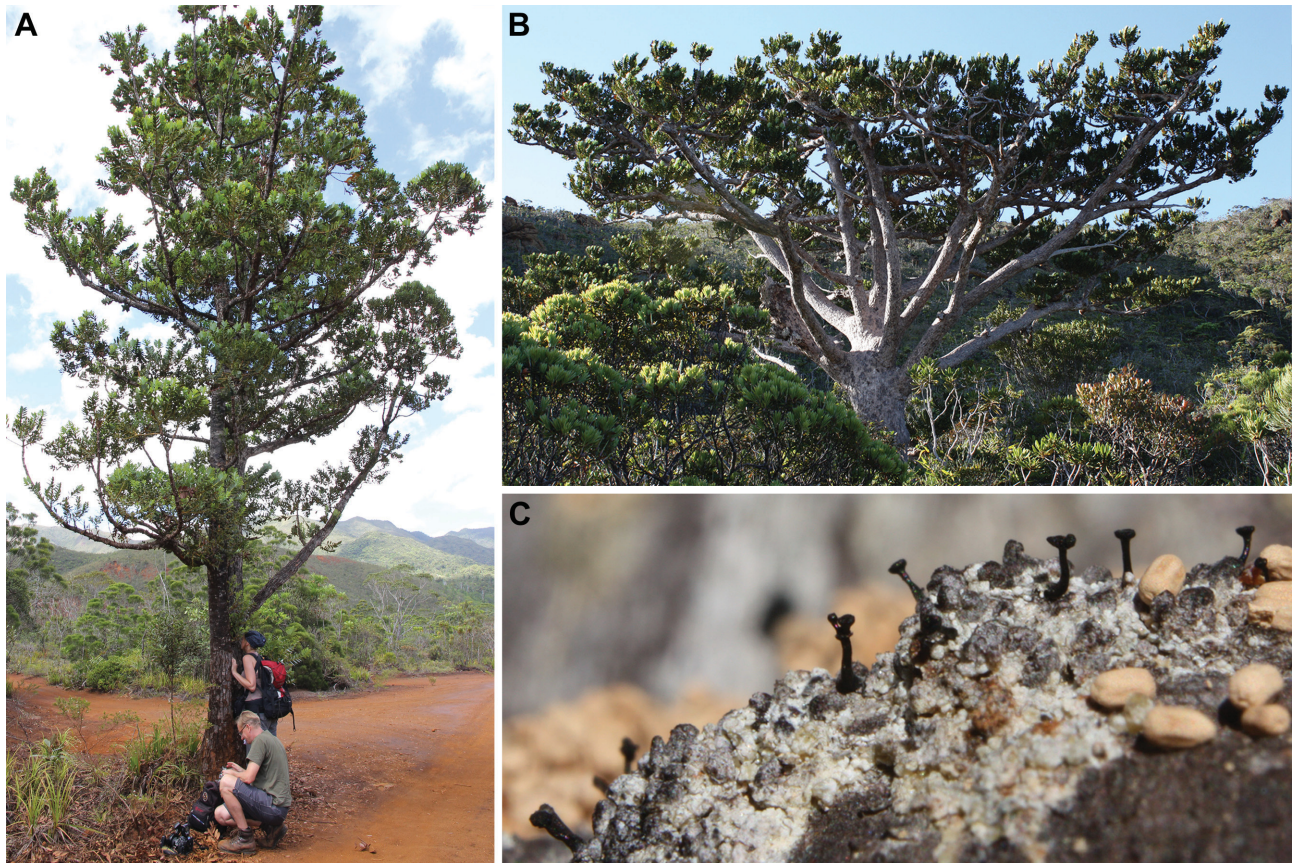
**Further specimens examined:**—NEW CALEDONIA, Province Sud: Yaté. Another *Agathis ovata* trunk at type locality, Rikkinen 010180 (paratype H). Along road GR NC1 in Blue River Provincial Park near the Refuge des Scientifiques, 180 m, 22°05'58.62"S, 166°39'00.12"E, 12. November 2011, Rikkinen 010273.

### Phylogenetic analysis

Both the ITS and LSU sequences from the two collection sites from Yaté were identical. GenBank accession numbers of all sequences are shown in Table 1. There were no major indels in the LSU and 5.8S sequences, so these regions could be unambiguously aligned. The ambiguously aligned regions were removed from ITS1 (32% of the total length) and ITS2 (31% of the total length). The resulting alignment has been uploaded to TreeBase, direct accession: <http://purl.org/phylo/treebase/phyloids/study/TB2:S14913>.

The results of the phylogenetic analysis are shown in Figure 6. The phylogeny is broadly consistent with previous results (e.g. Tibell & Vinuesa 2005, Tuovila *et al.* 2013). It places *Chaenothecopsis neocaledonica* together with several other *Chaenothecopsis* species with septate spores in clade A. This clade includes taxa that grow on conifer resins, conifer lignum, and also several species that are either lichen-parasitic or associate with free-living green algae.

While the crown clades of the tree are moderately or well supported, the deeper nodes of the tree tend to have only low support. The well-supported crown clades obtained in the analysis do not correspond with the generic delimitation of the classification in current use (e.g. Tibell 1999). However, the resinicolous species in each supported clade share a similar overall morphology and ecology. Clade A includes species with septate spores on conifer resin; Clade B includes species with aseptate spores on angiosperm exudates (see Tuovila *et al.* 2013); Clade C includes another group of species on conifer resin (see Tuovila *et al.* 2011b).

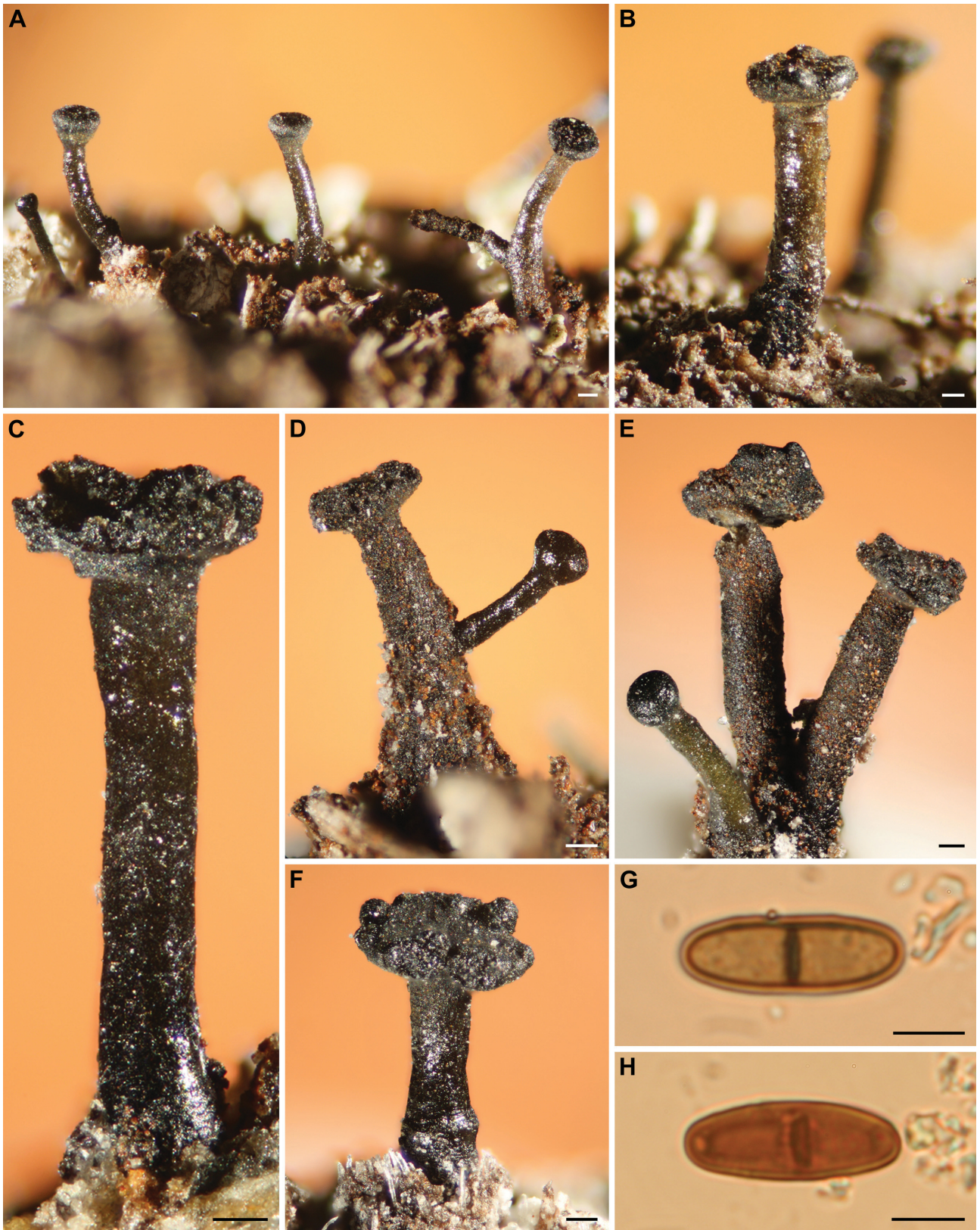


**FIGURE 2.** Habitat of *Chaenothecopsis neocaledonica* in southeastern New Caledonia. (A) Collecting specimens from a trunk of *Agathis ovata* in Blue River Provincial Park. (B) Large *Agathis ovata* tree west of Yaté which hosted the holotype specimen. (C) Ascomata of *C. neocaledonica* growing on a semi-solidified resin flow. Several termite fecal pellets are visible on the right.

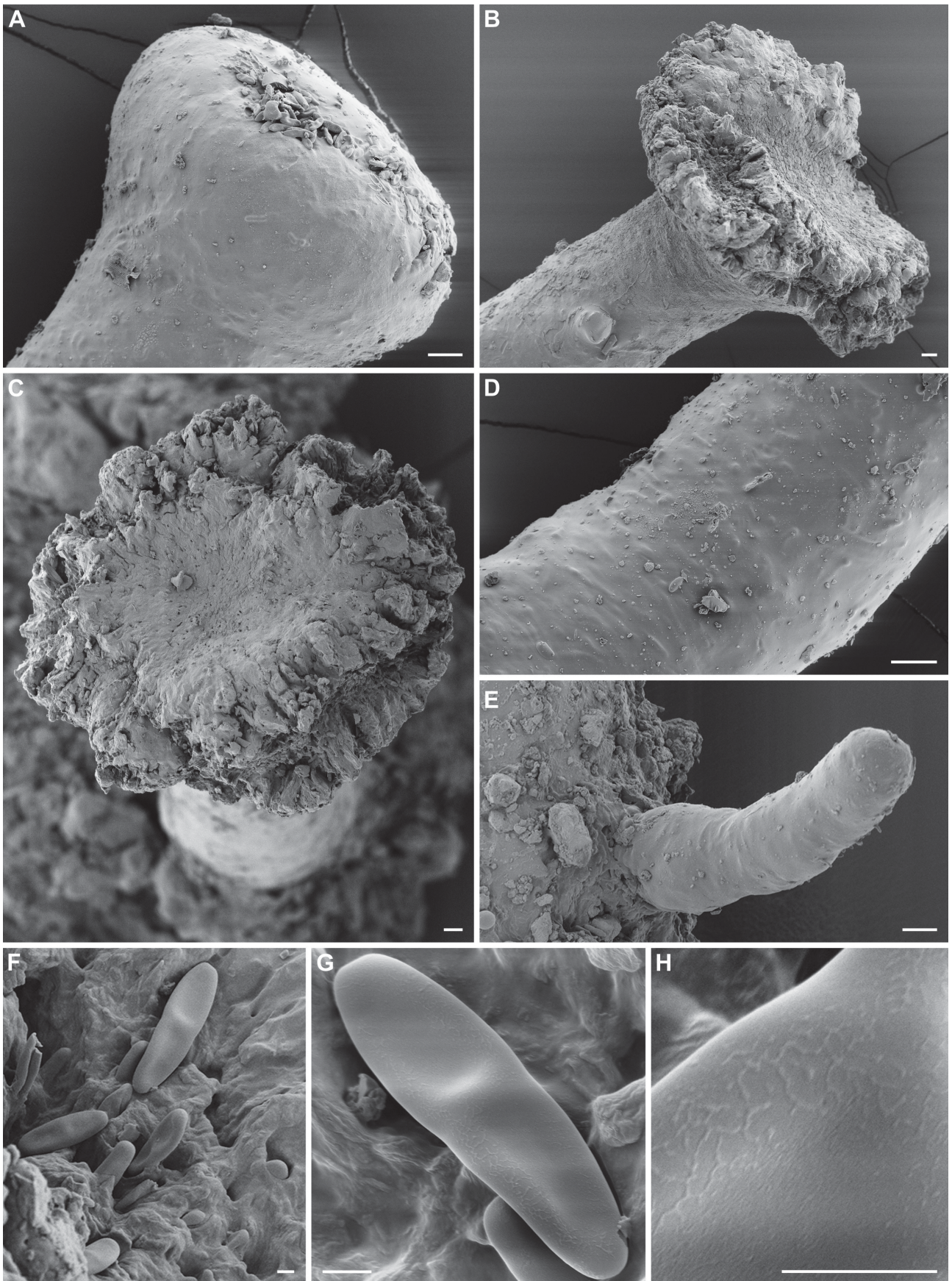
## Discussion

### Taxonomic relationships and morphological features

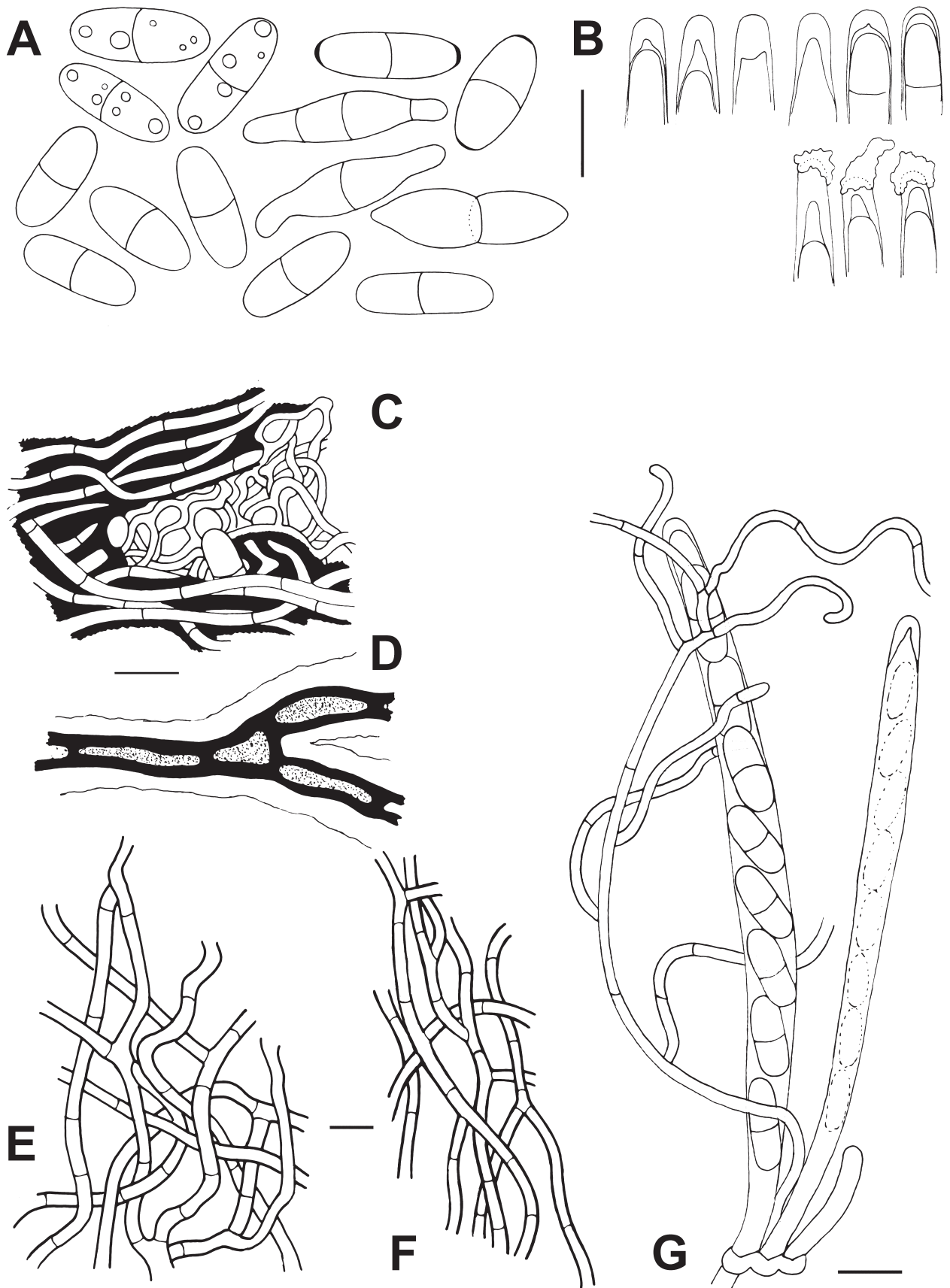
Our phylogenetic analysis grouped *Chaenothecopsis neocaledonica* together with several other resinicolous species from conifer resin. While all these species have one-septate ascospores and also share many other anatomical characters, the new species differs from its relatives in spore size and capitulum structure. The ascospores of *C. neocaledonica* are much larger than those of most other *Chaenothecopsis* species, which typically have spores ranging from 4 to ~10  $\mu\text{m}$  in length. Even *Chaenothecopsis nigripunctata* Rikkinen (2003b: 99) has somewhat smaller (13–15.5  $\mu\text{m}$ ) spores than *C. neocaledonica* (Rikkinen 2003b). The overall habit of mature *C. neocaledonica* ascomata is instantly recognizable. The ascomata are very robust and their capitula do not become (sub)hemispheric with age. In most *Chaenothecopsis* species the capitulum surface tends to become gradually more convex as more and more asci mature and expand the hymenium. In *C. neocaledonica* the epithecium in the central parts of the apothecial disk is very thick and this seems to prevent the expansion of the apothecium surface. Ascospores are mainly released from near the edge of the apothecial disk, where the epithecium is not as thickened as in the central parts.



**FIGURE 3.** Light micrographs of *Chaenothecopsis neocaledonica* (holotype, Rikkinen 010179). (A–F) Ascomata. (G–H) Ascospores. Scale bars = 100  $\mu\text{m}$  (A–F) and 5  $\mu\text{m}$  (G, H).

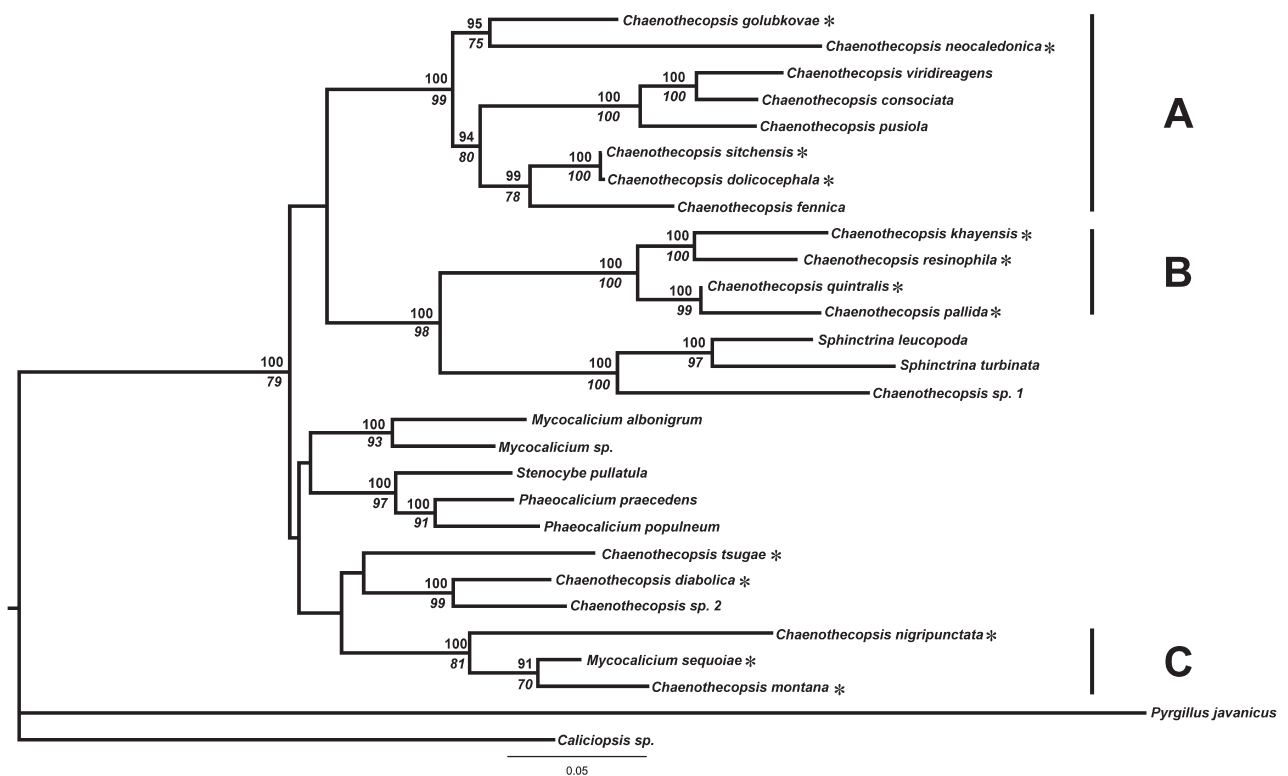


**FIGURE 4.** SEM micrographs of *Chaenothecopsis neocaledonica* (holotype, *Rikkinen* 010179). (A) Excipulum of young ascoma. (B–C) Thickened epithecium of mature ascoma. (D) Stipe surface. (E) Branch initial at base of mature stipe. (F–G) Ascospores. (H) Spore ornamentation. Scale bars = 20  $\mu\text{m}$  (A–E) and 2  $\mu\text{m}$  (F–H).



**FIGURE 5.** Line drawings of anatomical details of *Chaenothecopsis neocaledonica* (holotype, *Rikkinen 010179* A–D, F–G; *Rikkinen 010180* A, E) in water and CR. (A) Ascospores, (B) variation of ascus tip, (C) double layered structure of epithecium, (D) details of hyphae in the stipe, (E) general structure of stipe, (F) structure of excipulum, (G) asci and paraphyses. All scale bars 10 µm.





**FIGURE 6.** Phylogenetic relationship of *Chaenothecopsis neocaledonica* based on ML analysis of ITS and partial LSU sequences. Bootstrap values ( $\geq 70$ ) shown below the nodes. Bayesian posterior probabilities ( $\geq 90$ ) shown above the nodes. Resinicolous species are indicated by an asterisk.

Several species of *Chaenothecopsis* and *Mycocalicium* Vainio (1890: 182) develop distinct pruina on the exipulum and upper part of the stipe. Especially in young ascocarps the pruina may also extend to the lower parts of the stalk and sometimes the whole stalk remains pruinose even when mature. Depending on the species the pruina may be white, brown, yellow or greenish, and in the later case it often consists of vulpinic acid and/or other pulvinic acid derivatives (Bonar 1971, Tibell & Titov 1995). From this perspective it is important to note that the red brown particles seen on some ascocarps of *Chaenothecopsis neocaledonica* (Figures 3D–E and 4D–E) have not been produced by the fungus, but represent mineral dust from the ferruginous sediment which characterizes many ultramafic sites in southern New Caledonia (Figure 2A).

It is generally accepted that the genera of Mycocaliciaceae, as currently delimited, are not monophyletic and that generic delimitations within the family need to be revised (Hawksworth 1980, Tibell & Vinuesa 2005, Tibell 1984, Tuovila 2013). The overall topology of the phylogenetic tree is consistent to that of Tuovila *et al.* (2013) and reveals the same major clades which are also defined by consistent morphology and ecology. The phylogram demonstrates that the majority of genera in the Mycocaliciales (*Chaenothecopsis*, *Mycocalicium*, *Phaeocalicium* A.F.W. Schmidt (1970: 128)) are polyphyletic (see also Tibell & Vinuesa 2005). Unfortunately, the resolution in deep nodes is insufficient for resolving genus-level relationships. In spore and ascus size, *Chaenothecopsis neocaledonica* rather resembles some *Phaeocalicium* species than *Chaenothecopsis*, as traditionally conceived (Tibell 1996). Species of *Phaeocalicium* have been distinguished from species of *Chaenothecopsis* and *Mycocalicium* on the basis of their uniformly thickened ascus tip and large, < 10  $\mu\text{m}$  long spores. In addition, most *Phaeocalicium* species live on twigs or thin branches of angiosperms. *Chaenothecopsis neocaledonica* is yet another example of an ‘intermediate’ species that does not perfectly fit into *Chaenothecopsis* nor *Phaeocalicium* in a traditional sense. The spore and ascus size of *C. neocaledonica* are of the *Phaeocalicium* type, but all other characters, especially the structure of the stipe and excipulum place it within *Chaenothecopsis*.

### Resinicolous ecology

Many *Chaenothecopsis* species are resinicolous with different species colonizing terpenoid resins of conifers and phenolic resins of angiosperms. Many resinicolous *Chaenothecopsis* species appear to be quite host specific, with individual plant species hosting different fungi.

Most species on conifer resins from the Northern Hemisphere grow on different species of Pinaceae and Cupressaceae. Several fossilized specimens preserved in Oligocene and Eocene ambers demonstrate that the association

between mycocalicean fungi and conifer exudates is at least 35 to 50 million years old (Rikkinen & Poinar 2000, Tuovila *et al.* 2013). While the early evolution of the resinicolous ecology remains unclear, all the *Chaenothecopsis* clades with species on conifer resins are clearly distinct from the single clade of species from angiosperm exudates (Tuovila *et al.* 2011a, 2013).

We prospected for mycocalicioid fungi on several *Agathis* and *Araucaria* species in different locations (see Figure 1). *Agathis ovata* was checked near Yaté, in the Rivière Bleue Park, along Mont Dzumac road, and along the Mont Humboldt trail, however, *Chaenothecopsis neocaledonica* was only found on two trees in the Yaté site and on one tree trunk in Rivière Bleue. Although we were unable to find further *Chaenothecopsis* specimens, some localities harbour complex resinicolous communities that will be described in separate papers.

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