**MULTICLAVULA ICHTHYIFORMIS (FUNGI: BASIDIOMYCOTA: CANTHARELLALES: CLAVULINACEAE), A REMARKABLE NEW BASIDIOLICHEN FROM COSTA RICA**

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The new basidiolichen *Multiclavula icthyiformis* Nelsen, Lücking, Umaña, Trest & Will-Wolf is described from Costa Rica. The new species differs from other species of *Multiclavula* in having a basidiocarp with tomentose stipe and flattened lamina with nonamphigenous hymenium. Molecular sequence data (ITS) confirmed its placement within *Multiclavula* in the Clavulinaceae (Cantharellales, Agaricomycetes). The new lichen was discovered in a Central American paramo remnant, illustrating the importance of biotic inventories of fungi and lichens to increase our knowledge of the diversity of these groups in endangered tropical ecosystems. The new species was found as part of the TICOLICHEN project in Costa Rica.

**Key words:** Basidiomycota; Cantharellales; Clavulinaceae; Costa Rica; fungi; new species; TICOLICHEN biodiversity inventory.

Although lichenized fungi are believed to be most diverse in cool-temperate regions, recent studies indicate their diversity to be higher in the tropics, with estimates of up to 600 species found at a single rain forest site and a total of 3000–5000 species for megadiverse countries such as Mexico, Colombia, and Brazil (Lücking et al., 2004). Unfortunately, exact estimates of tropical lichen diversity are difficult because of the lack of detailed taxonomic knowledge in many groups. For this reason, the TICOLICHEN project was developed as an international collaboration to explore and describe the extraordinary lichen diversity expected in Costa Rica (between 2400 and 3000 species) and to enhance taxonomic knowledge of tropical lichens locally and globally (Gámez et al., 1997; Lücking et al., 2004; Lücking, 2006). In the numerous publications from this project so far (Chaves et al., 2004; Grube et al., 2004; Nelsen et al., 2006; Rivas Plata et al., 2006; Del Prado et al., 2006; Lücking et al., 2006; 2007a–d; Aptroot et al., 2006), four new genera and more than 50 new species of lichens from Costa Rica have been described, out of an estimated total of 300 new taxa.

Biotic inventories such as the TICOLICHEN project are important to increase our knowledge of the biodiversity and ecological role of little known organisms in tropical ecosystems, many of which potentially face extinction through extensive land use change. In addition, inventories of rare tropical lichens and their molecular analysis significantly contribute to our knowledge of fungal evolution, as shown by several recent studies mainly through the NSF-funded Assembling the Fungal Tree of Life (AFTOL) project (Lutzoni et al., 2004; Del Prado et al., 2006; James et al., 2006; Miadlikowska et al., 2006; Spatafora et al., 2006; Lücking et al., 2007d).

While over 99% of the lichenized fungi are Ascomycota, a few lichenized taxa of polyphyletic origins (Oberwinkler, 1984, 2001; Gargas et al., 1995; Moncalvo et al., 2002; Lücking et al., 2004) are in the Basidiomycota (Table 1). Lichenized Basidiomycota are thus far restricted to a small number of genera (Oberwinkler, 2001; Redhead et al., 2002a; Chaves et al., 2004; Palice et al., 2005). Many of these are tropical and also found in Costa Rica (Fig. 1A–I). Although few in species, lichenized Basidiomycota have received considerable attention in ecophysiological studies and as models for the evolution of the lichenized habit (Lange et al., 1994; Gargas et al., 1995; Lützoni and Vilgalys, 1995; Lützoni and Pagel, 1997; Zoller and Lützoni, 2003). Some species, such as *Dictyonema glabratum*, *Lepidostroma calocerum*, and *Multiclavula vernalis*, can dominate terricolous lichen communities with pioneer character especially at higher altitudes in the tropics.

Apart from ongoing changes in the systematics and nomenclature of lichenized Basidiomycota in the past decades (Hibbett and Thorn, 2001; Moncalvo et al., 2002; Redhead et al., 2002), recent new discoveries have been rare. Among these was the enigmatic basidiolichen "*Omphalina* foliacea" (Jørgensen, 1989), the first lichenized representative of the hymenochaetoid clade or Hymenochaetales confirmed by molecular data (Palice et al., 2005); *Cyphellostereum* is presumed to be another possible member of this lineage (Redhead et al., 2002b), but no publications have confirmed this hypothesis with molecular data. Through the TICOLICHEN project, we discovered two new species of *Dictyonema* (Chaves et al., 2004). Here, we describe the new species *Multiclavula icthyiformis*, a lichenized basidiomycete with a novel basidiocarp morphology that differs from other species...
of *Multiclavula* by the flattened, stipitate basidiocarp resembling a fishtail. Anatomically, the new taxon is characterized by its lack of an amphigenous hymenium, a crystalline layer covering the sterile upper part of the lamina, and the presence of numerous scale-like structures on the lower portions (stipes) of the basidiocarps, which include photobiont cells in bulbils. None of these characteristics are known from other species of *Multiclavula* (Petersen, 1967; Petersen and Kantvilas, 1986; Coste and Royaud, 1994), but molecular data suggest a close relationship between our new taxon and other *Multiclavula* species.

### MATERIALS AND METHODS

**Field work and microscopic studies**—Collections were made during two TICOLICHEN field campaigns in summer 2002 and spring 2004 from soil on a stream bank in a high altitude (2700 m a.s.l.) bog in Tapantí National Park in the province of San José, Costa Rica (83°51′ W, 9°40′ N). Specimens were examined both in fresh (fully hydrated) and dry (air dried) condition, using a Leica MS 5 stereomicroscope (Leica Microsystems GmbH, Wetzlar) and a Zeiss Axioskop 2 compound microscope (Carl Zeiss MicroImaging, Thornwood) connected to a DAGE MTI DC-330 3CCD Color Camera (Dage MTI, Michigan City, Michigan, USA). Images were also made with a Nikon F301 SLR camera and a Nikon Coolpix 5400 digital camera (Nikon Inc., Melville, Michigan City, Michigan, USA). Microscopic features, including basidia and basidiospores, were examined in the fresh state and after being rehydrated in water. Macroscopic measurements were made on dried samples. All measurements were examined in the fresh state and after being rehydrated in water. New York, USA). Microscopic features, including basidia and basidiospores, were examined in the fresh state and after being rehydrated in water. Images were also made with a Nikon F301 SLR camera and a Nikon Coolpix 5400 digital camera (Nikon Inc., Melville, Michigan City, Michigan, USA). Images were also made with a Nikon F301 SLR camera and a Nikon Coolpix 5400 digital camera (Nikon Inc., Melville, Michigan City, Michigan, USA). Images were also made with a Nikon F301 SLR camera and a Nikon Coolpix 5400 digital camera (Nikon Inc., Melville, Michigan City, Michigan, USA). Images were also made with a Nikon F301 SLR camera and a Nikon Coolpix 5400 digital camera (Nikon Inc., Melville, Michigan City, Michigan, USA).

**DNA extraction and molecular methods**—For one holotype sample (Nelsen 2255a), we extracted DNA following the methods of Grube et al. (1995) and used the ITS1F (Gardes and Bruns, 1996) and ITS4A (D. L. Taylor in Kroken and Taylor, 2001) PCR primers to amplify the fungal rDNA internal transcribed spacer (ITS), following Nelsen and Gargas (2006). Amplification began with an initial denaturation of 95°C for 5 min; followed by 10 cycles of 95°C for 1 min, 62°C for 1 min, and 72°C for 1 min; followed by 35 cycles of 95°C for 1 min, 53°C for 1 min, and 72°C for 1 min; and terminating with a final extension at 72°C for 7 min. The sample was cycle sequenced and cleaned (see Nelsen and Gargas, 2006) using the ITS1 and ITS4 primers (White et al., 1990).

**Phylogenetic analyses**—The sequence obtained for the newly described lichenized fungus was manually aligned with sequences from other taxa in the cantharelloid clade (Hibbett and Thorn, 2001; Lutzoni et al., 2004; Hibbett et al., 2005), and ambiguous regions were deleted. Based on the molecular phylogeny of the cantharelloid clade (Hibbett and Thorn, 2001; Lutzoni et al., 2004; Hibbett et al., 2005), *Hydnum rufescens* Schaeff. and *Sistotrema atholuteum* (Bourdot & Galzin) Bondartsev & Singer (Cantharellales: Clavulinaceae) were selected as outgroup. A maximum parsimony (MP) analysis was performed in PAUP* 4.0b10 (Swofford, 2002), using a heuristic search with random stepwise addition (100 replicates) and tree-bisection-reconnection (TBR) branch swapping, and support was assessed by performing 1000 bootstrap replicates. A Bayesian analysis was performed in MrBayes 3.0 (Huelsenbeck and Ronquist, 2001), using the best-fit model as determined by the Akaike information criterion (AIC) in MrModeltest (Nylander, 2004). The analysis was run for 3 000 000 generations with four chains at a “temperature” of 0.18, using default settings and sampling every 100 generations. A 50% majority-rule consensus of all sampled post-burn-in trees was constructed in PAUP*.

### RESULTS


**Type**—Costa Rica. Cartago. Tapantí National Park, Macizo de la Muerte Section, Tres de Junio (La Amistad Pacífico Conservation Area), Talamanca Ridge, km 68 on road (ruta 2) from Cartago to San Isidro, roadside, 83°51′ W, 9°40′ N, 2700 m, upper montane rainforest zone, disturbed upland peat bog with scattered shrubs and *Blechnum*, on soil along brook and road bank, 5 July 2002, *M. P. Nelsen 2255a* (INB, holotype; B,
Etymology—The name refers to the fishtail-like basidiocarps of the new taxon.

Description—Thallus terricolous (Fig. 1J), up to 50 mm across but very thin, barely visible as greenish layer, composed
of dense colonies of green algae associated with fungal hyphae. Photobiont *Coccomyxa* (Fig. 2A–D, G–K), cells broadly ellipsoid, 7–9 × 4–7 μm, each cell individually wrapped by a layer of 1.5–2.0 μm thick fungal hyphae (bulbils), algal cells frequently producing autospores (occasionally intermingled with prokaryotic cyanobacteria and other algae lack intimate contact with fungal hyphae and do not represent regular photobionts of this lichen).

Basidiocarps scattered, not aggregate (Fig. 1J–K), fleshy, unbranched, more or less lanceolate, with a basal stipe 2–3.3–5

Fig. 2. Light micrographs of *Multiclavula ichthyiformis*. (A–D) *Coccomyxa* photobiont from sections of terricolous thallus, showing hyphal wrap (bulbils) around each cell (arrows, enlarged in C; larger photobiont cells 9 × 6 μm) and autospore formation (D; daughter cells 4 × 3 μm). (E) Scales of basidiocarp stipes (largest scale on upper left 0.25 mm long). (F) Contextual hyphae of lamina (individual hyphae 3–4 μm wide). (G–H) Scales of basidiocarp stipes with individual photobiont cells wrapped on surface (arrows, enlarged in H; photobiont cells 8 × 5 μm). (I) Surface of basidiocarp lamina with bulbils including algal cells (larger photobiont cells 8 × 6 μm). (J) Section through upper sterile part of lamina showing densely arranged, parallel contextual hyphae (cont), thin cortical layer of compacted hyphae with gelatinized walls (cort; 10–15 μm diameter), and covering layer of crystalline clusters (crys). (K) Enlarged section through upper sterile part of lamina showing detail of crystalline clusters (large cluster on top 30 μm diameter). (L) Individual basidium with six sterigma and three attached basidiospores (largest basidiospore 5 μm diameter).
mm high and 0.5–0.55–0.7 mm in diameter and an upper widened, fishtail-like lamina 0.6–2.4–4.0 mm high and 0.9–1.4–1.9 mm wide. Stipe coarsely tomentose, covered by numerous tiny scales (Figs. 1K–L, 2E), pale flesh-colored when moist to translucent white when dry; scales 0.1–0.25–0.4 mm long, tapering, 30–40–50 μm broad at the base and 20–25–30 μm broad below the apex, formed by densely packed, parallel, sparsely branched hyphae. Both lamina, stipe, and scales frequently producing bulbils enclosing individual algal cells or algal colonies with the same morphology as the thallus algae; bulbils formed by a single layer of septate hyphae of same thickness as contextual hyphae and with cells not or very slightly inflated (Fig. 2G–I). Lamina dorsiventral; ventral side pale flesh-colored when moist to translucent white when dry, carrying the hymenium; dorsal side pale to tan-orange when moist, translucent to dark orange when dry, sterile; lamina in section 200–250–300 μm thick, for most part formed by parallel, densely arranged and sparsely branched, uniformly thin-walled contextual hyphae (Fig. 2F); upper sterile surface of lamina with a thin (10–15–20 μm) cortical layer of thick-walled, strongly compacted and gelatinized hyphae with yellowish walls (Fig. 2I), almost resembling a parenchyma, above with a layer of clusters of large (10–20–30 μm diameter), colorless to orange-brown crystals (Fig. 2K). Contextual hyphae densely packed (Fig. 2F), agglutinated, sparsely branched and anastomosing, hyaline, thin-walled, inconspicuously clamped, of two widths: algiferous thallus hyphae and those of external part of stipe and of scales 1.5–1.7–2.0 μm wide, those of internal parts of stipe and lamina 3–3.5–4 μm wide. Subhymenial hyphae parallel with contextual hyphae, 1.5–2–2.5 μm wide, producing basidia as side branches. Basidia suburniform with basal stalk, 25–30–35 × 7–8–10 μm, thin-walled, hyaline, clamped, (4–)6-sterigmate (Fig. 2L); sterigma narrowly flask-shaped, 6–7–8 × 2–2.5–3 (–4 at the base) μm. Basidiospores globose (Fig. 2L), 4–5–6 μm in diameter, hyaline, smooth, thin-walled, aguttulate, inamyloid.

Habitat and distribution—The new taxon is terricolous, occurring on moist soil along a brook and near a road bank among bryophytes in a high altitude bog in Costa Rica, in the upper montane cloud forest belt just below the paramo zone (Kappelle, 1995, 1996). Other common terricolous lichens found in this type of habitat were Cladia aggregata (Sw.) Nyl., Cladonia confusa R. Sant., Dictyonema glabratum (Spreng.) D. Hawksw., Icmadophila aversa (Nyl.) Rambold & Hertel, Phylobaeis imbricata (Hook.) Kalb and Gierl, and Siphula ceratites (Wahlenb.) Fr.

Phylogenetic analyses—The final alignment consisted of 571 characters, 257 of which were variable and 153 parsimony informative. The MP analysis recovered a single most
parsimonious tree, with a length of 487 steps, and revealed *M. ichthyiformis* to be sister to the three other species of *Multiclavula* studied (Fig. 3A). The GTR+G model was selected as the best fit model for the Bayesian analysis. The first 151 sampled trees were excluded, and a 50% majority-rule consensus tree was constructed (Fig. 3B). In this analysis, *M. ichthyiformis* was recovered as sister to the other three *Multiclavula* species with high support. Both analyses confirm the placement of *Multiclavula* in the Cantharellales or cantharelloid clade (Hibbett et al., 1997; Hibbett and Thorn, 2001) of the Homobasidiomycetes.

**DISCUSSION**

**Taxonomy**—The species at first glance resembles other species of *Multiclavula* but differs in several characters, in particular the dorsiventral, lanceolate basidiocarps with basal, tomentose stipe and fishtail-like lamina, and the globose rather than ellipsoid basidiospores. The hymenium is only developed on the ventral side, contrary to other species of *Multiclavula* where it is amphigenous on smooth, bisymmetrical to radiately symmetrical basidiocarps. Similarities with other *Multiclavula* species, in particular *M. coronilla* (G. W. Martin) R. H. Petersen, apart from the very general aspect and thallus structure, are seen in the contextual hyphae, the periclinal subhymenial hyphae producing basidia as side branches, the extranumerary (4–6) stigmates, and the lichenized bulbils. The bulbils in *M. ichthyiformis* agree with those of other species of *Multiclavula* in having thin walls (one layer of fungal hyphae of equal thickness as the contextual hyphae) but differ from the Botrydina-type bulbils of *Lichenomphalia* and *Semiomphalina*. The presence of basidia with 4–6 spores supports placement of the new species within *Multiclavula* and next to *Sistotrema*, basidia with 4–6 spores were also recently described for a new species of the non-lichenized genus *Clavulina* (Thacker and Henkel, 2004), which otherwise has 2–4 spores and which is sister to the *Sistotrema-Multiclavula* clade.

The tomentose stipe and dorsiventral basidiocarp of *M. ichthyiformis* are similar to those of *Semiomphalina leptoglossoides* (Corner) Redhead, a lichenized basidiomycete originally described in the genus *Pseudocraterellus* (Corner, 1966; Redhead, 1984). However, while the lamina in the new species remains completely flat, that of *Semiomphalina* is characterized by a thickening hymenium (Corner, 1966: 161, fig. 73). In addition, the bulbils of that taxon are Botrydina-like, with walls formed by fungal hyphae thicker than the contextual parent hyphae and featuring more numerous secondary septa and slightly inflated cells (Redhead, 1984). Also, the upper sterile surface of the lamina in *Semiomphalina* is composed of relatively thick, septate hyphae with inflated cells, while in *M. ichthyiformis* it produces a thin layer of strongly compacted and gelatinized hyphae covered by a layer of large crystals, which give the upper surface the characteristic orange color when dry. The nature of these crystals remains unknown; while lichenized Ascomycota frequently produce such crystals, we are unaware of any similar or related lichenized basidiomycete with similar crystals on the basidiocarp surface. The two taxa also differ in that the basidiocarp of *M. ichthyiformis* is more pointed, breaking up into smaller, jagged points, while the basidiocarp of *Semiomphalina* breaks up into more rounded sections. The ontogeny also differs in that *Semiomphalina* develops a split in the basidiocarp early in its development (Corner, 1966), while the basidiocarp of the new species only breaks up at maturity.

A few other non-lichenized basidiomycetes produce basidiocarps that are superficially similar to those of *M. ichthyiformis* but clearly differ in numerous morphological and anatomical details. *Rimbachia paradoxa* Pat. produces a flattened basidiocarp, but the pileus is attached to the stipe at an angle, the hymenial surface is reticulate and sublamellate, the basidiospores are ellipsoid, and it occurs on dead wood (Corner, 1966; Redhead, 1984). *Pterygellus armeniacus* Corner also bears some similarity in that it produces scale-like structures on the stipe and basidia with 4–6 spores, but its basidiocarps are radiately symmetrical except for the uppermost parts, and it produces a strongly lamellate hymenium and large squamules on the pileus.

**Systematic position**—Apart from the dorsiventral nature of the basidiocarps, the gross morphology, anatomical details, and molecular evidence place *M. ichthyiformis* within the genus *Multiclavula*. The latter is traditionally included in Clavariciaeae (part of which belongs to the Agaricales or euagarics clade; Larsson et al., 2004), but molecular phylogenetic analysis shows its close relationship with “Sistotrema” brinkmannii, on a clad sister to several *Clavulina* species in the cantharelloid clade of the Homobasidiomycetes (Pine et al., 1999; Hibbett and Thorn, 2001; Larsson et al., 2004; Hibbett et al., 2005).

The *Clavulina* sister clad forms the core of the family Clavulinaceae in the Cantharellales or cantharelloid clade (Hibbett and Thorn, 2001; Larsson et al., 2004; Thacker and Henkel, 2004; Hibbett et al., 2005), and it therefore seems appropriate to place the Sistotrema-Multiclavula clade in the same family and order. The systematics of the Basidiomycota is in flux, and many formal taxonomic and systematic changes will be necessary in the near future (Hibbett et al., 2005). At present, the clades emerging from various studies are therefore mostly given informal names (Hibbett et al., 1997; Hibbett and Thorn, 2001; Moncalvo et al., 2002; Larsson et al., 2004), but an attempt to use formal names at order level and above in the Basidiomycota is now being made with the Assembling the Fungal Tree of Life (AFTOL) classification project (Hibbett et al., 2007; http://www.clarku.edu/faculty/dhibbett/AFTOL/AFTOL.htm).

**Importance of the new discovery**—The habitat in which the new lichen was discovered, a high altitude bog in the transitional zone between the upper montane cloud forest and the paramo zone, forms part of an endangered ecosystem only found in disjunct patches along the Central American Cordilleras and best developed in the Cordillera de Talamanca in Costa Rica (Kappelle, 1995, 1996). In spite of its rareness, this habitat supports a high diversity of lichen species, including many rare taxa in the genera *Anzia*, *Graphis*, *Gyalidea*, *Icmadophila*, *Menegazzia*, *Pertusaria*, *Phaeographis*, and *Sticta*. Because of their proximity to the Interamerican Highway, the few remaining high altitude bogs in Costa Rica experience strong pressure due to local land use change, and the discovery of this new species exemplifies the importance of biotic inventories of endangered ecosystems to document organismic diversity before these habitats disappear from the landscape.

In terms of the evolution of fruit body types in Basidiomycota, the new taxon provides yet another example of how
cyphelloid (flattened, lobe- or cup-shaped) basidiocarps evolved independently within different lineages featuring a diversity of fruit body forms, thus confirming the notion that fruit bodies in Basidiomycota are much more plastic than implied by traditional classifications (Hibbett and Thorn, 2001; Moncalvo et al., 2002).

LITERATURE CITED


