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# First report of pleoanamorphy in *Gyrothrix verticiclada* with an Idriella-like synanamorph

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**Abstract** – Pleoanamorphy with two types of conidia is reported for the first time in *Gyrothrix verticiclada*. The first conidial type has the normal morphology already known for *G. verticiclada,* but the second conidial type is identified here as an *Idriella*-like synanamorph. Both conidial types were isolated, then cultivated separately, photographed, and deposited in the Mycothèque de l'Université Catholique de Louvain (BCCM™/MUCL). The morphological similarities of both types of conidia were examined in comparison with conidia in other species of *Idriella* and *Gyrothrix* guided by a phylogenetic analysis of DNA sequences from three gene regions and morphological data. Phylogenetic analyses were performed with maximum parsimony as the optimality criterion using the program TNT. Results indicated that one individual of *Gyrothrix verticiclada* is associated with *Idriella cubensis* and seven with species of both genera. Both types of conidia, *Gyrothrix verticiclada* (MUCL54065) and the *Idriella*-like synanamorph (MUCL54064) were recovered as homologous. In addition to knowledge about pleomorphism of *G. verticiclada*, a BLAST search was made using ITS and LSU sequences in order to find the teleomorphic relationship of *G. verticiclada*. The teleomorphic connection suggests a correspondence with named genera belonging to the order Xylariales.

**Anamorphic fungi / pleomorphism /phylogeny / ITS / LSU / tef-1alpha**

## INTRODUCTION

The most studied relationship among portions of the pleomorphic life cycle of fungi is the one that links sexual (teleomorph) to asexual (anamorph) phases (Shenoy *et al.,* 2007), although in the asexual stage, some fungi are synanamorphic. Synanamorphy applies when a single fungus concomitantly or successively produces two or more anamorphic forms (Ulloa & Hanlin, 2006). Morphologically distinct anamorphs have been described in species such as: *Cylindrodendrum album* (Buffin

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& Hennebert, 1984), *Basifimbria spinosa* (Buffin & Hennebert, 1985), *Phialophora cyanescens* synana. *Phaeosclera* (De Vries *et al.,* 1984), *Ceratosporium caribense* synana. *Selenosporella* (Holubová-Jechová, 1988), etc. These studies only provided the taxonomic descriptions but did not address other aspects such as the phylogeny of the species. However, the phylogenetic identity of some synanamorph life stages has been explored in *Aschernia insperata* (Liu *et al.,* 2005) and *Moelleriella zhongdongii* (Tadych *et al.,* 2009).

The anamorphic genus *Gyrothrix* Corda (Corda) is characterized by the production of long setae (simple or ramified). The conidiogenous cells are generally ampulliform, somewhat obclaviform, hyaline to subhyaline, and are forming a palisade at the bases of the setae. The conidia are cylindrical to falcate, non-septate, hyaline, and aggregated into a mucilaginous mass (Pirozynski, 1962). In *G. verticiclada* (Goid.) S. Hughes and Piroz. the setae are dichotomic or verticillate, the conidiogenous cells are ampulliform to lageniform and the conidias are falciform (Hughes & Pirozinski, 1971). Recently, an unusual specimen of *G. verticiclada* was collected in southeastern México. It forms the setae typical of the species, but the apices also present a second conidial form strongly reminiscent of those of genus *Idriella* P.E. Nelson and S. Wilh. In this study, we present morphological observations of the two types of conidia in *Gyrothrix verticiclada*, and phylogenetic analyses of morphology and DNA sequence data to identify the relationships between the different anamorphs. In addition, we report sexual connection information of *G. verticiclada*.

## MATERIALS AND METHODS

### *Fungus isolation and morphological data*

Decomposing leaf litter was collected from the archeological zone of Tulum, Quintana Roo, México as part of a study that explores phylogenetic relationships of species within *Gyrothrix*. Leaf litter was placed in humidity chambers for one month to allow the growth of fungal colonies on the leaves. Over the course of two months, leaves from each chamber were periodically examined under a stereoscopic microscope (Zeiss Stemi SV11) to find the colonies of *Gyrothrix*. The samples from colonies were mounted on slides in polyvinyl alcohol (Permanent slides are available upon request from the first author). The morphology of different structures (conidia, conidiogenous cells, and setae) was examined and measured at a magnification of × 1000 with an optical microscope (Nikon Eclipse 5O*i*). Micrographs were obtained with a digital camera mounted on the C-tube of the microscope (Nikon Digital Sight DS-2Mv). Specialized literature was consulted in order to identify the species of *Gyrothrix* (Pirozynski, 1962; Hughes & Pirozynski, 1971; Sutton, 1993) and *Idriella* (Ellis, 1971; Castañeda & Kendrick, 1991; Castañeda-Ruiz & Arnold, 1985). At the same time, colonies were inoculated separately in potato dextrose agar (PDA) and corn meal agar (CMA). Cultures were deposited at BCCM™/MUCL, Université Catholique de Louvain, Belgium. In addition to the Mexican strains collected for this study, we examined strains of the genera *Idriella* and *Gyrothrix* from Africa, Australia, Europe and America provided by BCCM™/MUCL to get morphological and molecular characters for phylogenetic analysis (Table 1).

Table 1. Species of *Gyrothrix* and *Idriella* used in this study

| *Collection code* | *Taxon* | *Strain data* | *GenBank accession number* | | |
|---|---|---|---|---|---|
| | | | *ITS* | *LSU* | *tef1a* |
| MUCL41095 | *Idriella cagnizarii* | Castañeda, R. F., Brazil | KC775732 | KC775707 | KJ476985 |
| MUCL39017 | *I. cubensis* | Castañeda, R. F., Cuba | KC775733 | KC775708 | KJ476986 |
| MUCL4103 | *I. lunata* | Barron, G., Canada | KC775734 | KC775709 | KJ476987 |
| MUCL7551 | *I. lunata* | Hennebert, G., Holland | KC775735 | KC775710 | KJ476988 |
| MUCL39857 | *I. ramos*a | Castañeda, R. F., Cuba | KC775736 | KC775711 | KJ476989 |
| MUCL40962 | *I. rara* | Castañeda, R. F., Cuba | KC775737 | KC775712 | KJ476990 |
| *BE03 | *I. sp.* | Becerra-Hernández, C.I., México | KC775739 | KC775714 | KJ476991 |
| *BE11 | *I. sp.* | Becerra-Hernández, C.I., México | KC775740 | KC775715 | KJ476992 |
| *MUCL54045 | *I. sp.* | Becerra-Hernández, C.I., México | KC775741 | KC775716 | KJ476993 |
| MUCL41006 | *I. uncinospora* | Castañeda, R. F., Brazil | KC775742 | KC775717 | KJ476994 |
| MUCL33100 | *Gyrothrix circinata* | Decock, C., Malawi | KJ476966 | KJ476962 | KJ476969 |
| *MUCL54042 | *G. circinata* | Becerra-Hernández, C.I, México | KJ476967 | KJ476963 | KJ476970 |
| MUCL54182 | *G. circinata* | Seifert, K.A. Australia | KC775744 | KC775719 | KJ476971 |
| MUCL54185 | *G. circinata* | Seifert, K.A. Australia | KJ476968 | KJ476964 | KJ476972 |
| *BE108 | *G. dichotoma* | Becerra-Hernández, C.I., México | KC775745 | KC775720 | KJ476973 |
| *BE74 | *G. inops* | Becerra-Hernández, C.I., Cuba | KC775746 | KC775721 | KJ476974 |
| *MUCL54061 | *G. ramosa* | Becerra-Hernández, C.I., Cuba | KC775747 | KC775722 | KJ476975 |
| MUCL40992 | *G. verticiclada* | Castañeda, R. F., Venezuela | KC775748 | KC775723 | KJ476976 |
| MUCL41076 | *G. verticiclada* | Castañeda, R. F., Venezuela | KC775749 | KC775724 | KJ476977 |
| MUCL41150 | *G. verticiclada* | Castañeda, R. F., Venezuela | KC775750 | KC775725 | KJ476978 |
| *MUCL52554 | *G. verticiclada* | Becerra-Hernández, C.I., México | KC775751 | KC775726 | KJ476979 |
| *MUCL54063 | *G. verticiclada* | Becerra-Hernández, C.I., México | KC775752 | KC775727 | KJ476980 |
| *MUCL54064 | *Idriella*-like syn. | Becerra-Hernández, C.I., México | KC775755 | KC775730 | KJ476984 |
| *MUCL54065 | *G. verticiclada* | Becerra-Hernández, C.I., México | KC775753 | KC775728 | KJ476981 |
| MUCL54181 | *G. verticiclada* | Seifert, K.A. Australia | KC775754 | KC775729 | KJ476982 |
| *MUCL54054 | *G. verticillata* | Becerra-Hernández, C.I., México | KC775756 | KC775731 | KJ476983 |
| MUCL39135 | *Vermiculariopsiella inmersa* | Castañeda, R.F. Cuba | KJ476965 | KJ476961 | KJ476995 |

* Species collected during field expeditions for this study.

*DNA extraction, amplification, and sequencing*

The cultures were transferred to liquid media (potato dextrose broth) for 3 weeks to determine the biomass of each colony. The mycelium was washed with distilled water and used for DNA extraction following the protocol of the DNeasy Plant Mini Kit column (Qiagen, Valencia, CA, USA). The polymerase chain reaction (PCR) was used to amplify two loci from the ribosomal DNA operon [a portion of the large subunit ribosomal DNA (LSU) and internal transcribed spacer region (ITS), and a portion of the nuclear encoded translation elongation factor 1 alpha (*tef1*$\alpha$). Primers and PCR cycling parameters followed those described in Castlebury *et al.* (2004). Amplified DNA was sequenced using forward and reverse primers. Sequencing reactions were performed using the ABI BigDye® Terminator kit v 3.1 (Applied Biosystems, Foster City, CA USA), and analyzed on an Applied Biosystems 310 Genetic Analyzer. Sequence reads were trimmed and assembled using Sequencher v. 5.1 (Gene Codes Corporation, Ann Arbor, MI, USA, reg. 2503030). The aligned were performed with the platform ClustalW implemented in the program Mega 6.06 (Tamura *et al.,* 2013). Due sequence divergence, alignments were performed for each gene and then joined for the analyses. All new DNA sequences produced in this study were submitted to the GenBank database (Table 1). The Basic Local Alignment Search Tool (BLAST) was used to know the maximum identity between all internal transcribed spacer region (ITS) sequences from this study and the GenBank database to find probable teleomorph sequences.

*Phylogenetic analysis of morphology and sequence data*

A total evidence matrix comprising morphological characters and three DNA loci (ITS, LSU and *tef1*$\alpha$) was generated using Mesquite v. 2.75 (Maddison & Maddison, 2011). This matrix was deposited in TreeBase (S15122). Data were analyzed using maximum parsimony as the optimality criterion. Analyses were performed using the TNT program v 1.1 (Goloboff *et al.,* 2003). A total of 10000 replicates were performed using a combination of the three new technologies Ratchet (20 iterations), Drift (20 cycles) and tree fusing (10 rounds), random seed of 15. The taxon *Vermiculariopsiella inmersa* was used as outgroup based on a previous phylogenetic study on the *Gyrothrix microsperma* complex performed with molecular and morphological data (Becerra-Hernández, 2010). This study recovered species of *Vermiculariopsiella* as sister taxa to a clade comprising species of *Gyrothrix*. The close relationship of both genera has been previously discussed by different authors (Pirozynski & Patil, 1970; Kendrick, 1980; Wu *et al.,* 1997). Branch support was determined using the Bremer support index (Bremer, 1994) and the Jackknife re-sampling method with 1000 replicates and 36% independent character removal.

*Teleomorph connection*

In addition to pleomorphism of *G. verticiclada*, we investigated the telemorphic connection of all exemplars of *G. verticiclada* using the LSU and ITS sequences data. A BLAST query of the GenBank database was performed using Megablast (Morgulis *et al.,* 2008). Only the sequences with the highest similarity are reported.

Table 2. Description of morphological characters used in the phylogenetic analysis

| *Characters* | *Character states* |
|---|---|
| **0. Colony** | 0 = Punctiform; 1 = Amphigenous; 2 = Hypophyllous, 3 = Effuse |
| **1. Appearance colony** | 0 = Pilose 1 = Irregular; 2 = Velutinate |
| **2. Type of setae** | 0 = Absent; 1 = Simple; 2 = Branched |
| **3. Walled setae** | 0 = Absent; 1=Smooth; 2 = Verruculose; 2 = Rough |
| **4. Position setae** | 0 = Absent; 1=Erect; 2 = Flexuous |
| **5. Primary branches** | 0 = Absent; 1 = Dichotomous; 2 = In one direction; 3 = In whorls; 4 = Alternate |
| **6. Secondary branches** | 0 = Absent; 1 = Dichotomous; 2 = Alternate; 3 = In one direction; 4 = In whorls |
| **7. Tertiary branches** | 0 = Absent; 1 = Dichotomous |
| **8. Position of apices of the branches** | 0 = Absent; 1 = Straight; 2 = Curved; 3 = Circinate |
| **9. Apices of the branches** | 0 = Absent; 1 = Sterile; 2 = Fertile |
| **10. Type of conidiogenous cells in the fertile branches of setae** | 0 = Absent; 1 = Integrated |
| **11. Number of points on setae** | 0 = Absent; 1=1; 2=2; 3=3 |
| **12. Number of branches for point in the setae** | 0 = Absent; 1=1; 2=2; 3=3; 4=4 |
| **13. Type of central stipites of the setae** | 0=Absent; 1 = Determinate; 2 = Indeterminate |
| **14. Type of conidiophore** | 0 = Absent; 1 = Simple; 2 = Branched |
| **15. Primary branches of conidiophores** | 0 = Absent; 1 = Dichotomous; 2 = In whorls |
| **16. Secondary branches of conidiophores** | 0 = Absent; 1 = Dichotomous; 2 = In whorls |
| **17. Type of conidiogenous cells** | 0 = Integrated; 1 = Discrete |
| **18. Disposition of conidiogenous cells** | 0 = Forming a palisade at the setae base; 1 = In the apices of conidiophores |
| **19. Shape of conidiogenous cells in the conidiophores** | 0 = Absent; 1 = Denticulate |
| **20. Shape of conidiogenous cells below the setae** | 0 = Absent; 1 = Lageniform; 2 = Ampulliform |
| **21. Shape of conidiogenous cells in the branches of the setae** | 0 = Absent: 1 = Denticulate |
| **22. Crystals** | 0 = Absent; 1 = Present |
| **23. Clamydospores** | 0 = Absent; 1 = Present |
| **24. Shape of conidia produced in the palisade of conidiogenous cells at the setae base** | 0 = Absent; 1=Fusiform; 2 = Falciform; 3 = Cylindrical |
| **25. Shape of conidia produced in the apices of conidiophores** | 0 = Absent; 1 = Lunate; 1 = Falciform; 3 = Fusiform; 4 = Cylindrical; 5 = Subfalcate |
| **26. Shape of conidia produced in the fertile apices of the branches of the setae** | 0 = Absent; 1 = lunate |
| **27. Septo in conidia** | 0 = Absent; 1 = Present |
| **28. Conidia aggregation** | 0 = Solitary; 1 = Aggregated into mucilaginous mass; 3 = Fasciculate; 3 = Equilateral |

## RESULTS AND DISCUSSION

### Taxonomy

After two months of periodic examination of the decomposing leaf litter from field expeditions, two colonies of the anamorphic fungi *G. verticiclada,* three of *Idriella* and six of *Gyrothrix* were found growing on an unidentified dead leaf (Table 1). In the natural substrate, one of the two colonies of *G. verticiclada* developed two different types of conidia. The anamorph type 1 was identified as the conidia normally known for *G. verticiclada* (MUCL54065); the anamorph type 2 was identified as *Idriella*-like conidia (MUCL54064). In fungi, synanamorphs play different roles in survival and dispersal strategies, perhaps with different sets of active genes as starting points (Carmichael, 1981; Seifert & Samuels, 2000). The conidia of the synanamorphs of *G. verticiclada* differ in size, form and type of aggregation. *G. verticiclada* conidia (anamorph type 1) are falcate, 15-17 × 1-1.5 µm and are aggregated into a mucilaginous mass suited to water-mediated dispersal, while the *Idriella*-like synanamorph (anamorph type 2) conidia are lunate, 7-9 × 1.5-2 µm and freely produced, thus being appropriate for dispersal by wind or by microfauna.

***Gyrothrix verticiclada*** (Goid.) S. Hughes and Piroz. 1971. N.Z. Jl Bot. 9(1): 42 **Figs 1, 4-5**

= *Peglionia verticiclada* Goid., 1934. Malpighia 33: 5.

*Anamorph type 1:* **Colonies** scattered, pilose. Mycellium immersed in the substrate. **Setae** erect, straight, smooth, determinate, verticillate in 4 branches, sometimes 2 branches, frequently present 3 or 4 branches fertile; when the synanamorph is absent the size is 80-125 × 3-4 µm; when the synanamorph is present the setae are longer 180-220 µm. **Branches** straight dichotomously, apices present small denticles. When the synanamorph is absent branch size is 13-30 × 3-5 µm; when it is present branches are longer (40-65 × 3-4 µm). **Conidiogenous cells** holoblastic, ampulliform to lageniform, hyaline to subhyaline, 8-10 × 1 µm wide. **Conidia** falcate, non-septate, hyaline, 15-17 × 1-1.5 µm, aggregated into a mucilaginous mass.

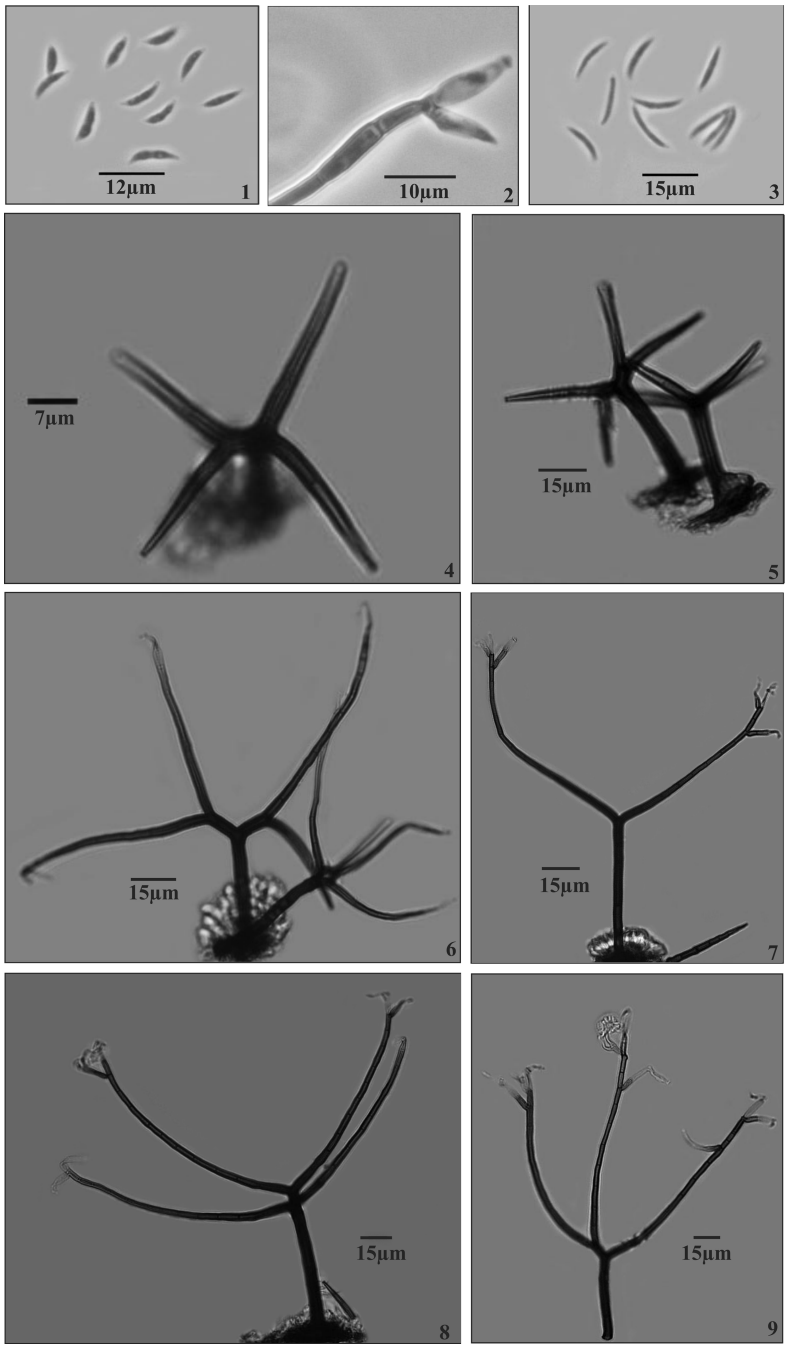
*Material examined:* MUCL54065. Quintana Roo, Tulum national Park. 20/12/2010. Leaf litter unknown. Col. *C. Becerra* BE16.

*Anamorph type 2:* **Synanamorph-like *Idriella*** **Figs 2-3, 6-9**

Conidiogenous cells integrate in the branch tips of the setae, cylindrical, polyblastic, denticulate, hyaline, 8-16 × 2-3 µm. Conidia lunate, non-septate, hyaline, 7-9 × 1.5-2 µm.

*Material examined:* MUCL54064. Quintana Roo, Tulum national Park. 20/12/2010. Leaf litter unknown. Col. *C. Becerra* BE15.

*Observations:* Colonies on corn meal agar-CMA begin the process of fructification after 10 days (with a diameter of 5-10 cm). The mycelium grows immersed and aerial. Both strains (MUCL54064 and MUCL54065) formed the two morphologically distinct asexual reproductive structures. Anamorph type 1 was typical of the specie *Gyrothrix verticiclada*: setae verticillate in 4 branches, conidiogenous cells ampulliform to lageniform, and conidia falcate. The anamorph type 2, were formed on three or four branches of a setae. Morphology of the anamorph type 2 presented denticulate conidiogenous cells and lunate conidia.




Figs 1-9. Morphology of the two anamorphs in *Gyrothrix verticiclada*. **3-5.** Anamorph type 1 of *Gyrothrix verticiclada* MUCL54065 showing the typical morphology. **3.** Conidia; **4** and **5.** Setae. 1-2; 6-9. Anamorph type 2, *Idriella*-like synanamorph MUCL54064. 1. Conidia; 2. Conidiogenous cell integrate in the branch tips of the setae. **6-9.** Setae with the *Idriella*-like synanamorph.

The individual MUCL54063 of *G. verticiclada* also formed the anamorph type 2, when grown on agar-CMA. When this individual was found in natural substrate the synanamorph was absent.

*Distribution:* England (Kirk, 1992), Argentina (Allegrucci *et al.,* 2005), Brazil (Grandi & Gusmão, 1995; Barbosa *et al.,* 2009a; Barbosa *et al.,* 2009b; Argôlo *et al.,* 2011), New Zealand (Hughes & Pirozynski, 1971; McKenzie, 1981), South Africa (Crous *et al.,*1996), Italy (Hughes & Pirozynski, 1971; Rambelli *et al.,* 2008; Lunghini *et al.,* 2013), México (Heredia-Abarca *et al.,* 1997), Cuba (Delgado-Rodriguez *et al.,* 2002), Venezuela (Castañeda-Ruiz *et al.,* 2003).

*Substrate: Gyranthera caribensis* (Castañeda-Ruiz *et al.,* 2003), *Knightia excelsa* (Hughes & Pirozinsky, 1971), *Cedrela fissilis* (Grandi & Gusmão, 1995), *Clusia melchiorii* (Barbosa *et al.,* 2009a; 2009b), *Clusia nemorosa* (Barbosa *et al.,* 2009a; 2009b), *Laurus nobilis* (Kirk, 1992), *Myrsine chathamica* (McKenzie, 1981), *Smilax aspera* (Rambelli *et al.,* 2008) *Cistus* sp. (Lunghini *et al.,* 2013), *Phillyrea angustifolia* (Lunghini *et al.,* 2013), *Pistacia lentiscus* (Lunghini *et al.,* 2013), *Quercus ilex* (Lunghini *et al.,* 2013) and unknown leaf litter (Heredia-Abarca *et al.,* 1997; Delgado-Rodríguez *et al.,* 2002).

Morphological polymorphism in *G. verticiclada* has been documented previously. The first report of this variability was based on one individual growing on *Podocarpus elongus* in Western Cape Province (Crous *et al.,* 1996). The morphological description of this exemplar is comparable with the individuals MUCL54064 and MUCL54065. Individual on *Podocarpus elongus* and our study form the typical morphology of *G. verticiclada*, setae branched verticillate in 4 branches, and the conidia are falcate. But it differs in two aspects:

– **Branch Apices.** The original description of *G. verticiclada* (Hughes & Pirozinski, 1971) defined the branch apices as often fractured and appearing flattened, but did not state whether these were fertile. Furthermore, the individual reported in Crous *et al.* (1996) presented a swollen and fertile apex featuring small denticles or bumps when young, that burst open resembling collarettes typical of the phialides formed by enteroblastic conidiogenesis on the genus *Dictyochaeta*. However, the conidia were rarely formed on the branch apices. Similarly, the individuals reported in our study present fertile denticles at the tips of the branches, but the branch tips are differentiated and branched, so that they never burst and always form conidia.

– **Conidia.** The original description reported that conidia are falcate, 0-septos, hyaline and produced continually on the conidiogenous cells (at the setae base). Crous *et al.* (1996) described the typical morphology, but also found that the conidia were formed at the apices of the branches. In this study, the individual formed the conidia as individual collected by Crous *et al.* (1996) however, morphologically different conidia were produced. Conidia that formed on the conidiogenous cells at the setae base presented the same morphology as those described for *G. verticiclada*: falcate, 0-septos, hyaline, 17-21 × 2 µm. Furthermore, conidia produced in the fertile branch tips are lunate and smaller (7-9 × 1.5-2 µm), and appear similar to conidia produced by the genus *Idriella.*

Other studies have described the typical morphology of *G. verticiclada* (McKenzie, 1981; Kirk, 1992; Grandi & Gusmão, 1995; Heredia *et al.,* 1997; Delgado *et al.,* 2002; Castañeda-Ruiz *et al.,* 2003; Allegrucci *et al.,* 2005; Barbosa *et al.,* 2009a; 2009b; Argôlo *et al.,* 2011; Lunghini *et al.,* 2013); however, one specimen collected in Pantelleria, Italy (Rambelli *et al.,* 2008) presented another type of variation. The colony had simple setae, but since other morphological characters coincided with the description of *G. verticiclada sensu stricto*, the authors decided to keep the species as *G. verticiclada* (Rambelli *et al.,* 2008).

## Phylogenetic analyses

The total evidence matrix included twenty-nine morphological characters and 2106 nucleotides for 27 taxa. From the 2135 characters, 709 were parsimony-informative. Parsimony analysis yielded one optimal tree of 2777 steps, CI = 78, and RI = 93 (Fig. 10). Our analysis indicated that neither *Gyrothrix* nor *Idriella* formed monophyletic entities, as their sequences were scattered over various part of the tree (Fig. 10). The type species of *Idriella* (*I. lunata*) was recovered in a clade containing four species of *Gyrothrix* (*G. ramosa*, *G. inops, G. verticillata* and *G. circinata*). The two anamorphic types of conidia, *G. verticiclada* (anamorph type 1, MUCL54065) and the *Idriella*-like synanamorph (anamorph type 2, MUCL54064) were recovered as homologous with almost no support and resulted sister to a clade containing *I. uncinospora*, *G. dichotoma,* and two unidentified species of *Idriella* (Fig. 10).

The generic circumscription of *Idriella* and *Gyrothrix* has mainly been based on morphological characters without consideration of their phylogenetic

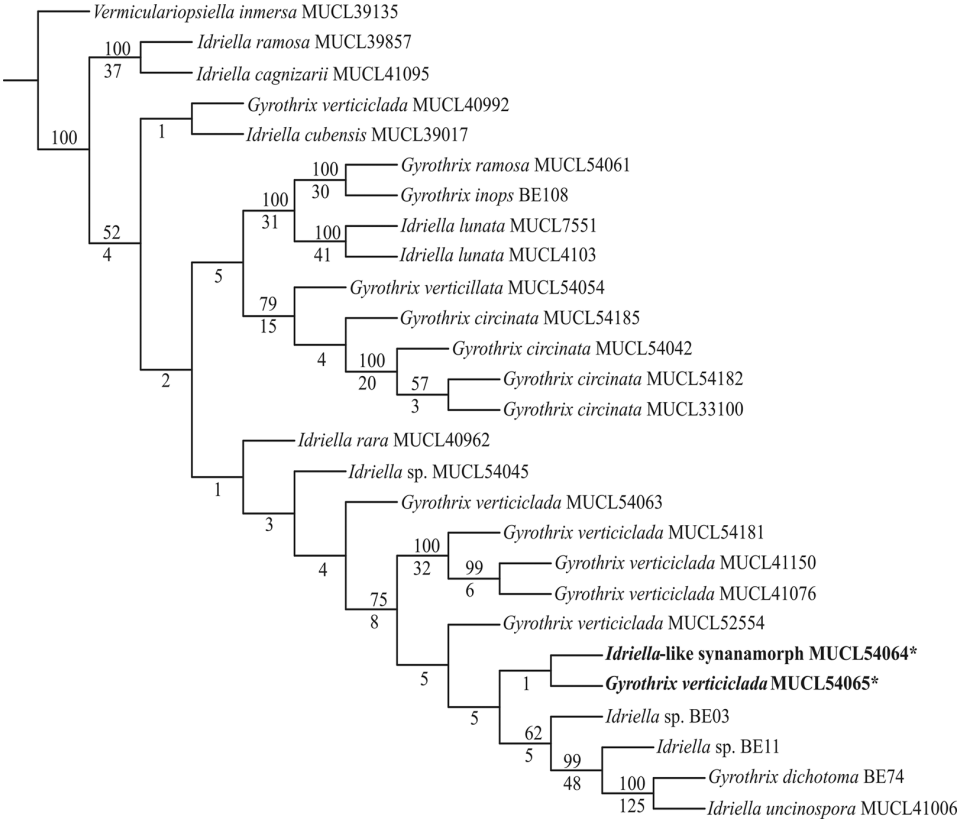



Fig. 10. Most parsimonious tree for phylogenetic relationships of *G. verticiclada* and other species of *Gyrothrix* and *Idriella* inferred from the combined analysis of morphological and molecular data (ITS, LSU and *tef1*$\alpha$). Jacknife values are indicated above branches, while Bremer support is indicated below.

Table 3. Teleomorph sequences with maximum identity after the BLAST search of ITS and LSU sequences for eight individuals of *G. verticiclada*

| *Strain* | *ITS* | *GenBank accession number* | *% of Identity* | *LSU* | *GenBank accession number* | *% of Identity* |
|---|---|---|---|---|---|---|
| MUCL40992 | *Hypoxylon stygium* | JX997755.1 | 99 | *Pseudomassaria carolinensis* | DQ810233 | 96 |
| MUCL41076 | *Microdochium bolleyi* | KC989068.1 | 95 | *Xylaria papulis Anthostomella eucalyptorum* | DQ890026.1 | 94 |
| MUCL41150 | *Microdochium bolleyi* | KC989068.1 | 91 | *Xylaria papulis Anthostomella eucalyptorum* | DQ890026.1 | 94 |
| * MUCL52554 | *Microdochium nivale* | AB272124.1 | 91 | *Microdochium phragmitis* | EU926218.1 | 95 |
| * MUCL54063 | *Microdochium* sp. | KJ188679.1 | 86 | *Microdochium phragmitis* | EU926218.1 | 95 |
| * MUCL54064 | *Microdochium phragmitis* | EU926218.1 | 96 | *Microdochium phragmitis* | EU926218.1 | 96 |
| * MUCL54065 | *Microdochium nivale* | AB586986.1 | 91 | *Microdochium phragmitis* | EU926218.1 | 95 |
| MUCL54181 | *Microdochium bolleyi* | KC989068.1 | 90 | *Microdochium bolleyi Xylaria papulis* | HM216199.1 JQ862641.1 | 93 |

* Species collected during field expeditions for this study.

position. Recent molecular phylogenetic studies have shown that several anamorphic fungi are polyphyletic such as: *Sporidesmium, Ellisembia*, *Linkosia*, *Repetophragma*, *Sporidesmiella*, *Stanjehughesia*, *Diploccium* and *Spadicoides* (Shenoy *et al.,* 2006; 2010); *Chalara* and *Volutella* (Fernández *et al.,* 2006); and *Fusarium* (Gräfenhan *et al.,* 2011). Therefore, for solving the phylogenetic position of *Gyrothrix* and *Idriella* it would also be necessary to include in a phylogenetic analysis the type species of *Gyrothrix* and most if not all species for both genera. The highest identity in the Megablast search of the LSU and ITS sequences of *G. verticiclada* are presented in Table 3. Based on both loci, five individuals of *G. verticiclada* included for this study from México, Venezuela and Cuba are related with different species of the genus *Microdochium,* while three individuals provided by BCCM™/MUCL were related to different ascomycete genera (Table 3). Nonetheless, both markers suggest that all individuals of *G. verticiclada* included in this study are associated with the order Xylariales (Table 3). The Mycobank database does not establish any sexual connection to the *Gyrothrix* genus (Crous *et al.,* 2004; Robert *et al.,* 2005), while the *Dictionary of the fungi* places the *Gyrothrix* genus in the Ascomycota phylum, but does not refer it to a specific order (Kirk *et al.,* 2001). The ITS sequences for MUCL40992 had the highest similarity (99%) with *Hypoxylon stygium,* while the LSU sequences had the highest similarity (96%) with *Pseudomassaria carolinensis* (Table 3). In aquatic hyphomycetes the ITS region has been proposed as barcode for identifying species (Seena *et al.,* 2010), while LSU sequences have been used in the *Chaetosphaeriaceae* to identify the teleomorph connection for some anamorphic genera (Shenoy *et al.,* 2006).

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