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***Taitaia*, a novel lichenicolous fungus in tropical montane forests in Kenya (East-Africa)**

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Abstract: During lichenological explorations of tropical montane forests in Kenya, a remarkable new lichenicolous fungus was repeatedly found growing on thalli of the epiphytic tripartite cyanolichen *Crocodia* cf. *clathrata*. Molecular phylogenetic analyses placed the fungus within *Gomphillaceae* (*Ostropales*, *Lecanoromycetes*), a family mainly of lichen-symbiotic species in the tropics. The anatomical features (unitunicate, non-amyloid asci and simple, septate paraphyses) as well as the hemiangiocarpic ascoma development confirm its taxonomic affinity. DNA sequence data showed the closest relationship was with *Gyalidea fritzei*, followed by *Corticifraga peltigerae*. A monotypic genus, *Taitaia*, is introduced to incorporate a single species, *T. aurea*. The new fungus is characterized by aggregated ascomata with yellow margins and salmon red discs developing from a single base.

Key words: Ascomycota, *Corticifraga*, *Gyalidea*, lichen-inhabiting fungi, Taita Hills, taxonomy

Introduction

The diversity of lichenicolous fungi in Africa is largely underexplored and the limited information available is scattered in the literature (e.g. Bock et al. 2007; Ertz 2009; Fryday 2015). Thus, we can expect to find a plethora of taxa among the lichenized and lichen-inhabiting fungi that live in the many habitat types unique to Africa. The Taita Hills in south-eastern Kenya form the northernmost section of the Eastern Arc Mountains which, together with nearby coastal forests, represent a hotspot of global biodiversity (Myers et al. 2000; Burgess et al. 2007; Dimitrov et al. 2012). The moist and relatively cool conditions support a high diversity and considerable biomass of cryptogamic epiphytes (e.g. Enroth et al. 2013; Malombe et al. 2016; Stam et al. 2017). Even among macrolichens, many local taxa cannot be readily identified with the keys currently available for East Africa (Swinscow & Krog 1988).

In the framework of lichenological exploration of tropical montane forests in Kenya, a remarkable lichenicolous fungus was repeatedly found growing out of the thalli of the tripartite cyanolichen *Crocodia* cf. *clathrata* (Peltigerales, Lecanoromycetes). Species of Peltigerales are known to host a large diversity of lichenicolous fungi of different phylogenetic origins (e.g. Kondratyuk & Galloway 1995; Hawksworth & Miadlikowska 1997; Lawrey & Diederich 2016) but none of the available descriptions correspond with our new fungus. To clarify the systematic position of the unknown fungus, we analyzed its morphology as well as slow-evolving ribosomal and mitochondrial markers, and describe a new genus and species based on the results.

Material and methods

Sampling sites

Cyanolichen specimens infected by the new fungus were collected from montane forests in the Taita Hills and the western part of the Rift Valley. The Taita Hills rise abruptly from the surrounding dry plains at c. 600–1000 m to a series of mountain ridges, reaching 2208 m at the highest peak Vuria. The upper slopes of East African mountains benefit from moisture brought by the trade winds and capture enough of this to sustain moist evergreen montane forests. The moist and relatively cool climate provides favourable conditions for the development of species-rich bryophyte and lichen communities and considerable epiphyte biomass (Fig. 1). The type locality of the new fungus is located close to the summit of Vuria, in a dense

indigenous forest dominated by *Maesa lanceolata*, *Nuxia congesta* and *Dracaena afromontana*.

Morphology

Light microscopy was carried out using hand-cut sections mounted in either tap water, a 10% aqueous solution of potassium hydroxide (KOH; K), Cresyl blue (CRB), Congo red or in Lugol's solution (I). The apical apparatus of the ascus was observed in Lugol's solution (I) pretreated with K (denoted as K/I). The measurements were made in water and the sizes are presented as minimum–(mean±1SD)–maximum value. The close-up photographs of ascomata and thallus cross-sections were taken using a microscope equipped with a Canon 60D digital camera. For the images, a series of up to 57 photomicrographs at different focal planes were stacked using HeliconFocus 4.45. The fluorescence microscopy, used to observe the pigments in the host thallus and in ascomata of the lichenicolous fungus, was carried out with a Zeiss Axioskop 40 FL microscope, an AxioCam MRc camera and the AxioVision 1.6. For the scanning electron microscopy (SEM), pieces were fixed onto an SEM-mount, sputter-coated with a 12 nm thick coating of Pt/Pd using an Automatic Sputter Coater (Canemco Inc.), and examined and imaged with a Carl Zeiss LEO 1530 Gemini field emission scanning electron microscope.

The material studied is deposited in the herbarium of the National Museums of Kenya (EA) and in the lichen herbarium of the Natural History Museum at the University of Tartu (TU).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from ascomata of specimens using High Pure PCR Template Preparation Kit (Roche Applied Science®) and following the protocol provided by the manufacturer with minor modifications. We amplified three gene loci: the internal transcribed spacer (ITS), the large subunit nuclear ribosomal RNA gene (nuLSU), and the mitochondrial small subunit ribosomal RNA gene (mtSSU). The primers used for amplification and sequencing were ITS0F, LA-W (Tedersoo et al. 2008), ITS4 and ITS5 (White et al. 1990) for ITS, LROR and LR5 (Vilgalys & Hester 1990) and CTB6 (Garbelotto et al. 1997) for nuLSU, and mrSSU1 and mrSSU3R (Zoller et al. 1999) for mtSSU. The PCR reaction mix (25 µl) consisted of 5 µl 5× HOT FIREPol Blend Master Mix (Solis BioDyne, Tartu, Estonia), 0.5 µl of

20 μ M of both primers, 1–8 μ l of target-DNA and distilled water. The PCR products were visualized in a 1% agarose gel stained with ethidium bromide. For the purification of PCR products, 1 μ l of FastAP and 0.5 μ l of Exonuclease I (Thermo Scientific, Waltham, MA, USA) were added to each tube per 20 μ l of product. Both complementary strands were sequenced by Macrogen Inc. (Amsterdam, The Netherlands). Sequencher 4.10.1 (GeneCodes Corp.®, Ann Arbor, MI, USA) was used to check, assemble and manually adjust the resulting sequence fragments. The consensus sequences were compared with those publicly available in NCBI (<https://www.ncbi.nlm.nih.gov/genbank>) using a BLAST search to confirm their identity. For each gene, the top-scoring match was a member of Ostropomycetidae, even if the similarity did not exceed 90%. The newly generated sequences are available in NCBI (<https://www.ncbi.nlm.nih.gov/genbank>) under Accession numbers MF372796–MF372801 and MF509277 (Table 1).

Phylogenetic analyses

The newly generated nuLSU and mtSSU sequences were aligned with sequences downloaded from NCBI (Table 1) using MUSCLE (Edgar 2004). We did not use ITS sequences in the analyses due to their high variability at higher taxon levels. The alignments were manually checked and trimmed with SeaView v.4.6 (Gouy et al. 2010). The first analysis included representatives of major classes of Ascomycota and subclasses of Lecanoromycetes, but focusing on Ostropomycetidae (data not shown). After the first analysis, we excluded sequences with ambiguous alignment and reduced the dataset, but left a representation of families of Ostropales. The online version of Gblocks v.0.91b (Talavera & Castresana 2007) run at http://molevol.cmima.csic.es/castresana/Gblocks_server.html was used to eliminate poorly aligned positions and divergent regions of the alignment while allowing gap positions within the final blocks. For each gene, the best-fit nucleotide substitution model was calculated and selected based on the lowest value of AIC criterion with jModelTest v.2.1.6. (Darriba et al. 2012). The best-fit model for nuLSU was TIM+I+G and for mtSSU it was TVM+I+G. Each gene locus was aligned and analyzed separately with a maximum likelihood (ML) approach using PHYML (Guindon et al. 2010) and, as no topological conflict was detected in supported clades (bootstrapping over 100 replicates) by visual inspection (data not shown), nuLSU and mtSSU alignments were concatenated. The Markov chain Monte Carlo (MCMC)

approach using MrBayes v.3.2.1 (Ronquist et al. 2012) was applied to reconstruct phylogeny. For the final partitioned analysis, two parallel simultaneous runs with four chains run were applied over 7 M generations until convergence of the chains was confirmed by the standard deviation of split frequencies reaching 0.01. The first 25% of saved data was discarded as burn-in, and the 50% majority-rule consensus tree and posterior probabilities (PP) were calculated from the rest. As an alternative, a maximum likelihood (ML) analysis using GTR+G as the substitution model was implemented with RAxML v.8.1.10 (Stamatakis 2014) at the CIPRES Science Gateway (Miller et al. 2010). Bootstrap support (BS) was calculated from over 500 pseudoreplicates. The outgroup included *Loxosporopsis corallifera* Brodo et al., *Dibaeis baeomyces* (L. f.) Rambold & Hertel (Pertusariales, Lecanoromycetes), *Peltula auriculata* Büdel et al. (Lichinomycetes) and *Geoglossum nigratum* (Pers.) Cooke (Geoglossomycetes).

The phylogenetic tree was visualized with FigTree v.1.4.2 (<http://tree.bio.ed.ac.uk/>) and Adobe Illustrator CS3® was used for artwork.

Results and Discussion

The combined nuLSU + mtSSU dataset consisted of 912 characters, 366 of which were nuLSU and 530 mtSSU. The singlegene and concatenated nuLSU + mtSSU analyses placed the sequences within Gomphillaceae, Ostropales (PP= 1, BS =100; Fig. 2). The combination of phenotypic characters (hemiangiocarpic ascomata with non-amyloid asci without distinct apical apparatus; see Taxonomy, Fig. 4) supports the phylogenetic placement within Ostropales (Lumbsch et al. 2007; Jaklitsch et al. 2016), an ascomycete order of various lifestyles, including symbiotic, optionally lichenized and lichen-dwelling fungi (Baloch et al. 2010).

The family Gomphillaceae includes mainly lichen-symbiotic species distributed in the tropics and variously growing on, for example, living leaves, tree bark, bryophytes and soil or rock (Lücking et al. 2004; Baloch et al. 2010; Jaklitsch et al. 2016). In addition to the new fungus, only two other genera of lichen-inhabiting fungi are currently known in the family, *Gyalideopsis* Vězda (Etayo & Diederich 2001; Etayo 2010) and *Corticifraga* D. Hawksw. & R. Sant. (Pino-Bodas et al. 2017). The phylogenetic concept of Gomphillaceae itself has remained obscure and unresolved (Lücking et al. 2004). The family includes taxa previously placed in a separate family, Asterothyriaceae, distinguished by simple, unbranched paraphyses in

the hamathecium and by the lack of hyphophores (Vězda 1987; Henssen & Lücking 2002). Both of these characters are also observed in our new fungus (see Taxonomy). Considering the proportion of publicly available sequences (Table 1) and the number of species and genera currently assigned to Gomphillaceae (Jaklitsch et al. 2016), a re-evaluation of these two families can be expected in the future.

The new fungus, represented in the phylogeny by three specimens, showed the closest relationship to *Gyalidea fritzei* (Stein) Vězda (PP= 0.98, BS =78; Fig. 2). The two fungi have different ecologies, with the latter species being lichenized and growing on lime-rich stone (Gilbert et al. 2009). Both species in turn are sister to *Corticifraga peltigerae* (PP= 0.98, BS = 80; Fig. 2), another lichenicolous fungus which was recently shown to be a member of Gomphillaceae (Pino-Bodas et al. 2017). Similar to the new fungus, the species of *Corticifraga* live on cyanolichens of the Peltigerales (e.g. Hawksworth & Santesson 1990; Zhurbenko 2007). All three genera share similar ascoma development, ascus arrangement, simple, septate paraphyses and colourless, ellipsoid to fusiform septate ascospores (Hawksworth & Santesson 1990; Gilbert et al. 2009). Both the new fungus and *Corticifraga* have a similar type of exciple consisting of more or less globular cells (i.e. textura globularis-type) (Hawksworth & Santesson 1990); no information is provided about the exciple type of *Gyalidea* (e.g. Gilbert et al. 2009). Both *Corticifraga* and *Gyalidea* differ from the new fungus by having ascomata that arise singly, that are immersed, pale cream to almost black and with discs that are deeply concave (urceolate) to plane when mature (Hawksworth & Santesson 1990; Gilbert et al. 2009).

Taxonomy

Taitaia Suija, Kaasalainen, Kirika & Rikkinen, gen. nov.

MycoBank No.: MB 821739 – Type species: *Taitaia aurea* Suija, Kaasalainen & Rikkinen

Taitaia aurea Suija, Kaasalainen & Rikkinen, sp. nov.

MycoBank No.: MB 821740

DNA barcode/reference sequence (rDNA ITS): MF372800 (*from holotype: Rikkinen 16258, DNA sample: AF259*)

Lichenicolous fungus. Ascomata apothecioid, hemiangiocarpic, breaking through cortex of host thallus, sometimes with ten or more ascomata arising from a single base, margins yellow and disc salmon red. Hymenium with unitunicate non-amyloid asci and simple, septate paraphyses. Ascospores fusiform and 1-septate.

Type: Kenya, Taita-Taveta Province, near summit of Vuria, dense moist forest with *Maesa lanceolata*, *Nuxia congesta* and *Dracaena afromontana*, UTM 421443, 9623094, 2072 m, on thallus of *Crocodia* cf. *clathrata* growing on stem of woody climber, 15 January 2015. J. Rikkinen 16258 (TU56326—holotype; DNA sample: AF259)

(Figs 3–5)

Lichenicolous ascomycete, producing ascomata through the upper and lower surface and margins of the thallus of *Crocodia* cf. *clathrata* (Figs 1C, 3A & B).

Ascomata apothecioid, sessile, the developing fruiting bodies bursting through the cortex of the host thallus; grouped, sometimes ten or even more ascomata starting from a single base, basally constricted (Fig. 3C & D); diameter of aggregations of immature ascomata 0.4–(0.7±0.3)–0.8mm (n=19), and aggregations of mature, opened ascomata 0.6– (1.4±0.4)–2.4mm (n=16; Fig. 3C); hemiangiocarpic, at first closed (Fig. 4C), then opening with the edge rolling outwards exposing a salmon red disc (Fig. 3C), 0.2– (0.3±0.1)–0.7mm (n=20). *True exciple* well developed, more-or-less concolorous with the yellow medulla of the host lichen (Fig. 3D), *textura globularis*-type (Fig. 4E), c. 10–15 µm wide. *Exciple* surrounding hymenium and hypothecium impregnated with dark yellowish to brownish pigment grains (Fig. 4C, D & G), this pigment does not dissolve in either Kor N, and does not autofluoresce, (Fig. 4A), and is distinct from pulvinic acid that gives the yellow colour to the host thallus (pinkish fluorescence in Fig. 4A); the hyaline hyphal tips of the external excipular cells extend out of the margin (Figs 4D & 5A) revealing the mealy appearance of the ascomatal margin (Figs 3D & 5A). *Hymenium* c. 35µm high, hyaline (Fig. 4F), without hymenial gel, the upper part covered by an amorphous hyaline layer, K–, K/I–, CRB+ dark blue; *subhymenium* slightly yellowish, *textura intricata* (Fig. 4F). *Hypothecium* hyaline, c. 160 µm (until the pigmented rim), similar in structure to exciple (i.e. *textura globularis-angularis* type) (Fig. 4E), K–, K/I–,

CRB+ pale blue; sometimes with clumps of yellow pigment grains (Fig. 4C & E). *Paraphyses* irregularly septate, unbranched (Fig. 4H & L) or only dichotomously branched at the base, lax (no hymenial gel), *c.* 1 µm wide, not or only very slightly widened at tips, remaining hyaline, but tips surrounded by a hyaline amorphous substance (forming a layer over the hymenium), CRB+ dark blue; contents of paraphyses in Congo red+ reddish (Fig. 4H & L), CRB+ blue. *Asci* functionally unitunicate, clavate, 40–(44.8±5.6)–55×10–(13.2±3.5)– 20 µm (n=10); apical ascus wall not widened or slightly widened, non-amyloid (K/I–) without distinct apical apparatus; the ascus base distinctly narrowed; 8 biseriate spores (Fig. 4H & K). *Ascospores* 2-celled, hyaline, fusiform, spore tips attenuated, not constricted or only slightly constricted at septum, symmetrical or sometimes asymmetrical (one cell longer than the other), 14–(15.8±1.5)–19×4– (4.8±0.8)–7 µm, l/w=2.1–4.5 (n=20), with perispore <1 µm, which is Congo red– and CRB– (Fig. 4I & J); intracellular matrix of ascospores Congo red+, CRB+ (Fig. 4I–K). *Vegetative hyphae* hyaline, non-amyloid, I–, K/I–, protruding between the medullary hyphae of the host (Fig. 4B). Asexual morph not observed.

Etymology. The genus name refers to the type locality, Taita Hills in Kenya; the epithet ‘aurea’ refers to the golden yellow colour of the ascoma margin.

Taxonomy. Monotypic genus in Gomphillaceae (Ostropales, Ostropomycetidae, Lecanoromycetes, Ascomycota).

Ecology and distribution. Obligately lichenicolous, growing on thalli of *Crocodia cf. clathrata* (Peltigerales). The ascomata can burst through the host thallus but no obvious damage to mycobiont or photobiont was detected by microscopic inspection. On the lower surface of the host the ascomata usually develop through pseudocyphellae (pores); however, on the upper surface and thallus margins they might develop preferentially at locations where the cortex of the host has been damaged. Inside the host, the vegetative hyphae of *Taitaia* are slightly narrower than those of the host (Fig. 5A & B) and can be followed for some distance between the medullary hyphae of the host (Fig. 4B). The ascomata of *Taitaia* are closely associated with the internal cephalodia of the host (Figs 3D & 5A) that contain a nitrogen-fixing cyanobacterium (*Nostoc*). In cephalodiate lichens, the green-algal photobiont typically produces most photosynthate and the cephalodial cyanobiont mainly fixes nitrogen (Cornejo & Scheidegger 2013; Rikkinen 2017). While

vegetative hyphae of the fungus are seen on cephalodial surfaces, they do not appear to penetrate into these structures.

The new taxon is known from two localities in tropical lower-mountain forests in Kenya (East Africa).

Additional specimen examined. **Kenya:** Taita-Taveta Province: near summit of Vuria, dense moist forest with *Maesa lanceolata*, *Nuxia congesta* and *Dracaena afromontana*, UTM 421443, 9623094, 2072 m, on thallus of *Crocodia* cf. *clathrata* growing on stem of woody climber, 2015, J. Rikkinen 16260 (TU 56327; DNA sample: AF260). Rift Valley Province: Cherangani Hills, Kapcherop, along Chepkait River, riverine in disturbed montane forest, 01°02'N, 35°19'E, 2200 m, on *Crocodia* cf. *clathrata*, on bark, 2016, P. Kirika 5103 (EA; DNA sample: AF275).

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REFERENCES

- Baloch, E., Lücking, R., Lumbsch, H. T. & Wedin, M. (2010) Major clades and phylogenetic relationships between lichenized and non-lichenized lineages in Ostropales (Ascomycota: Lecanoromycetes). *Taxon* **59**: 1483–1494.
- Bock, C., Hauck, M. & Fischer, E. (2007) The lichen flora of Rwanda: an annotated checklist. *Willdenowia* **37**: 563–575.

- Burgess, N. D., Butynski, T. M., Cordeiro, N. J., Doggart, N. H., Fjeldsa, J., Howell, K. M., Kilahama, F. B., Loader, S. P., Lovett, J. C., Mbilinyi, B., Menegon, M., Moyer, D. C., Nashanda, E., Perkin, A., Rovero, F., Stanley, W. T. & Stuart, S. N. (2007) The biological importance of the Eastern Arc Mountains of Tanzania and Kenya. *Biological Conservation* **134**: 209–231.
<https://doi.org/10.1016/j.biocon.2006.08.015>
- Cornejo, C. & Scheidegger, C. (2013) New morphological aspects of cephalodium formation in the lichen *Lobaria pulmonaria* (Lecanorales, Ascomycota). *The Lichenologist* **45**: 77–87.
<https://doi.org/10.1017/S0024282912000631>
- Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772.
[doi:10.1038/nmeth.2109](https://doi.org/10.1038/nmeth.2109)
- Dimitrov, D., Nogués-Bravo, D. & Scharff, N. (2012) Why Do Tropical Mountains Support Exceptionally High Biodiversity? The Eastern Arc Mountains and the Drivers of *Saintpaulia* Diversity. *PLoS ONE* **7**: e48908.
[doi:10.1371/journal.pone.0048908](https://doi.org/10.1371/journal.pone.0048908)
- Edgar, R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.
[doi:10.1093/nar/gkh340](https://doi.org/10.1093/nar/gkh340)
- Enroth, J., Nyqvist, P., Malombe, I., Pellikka, P. & Rikkinen, J. (2013) Additions to the moss flora of Kenya and Taita Hills and Mount Kasigau, Kenya. *Polish Botanical Journal* **58**: 495–510.
[doi: 10.2478/pbj-2013-0062](https://doi.org/10.2478/pbj-2013-0062)
- Ertz, D. (2009) Revision of the Corticolous *Opegrapha* Species from the Paleotropics. *Bibliotheca Lichenologica* 102. J. Cramer in der Gebrüder Borntraeger Verlagsbuchhandlung, Berlin and Stuttgart. 176 pp.
- Etayo, J. (2010) Hongos liquenícolas de Perú: Homenaje a Rolf Santesson. *Bulletin de la Société linnéenne de Provence* **61**: 1–46.
- Etayo, J. & Diederich, P. (2001) *Gyalideopsis floridae*, sp. nov. a new lichenicolous lichen from Florida (Gomphillaceae, Ascomycetes). *The Bryologist* **104**: 130–133.
- Fryday, A. M. (2015) A new checklist of lichenised, lichenicolous and allied fungi reported from South Africa. *Bothalia* **45**: 59–122.

<http://dx.doi.org/10.4102/abc.v45i1.148>

- Garbelotto, M. M., Lee H. K., Slaughter, G., Popenuck, T., Cobb, F. W. & Bruns, T. D. (1997) Heterokaryosis is not required for virulence of *Heterobasidion annosum*. *Mycologia* **89**: 92–102.
- Gilbert, O. L., James, P. W. & Woods, R. G. (2009) *Gyalidea* Lettau (1937). In *The Lichens of Great Britain and Ireland* (C. W. Smith, A. Aptroot, B. J. Coppins, A. Fletcher, O. L. Gilbert, P. W. James & P. A. Wolseley (eds.)): 421–423.
- Gouy, M., Guindon, S. & Gascuel, O. (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* **27**: 221–224.
doi:10.1093/molbev/msp259
- Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010) New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Systematic Biology* **59**: 307–321.
doi: 10.1093/sysbio/syq010
- Hawksworth, D. L. & Miadlikowska, J. (1997) New species of lichenicolous fungi occurring on *Peltigera* in Ecuador and Europe. *Mycological Research* **101**: 1127–1134.
- Hawksworth, D. L. & Santesson, R. (1990) A revision of the lichenicolous fungi previously referred to *Phragmonaevia*. In *Contributions to Lichenology in Honour of A. Henssen* (H. M. Jahns (ed.)) *Bibliotheca Lichenologica* **38**. J. Cramer, Berlin-Stuttgart, pp. 121–143.
- Henssen, A. & Lücking, R. (2002) Morphology, anatomy, and ontogeny in the Asterothyriaceae (Ascomycota: Ostropales), a misunderstood group of lichenized fungi. *Annales Botanici Fennici* **39**: 273–299.
- Jaklitsch, W., Baral, H.-O., Lücking, R. & Lumbsch, H. T. (2016) *Syllabus of Plant Families, Volume 1/2: Ascomycota*. Gebrüder Borntraeger Verlag. 322 pp.
- Kondratyuk, S. Y. & Galloway D. J. (1995) Lichenicolous fungi and chemical patterns in *Pseudocyphellaria*. In *Studies in Lichenology with Emphasis on Chemotaxonomy, Geography and Phytochemistry. Festschrift Christian Leuckert* (J.-G. Knoph, K. Schrüfer & H. J. M. Sipman (eds.)). *Bibliotheca Lichenologica*. J. Cramer, Berlin, Stuttgart, pp. 327–345.

- Lawrey, J. D. & Diederich, P. (2016) Lichenicolous fungi – worldwide checklist, including isolated cultures and sequences available.
URL: <http://www.lichenicolous.net> [accessed: 9/5/2017].
- Lücking, R., Stuart, B. L. & Lumbsch, H. T. (2004) Phylogenetic relationships of Gomphillaceae and Asterothyriaceae: evidence from a combined Bayesian analysis of nuclear and mitochondrial sequences. *Mycologia* **96**: 283–294.
<http://dx.doi.org/10.1080/15572536.2005.11832978>
- Lumbsch, H. T., Schmitt, I., Lücking, R., Wiklund, E. & Wedin, M. (2007) The phylogenetic placement of Ostropales within Lecanoromycetes (Ascomycota) revisited. *Mycological Research* **111**: 257–267.
<https://doi.org/10.1016/j.mycres.2007.01.006>
- Malombe, I., Matheka, K. W., Pócs, T. & Patiño, J. (2016) The ecological response of epiphyllous bryophytes to human-induced edges in Afromontane fragmented forests. *Journal of Bryology* **38**: 33–46.
doi: 10.1080/03736687.2015.1122360
- Miller, M. A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees In *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010*, New Orleans, LA, pp. 1–8.
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature* **403**: 853–858.
- Pino-Bodas, R., Zhurbenko, M. P. & Stenroos, S. (2017) Phylogenetic placement within Lecanoromycetes of lichenicolous fungi associated with *Cladonia* and some other genera. *Persoonia* **39**: 91–117.
<https://doi.org/10.3767/persoonia.2017.39.05>
- Rikkinen, J. (2017) Cyanobacteria in terrestrial symbiotic systems. In *Modern topics in the Phototrophic Prokaryotes – Environmental and Applied Aspects* (P. Hallenbeck (ed.)). Springer, Cham, Switzerland: pp. 243–294
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology* **61**: 539–542.
doi: 10.1093/sysbio/sys029

- Stam, Å., Enroth, J., Malombe, I., Pellikka, P. & Rikkinen, J. (2017) Experimental transplants reveal strong environmental effects on the growth of non-vascular epiphytes in Afrotropical Forests. *Biotropica*, in press.
doi: 10.1111/btp.12472
- Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.
doi: 10.1093/bioinformatics/btu033.
- Swinscow, T. D. V. & Krog, H. (1988) *Macrolichens of East Africa*. London: British Museum (Natural History).
- Talavera, G., & Castresana, J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* **56**: 564–577.
doi: <https://doi.org/10.1080/10635150701472164>
- Tedersoo, L., Jairus, T., Horton, B.M., Abarenkov, K., Suvi, T., Saar, I. & Kõljalg, U. (2008) Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. *New Phytologist* **180**: 479–490.
doi: 10.1111/j.1469-8137.2008.02561.x
- Vězda, A. (1987) Flechtensystematische Studien XII. Die Familie Gomphillaceae und ihre Gliederung. *Folia Geobotanica et Phytotaxonomica* **22**: 179–198.
- 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.
- White, T. M., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA for phylogenetics. In *PCR protocols: a guide to methods and applications* (M. A. Innis, D. H Gelfand, J. J. Sninsky & T. J. White (eds.)). Academic Press, New York, pp. 315–321,
- Zhurbenko, M. (2007) *Corticifraga santessonii* and *C. chugachiana* (Lecanoromycetes, Ascomycota), new species of lichenicolous fungi from the Holarctic. *The Lichenologist* **39**: 221–226.
doi: <https://doi.org/10.1017/S0024282907006317>
- Zoller, S., Scheidegger, C. & Sperisen, C. (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. *The Lichenologist* **31**: 511–516.

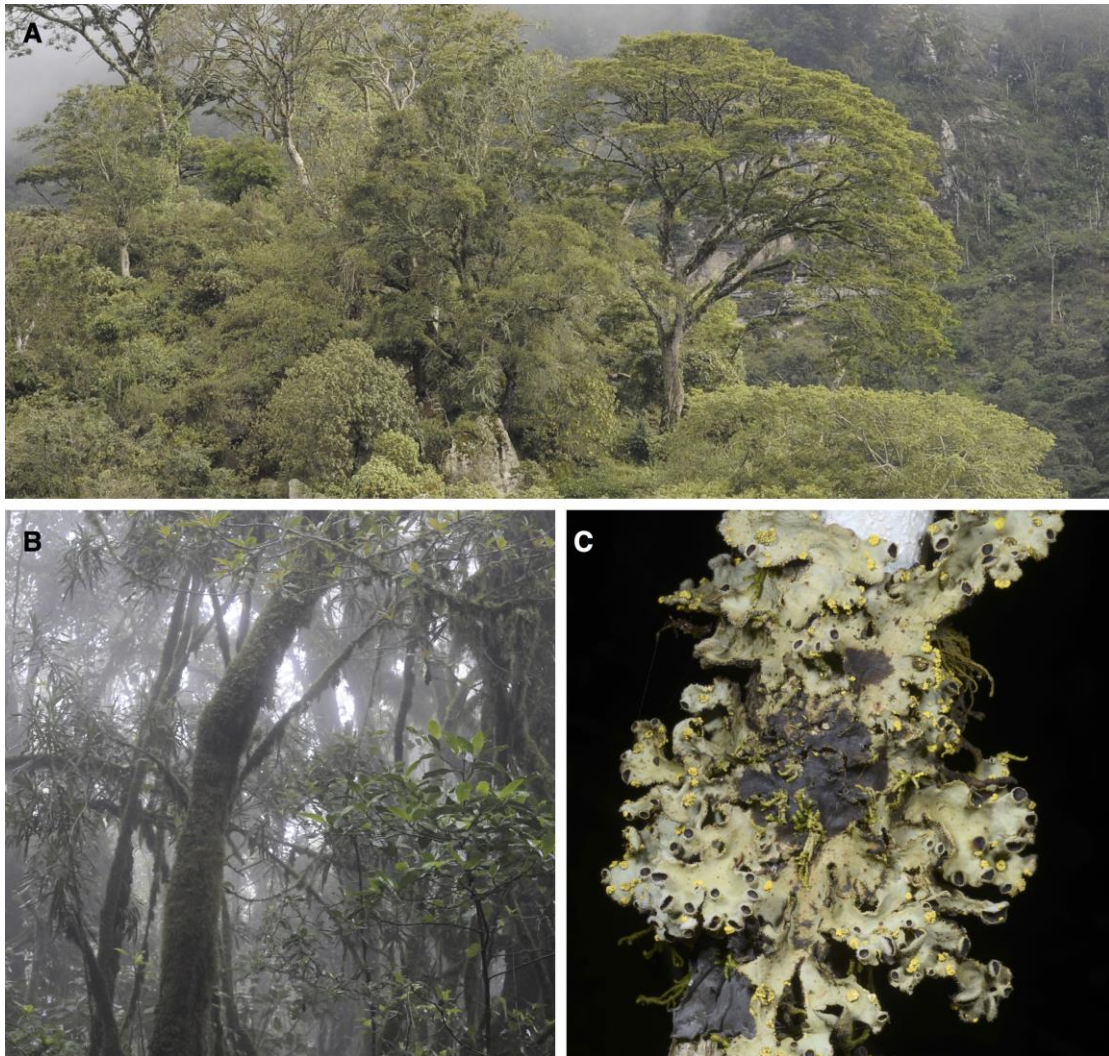


Figure 1. Habitat of *Taitaia aurea* in the Taita Hills, south-eastern Kenya. A, indigenous montane forest on the northfacing slope of Vuria, part of the Dawida massif; B, moss-rich forest at the type locality; C, *Crocodia* cf. *clathrata* with yellow outgrowths of *T. aurea* on the upper surface and thallus margins (holotype, Rikkinen 15258).

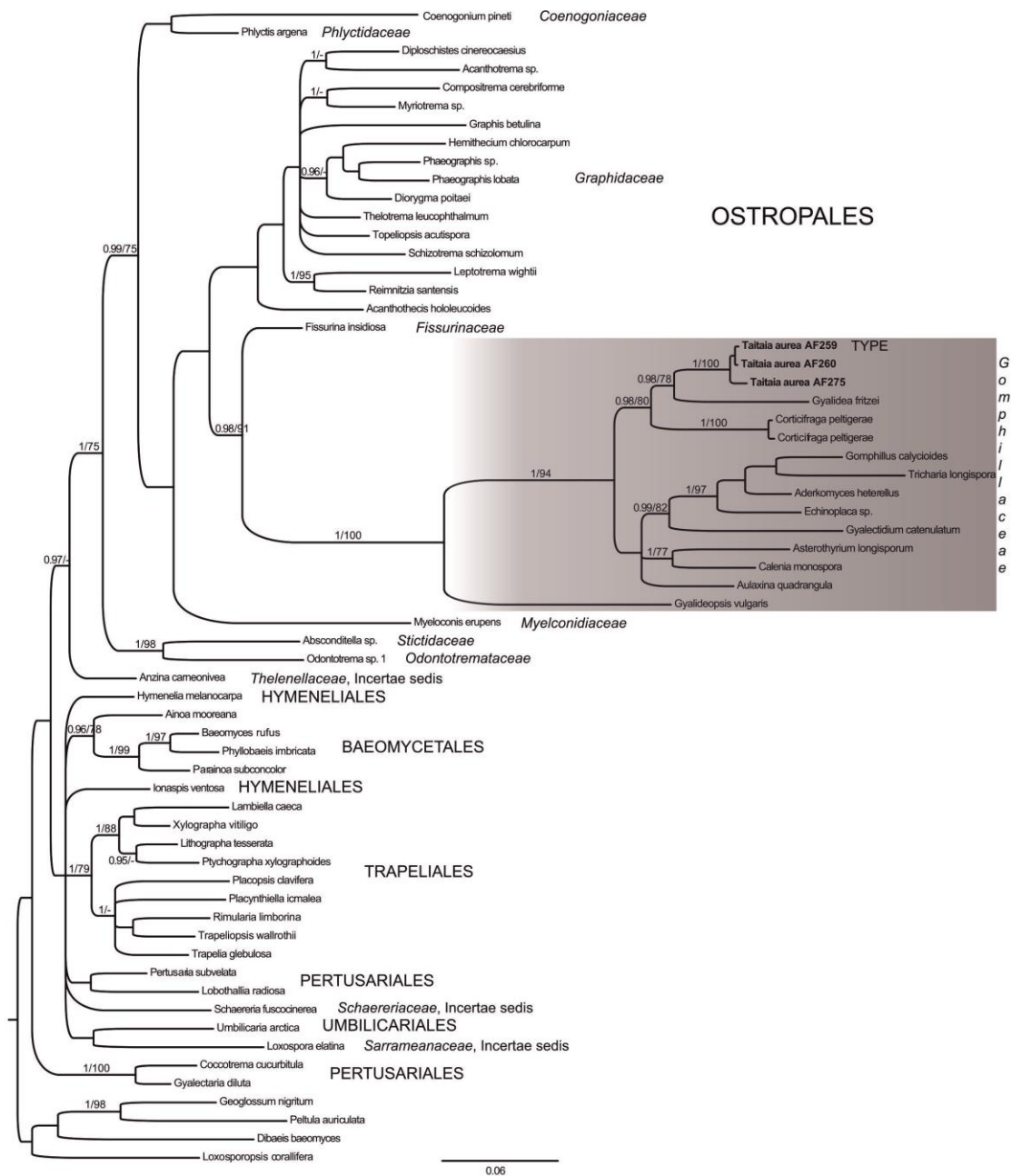


Figure 2. 50% majority-rule consensus tree using two loci (nuLSU + mtSSU) based on Bayesian approach showing the position of *Taitaia aurea* within Gomphillaceae, Ostropales (grey box). Numbers at nodes indicate phylogenetic relationships which are considered to be supported when posterior probabilities (PPs) are ≥ 0.95 (before slash) and bootstrap values (BS) ≥ 75 (after slash).

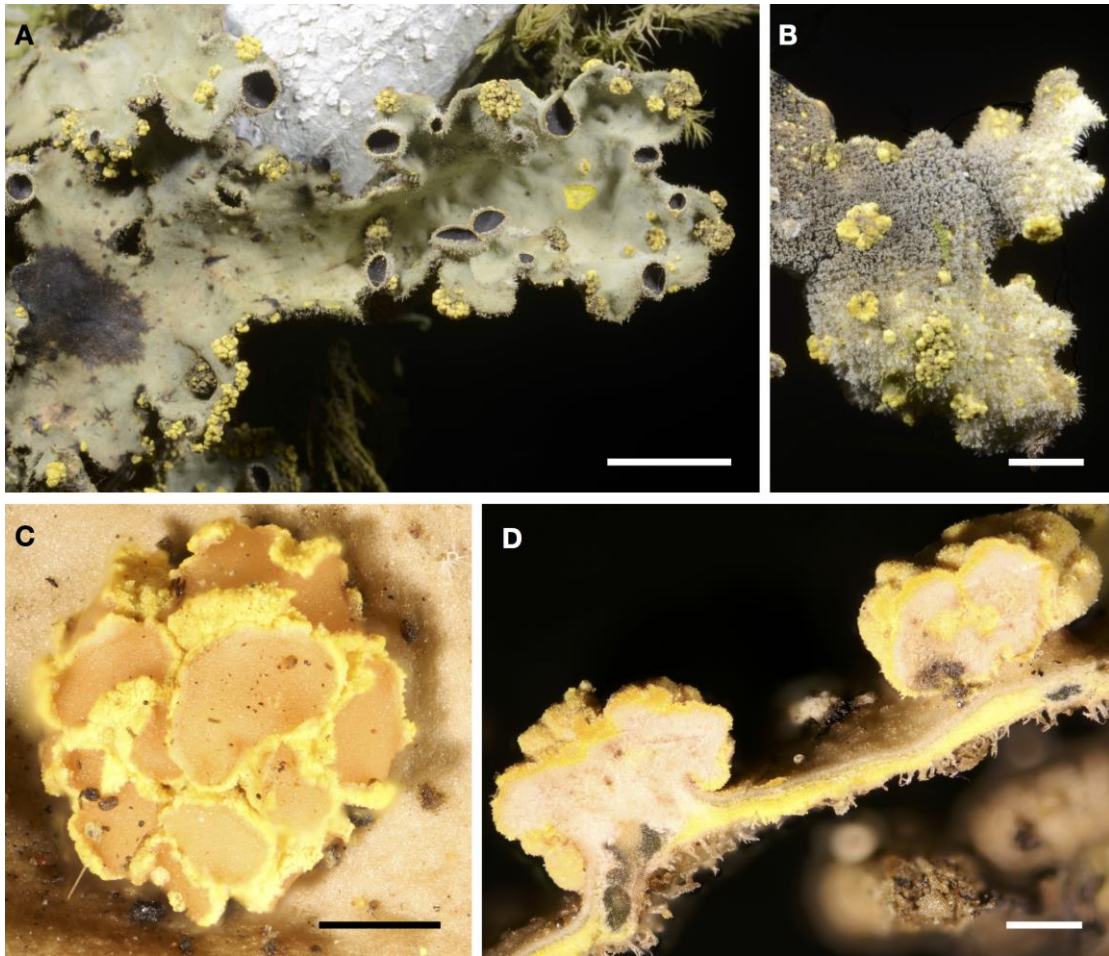


Figure 3. *Taitaia aurea*. A, ascomata developing on the upper surface and thallus margins of *Crocodia* cf. *clathrata*; B, ascomata developing through pseudocyphellae on the lower surface of the host; C, group of mature ascomata; D, cross-section through two ascomata and the host thallus. A–C, Rikkinen 15258 (holotype), D, Rikkinen 15260. Scales: A=5mm; B=2 mm; C & D=0.5mm.

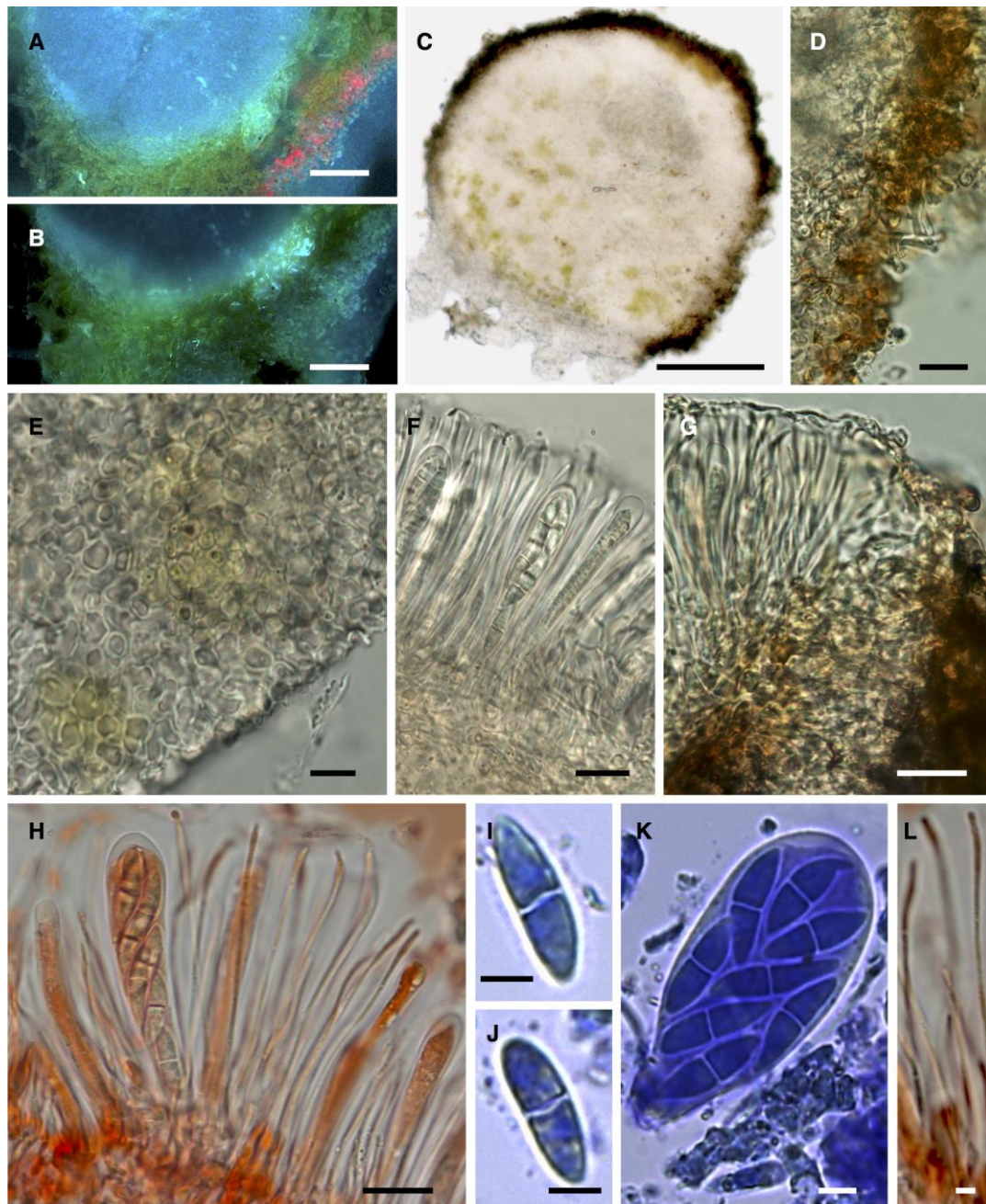


Figure 4. *Taitaia aurea* (Kirika 5103). A, lower part of ascoma immersed within the host thallus (note fluorescing pulvinic acid crystals using blue fluorescence filter (arrows)); B, same as A, but showing lichenicolous fungal hyphae (arrows) intruding between the hyphae of host fungus; C, cross-section through immature ascoma; D, section of exciple showing protruding hyphal tips; E, section of hyaline hypothecium of textura globulosa type; F, section of hymenium with asci and paraphyses; G, section of borderline between hymenium and exciple; H, section of hymenium with asci and unbranched paraphyses; I & J, ascospores; K, ascus; L, simple septate paraphyses. A–F, in water; G, H & L, in Congo red; I–K, in Cresyl blue. Scales: A, B, D, F–H=10 μm ; C=40 μm ; E, I–K=5 μm ; L= 2 μm .

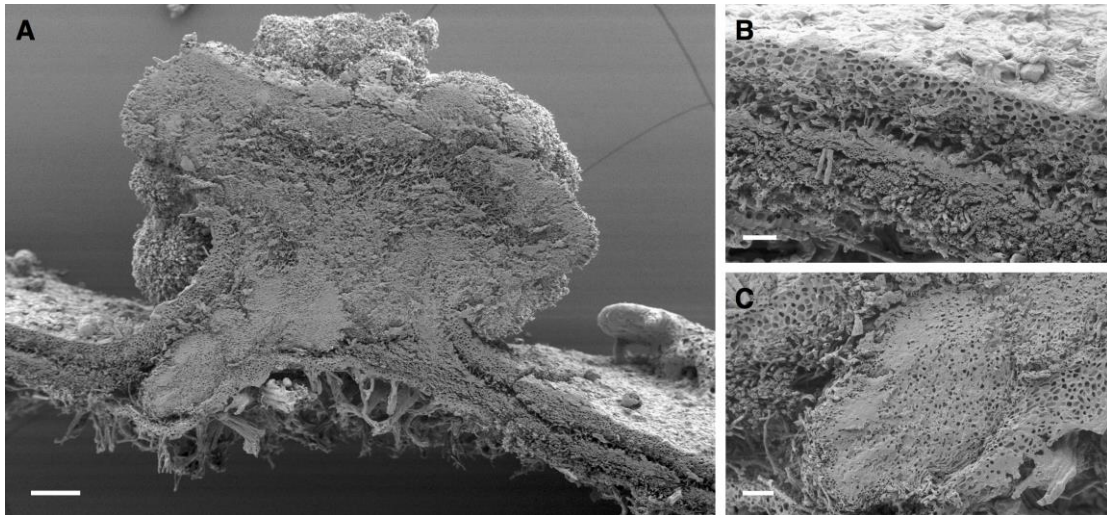


Figure 5. SEM of *Taitaia aurea* (Rikkinen 15260; see Fig. 3D). A, cross-section through young ascomata and host lichen. Note that the ascomata of *Taitaia* associate with internal cephalodia (*Nostoc*) of the host (arrow) and develop through its upper cortex; B, cross-section of the host showing upper cortex (c), green algal photobiont layer (p), medulla (m) and lower cortex; C, cross-section of internal cephalodium (ceph) showing superficial hyphae (arrow) which do not penetrate into the central parts of the cephalodium. Scales: A=100 μm ; B & C=20 μm .

TABLE 1. GenBank Accession numbers for sequences used in this study. Newly generated sequences are in bold. Numbers in brackets are laboratory codes. The taxonomy of families follows Index Fungorum (<http://www.indexfungorum.org/>).

Species	Taxonomy	GenBank Accession numbers		
		mtSSU	nuLSU	ITS
<i>Absconditella</i> sp.	<i>Stictidaceae, Ostropales</i>	KR017331	KR017188	-
<i>Acanthothecis hololeuroides</i>	<i>Graphidaceae, Ostropales</i>	JX420952	JX421423	-
<i>Acanthotrema</i> sp.	<i>Graphidaceae, Ostropales</i>	KF688506	KF688492	-
<i>Aderkomyces heterellus</i>	<i>Gomphillaceae, Ostropales</i>	KF833342	KF833330	-
<i>Ainoa mooreana</i>	<i>Baeomycetaceae, Baeomycetales</i>	KJ462394	KJ462339	-
<i>Anzina carneonivea</i>	<i>Thelenellaceae, Incertae sedis</i>	AY212851	AY212829	-
<i>Asterothyrium longisporum</i>	<i>Gomphillaceae, Ostropales</i>	AY341363	AY341349	-
<i>Aulaxina quadrangula</i>	<i>Gomphillaceae, Ostropales</i>	AY341364	AY341350	-
<i>Baeomyces rufus</i>	<i>Baeomycetaceae, Baeomycetales</i>	KJ462396	KJ462341	-
<i>Calenia monospora</i>	<i>Gomphillaceae, Ostropales</i>	KF833339	KF833327	-
<i>Coccotrema cucurbitula</i>	<i>Coccotremataceae, Pertusariales</i>	AF329161	AF274092	-
<i>Coenogonium pineti</i>	<i>Coenogoniaceae, Ostropales</i>	KR017337	KR017237	-
<i>Compositrema cerebriforme</i>	<i>Graphidaceae, Ostropales</i>	JX421017	JX421471	-
<i>Corticifraga peltigerae</i>	<i>Gomphillaceae, Ostropales</i>	-	KY462801	-
<i>Corticifraga peltigerae</i>	<i>Gomphillaceae, Ostropales</i>	KY661684	KY661661	-
<i>Dibaeis baeomyces</i>	<i>Icmadophilaceae, Pertusariales</i>	KJ462397	KJ462342	-
<i>Diorygma poitaei</i>	<i>Graphidaceae, Ostropales</i>	HQ639596	HQ639627	-
<i>Diploschistes cinereocaesius</i>	<i>Graphidaceae, Ostropales</i>	DQ912306	DQ883799	-
<i>Echinoplaca</i> sp.	<i>Gomphillaceae, Ostropales</i>	KF833340	KF833328	-
<i>Fissurina insidiosa</i>	<i>Fissurinaceae, Ostropales</i>	KR017325	KR017185	-
<i>Geoglossum nigratum</i>	<i>Geoglossomycetes</i>	AY544740	AY544650	-
<i>Gomphillus calycioides</i>	<i>Gomphillaceae, Ostropales</i>	KF833341	KF833329	-
<i>Graphis betulina</i>	<i>Graphidaceae, Ostropales</i>	KF875562	KF875541	-
<i>Gyalectaria diluta</i>	<i>Coccotremataceae, Pertusariales</i>	KR017332	KR017189	-
<i>Gyalectidium catenulatum</i>	<i>Gomphillaceae, Ostropales</i>	KF833335	KF833323	-
<i>Gyalidea fritzei</i>	<i>Gomphillaceae, Ostropales</i>	HM244744	HM244767	-
<i>Gyalideopsis vulgaris</i>	<i>Gomphillaceae, Ostropales</i>	AY584618	AY584649	-
<i>Hemithecium chlorocarpum</i>	<i>Graphidaceae, Ostropales</i>	HQ639595	HQ639651	-
<i>Hymenelia melanocarpa</i>	<i>Hymeneliaceae, Hymeneliales</i>	KJ462398	KJ462343	-
<i>Ionaspis ventosa</i>	<i>Hymeneliaceae, Hymeneliales</i>	KR017322	KR017181	-
<i>Lambiella caeca</i>	<i>Xylographaceae, Baeomycetales</i>	KR017338	KR017216	-
<i>Leptotrema wightii</i>	<i>Graphidaceae, Ostropales</i>	EU075574	EU075622	-
<i>Lithographa tesserata</i>	<i>Trapeliaceae, Trapeliales</i>	KR017327	KR017186	-
<i>Lobothallia radiosa</i>	<i>Megasporaceae, Pertusariales</i>	KJ766430	KJ766596	-
<i>Loxospora elatina</i>	<i>Sarrameanaceae, Incertae sedis</i>	KR017350	KR017192	-
<i>Loxosporopsis corallifera</i>	<i>Pertusariaceae, Pertusariales</i>	KR017381	KR017219	-
<i>Myeloconis erumpens</i>	<i>Myeloconidiaceae, Ostropales</i>	KJ449328	KJ449338	-
<i>Myriotrema</i> sp.	<i>Graphidaceae, Ostropales</i>	JX421091	JX421522	-
<i>Odontotrema</i> sp. 1	<i>Odontotremataceae, Ostropales</i>	HM244751	HM244771	-
<i>Parainoa subconcolor</i>	<i>Incertae sedis, Baeomycetales</i>	KR017412	KR017236	-

<i>Peltula auriculata</i>	<i>Lichinomycetes</i>	DQ922953	DQ832330	-
<i>Pertusaria subvelata</i>	<i>Pertusariaceae, Pertusariales</i>	KR017389	KR017227	-
<i>Phaeographis lobata</i>	<i>Graphidaceae, Ostropales</i>	DQ431984	DQ431944	-
<i>Phaeographis</i> sp.	<i>Graphidaceae, Ostropales</i>	DQ431959	DQ431959	-
<i>Phlyctis argena</i>	<i>Phlyctidaceae, Ostropales</i>	DQ986880	DQ986771	-
<i>Phyllobaeis imbricata</i>	<i>Baeomycetaceae, Baeomycetales</i>	DQ986895	DQ986781	-
<i>Placopsis clavifera</i>	<i>Trapeliaceae, Trapeliales</i>	KU844527	KU844591	-
<i>Placynthiella icmalea</i>	<i>Trapeliaceae, Trapeliales</i>	AY212870	EU940160	-
<i>Ptychographa xylographoides</i>	<i>Trapeliaceae, Trapeliales</i>	KJ462403	KJ462348	-
<i>Reimnitzia santensis</i>	<i>Graphidaceae, Ostropales</i>	HQ639622	HQ639664	-
<i>Rimularia limborina</i>	<i>Trapeliaceae, Trapeliales</i>	KR017368	KR017215	-
<i>Schaereria fuscocinerea</i>	<i>Schaereriaceae, Incertae sedis</i>	KR017384	KR017225	-
<i>Schizotrema schizolomum</i>	<i>Graphidaceae, Ostropales</i>	JX421328	FJ708492	-
<i>Taitaia aurea</i> (AF259)	<i>Gomphillaceae, Ostropales</i>	-	MF372796	MF372800
<i>Taitaia aurea</i> (AF260)	<i>Gomphillaceae, Ostropales</i>	MF372798	MF509277	MF372801
<i>Taitaia aurea</i> (AF275)	<i>Gomphillaceae, Ostropales</i>	MF372799	MF372797	-
<i>Thelotrema leucophthalmum</i>	<i>Graphidaceae, Ostropales</i>	JX421374	JX421658	-
<i>Topeliopsis acutispora</i>	<i>Graphidaceae, Ostropales</i>	KF875557	JX421675	-
<i>Trapelia glebulosa</i>	<i>Trapeliaceae, Trapeliales</i>	KR017354	KR017159	-
<i>Trapeliopsis wallrothii</i>	<i>Trapeliaceae, Trapeliales</i>	KR017410	KR017235	-
<i>Tricharia longispora</i>	<i>Gomphillaceae, Ostropales</i>	AY341374	AY341360	-
<i>Umbilicaria arctica</i>	<i>Umbilicariaceae, Umbilicariales</i>	DQ986872	DQ986772	-
<i>Xylographa vitiligo</i>	<i>Xylographaceae, Baeomycetales</i>	KJ462458	KJ462391	-