

***Cladobotryum semicirculare* sp. nov. (Hyphomycetes)
from commercially grown *Ganoderma tsugae* in Taiwan
and other Basidiomycota in Cuba**

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Cladobotryum semicirculare sp. nov. stopping growth and development of basidiomata of *Ganoderma tsugae* in a Taiwanese mushroom farm was also isolated from a polypore and an agaric in Cuba. The species differs from other species in the genus by strongly curved, 0–3-septate conidia. This species concept is supported by an analysis of partial LSU rDNA sequences from three strains of the new species and other species of *Cladobotryum* and *Hypomyces* (Ascomycota, Hypocreales).

Keywords: fungicolous, Hymenomycetes, mushroom diseases, mycoparasitic, systematics.

Species of *Cladobotryum* Nees are considered anamorphs of species of *Hypomyces* (Fr.) Tul. (Ascomycota, Hypocreales) that are found typically on fruit-bodies of different groups of fungi (Pöldmaa *et al.* 1997, Rogerson & Samuels 1985, 1993a, b, c). The anamorphs are diagnostic of *Hypomyces* species even in the absence of the perithecia, and in some cases the anamorph forms on a wider range of substrata than the teleomorph.

With respect to economic relevance, these species include destructive parasites in mushroom farms, like *C. asterophorum* de Hoog, *C. dendroides* (Bull.) W. Gams & Hooz. (anamorph of *Hypomyces rosellus* (Alb. & Schwein.) Tul. & C. Tul.), and *C. mycophilum* (Oudem.) W. Gams & Hooz. (anamorph of *H. odoratus* G. R. W. Arnold) which are associated with cobweb disease of *Agaricus bisporus* (J. E. Lange) Pilát worldwide (Hoog 1978, McKay *et al.* 1999), and *C. varium* Nees (anamorph of *H. aurantius* Plowr.) on *Flammu-*

lina velutipes (Curtis) Singer in Asia (Kim *et al.* 1999). On the other hand, some species of *Cladobotryum* are antagonistic to plant pathogenic fungi, e. g. *C. amazonense* C. N. Bastos, H. C. Evans & Samson against fungal pathogens of cocoa (*Theobroma cacao* L.) (Bastos *et al.* 1981).

During the last two decades, our knowledge about the global diversity of *Cladobotryum* species and their teleomorphic *Hypomyces* stages has increased considerably (Pöldmaa 2003, Pöldmaa & Samuels 1999, 2003, Pöldmaa *et al.* 1997, 1999, Rogerson & Samuels 1985, 1993a, b, c). Many additional *Cladobotryum* species have been described from a broad range of host fungi, several, however, as unnamed anamorphs of *Hypomyces* species. Due to the infraspecific morphological variability of *Cladobotryum* species, the morphological similarities between the species, and the not yet well-understood host specificities, the separation of species is difficult. If morphological studies and analyses of DNA sequences are, however, combined, different strains can be assigned on the species level with high certainty (McKay *et al.* 1999, Pöldmaa *et al.* 1999, Pöldmaa & Samuels 2003). Analyses are hitherto mainly based on LSU rDNA sequences (Pöldmaa *et al.* 1999, Pöldmaa & Samuels 2003), whereas ITS sequences are available only of a few species (McKay *et al.* 1999).

A distinctive species of *Cladobotryum* having curved conidia was found independently in Cuba by G. R. W. Arnold in 1985 and in Taiwan by R. Kirschner and C.-J. Chen in 2002. In Taiwan, the fungus caused a dramatic decline of commercially grown basidiomata of *Ganoderma tsugae* Murrill in a mushroom farm and could be controlled only by strict removal of contaminated material and cleaning of the surfaces. On the basis of phenotype and DNA sequences, these collections were found to represent the same, undescribed species.

Materials and Methods

Specimens of *Cladobotryum* were collected on the natural substrate and cultivated on 2 % malt extract agar (MEA, Roth, Germany). For describing colony characteristics from cultures, cultures were grown on MEA in 90 mm diameter Petri dishes in diffuse day light and at approx. 22 °C. For induction of perithecium development, three strains (two from Cuba, one from Taiwan) of the new species were pairwise inoculated onto 3 % malt extract agar (Bio-malz-Backmittel GmbH Teltow, Germany). Dried material was deposited in the herbaria FR, HAJB, IMI, and TNM and cultures at the American Type Culture Collection (ATCC), Manassas, USA, the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands, and the German National Resource Centre for Biological Material

(DSMZ), Braunschweig, Germany. Microscopic examinations and measurements were performed using an oil immersion objective (100×) with material growing on basidiomata mounted in 5 % aqueous potassium hydroxide (KOH) solution and 1 % aqueous phloxine solution. Measurements of microscopic structures are given as ranges from minimum to maximum values directly or as mean values ± standard deviation with extreme values in brackets (n = 30). For isolation of nuclear DNA with the PeqLab E. Z. N. A.[®] Fungal DNA kit, for amplification with primers NL1 and NL4, and purification of the PCR products of partial LSU rDNA with PeqLab E. Z. N. A.[®] Cycle-Pure kit, the protocols were used as described in Kirschner & Yang (2005). Purified double-stranded DNA was sent to Scientific Research & Development (SRD, Oberursel, Germany) for sequencing and primary editing. Sequences were additionally edited with CodonCode Aligner (CodonCode Corporation). New sequences (deposited in GenBank with numbers beginning with DQ) were obtained from cultures of own collections as given below:

Cladobotryum asterophorum de Hoog: Taiwan, Taitung, Zhiben, ca. 100 m, on old polypore and adjacent bark on dead wood, 15 Aug 2002, R. Kirschner & H.-C. Kuo 1422 (TNM), CBS 118999 (living strain), DQ376085.

Cladobotryum cf. *multiseptatum* de Hoog: Germany, Nordrhein-Westfalen, Eifel, Nettersheim, ca. 500 m, on *Hygrocybe pratensis* (Pers.) Murrill var. *pratensis* (Agaricales), 23 Oct 2004, R. Kirschner et al. 2228 (FR), CBS 118756 (living strain), DQ376086.

Cladobotryum semicirculare (described in this publication): see “material examined” in the Results Section.

Cladobotryum varium Nees: Austria, Kleinwalsertal, Riezlern, ca. 1400 m, on *Tremiscus helvelloides* (D.C.: Pers.) Donk (Auriculariales), 19 Sep 2004, R. Kirschner et al. 2195 (FR), CBS 118755 (living strain), DQ376087.

Gliocladium sphaerosporum Matsush.: Taiwan, Taipei, Peitou, bark of *Pinus luchuensis* Hayata, 10 Jul 1999, R. Kirschner 787 (TNM), DQ376088.

Tubercularia vulgaris Tode: Germany, Baden-Württemberg, Tübingen, Morgenstelle, branch of deciduous tree, 1 Mar 2000, R. Kirschner 620 (FR), DQ376089.

Tab. 1. – GenBank accession numbers and origins of species of *Cladobotryum*, *Hypomyces* and other fungi used in the phylogenetic analyses.

| Species | Origin of isolates used for new sequences* | GenBank no. | Reference |
|--|--|-------------|--------------------------|
| <i>Aspergillus sparsus</i> Raper & Thom | | AF33090 | Peterson (2000) |
| <i>C. apiculatum</i> (Tubaki) W. Gams & Hooz. | | AF213025 | Pöldmaa (2000) |
| <i>C. arthrobotryoides</i> K. Pöldmaa | | AJ583468 | Pöldmaa & Samuels (2003) |
| <i>C. asterophorum</i> de Hoog | polypore, Taiwan, TNM, CBS 118999 | DQ376085 | |
| <i>C. asterophorum</i> de Hoog | | AJ583469 | Pöldmaa & Samuels (2003) |
| <i>C. cubitense</i> R.F. Castañeda & G. R. W. Arnold | | AJ583470 | Pöldmaa & Samuels (2003) |
| <i>C. dimorphicum</i> K. Pöldmaa | | AJ583471 | Pöldmaa & Samuels (2003) |

| Species | Origin of isolates used for new sequences* | GenBank no. | Reference |
|--|--|-------------|--------------------------------|
| <i>C. gracile</i> K. Pöldmaa | | AF213026 | Pöldmaa (2000) |
| <i>C. multiseptatum</i> de Hoog | | AJ583472 | Pöldmaa & Samuels (2003) |
| <i>C. cf. multiseptatum</i> de Hoog | <i>Hygrocybe pratensis</i> , Germany, FR, CBS 118756 | DQ376086 | |
| <i>C. odorum</i> G.R.W. Arnold | | AJ583473 | Pöldmaa & Samuels (2003) |
| <i>C. rubrobrunnescens</i> W. Helfer | | AF160228 | Pöldmaa <i>et al.</i> (1999) |
| <i>C. semicircularis</i> G.R.W. Arnold, R. Kirschner & Chee J. Chen | <i>Ganoderma tsugae</i> , Taiwan, TNM | DQ376084 | |
| <i>C. semicircularis</i> G. R.W. Arnold, R. Kirschner & Chee J. Chen | polypore, Cuba, IMI, CBS 119123 | DQ376082 | |
| <i>C. semicircularis</i> G.R.W. Arnold, R. Kirschner & Chee J. Chen | agaric, Cuba, CBS 1191122 | DQ376083 | |
| <i>C. stercicola</i> G.R.W. Arnold | | AF160229 | Pöldmaa <i>et al.</i> (1999) |
| <i>C. varium</i> Nees | <i>Tremiscus hellvelloides</i> , Austria, FR, CBS 118755 | DQ376087 | |
| <i>Cordyceps ophioglossoides</i> (Ehrh.) Link | | U47827 | Spatafora <i>et al.</i> (1998) |
| <i>Gliocladium sphaerosporum</i> Matsush. | bark, Taiwan, TNM | DQ376088 | |
| <i>H. armeniacus</i> Tul. | | AF160239 | Pöldmaa <i>et al.</i> (1999) |
| <i>H. aurantius</i> (Pers.: Fr.) Tul. | | AF160230 | Pöldmaa <i>et al.</i> (1999) |
| <i>H. mycophilus</i> Rogerson & Samuels | | AF160238 | Pöldmaa <i>et al.</i> (1999) |
| <i>H. odoratus</i> G.R.W. Arnold | | AF160240 | Pöldmaa <i>et al.</i> (1999) |
| <i>H. orthosporus</i> K. Pöldmaa | | AF160241 | Pöldmaa <i>et al.</i> (1999) |
| <i>H. polyporinus</i> Peck | | AF049133 | Spatafora <i>et al.</i> (1999) |
| <i>H. rosellus</i> (Alb. & Schwein.: Fr.) Tul. | | AF160242 | Pöldmaa <i>et al.</i> (1999) |
| <i>H. semitranslucens</i> G.R.W. Arnold | | AJ459303 | Pöldmaa (2003) |
| <i>H. symodiophorus</i> Rogerson & Samuels | | AJ459308 | Pöldmaa (2003) |
| <i>H. thailandicus</i> K. Pöldmaa & Samuels | | AJ459310 | Pöldmaa (2003) |
| <i>H. xyloboli</i> K. Pöldmaa & Samuels | | AJ459300 | Pöldmaa (2003) |
| <i>Hypocrea jecorina</i> Berk. & Broome | | AY281997 | Réblová & Seifert (2004) |
| <i>Hypocrea schweinitzii</i> (Fr.) Sacc. | | AY281095 | Réblová & Seifert (2004) |
| <i>Tubercularia vulgaris</i> Tode | bark, Germany, FR | DQ376089 | |

*Acronyms of herbaria: FR, Forschungsinstitut Senckenberg, Frankfurt am Main, Germany; IMI, International Mycological Institute, Egham, Surrey, UK; TNM, National Museum of Natural Sciences, Taichung, Taiwan.

An alignment with MEGALIGN of the Lasergene package (DNASTAR, Inc. 1997) over a length of 555 bp positions was created with sequences from GenBank and with new sequences of own isolates without manual manipulations. The accession numbers of sequences of selected species of Hypocreales with focus on species of *Cladobotryum* and *Hypomyces* from GenBank used for this study were published with details of the respective origins by Pöldmaa (2000, 2003), Pöldmaa & Samuels (2003), and Pöldmaa *et al.* (1999).

The GenBank accession numbers and references of all species included in our study are given in Tab. 1.

The PHYLIP package, version 3.5c (Felsenstein 1993) was used to perform a neighbour-joining analysis (Kimura two-parameter distances, transition/transversion ratio 2.0), followed by a bootstrap analysis with 1000 replicates. *Aspergillus flavus* Raper & Thom was chosen as outgroup.

Results

Cladobotryum semicirculare G.R.W. Arnold, R. Kirschner & Chee J. Chen, **sp. nov.** – Figs. 1, 2

Colonia in substrato naturali effusa, cottonea, primum alba, deinde luteola vel rosea. Hyphae hyalinae, laeves, usque 13 μm latae. Conidiophora adscendentia vel erecta, recta vel leniter sinuata, laevia, septata, hyalina, ramosa, ad 460 μm longa, ad basim 8–10 μm lata. Rami irregulariter dispositi vel in verticillis. Cellulae conidiogenae 2-5-verticillatim dispositae, subulatae, rectae vel curvatae, 28–48 μm longae, prope basim 4–6 μm latae, unum locum conidiogenum ferentes. Conidia cylindrica, fortiter curvata, ad apicem rotundata, ad basim hilo conspicuo, tenuitunicata, laevia, 18–32 \times 3.5–5 μm .

Holotype. – Cuba, Prov. Ciudad de La Habana, Santiago de las Vegas, Instituto de Investigaciones Fundamentales en Agricultura Tropical Alejandro de Humboldt, on old polypore, 9 Jan 1985, G. R. W. Arnold A 85/185 (IMI 394236).

Isotype. – HAJB.



Fig. 1. Mycelium of *Cladobotryum semicirculare* on a basidioma of *Ganoderma tsugae* from a mushroom farm in Taiwan (same locality as R. Kirschner & C.-J. Chen 1301, 30 Sep 2001). Bar = 3 cm.

Mycelium on the natural substrate and on 2 % malt extract agar (MEA) lanose, white, yellowish to pinkish-violet (Fig. 1). Growth on MEA rapid, 30–80 mm in 7 days at approx. 22 °C, aerial mycelium white, lanose with the texture denser and reverse yellow in the strains from Cuba, texture loser and reverse brown in the strain from Taiwan, mycelium loosing the ability to sporulate on MEA, chlamydospores, crystals and odour absent in cultures on MEA.

Hyphae smooth, hyaline, up to 13 μm wide. – Conidiophores arising from aerial hyphae covering the basidiomata of host fungi, prostrate or erect, straight or slightly sinuous, irregularly or verticillately branched, with few septa, smooth, hyaline, up to 460 μm long and 8–10 μm wide at the base (Fig. 2). – Conidiogenous cells in whorls of 2–5, flask-shaped, 28–48 μm long and 4–6 μm wide at the base in the material from Cuba, smaller in the material from Taiwan, (10) 11–20 (33) \times 2.5–3 (4) μm ($n = 30$), narrowed at the tip and mostly appearing truncate at the single apical conidiogenous locus. – Conidia slightly curved to hook-like or semicircular, rarely sigmoid or straight, 0–3-septate, smooth, hyaline, with rounded apex and basal centric or asymmetrical apiculus, 18–32 \times 3.5–5 μm in the material from Cuba, smaller in the material from Taiwan, (9) 11.5–17.5 (22) \times (3) 3.5–5 (6) μm ($n = 30$; Fig. 2).

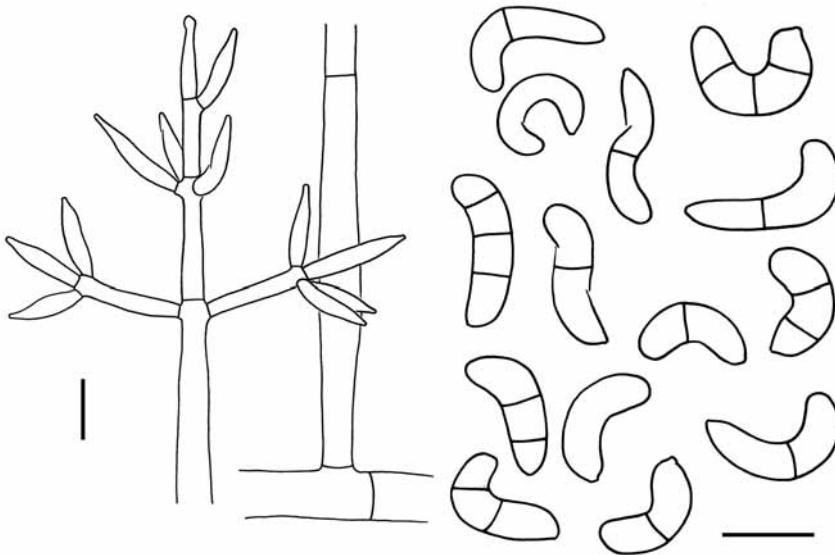


Fig. 2. Apex and base of a conidiophore and conidia of *Cladobotryum semicirculare* (R. Kirschner & C.-J. Chen 1301, TNM). The morphology of the type from Cuba is identical except for sizes. Bars = 10 μm .

Teleomorph. – Presumably a species of *Hypomyces*. Perithecial primordia were present on a reddish subiculum in the type material of *C. semicirculare*. All three available strains (two from Cuba, one from Taiwan) were combined with each other, without resulting in the development of perithecia. In the phylogenetic analysis, the three strains form a well supported clade (Fig. 3). There are smaller distances between both strains from Cuba than between these both strains and the strain from Taiwan.

Etymology. – *semicircularis*, referring to the strongly curved conidia.

Habitat. – Apparently parasitic on basidiomata of polypores (including *Ganoderma* species) and agarics (including pleurotoid taxa).

Distribution. – Cuba, Taiwan.

Material examined (including cultures and GenBank numbers of LSU rDNA sequences). – Cuba, Prov. Ciudad de La Habana, Santiago de las Vegas, Instituto de Investigaciones Fundamentales en Agricultura Tropical “Alejandro de Humboldt” (INIFAT), on old polypore, 9 Jan 1985, G.R.W. Arnold A 85/185 (IMI 394236, holotype; HAJB, isotype; ATCC 66123, CBS 119123 = CBS 705.88, DSM 12235 = living ex-type strain), DQ376082; Cuba, same locality, on old agaric, 28 Jul 1985, G.R.W. Arnold A 85/380 (not preserved in a herbarium, CBS 119122), DQ376083; Cuba, same locality, on pleurotoid mushroom, 31 Jul 1984, G.R.W. Arnold A 84/826 (private collection G.R.W. Arnold); Cuba, same locality, on *Lentinus scleropus* (Pers.) Fr., 12 Aug 1987, R. Castañeda Ruiz C 87/249 (private collection G.R.W. Arnold, CBS 533.88); Cuba, Ciudad La Habana, Bosque del Almendares, on *Pleurotus* sp., 8 Sep 1985, G.R.W. Arnold A 85/959-5 (private collection G.R.W. Arnold); Taiwan, Chia Yi, Zhongpu, on a basidioma of a *Ganoderma* species distributed as *Ganoderma tsugae* Murrill in a commercial mushroom farm, 8 Jun 2002, R. Kirschner & C.-J. Chen 1301 (TNM, no living strain preserved), DQ376084.

Discussion

Conidia in species of *Cladobotryum* are generally straight, often only with an asymmetrical basal hilum and with few slightly curved conidia among straight ones. Almost curved conidia are described in *C. curvatum* de Hoog & W. Gams and *C. curvididymum* Matsush., which differ by the clavate shape and less strong curvature of the conidia from *C. semicircularis* (Hoog 1978, Matsushima 1996). In *C. curvididymum*, conidia are one-septate in contrast to the 0–3-septate conidia of *C. semicircularis* (Matsushima 1996). Conidia are not produced in imbricate chains in *C. semicircularis*, whereas the process of conidium production could not be resolved in the dried material of *C. curvatum* (Hoog 1978). *Cladobotryum curvatum* and *C. cubitense* R.F. Castañeda & G. R. W. Arnold are considered close relatives of the anamorph of *Hypomyces khaoyaiensis* K. Pöldmaa & Samuels because of similar conidia that are held in imbricate chains in *C. cubitense* and the anamorph of *H. khaoyaiensis* (Pöldmaa & Samuels 2003).

The strains of *Cladobotryum semicircularis* from Cuba and Taiwan differ morphologically from each other by the smaller dimensions of the conidiogenous cells and conidia as well as cultural characteristics like the brown reverse of cultures in the strain from Taiwan in contrast to the larger dimensions and the yellow reverse of the strains from Cuba. These morphological differences appear to be reflected in a certain genetic distance between the analysed LSU

rDNA sequences of the two strains from Cuba on the one hand and the strain from Taiwan on the other. Because of the identical shape of the conidia, similar structure of the conidiophores, and the close genetic relationship between the strains from Cuba and Taiwan, however, we conclude that these morphological and genetic differences are correlated to an intraspecific variability.

The position of the clade formed by the three strains of *Cladobotryum semicirculare* is, although with a low support, close to the

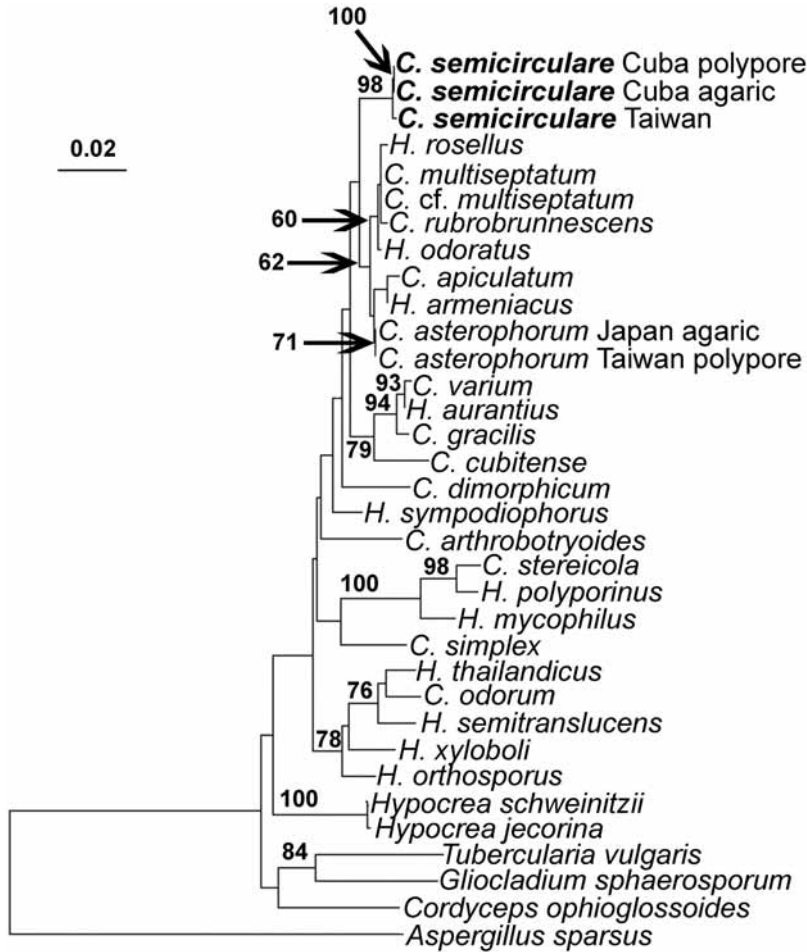


Fig. 3. Phylogenetic hypothesis derived from neighbour-joining analysis of partial large subunit RNA gene sequences of species of *Cladobotryum*, *Hypomyces* and other Ascomycota. The topology is rooted with *Aspergillus sparsus*. Bootstrap values are given as numbers (in percentages) on branches based on 1000 replicates (values smaller than 60 % not shown). Branch lengths are scaled in terms of expected numbers of nucleotide differences per site.

clade composed of *C. apiculatum*, *C. asterophorum*, *C. multiseptatum*, *C. rubrobrunnescens*, *Hypomyces armeniacus*, *H. odoratus*, and *H. rosellus*. This clade corresponds to “clade I” designated by Pöldmaa & Samuels (2003) comprising *C. asterophorum*, *C. multiseptatum*, *C. rubrobrunnescens*, *H. armeniacus*, and *H. rosellus* as well as to a subclade within another clade I designated by Pöldmaa (2003) comprising *C. apiculatum*, *C. rubrobrunnescens*, *H. armeniacus*, *H. odoratus*, and *H. rosellus*. In our clade, all species of the two cited published clades are combined in a single clade, which also includes our strains of *C. asterophorum* and *C. cf. multiseptatum*. In species of this clade, the morphological species concepts appear considerably narrower than in certain other clades. Anamorphic strains are difficult to distinguish morphologically and show 1–2 % divergence of the analysed sequences between species, whereas in other species wider concepts are applied with respect to morphological and genetic differences, e. g. in the anamorphs of *H. australalbidus* Pöldmaa & Samuels and *H. lateritius* (Fr.: Fr.) Tul. (Helfer 1991, Pöldmaa & Samuels 2003). Within these and other species, conidial dimensions and cultural characteristics can differ considerably between strains of a single species, as it is the case in our strains of *C. semicircularare*. When sequences of the ITS region of rDNA or other DNA regions will be generated for more *Cladobotryum* species than presently available, analyses might provide a higher resolution for separating closely related species and narrower morphological species concepts. A similar development has occurred in the taxonomy of *Trichoderma* species (Samuels 2006).

Cladobotryum semicircularare appears to have a broad range of host fungi within the Agaricomycotina (Basidiomycota): agarics, pleurotoid mushrooms, *Ganoderma* species, polypores. A similarly wide host range could be shown for *C. asterophorum* with our own isolate growing on a polypore and adjacent bark in contrast to the strain from an agaric (Hoog 1978) used in the phylogenetic analysis, as well as for our strain of *C. varium* isolated from *Tremiscus helvelloides* (Auriculariales) and the strain of *H. aurantius*, the teleomorph of *C. varium*, from the polypore *Bjerkandera adusta* (Willd.: Fr.) P. Karst (Pöldmaa *et al.* 1999) used in the phylogenetic analysis. Whereas in some groups of *Cladobotryum* species, identification of species is now possible only by a combination of cultural, microscopic, host and molecular data, *C. semicircularare* can be identified by its exceptional conidial morphology.

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ing some of the strains included in this study, H.-C. Kuo for assisting in collecting species of *Cladobotryum* in Taiwan and Germany, B. Oertel for arranging a collecting trip to sites with *Hygrocybe* species including identification of *Hygrocybe* species in Germany, and the owner of the mushroom farm in Taiwan for allowing us to use the parasitized basidioma for study. We thank W. Gams for communicating the existence of Cuban strains of *C. semicirculare* to the first author, K. Pöldmaa for confirming the assumed conspecificity of the *C. semicirculare* strains, and G. Samuels for critically reading the manuscript. Studies in Taiwan were supported by a grant of the Alexander von Humboldt Foundation, by the German Academic Exchange Service (DAAD), and the National Science Council of Taiwan.

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