

A new species of *Micarea* (*Pilocarpaceae*) from Madeira growing on *Usnea*

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Abstract: The new species *Micarea usneae* is described from Madeira. It has been found at two localities where it grew on epiphytic *Usnea* thalli. *Micarea usneae* is distinguished by a very thin pale brownish thallus, pallid to greyish black or bluish subglobose apothecia of 0.1–0.3 mm diameter, very narrow (1.5–2.5 µm) ascospores, black pycnidia and strongly curved to sigmoid macroconidia. Phylogenetic analyses using mtSSU sequences place the new species in the *Micarea peliocarpa* group. *Catillaria usneicola* and *Cladonia parasitica* are recorded for the first time from Madeira. A key to the lichenicolous species on *Usnea* in Macaronesia is provided.

Key words: lichenized ascomycete, *Lecanorales*, Macaronesia, new records, phylogeny

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Introduction

As a result of studies on lichens and lichenicolous fungi on the island of Madeira by the first author, several lichenicolous fungi on *Usnea* were collected, including the common and widespread *Biatoropsis usnearum* Räsänen and *Lichenostigma maureri* Hafellner. In two localities on the western part of the island, an unknown lichenicolous lichen was also found on a few *Usnea* thalli. It belongs to the genus *Micarea* and is most closely related to *M. peliocarpa* (Anzi) Coppins & R. Sant. The aim of the present paper is to describe this species and provide a review of lichenicolous species on *Usnea* in Macaronesia. Two further species growing near the type are recorded for the first time from Madeira: *Catillaria usneicola* Etayo, growing on *Usnea articulata* (L.) Hoffm., and *Cladonia parasitica* (Hoffm.) Hoffm., growing epiphytically.

Material and Methods

Hand-cut sections were investigated microscopically in water and 5% KOH. Microscopical measurements all

refer to material examined in water. Chemical reactions were tested using 10–15% KOH (K) and Lugol's reagent without (I) or with (K/I) pretreatment with K. Microcrystallization, used to identify gyrophoric acid, follows Orange *et al.* (2001: 42). This method was required because of the very thin thallus and very small apothecia.

The method of direct PCR as explained in Lawrey *et al.* (2007: 780) was performed on thin apothecial slices of the new *Micarea* (*van den Boom* 48057), using the primers mrSSU1 and mrSSU3R (Zoller *et al.* 1999) for the amplification and sequencing of mtSSU. Amplicons were sequenced by Macrogen®. Sequence fragments were assembled with Sequencher version 4.9 (Gene Codes Corporation, Ann Arbor, Michigan) to obtain a consensus sequence of 714 nucleotides.

We included our sequence (GenBank accession number KF569511) in the alignment of Andersen & Ekman (2005) retrieved from the TreeBASE website (<http://treebase.org/treebase-web/home.html>) [S1258; the name '*Micarea prasina*' was changed to '*Micarea micrococca*' to agree with the published trees of Andersen & Ekman (2005)] to determine which group of *Micarea* species our new species belongs to. The sequence was aligned manually using MacClade 4.05 (Maddison & Maddison 2002). Ambiguous regions representing a total of 62 bp were delimited on the alignment and excluded from the analyses. The final data set consisted of 82 taxa and 827 unambiguously aligned sites.

A Maximum Likelihood (ML) analysis was performed on the mtSSU data set using GARLI (Zwickl 2006, v.0.951 for OSX) with default settings. *Physcia adscendens* was chosen as the outgroup. A single most likely tree was produced (–lnL = 13919.25867) (Fig. 1). The phylogenetic tree was visualized using FigTree v1.3.1 (Rambaut 2012). Five hundred bootstrap pseudoreplicates were used to calculate a majority-rule consensus tree in

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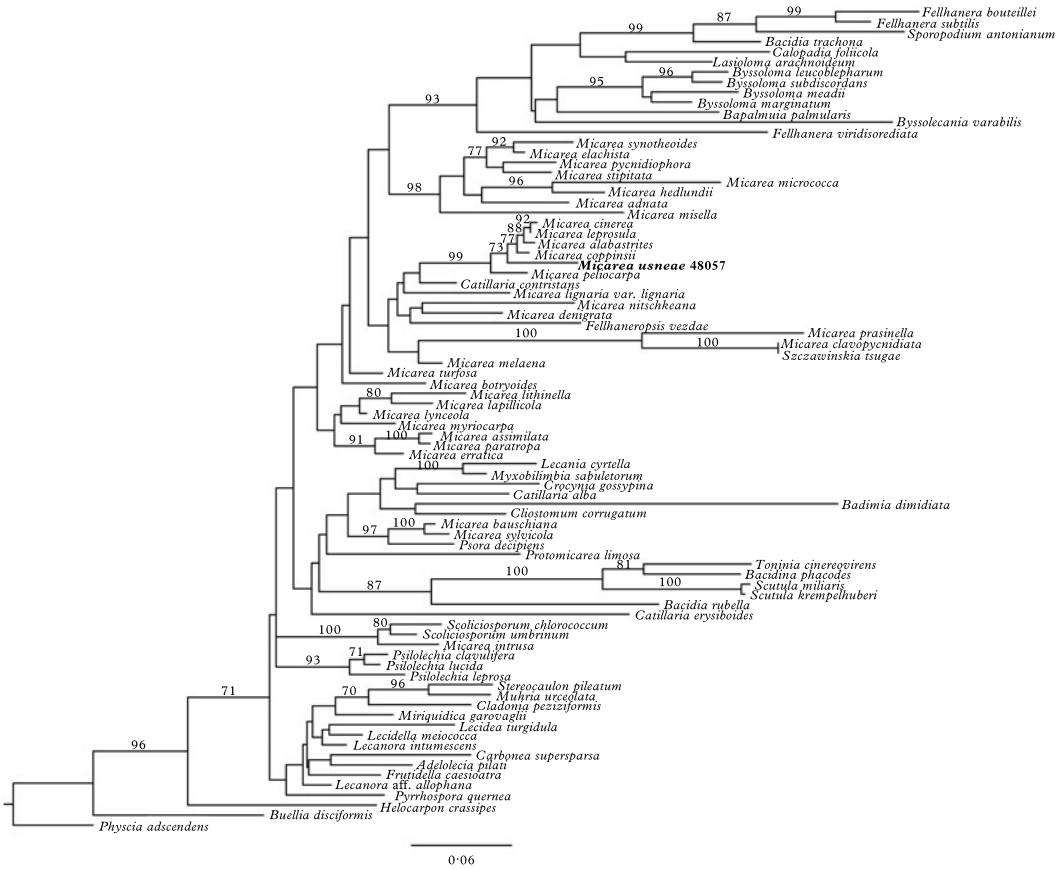


FIG. 1. Phylogenetic relationships among 82 taxa based on mtSSU sequences that resulted from a Maximum Likelihood analysis. Maximum Likelihood bootstrap values ≥ 70 are shown above internal branches. The new species, *Micarea usneae*, is in bold followed by the collecting number.

PAUP* 4.0b10 (Swofford 2002) to assess the Maximum Likelihood bootstrap values (ML-bs). ML-bs $\geq 70\%$ were considered to be significant and added to the tree (Fig. 1).

Comparative material examined. *Micarea denigrata* (lichenicolous): **The Netherlands: Noord-Brabant:** Stiphout, churchyard, vertical on a gravestone, on *Physcia dubia*, 2012, P. & B. van den Boom 48161 (hb. van den Boom).

***Micarea coppinsii*:** **Madeira:** N of Funchal, E of Poiso, along road (ER 202) to Santo da Serra, (halfway), near João do Prado, edge of *Pinus* forest, open area with low (basalt) outcrops, 1240 m, 2012, P. & B. van den Boom 47615 (hb. van den Boom).

***Micarea alabastrites*:** **Madeira:** NE of Funchal, W of Portela, trail to the west, along levada, in laurisilva area with mature *Acer* and *Cryptomeria* trees along the trail, 665 m, 2012, P. & B. van den Boom 47682 (hb. van den Boom).

Results

Phylogenetic analysis

Our phylogenetic tree is congruent with the well-supported lineages in Andersen & Ekman (2005) (Fig. 1). Our sequence of *Micarea usneae* is resolved in a strongly-supported clade (ML-bs = 99) with *Micarea peliocarpa*, *M. coppinsii* Tønsberg, *M. alabastrites* (Nyl.) Coppins, *M. leprosula* (Th. Fr.) Coppins & A. Fletcher and *M. cinerea* (Schaer.) Hedl. Our new taxon fits well in this group of species characterized by pallid or bluish apothecia, mostly 3-septate ascospores and the presence of gyrophoric acid. Their pycnidia are either large and immersed,

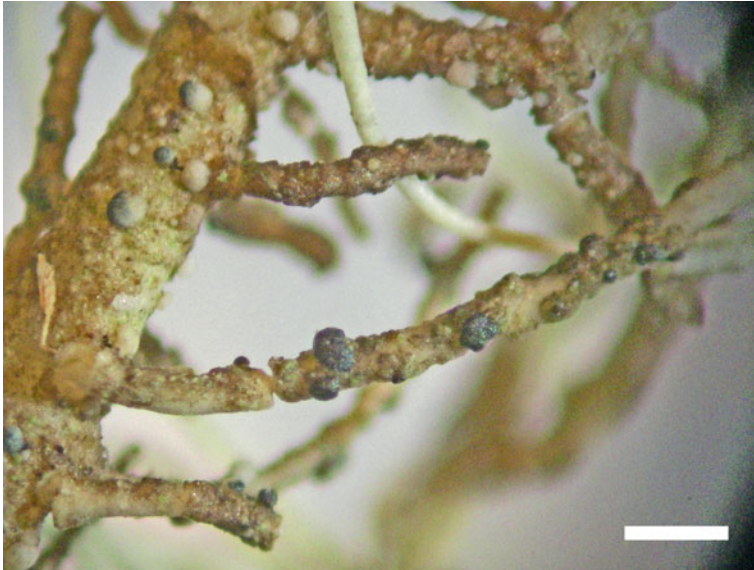


FIG. 2. *Micarea usneae* (holotype) on *Usnea*, habitus. Scale = 1 mm.

or small and sessile, with both micro- and macroconidia (Andersen & Ekman 2005).

Taxonomy

Micarea usneae van den Boom & Ertz sp. nov.

Mycobank No.: MB805217

Lichenicolous lichen on thallus of *Usnea* sp. Thallus \pm effuse, very thin, pale brownish to pale greenish grey, up to 2 mm wide; apothecia immarginate, dull brownish to bluish; ascospores slightly clavate, straight to curved, 0–3-septate, (10–)12–18 \times 1.5–2.5 μ m; macrospores abundantly present, dark brown to black, immersed to sessile, 40–70 μ m diam., K+ greenish, ostiole up to 30 μ m wide with a small white blob, macroconidia 15–30 \times 1.0–1.8 μ m, 0–1(–3)-septate, curved to sigmoid. Thallus and apothecia with gyrophoric acid.

Type: Portugal, Madeira, road from Paul da Serra to Ribeira da Janela, W of Fanal, open area with *Laurus* and *Erica* trees, 32°49.1'N, 17°09.35'W, 1100 m, 2 May 2012, P. & B. van den Boom 48057 (BR—holotype; hb. van den Boom—istotype).

(Fig. 2)

Thallus, \pm effuse, very thin, not evanescent, up to 20 μ m thick, smooth up to 2 mm wide, coalescing and forming a contiguous crust, or scattered patches, not forming goniocysts

or areoles, greenish grey to brownish grey to pale brown, matt to weakly shiny, mostly thinly coated by gelatinous algae. *Photobiont* micareoid, algal cells globose, 4–8 μ m diam.

Apothecia white, cream, pale dull brown, to greyish black, or bluish, sometimes piebald, 0.1–0.3 mm diam., immarginate, slightly to strongly convex, or hemispherical, scattered, sometimes tuberculate and 0.35 mm wide. *Hymenium* hyaline, (25–)30–40 μ m; *hamathecium* of abundantly branched paraphyses c. 1 μ m wide, septate, tips not or sometimes slightly widened and 1.5 μ m wide, not pigmented; *epithecium* greenish, K–, N+ reddish; *hypothecium* hyaline. *Asci* with a strongly amyloid apical dome, sometimes an apical cushion is visible, slightly clavate, 20–27 \times 10–14 μ m, 8-spored; *ascospores* slightly clavate, straight to curved 0–3-septate, (10–)12–18 \times 1.5–2.5 μ m.

Macrospores abundantly present, dark brown to black, immersed to slightly sessile, 40–70 μ m diam., in section pale brownish to greenish, K– ostiole up to 30 μ m wide with a small white blob; *macroconidia* 15–30 \times 1.0–1.8 μ m, 0–1(–3)-septate, strongly curved to sigmoid.

Chemistry. Thallus and apothecia K–, C+ red, KC–, P–; gyrophoric acid (detected by microcrystal test).

Habitat and distribution. *Micarea usneae* is known from only two localities, in the NW part of Madeira, in a rather open forest with smaller to mature *Laurus* trees and *Erica* trees and shrubs. It occurred on an unidentified *Usnea* species, with a pendulous thallus without isidia, papillae or pseudocyphellae, but with fibrils and poorly developed soralia that could be referable to *Usnea geissleriana* P. Clerc. In the type locality, the *Usnea* sp. was growing on a small *Erica* tree, in a rather disturbed roadside spot, not rich in epiphytic lichen communities. Several *Usnea* species were found and a few well-developed macrolichens such as *Cladonia parasitica*, *C. ramulosa* (With.) J. R. Laundon, *Hypotrachyna taylorensis* (M. E. Mitch.) Hale, *H. rockii* (Zahlbr.) Hale and *Parmeliella parvula* P. M. Jørg., as well as a few microlichens such as *Bacidia laurocerasi* (Delise ex Duby) Zahlbr., *Lecidea* sp., *Pertusaria* sp. and *Pyrenula dermatodes* (Borrer) Schaer.

Notes. *Micarea usneae* is characterized by the lichenicolous habit, the very thin filmy pale brownish thallus, the brownish to bluish subglobose apothecia 0.1–0.3 mm diam., black pycnidia and strongly curved to sigmoid macroconidia 15–30 × 1.0–1.8 µm, 0–1(–3)-septate.

Although the first author has collected *Micarea* material all over Madeira and on all the Canary Islands, with special attention paid to that genus, the new species was collected only twice. Many *Usnea* specimens were also collected, especially when bearing lichenicolous fungi. When sterile, *Micarea usneae* must be very inconspicuous and although we have checked many *Usnea* specimens from Madeira, we failed to find further collections that could refer to *M. usneae*.

Species of *Micarea* sometimes grow over other lichens. *Micarea peliocarpa* is usually corticolous but when saxicolous it might grow over *Aspicilia*, *Neofuscelia* etc. (e.g. Diederich & Sérusiaux 2000), and we have seen *Micarea demigrata* (Fr.) Hedl. clearly growing on saxicolous *Physcia dubia*. *Micarea inquinans* (Tul.)

Coppins is lichenicolous on *Dibaeis baeomyces* but has simple ascospores and no chemical compounds. Future studies should check if the new species is host-specific or if it might also grow over other lichens and other substrata.

Comparison with other species. The new species is easily mistaken in the field for *Micarea peliocarpa*, a species that also occurs on Madeira. This latter species usually has a much more granular thallus, with granules up to 0.2 mm diam.; ascomata can be as dark as in the new species, but asci are longer (25–55 µm), ascospores are wider (2.3–)3.0–4.0(–5.5) µm and not or rarely slightly constricted at septum, macroconidia are mostly whitish, to pale greenish up to 0.2 mm wide and usually immersed, with gaping ostioles and the macroconidia are 21–40 × 1.0–1.5 µm, 1–5-septate (Coppins 2009). *Micarea alabastrites* is also similar to *M. usneae* in having a thin and smooth thallus, but apothecia are whitish, cream- or ivory- white, ascospores (16–)18–26(–29) µm and macroconidia 21–55 µm long (Coppins 1983).

In the phylogenetic tree, *Micarea coppinsii* Tønsberg, which is also known from Madeira, is a closely related species. It has 3-septate ascospores 20–28 × 4(–5) µm, only microconidia are known and it is sorediate (Coppins 2009). A recent Madeira collection of this latter species (*P. & B. van den Boom* 47615) is fertile and grew on a mature *Pinus* trunk.

Micarea synotheoides (Nyl.) Coppins also occurs on Madeira and resembles the new species in habitus, but differs in having longer ascospores with more septa, a K+ purple epihymenium, a different chemistry (no compounds) and macroconidia are unknown. This species also belongs to a different lineage in our phylogenetic tree (Fig. 1).

Subsequent to the monograph of *Micarea* in Europe (Coppins 1983), many new species of *Micarea* have been described from the Northern Hemisphere, but only two further species have 3-septate ascospores. *Micarea subcinerea* Brand & van den Boom has macroconidia that are nearly straight and 65–100 × 1.2–1.7 µm, much longer than in the new species (van den Boom & Brand 2004);

whereas *Micarea neostipitata* Coppins & May, which is known from conifers in North America, has similar 3-septate ascospores $17\text{--}25 \times 1.7\text{--}2.5 \mu\text{m}$, but has pale stalked pycnidia and conidia $5.5\text{--}6.7 \times 1.8\text{--}3.0 \mu\text{m}$ (Coppins & May 2001).

In the Southern Hemisphere, other *Micarea* species with 3-septate ascospores have been described. Neither *M. austroternaria* Coppins & Kantvilas [a synonym of *M. magellanica* (Müll. Arg.) Fryday] or *M. isabellina* Coppins & Kantvilas contain gyrophoric acid and they either have small conidia or conidia are unknown and are mainly terricolous, not lichenicolous. *Micarea flagellispora* Coppins & Kantvilas also has 3-septate ascospores but they are much longer, $60\text{--}85 \times 1.5\text{--}1.7 \mu\text{m}$ (Coppins & Kantvilas 1990). *Micarea pannarica* Fryday has 3-septate ascospores *c.* $17.5 \times 5.7 \mu\text{m}$ (much wider than the new species), the chemistry is different (pannarin) and it is terricolous (Fryday 2004).

In a recent study by the first author and co-workers on the genus *Micarea* on the island of Réunion in the Indian Ocean (Brand *et al.* 2014), several new species were detected. One of them, *Micarea borbónica* Brand *et al.*, is corticolous and related to *M. peliocarpa* but differs from the new species by a granular thallus, apothecia up to 0.5 mm, ascospores $15\text{--}27 \times 3.0\text{--}4.1 \mu\text{m}$ and macroconidia $30\text{--}42 \times 1.0\text{--}1.3 \mu\text{m}$ that are indistinctly 3-septate.

Several lichenicolous fungi growing on *Usnea* are known from Macaronesia, and it is possible to mistake these species for *Micarea usneae*. The heterobasidiomycete *Biatoropsis usnearum*, which is common on the island and with which the new species sometimes grows, has biatorine basidiomata that can have the same colour as the new species, but they are often much larger, up to 2.5 mm

diam, and have neither asci nor ascospores. Some species with apothecia are also similar in habitus. *Abrothallus usneae* is known from Madeira (Hafellner 1995) and has dark brown-black apothecia 0.3–0.7 mm diam., but 1-septate ascospores of $14\text{--}18 \times 5\text{--}7 \mu\text{m}$. In the two localities where *M. usneae* occurs, we have found *Catillaria usneicola* on a different *Usnea* species: *U. articulata*. It has very small black apothecia and is easily mistaken for *M. usneae*. The apothecia have a paler disc, so it resembles the pycnidia of *M. usneae*, although these have a small white blob at their apex. It also differs in that it has no thallus, the ascospores are $4.5\text{--}6.0 \times 1.5\text{--}2.0 \mu\text{m}$, and pycnidia are black and *c.* $30 \mu\text{m}$. It is reported from Gomera in the original description (Etayo 2000) and from El Hierro (van den Boom & Ertz 2012), and is here newly reported for Madeira. *Lichenostigma maureri* is also known from Madeira (Hafellner 1995). It has small black apothecia *c.* 0.1 mm diam., but has brown, 1-septate ascospores and is not confusable with the new species. In addition, the following coelomycetes that are known from the Canary Islands have conidiomata that can easily be mistaken for those of *M. usneae*: *Pseudoseptoria usneae* (Vouaux) D. Hawksw. has pycnidia 0.1–0.2 mm diam. and conidia $10\text{--}16 \times 3\text{--}4 \mu\text{m}$; *Lichenoconium usneae* (Anzi) D. Hawksw. has pycnidia of <0.1 mm and brown conidia $2\text{--}5 \mu\text{m}$ diam.; and *Phaeosporobolus usneae* D. Hawksw. & Hafellner has black conidiomata 50–75 μm diam., and brown conidia, 4–6 μm diam.

Additional specimen examined. Madeira: W side of island, road (ER209) from Paul da Serra to Ribeira da Janela, NW of Fanal, trail to the east, open area with *Laurus* trees and mature *Erica* among ferns, on *Erica*, 1080 m, 2012, P. & B. van den Boom 47988 (hb. van den Boom).

Key to the lichenicolous fungi and lichens growing on *Usnea* in Macaronesia

A = Azores, C = Canary Islands, M = Madeira

- | | | |
|------|------------------------------------|-----------------------------|
| 1 | Apothecia present | 2 |
| | Apothecia lacking | 5 |
| 2(1) | Ascospores simple (C, M) | Catillaria usneicola |
| | Ascospores 1–3-septate | 3 |

- 3(2) Ascospores hyaline, 3-septate (M) **Micarea usneae**
 Ascospores brown, 1-septate 4
- 4(3) Ascospores 14–18 × 5–7 µm (C, M) **Abrothallus usneae**
 Ascospores 8–10 × 3–4 µm (A) **Abrothallus chrysanthus**
- 5(1) Bacidiomata present (C, M) **Biatoropsis usnearum**
 Ascomata or pycnidia present, or conidia present without pycnidia 6
- 6(5) Ascomata present, superficial, black, up to 0.1 mm, ascospores 1-septate (C, M) . .
 **Lichenostigma maureri**
 Ascomata lacking 7
- 7(6) Pycnidia unknown (Deuteromycete), conidiomata 50–75 µm diam., conidia brown,
 4–6 µm diam. (C) **Phaeosporobolus usneae**
 Pycnidia present 8
- 8(7) Pycnidia 0.1–0.2 mm, conidia hyaline, 10–16 × 3–4 µm (C)
 **Pseudoseptoria usneae**
 Pycnidia 50–75 µm, conidia brown, 2–5 µm diam. (C) . . **Lichenonium usneae**

Other species reported as new to Madeira

Catillaria usneicola Etayo

Madeira, road (ER209) from Paul da Serra to Ribeira da Janela, NW of Fanal, trail to the east, open area with *Laurus* trees and mature *Erica* among ferns, on *Erica*, 1080 m, 2012, P. & B. van den Boom 47981 (hb. van den Boom).

Cladonia parasitica (Hoffm.) Hoffm.

Madeira, road (ER209) from Paul da Serra to Ribeira da Janela, W of Fanal, W side of the road, open area with *Laurus* trees and *Erica* shrubs among ferns, on *Erica*, 1100 m, 2012, P. & B. van den Boom 48060 (hb. van den Boom).

We are greatly honoured to publish this work in the special issue of *The Lichenologist* for the 65th birthday of our most distinguished colleague and friend, Dr Brian J. Coppins.

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