


Standard Paper

Two new species of *Lichenotubeufia* (Dothideomycetes, Tubeufiales) from Chile and New Zealand, with a revised key to the genus

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Abstract

Two species of lichenicolous fungi are described as new to science: *Lichenotubeufia etayoi* Zhurb. (on *Trachyderma*), with light orange perithecia up to 360 µm diam., non-fasciculate excipular hairs, and 10–13-septate ascospores, 110–162 × 3–5 µm; and *L. tibellii* Zhurb. (on *Coccocarpia*), with light orange perithecia up to 275 µm diam., non-fasciculate excipular hairs, and 5–12-septate ascospores, 50–100 × 3–4.5 µm. An updated key to the species of *Lichenotubeufia* is provided.

Key words: Australasian biogeographical realm, lichen parasites, taxonomy, Valdivian temperate rainforests

(Accepted 25 November 2021)

Introduction

In 2017, I was invited to the Museum of Evolution of Uppsala University to sort through a collection of lichenicolous fungi left by the late Rolf Santesson. This collection contained, among others, specimens on *Coccocarpia* and *Trachyderma* hosts annotated as ‘*Baryonectria coccocarpiae* R. Sant. ined.’. Subsequent examination of these specimens revealed that they belong to the lichenicolous genus *Lichenotubeufia* Etayo (Etayo 2017), and are hitherto unknown to science. The aims of the present paper are: 1) to describe two new species of *Lichenotubeufia*; 2) to present a comparative table of diagnostic characters of species of this genus and an updated key for their identification.

Materials and Methods

Microscopy was carried out, and images were captured, using a Zeiss Axio Zoom.V16 microscope and a Zeiss Axio Imager.A1 microscope equipped with Nomarski differential interference contrast optics and fitted with a Zeiss AxioCam MRc5 digital camera. Microscopic characters were studied using sections hand-cut with a razor blade and mounted in water, 10% potassium hydroxide (K), Lugol’s iodine directly (I) or after a K pretreatment (K/I), phloxine directly or after K pretreatment. Measurements were taken from water mounts unless otherwise indicated. The length, width and length/width ratio (l/w) of the ascospores, as well as the size of the perithecia, excipular hairs and asci, are given as (min)-(x̄ - SD)-(x̄ + SD)(-max), where ‘min’ and ‘max’

are the extreme values observed, x̄ the arithmetic mean and SD the corresponding standard deviation. Colours were named according to Kornerup & Wanscher (1978). Voucher specimens are held at UPS.

Results

The new species

Lichenotubeufia etayoi Zhurb. sp. nov.

MycoBank No.: MB 841975

Differs from *Lichenotubeufia cryptica* mainly in the larger ascospores (150–360 µm vs 130–200 µm diam.), sometimes with a distinct papilla, a wider exciple (30–50 µm vs 15–20 µm wide) and slightly wider ascospores (3–5 µm vs 2–3.5 µm wide).

Type: Chile, Valdivia Province, Lake Rinihue, Enco, edge of the rainforest on the lake shore, on the thallus of *Trachyderma gayanum* growing on *Ugni molinae* and other shrubs, 12 October 1940, R. Santesson S422 (UPS F-857602—holotype).

(Fig. 1)

Lichenicolous ascomycete growing on *Trachyderma*. Ascospores light orange, subglobose ovoid or narrowly ampulliform, (150–)180–300(–360) µm diam. ($n = 34$), with a central ostiole 20–45 µm diam., sometimes with a distinct papilla 100–150 µm diam., not collapsing when dry, entirely covered with subhyaline excipular hairs; superficial, scattered or in groups, occasionally adjacent, mainly growing on the upper surface of the host lobes, often along their edge, where they also rarely occur on the lower surface, including rhizines. Excipular hairs colourless,

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Cite this article: Zhurbenko MP (2022) Two new species of *Lichenotubeufia* (Dothideomycetes, Tubeufiales) from Chile and New Zealand, with a revised key to the genus. *Lichenologist* 54, 117–122. <https://doi.org/10.1017/S0024282922000081>

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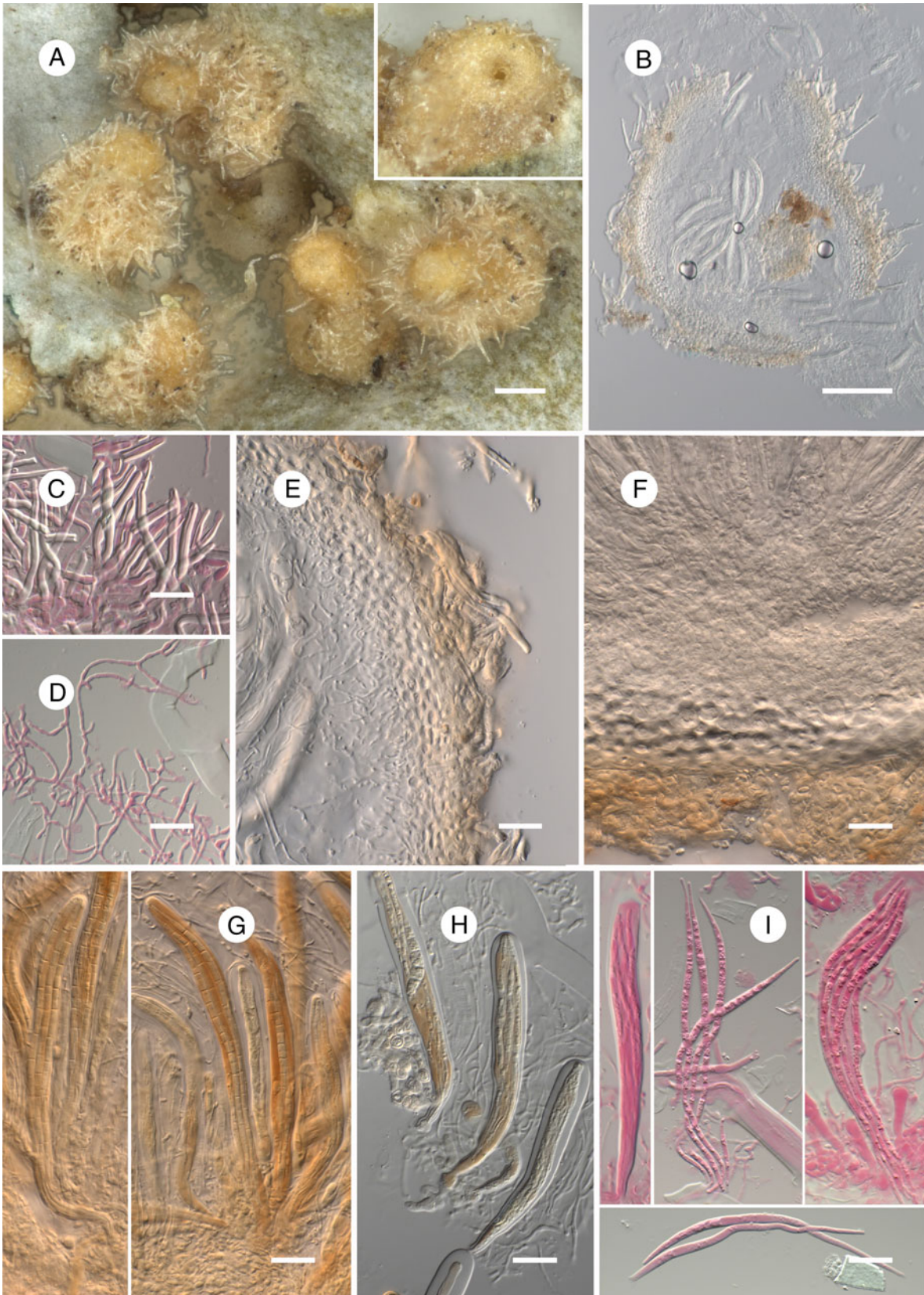


Fig. 1. *Lichenotubeufia etayoi* (holotype). A, habitus of ascomata on the thallus of *Trachyderma gayanum*. B, ascoma in cross-section. C, excipular hairs. D, paraphysoids. E, lateral exciple in cross-section. F, basal exciple and hypothecium-like layer in cross-section. G, asci with spores. H, asci with spores. I, ascus and ascospores. B, E & F, in water; C, D & I in phloxine after K; G in I; H in K/I. Scales: A & B = 100 μ m; C–I = 20 μ m. In colour online.

with a thin translucent lumen, sometimes undeveloped in places, and a thick opaque wall, straight, $(18-)\text{40-84}(-113) \times (3-)\text{4-5}(-5.5) \mu\text{m}$ ($n = 45$), isodiametric or somewhat gradually tapering above, with a rounded, not enlarged apex, rarely branched below, occasionally with septa or possibly with septum-like connectors, not gathered in fascicles. *Exciple* continuous, same structure throughout, in surface view orange-white/very pale orange (slightly decolourizing in K), pseudoparenchymatous, composed of cells 5–14 μm diam., with walls 1.5–6 μm wide; in cross-section hyaline (inside) to orange-white (outside), 30–50 μm wide, composed of isodiametric or tangentially elongated cells 2–15 μm long, with walls 1–7.5 μm wide. *Hymenium* not interspersed, I–, K/I–. *Hamathecium* composed of abundant, persistent, branched, anastomosed paraphysoids, 1–3.5 μm wide, attached to the subhymenium and lateral exciple (along its entire length) and filling the space between the asci and the entire upper part of the perithecium. *Subhymenium* indistinct. *Hypothecium-like layer* between subhymenium and basal exciple hyaline, 35–85 μm tall, of an unclear structure partly resembling *textura globulosa*, composed of cells 1.5–8 μm long. *Asci* 8-spored, subcylindrical, gradually tapering below, short pedicellate, rounded at the apex, without any distinct apical apparatus, wall not thickened at the apex (in water), $(120-)\text{133-169}(-181) \times (10-)\text{11-14}(-16) \mu\text{m}$ ($n = 35$; in water, I or phloxine), I–, K/I– except for the orange-coloured plasma. *Ascospores* hyaline, acicular, gradually tapering at both ends, straight, $(110-)\text{128-157}(-162) \times (3-)\text{3.5-4.5}(-5) \mu\text{m}$, l/w = $(28-)\text{32-42}(-45)$ ($n = 15$, in water or I), 10–13-septate, not constricted at the septa, smooth-walled, without perispore, with many small guttules as seen in K, arranged in the ascus in one bundle.

Conidiomata not observed.

Etymology. The species is dedicated to the eminent Spanish lichenologist Javier Etayo who has made an enormous contribution to the study of the lichenicolous fungi of South America.

Distribution and host. The new species is known from the type locality in the Valdivian temperate rainforests of Chile, growing on the thallus of *Trachyderma gayanum*, not visibly damaging the host.

Notes. Morphologically *Lichenotubeufia etayoi* strongly resembles *L. cryptica* Etayo & Flakus growing on *Lobariella*; however, the latter differs in having smaller perithecia, 130–200 μm diam., a thinner exciple, 15–20 μm wide, and slightly narrower ascospores, 2–3.5 μm wide (Flakus *et al.* 2019). Additionally, *L. cryptica* is characterized by wider excipular hairs, 3–8 μm wide, a K+ slightly yellowish green exciple, the absence of a distinct hypothecium, and narrower paraphysoids, 0.5–1 μm wide (Flakus *et al.* op. cit.). In terms of ascospore size, it is also similar to *Lichenotubeufia eriodermatis* (Etayo) Etayo and *L. heterodermiae* (Etayo) Etayo. However, the former is well distinguished by its white perithecia, fasciculate excipular hairs and a soon disappearing hamathecium (Etayo 2002a), while the latter has white and smaller perithecia, 100–200 μm diam., and ascospores with more (16–22) septa (Etayo 2002b).

Lichenotubeufia tibellii Zhurb. sp. nov.

Mycobank No.: MB 841976

Differs from *Lichenotubeufia boomiana* mainly in having shorter ascospores, 50–100 μm vs 95–112 μm long, with 5–12 vs 10–15 septa.

Type: New Zealand, North Island, Taranaki, Mount Egmont National Park, at North Egmont, along Veronica Walk, 39°16'S, 174°05'E, 990 m, on the thallus of *Coccocarpia palmicola* growing on a decorticated stump, 26 November 1983, L. Tibell 15036 (UPS F-857606—holotype).

(Fig. 2)

Lichenicolous ascomycete growing on *Coccocarpia*. *Ascomata* perithecia, light orange, subglobose to broadly ovoid, $(95-)\text{160-255}(-275) \mu\text{m}$ diam. ($n = 18$), with a central ostiole 25–55 μm diam., without a papilla, not collapsing when dry, entirely covered with subhyaline excipular hairs; superficial, scattered or in groups, almost always growing on the upper surface of the host lobes, often along their edges, where they also rarely occur on the lower surface. *Excipular hairs* colourless, with a thin translucent lumen, sometimes underdeveloped in places, and a thick opaque wall, straight, $(40-)\text{55-95}(-120) \times 3-5.5 \mu\text{m}$ ($n = 19$), isodiametric or somewhat gradually tapering above, with a rounded, not enlarged apex, not or very rarely branched at the base, aseptate, not gathered in fascicles. *Exciple* continuous, same structure throughout, in surface view orange-white/very pale orange (slightly decolourizing in K), pseudoparenchymatous, composed of cells 2.5–11.5 μm diam., with walls 1–6.5 μm wide; in cross-section hyaline (inside) to orange-white (outside), 20–40 μm wide; inner cells strongly tangentially elongated, thin-walled, and outer cells isodiametric or slightly tangentially elongated, 2–10.5 μm long, with walls 1.5–6 μm wide. *Hymenium* not interspersed, I–, K/I–. *Hamathecium* composed of abundant, persistent, branched, anastomosed paraphysoids, 0.5–2.5 μm wide, attached to the subhymenium and lateral exciple (along its entire length) and filling the space between the asci and the entire upper part of the perithecium. *Subhymenium* indistinct. *Hypothecium-like layer* between subhymenium and basal exciple hyaline, 10–40 μm tall, of an unclear structure partly resembling *textura globulosa*, composed of cells 1–6 μm long. *Asci* 8-spored, subcylindrical, gradually tapering below, short pedicellate, rounded at the apex, without any distinct apical apparatus, wall not or slightly thickened at the apex (in water), $(72-)\text{86-114}(-120) \times 12-16(-19) \mu\text{m}$ ($n = 19$; in water, I or phloxine), I–, K/I– except for the orange-coloured plasma. *Ascospores* hyaline, acicular to long subfusiform, gradually tapering at both ends, straight, $(50-)\text{60-85}(-100) \times (3-)\text{3.5-4}(-4.5) \mu\text{m}$, l/w = $(11-)\text{16-24}(-30)$ ($n = 22$; in water, I or phloxine), 5–12-septate, not or only slightly constricted at the septa, smooth-walled, without perispore, with many small guttules as seen in K, arranged in the ascus in one bundle.

Conidiomata not observed.

Etymology. The species is dedicated to the eminent Swedish lichenologist Leif Tibell who collected the holotype.

Distribution and host. The new species is known from the type locality in New Zealand, growing on the thallus of *Coccocarpia palmicola*, not visibly damaging the host.

Notes. In terms of the length of the ascospores, the new species differs greatly from all other known species of *Lichenotubeufia* Etayo (Table 1). Morphologically it possibly most resembles *L. boomiana* Etayo, although this differs in having shorter excipular hairs, 45–80 μm long, an occasionally evanescent hamathecium, and on average longer ascospores, 95–112 μm long, with more (10–15) septa (Etayo 2017). Remarkably, this is

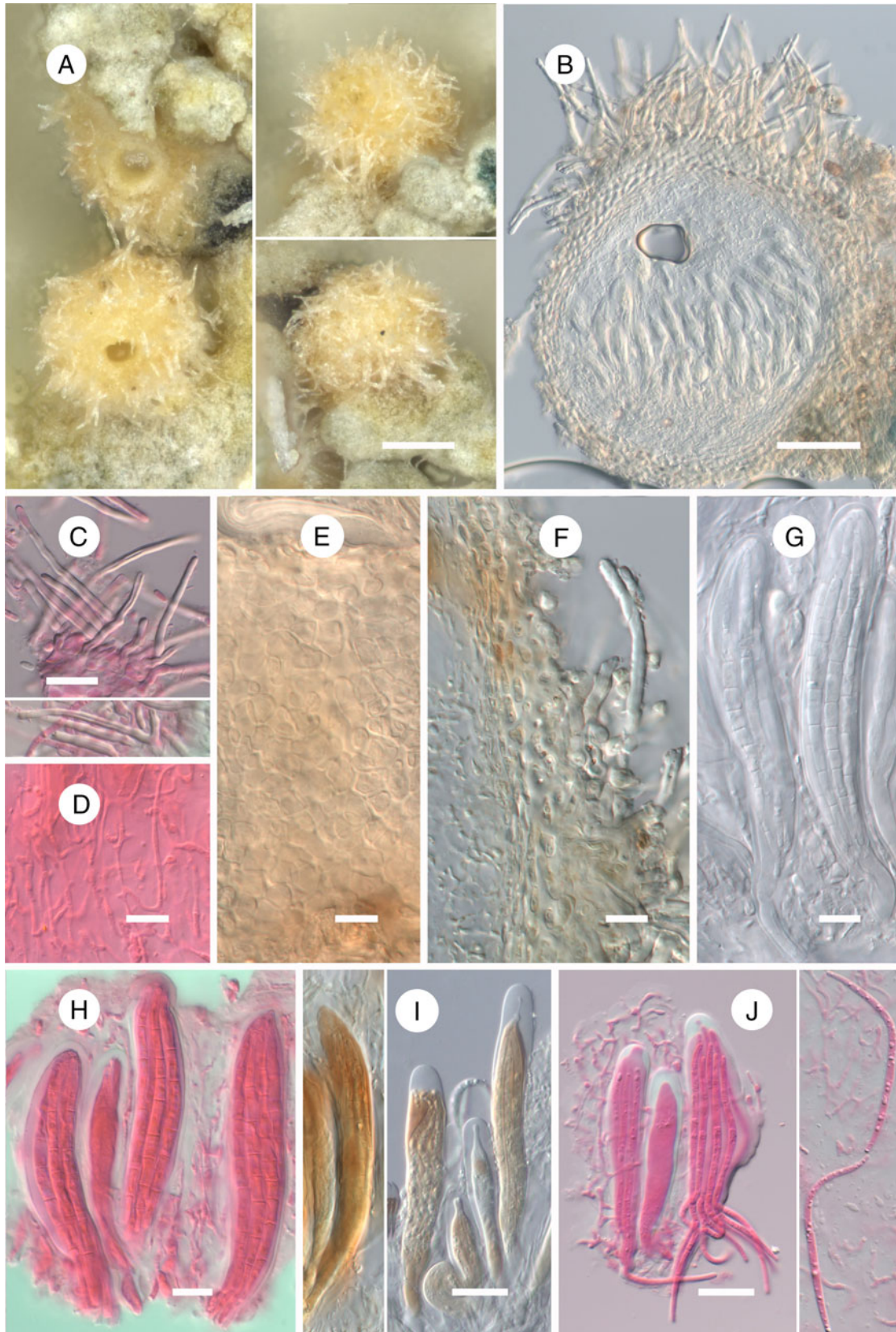


Fig. 2. *Lichenotubeufia tibellii* (holotype). A, habitus of ascomata on the thallus of *Coccocarpia palmicola*. B, ascoma in cross-section. C, excipular hairs; note the branched hair below. D, paraphysoids. E, exciple in surface view. F, exciple in cross-section. G, asci with spores. H, asci with spores. I, asci in I (on the left) and in K/I (on the right). J, asci, ascospores and paraphysoids. B, E & G in water; C & J in phloxine after K; D & H in phloxine; F in I. Scales: A = 100 μ m; B = 50 μ m; C, I & J = 20 μ m; D–H = 10 μ m. In colour online.

Table 1. Distinguishing characters of *Lichenotubeufia* species.

<i>Lichenotubeufia</i> species	Perithecia colour / diameter (µm) / presence of papilla	Excipular hairs length (µm) / occurrence in bundles	Exciple thickness (µm)	Hamathecium	Ascus length (µm)	Ascospore size (µm) / shape / number of septa	Host / known distribution
⁴ <i>boomiana</i>	whitish to cream / 100–200 / no	45–80 / no	30–35	occasionally evanescent	100–115	95–112 × 3–4 / acicular / 10–15	<i>Sticta</i> / Ecuador, Guatemala
⁵ <i>cryptica</i>	beige to cream / 130–200 / no	35–80 / no	15–20	persistent	105–165	100–150 × 2–3.5 / acicular / multiseptate (number of septa not specified)	<i>Lobariella</i> / Bolivia
² <i>eriodermatis</i>	white / 200–300 / no	100–150 / yes	c. 30	evanescent	c. 180	105–145 × 4–5 / acicular / 17–19	<i>Erioderma</i> / Colombia
⁶ <i>etayoi</i>	light orange / 150–360 / yes	18–113 / no	30–50	persistent	120–181	110–162 × 3–5 / acicular / 10–13	<i>Trachyderma</i> / Chile
^{1, 3, 4} <i>heterodermiae</i>	white / 100–200 / no	50–100 / no	not specified	persistent	135–160	130–150 × 3–4 / acicular / 16–22	<i>Heterodermia</i> , <i>Physcia</i> and <i>Xanthoria</i> / Central and Southern Europe, Ecuador
² <i>pannariae</i>	light yellow to cream / 120–200 / no	10–55 / no	c. 20	persistent	75–85	40–50 × 3.5–4.5 / very narrowly clavate / 5–8	<i>Pannaria</i> / Colombia
⁴ <i>tafallae</i>	white / 100–150 / no	200–250 / yes	not specified	persistent	65–85	16–20 × 4–4.5 / fusiform / 3	<i>Leptogium</i> / Ecuador
⁶ <i>tibellii</i>	light orange / 95–275 / no	40–120 / no	20–40	persistent	72–120	50–100 × 3–4.5 / acicular to long subfusiform / 5–12	<i>Coccocarpia</i> / New Zealand

¹Brackel (2014); ²Etayo (2002a); ³Etayo (2002b); ⁴Etayo (2017); ⁵Flakus *et al.* (2019); ⁶present paper

the first species of *Lichenotubeufia* reported from the Australasian biogeographical realm.

Discussion

The genus *Tubeufia* Penz. & Sacc. (*Dothideomycetes*, *Tubeufiales*, *Tubeufiaceae*) was introduced to accommodate saprobic pyrenomycetes growing on decorticated or decaying woody substrata, characterized by fleshy, tender, solitary, superficial, mostly white, basally dark, glabrous, vertically oblong, non-papillate perithecia that are indefinitely split at the apex; clavate, 4–8-spored asci, extending from the base of the perithecium and usually surrounded by paraphysoids; and hyaline, cylindrical-bacilliform, pluriseptate ascospores, usually extending the full length of the ascus (Penzig & Saccardo 1898). *Tubeufia* was typified on *T. javanica* Penz. & Sacc., a detailed modern description of which is presented in Boonmee *et al.* (2014).

Etayo (2002a, b) described three lichenicolous species of *Tubeufia* that he later transferred to the new genus *Lichenotubeufia* typified on *L. eriodermatis* (Etayo 2017). According to the diagnosis, *Lichenotubeufia* differs from

Tubeufia in its lichenicolous lifestyle and perithecia covered by hyaline to subhyaline thick-walled hairs (Etayo 2017). Comparing the types of these genera, *Tubeufia* is additionally distinct in its oblong, subclavate to obclavate ascomata that collapse when dry and are seated on a subiculum, an exciple that is much darker at the base, asci with a distinct ocular chamber, and hyaline to pale yellow or brown ascospores (Boonmee *et al.* 2014).

So far, six species of *Lichenotubeufia* have been described (Table 1). The two new species presented here match the generic concept well, although the type species differs from them in having fasciculate excipular hairs and an evanescent hamathecium (Etayo 2017). It is also noteworthy that papillate perithecia, as occur in *Lichenotubeufia etayoi*, have previously not been reported in this genus.


Six of the eight species of *Lichenotubeufia* are so far known from single or few finds in South and Central America, one species is known from a single find in New Zealand, and one species (*L. heterodermiae*) is known from many records in Europe and also from South America (Table 1). The latter has also been observed on three host genera (two of which belong to the same family), while the other species are confined to a single host genus (Table 1).

Key to the species of *Lichenotubeufia*

This key is based on data from Etayo (2002a, b, 2017), Flakus *et al.* (2019) and the present paper.

- 1 Ascospores up to 100 µm long 2
 At least some ascospores more than 100 µm long 4
- 2(1) Ascospores 50–100 µm long, acicular to long subfusiform, 5–12-septate; on *Coccocarpia* ... **L. tibellii**
 Ascospores up to 50 µm long, not acicular, 3–8-septate 3
- 3(2) Ascospores 16–20 µm long, fusiform, 3-septate; on *Leptogium* **L. tafallae**
 Ascospores 40–50 µm long, very narrowly clavate, 5–8-septate; on *Pannaria* **L. pannariae**
- 4(1) Perithecia light orange, up to 360 µm diam., some with a distinct papilla; on *Trachyderma* **L. etayoi**
 Perithecia white, beige or cream, smaller, without papilla 5
- 5(4) Perithecia white, up to 300 µm diam.; excipular hairs 100–150 µm long, fasciculate; hamathecium evanescent; on *Erioderma* ..
 **L. eriodermatis**
 Perithecia white, beige or cream, up to 200 µm diam.; excipular hairs up to 100 µm long, not fasciculate; hamathecium usually
 persistent 6
- 6(5) Ascospores 95–112 µm long; on *Sticta* **L. boomiana**
 Ascospores longer 7
- 7(6) Perithecia beige to cream, growing on the underside of the host thallus; ascospores 100–150 × 2–3.5 µm; on *Lobariella*
 **L. cryptica**
 Perithecia white, growing on the upper side of the host thallus; ascospores 130–150 × 3–4 µm; on *Heteroderma*, *Physcia* and
Xanthoria **L. heterodermiae**

Acknowledgements. This study was carried out within the framework of the research project of the V. L. Komarov Botanical Institute of the Russian Academy of Sciences ‘Biodiversity, ecology, structural and functional features of fungi and fungus-like protists’ (AAAA-A19-119020890079-6) using the equipment of its Core Facility Center ‘Cell and Molecular Technologies in Plant Science’. The author’s visit to the UPS herbarium was financially supported by the Rolf Santesson foundation. Roland Moberg, Stefan Ekman and Martin Westberg are thanked for various kinds of help and support during his stay in Uppsala. Javier Etayo, Paul Diederich and an anonymous reviewer provided valuable comments on the manuscript.

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