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Short Communication

The phylogenetic position of *Culbersonia* is in the *Caliciaceae* (lichenized ascomycetes)

Culbersonia Essl. is a monotypic genus originally based on *C. americana* Essl., a taxon described from Arizona, United States of America (Esslinger 2000). Soon after publication, it was realized that this species had already been described in 1980, as *Pyxine nubila* Moberg, from Africa (Moberg 1980). Consequently, the combination *Culbersonia nubila* (Moberg) Essl. was made (Nash *et al.* 2002). The species combines characters of *Pyxine* Fr. with those of *Physconia* Poelt. It is widely distributed in dry subtropical regions around the world but is most common in Africa and Central America, where it grows on trees and rocks (Swinscow & Krog 1988; Moberg 2004; Obermayer *et al.* 2009).

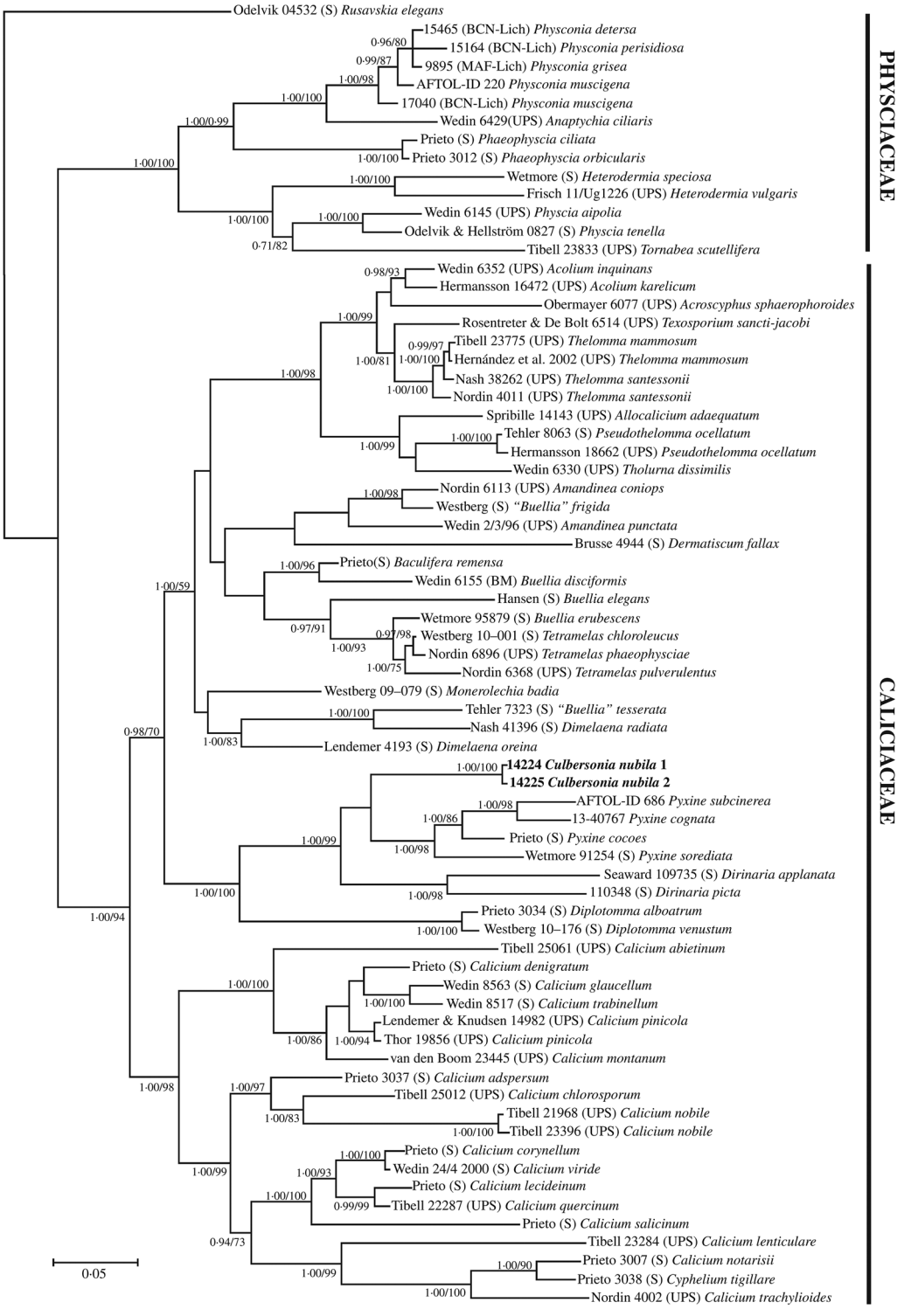
Culbersonia is currently thought to belong in the *Physciaceae* Zahlbr. because it morphologically resembles the genus *Physconia* Poelt (Esslinger 2000), but this position has not yet been confirmed by genetic analysis (Lücking *et al.* 2016). The type species was originally described in the genus *Pyxine* Fr., which is currently classified in a different family, *Caliciaceae* Chevall (Prieto & Wedin 2017). These two families constitute the order *Caliciales* Bessey in the current sense. Almost all species in both families are lichenized. Both families contain crustose, foliose and a small number of fruticose growth forms, and together contain *c.* 600 known species. Non-mazaedioid genera, previously classified in the *Physciaceae* or *Buelliaceae* Zahlbr., have been added to the family *Caliciaceae* (which previously contained only mazaedioid taxa) after molecular work (Wedin *et al.* 2002). Many genera classified for a long time in the *Caliciales* (see e.g. Tibell 1984) are now relocated in other families, orders, and classes following molecular work. However, it is worth noting that all six genera that Tibell (1984) classified in the *Caliciaceae* still remain in that family in the present sense.

Culbersonia nubila was identified by us among lichens collected in South Africa (Maphangwa *et al.* 2018). As the phylogenetic position of this monotypic genus is uncertain, we produced genetic data from our specimens and generated a phylogenetic tree to establish the most suitable taxonomic status of *Culbersonia*.

Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). PCR amplification was performed with the primers ITS1F and ITS4 (White *et al.* 1990; Gardes & Bruns 1993) for the rDNA internal transcribed spacer 1, 5.8S and internal transcribed spacer 2 (collectively referred to as ITS), and LR0R and LR5 (Vilgalys & Hester 1990; Cubeta *et al.* 1991) were used to amplify the 28S rDNA of the nuclear ribosomal repeat. PCR reactions were performed under a program consisting of a hot start at 95 °C for 5 min, followed by 35 cycles at 94 °C, 54 °C and 72 °C (45, 30 and 45 s respectively) with a final 72 °C step for 10 min. PCR products were checked in 1% agarose gels and positive reactions were sequenced with one of the PCR primers.

BLAST (Altschul *et al.* 1997) of 5.8S-ITS2 and 28S rDNA sequences was used to select the most closely related taxa; 5.8S-ITS2 and 28S rDNA were the only regions amplified from the samples. ITS only or 28S rDNA only phylogenies produced significant support for some clades, such as that including *Culbersonia*, *Dirinaria* and *Pyxine*, but failed to recover significant support values at the family level. Therefore, a 5-gene phylogeny was produced to add some resolution at the supraspecific level. Thus, 5.8S-ITS2, 28S rDNA, mtSSU rDNA, *Mcm7* and β-tubulin sequences of representative members of the *Caliciaceae* were downloaded from GenBank, mainly originating from Schmull *et al.* (2011) and Prieto & Wedin (2017), including most sequences available for the genus *Pyxine* when all five loci were available for the species. *Rusavskia elegans* was used as outgroup, following Prieto & Wedin (2017). Sequences were first aligned

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in MEGA 5.0 (Tamura *et al.* 2011) with the ClustalW application and then corrected manually. Ambiguous regions were not removed from the alignment. ITS1 was excluded because of the insertions/deletions making alignment difficult. Gblocks (Castresana 2000) was employed to remove ambiguous positions from 5.8S-ITS2, 28S rDNA and mtSSU, while introns were manually removed from *Mcm7* and β-tubulin datasets. The final alignment included 116/254 variable sites in the 5.8S and ITS2 regions, 219/642 in the 28S rDNA, 212/513 in the mtSSU rDNA, 221/444 in the *Mcm7* gene, and 228/624 in the β-tubulin gene. The aligned loci were loaded as independent partitions (*Mcm7* and β-tubulin each split into three partitions, one for each codon position) in PAUP* 4.0b10 (Swofford 2001) and subjected to MrModeltest 2.3 (Nylander 2004). The model GTR+Γ+I was implemented for all partitions (except the 2nd position of β-tubulin, for which HKY+Γ+I was employed) in MrBayes 3.1 (Ronquist & Huelsenbeck 2003), where a Bayesian analysis was performed (two simultaneous runs, six chains, temperature set to 0·2, sampling every 100th generation) until convergence parameters were met after *c.* 890 000 generations (on which the Bayesian analysis is based), standard deviation having fallen below 0·01. A total of 8900 trees were sampled but the first 25% (2225) was discarded as burn-in; the others were used to produce a consensus tree. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML (Stamatakis 2006) using the standard search algorithm (data partitioned, GTRMIX model, 2000 bootstrap replications). The significance threshold was set above 0·95 for posterior probability (PP) and 70% bootstrap proportions (BP).

*Specimens sequenced (*Culbersonia nubila*).* **South Africa:** *Gauteng Province*: Pretoria, Arcadia, Stanza Bopape Street, *c.* 25°44′41″S, 28°12′30″E, 1332 m, 20 vii 2016, *Maphangwa & Zedda* [KWM_0205] (PRE), no. 205 = ALV 14225, GenBank MH121318 (ITS) & MH121320 (LSU); Pretoria, Pionier Museum, Keuning Dr, *c.* 25°44′07″S, 28°18′36″E, 1317 m, 14 xi 2016, *Maphangwa* [KWM_0103] (PRE), no. 103 = ALV 14224, GenBank MH121317 (ITS) & MH121319 (LSU).

The two analyzed specimens of *Culbersonia nubila* clustered together with only a small number of different base pairs (Fig. 1). They clustered with significant statistical support with the monophyletic genera *Pyxine* and *Dirinaria* (Tuck.) Clem., nested deep within the family *Caliciaceae*, and they are unrelated to the *Physciaceae*. Therefore, we propose to classify the genus *Culbersonia* in the *Caliciaceae* in the current sense.

Morphologically, *Culbersonia* is quite distinct from other foliose lichens in the *Caliciales*. The upper surface is thickly pruinose, grey with a bluish tint, K+ rose-violet, and green when wet. The lower surface is pale tan, with the same bluish tint as on the upper surface in a broad marginal zone. *Culbersonia* shares with *Pyxine* the presence of an internal stipe in the apothecia. However, apothecia are extremely rare in *Culbersonia* and currently only one apothecium has been reported (Moberg 1980). Conidia are cylindrical to fusiform, but also rare and again only reported from one specimen (Nash *et al.* 2002). The overall aspect of the thallus, however, mostly resembles that of the genus *Physconia*, with which it also shares the pale lower surface which is almost invariably black in all species of *Pyxine* and *Dirinaria*.

The monophyletic group formed by *Culbersonia*, *Pyxine* and *Dirinaria* is morphologically distinguished from the other *Caliciaceae* by the appressed foliose growth form and the absence of a mazaedium, and ecologically by its predominance in the (sub-) tropics.

Almost all other genera in the two families *Physciaceae* and *Caliciaceae* that are represented in Fig. 1 are shown to be monophyletic. These results agree with those produced by Wedin *et al.* (2002), Helms *et al.* (2003), Gaya *et al.* (2012), Miadlikowska *et al.* (2014) and Prieto & Wedin (2017), where the lineages comprising the families of *Physciaceae* and *Caliciaceae* were shown to be significantly different. Three main clades were found within *Caliciaceae*, those of *Calicioideae*, *Buellioideae* s. str. and the clade containing *Dirinaria*, *Pyxine* and *Diplotomma* Flot., also in agreement with previous authors. The

FIG. 1. Consensus phylogram produced in MrBayes after the analysis of a combined ITS + nuLSU rDNA + mtSSU rDNA + *Mcm7* + β-tubulin-alignment of species in the families *Caliciaceae* and *Physciaceae* demonstrating the phylogenetic position of *Culbersonia nubila* nested within the *Caliciaceae*. Values next to nodes represent Bayesian PP and maximum likelihood bootstrap proportions (BP); only those nodes supported by >0·95 PP or >70% BP are annotated. Taxon names are preceded by the collectors of the voucher specimen and herbarium location for specimens not mentioned in the text. Further details of the species included in the tree can be found in Schmull et al. (2011) and Prieto & Wedin (2017).

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exception is the genus *Buellia* De Not., which contains many species that should be reclassified into other genera. Such species are marked as "*Buellia*" in Fig. 1.

Author ORCIDs

André Aptroot 0000-0001-7949-2594.

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References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1997) Basic local alignment search tool. *Journal of Molecular Biology* **215:** 403–410.

Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17:** 540–552.

Cubeta, M. A., Echandi, E., Abernethy, T. & Vilgalys, R. (1991) Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. *Phytopathology* **81:** 1395–1400.

Esslinger, T. L. (2000) *Culbersonia americana*, a rare new lichen (Ascomycota) from western America. *Bryologist* **103:** 771–773.

Gardes, M. & Bruns, T. D. (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2:** 113–118.

Gaya, E., Högnabba, F., Holguin, Á., Molnar, K., Fernández-Brime, S., Stenroos, S., Arup, U., Søchting, U., van den Boom, P., Lücking, R., *et al.* (2012) Implementing a cumulative supermatrix approach for a comprehensive phylogenetic study of the *Teloschistales* (Pezizomycotina, Ascomycota). *Molecular Phylogenetics and Evolution* **63:** 374–387.

Helms, G., Friedl, T. & Rambold, G. (2003) Phylogenetic relationships of the *Physciaceae* inferred from rDNA sequence data and selected phenotypic characters. *Mycologia* **95:** 1078–1099.

Lücking, R., Hodkinson, B. P. & Leavitt, S. D. (2016) The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – approaching one thousand genera. *Bryologist* **119:** 361–416.

Maphangwa, K. W., Sipman, H. J. M., Tekere, M. & Zedda, L. (2018) Epiphytic lichen diversity on *Jacaranda* and *Acacia* trees in Pretoria (Tshwane, Republic of South Africa). *Herzogia* **31:** 949–964.

Miadlikowska, J., Kauff, F., Högnabba, F., Oliver, J. C., Molnár, K., Fraker, E., Gaya, E., Hafellner, J., Hofstetter, V., Gueidan, C., *et al.* (2014) A multigene phylogenetic synthesis for the class Lecanoromycetes (Ascomycota): 1307 fungi representing 1139 infrageneric taxa, 317 genera and 66 families. *Molecular Phylogenetics and Evolution* **79:** 132–168.

Moberg, R. (1980) Studies on *Physciaceae* (Lichens) 1. A new species of *Pyxine*. *Norwegian Journal of Botany* **27:** 189–191.

Moberg, R. (2004) Notes on foliose species of the lichen familiy *Physciaceae* in southern Africa. *Symbolae Botanicae Upsalienses* **34:** 257–288.

Murray, M. G. & Thompson, W. F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* **8:** 4321–4325.

Nash, T. H., III, Ryan, B. D., Gries, C. & Bungartz, F. (eds) (2002) *Lichen Flora of the Greater Sonoran Desert Region, Vol. 1*. Tempe, Arizona: Lichens Unlimited, Arizona State University.

Nylander, J. A. A. (2004) *MrModeltest v2*. Program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University.

Obermayer, W., Kalb, K., Sipman, H. J. M. & Nash III, T. H. (2009) New reports of *Culbersonia nubila* (Moberg) Essl. from the Tibetan Region, Bolivia, Argentina, Lesotho and South Africa. *Lichenologist* **41:** 683–687.

Prieto, M. & Wedin, M. (2017) Phylogeny, taxonomy and diversification events in the *Caliciaceae*. *Fungal Diversity* **82:** 221–238.

Ronquist, F. & Huelsenbeck, J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Schmull, M., Miadlikowska, J., Pelzer, M., Stocker-Wörgötter, E., Hofstetter, V., Fraker, E., Hodkinson, B. P., Reeb, V., Kukwa, M., Lumbsch, H. T., *et al.* (2011) Phylogenetic affiliations of members of the heterogeneous lichen-forming fungi of the genus *Lecidea sensu* Zahlbruckner (Lecanoromycetes, Ascomycota). *Mycologia* **103:** 983–1003.

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.

Swinscow, T. D. V. & Krog, H. (1988) *Macrolichens of East Africa*. London: British Museum (Natural History).

Swofford, D. L. (2001) *PAUP*4.0b10: Phylogenetic Analysis Using Parsimony (and Other Methods)*. Sunderland, Massachusetts: Sinauer Associates.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28:** 2731–2739.

Tibell, L. (1984) A reappraisal of the taxonomy of *Caliciales*. *Beiheft zur Nova Hedwigia* **79:** 597–713.

Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172:** 4238–4246.

Wedin, M., Baloch, E. & Grube, M. (2002) Parsimony analyses of mtSSU and nITS rDNA sequences

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reveal the natural relationships of the lichen families *Physciaceae* and *Caliciaceae*. *Taxon* **51:** 655–660.

White, T. J., Bruns, T., Lee, S. & Taylor, J. W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications* (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 315–322. New York: Academic Press.

André Aptroot, Khumbudzo Walter Maphangwa, Luciana Zedda, Memory Tekere, Pablo Alvarado and Harrie J. M. Sipman

A. Aptroot: Laboratório de Botânica/Liquenologia, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Avenida Costa e Silva s/n, Bairro Universitário, CEP 79070-900, Campo Grande, Matto Grosso do Sul, Brazil. Email: andreaptroot@gmail.com.

K. W. Maphangwa and M. Tekere: Department of Environmental Sciences, University of South Africa (Science Campus), P.O. Box X6, Florida, 1710, South Africa.

L. Zedda: BIO-Diverse, Ließemer Straße 32a, 53179 Bonn, Germany.

P. Alvarado: ALVALAB, Av. Bruselas 2 3B, 33011 Oviedo, Spain.

H. J. M. Sipman: Botanischer Garten und Botanisches Museum, Freie Universität, Königin-Luise-Straße 6–8, 14195 Berlin, Germany.

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