The Lichenologist (2024), **56**, 371–377
doi:10.1017/S0024282924000380

CAMBRIDGE UNIVERSITY PRESS

Standard Paper

Apothecia trump setae: *Paratricharia* belongs in the *Aulaxina* clade and is distant from *Tricharia* (lichenized *Ascomycota*: *Gomphillaceae*)

Robert Lücking[1], José Luis Chaves-Chaves[2] and Bibiana Moncada[1,3]

[1]Botanischer Garten und Botanisches Museum Berlin, Freie Universität Berlin, 14195 Berlin, Germany; [2]Parcelas Quebrada Azul, Tilarán, Guanacaste, Costa Rica and [3]Licenciatura en Biología, Universidad Distrital Francisco José de Caldas, Torre de Laboratorios, Herbario, Bogotá, Colombia

Abstract

The phylogenetic relationships of the monospecific genus *Paratricharia*, with the single species *P. paradoxa*, within the family *Gomphillaceae* are resolved using newly generated sequences of the mtSSU and nuLSU markers for three specimens collected in Costa Rica. The results support placement as sister to the genus *Caleniopsis*, the two genera sister to a clade containing the genera *Aulaxina* and *Aulaxinella*. This placement confirms earlier studies based on cladistic analysis of phenotype characters and phenotype-based phylogenetic binning, suggesting that apothecial features are more informative for the phylogenetic placement of taxa within *Gomphillaceae* than thallus characteristics.

Keywords: *Batistomyces*; *Graphidales*; *Microxyphiomyces*; *Santricharia*; *Tricharia*

(Accepted 4 September 2024; first published online 31 December 2024)

Introduction

Gomphillaceae is the dominant element of foliicolous lichen communities in tropical rainforests, with well over 300 leaf-dwelling species known (Lücking *et al.* 2005, 2017; Lücking 2008; Xavier-Leite *et al.* 2022). The family is uniquely characterized by peculiar conidiomata, the so-called hyphophores, which are mostly setiform and produce hyphal drops that serve as asexual propagules (Vězda 1979; Sérusiaux & De Sloover 1986; Vězda & Poelt 1987; Lücking 1997, 2008; Lücking *et al.* 2005). Several lineages have also evolved sterile thallus setae, probably derived from setiform hyphophores; these were previously included in the collective genus *Tricharia* Fée s. lat. (Santesson 1952; Vězda & Poelt 1987; Lücking 1997).

The circumscription of the family, as well as the delimitation of genera contained within it, has undergone substantial changes since it was first described by Watson (1929) for the single genus, *Gomphillus* Nyl., and in its current sense by Vězda & Poelt (1987), who recognized ten genera. Given the sparse molecular data at the time (Lücking *et al.* 2004), Dennetière & Péroni (1998) and Lücking *et al.* (2005) made an attempt to define genera based on a quantitative, cladistic analysis, and the latter authors increased the number of genera to 19. Until the year 2018, this number had increased to 27 (Lücking *et al.* 2017; Diederich *et al.* 2018). Recently, Xavier-Leite *et al.* (2022) provided the first broad molecular phylogenetic approach for the *Gomphillaceae*, confirming most of the phenotypically defined genera but identifying an additional 19 genus-level clades which were subsequently formally instated (Xavier-Leite *et al.* 2023, 2024). The underlying phylogenetic study supported the notion that a combination of apothecial and hyphophore features characterizes genera in this family.

The genus *Paratricharia* Lücking (Lücking 1997) was established to accommodate a single species originally described in *Tricharia* Fée, *T. paradoxa* Lücking (Lücking 1991), which deviated from all other *Gomphillaceae* known at the time by forming apothecia with a carbonized zeorine margin, such as in the genus *Aulaxina* Fée, combined with robust, sterile black thallus setae, seen for example in the genus *Tricharia* as defined by Lücking *et al.* (2005). The new taxon further differed from other genera by producing a sterile columella in the centre of the apothecial disc, a feature otherwise known from the genus *Ocellularia* G. Mey. and its relatives in the sister family *Graphidaceae* (Kraichak *et al.* 2015).

Thus far, the phylogenetic relationships of *Paratricharia* have been unresolved. Phenotype-based cladistic analysis predicted the placement in a clade containing the genera *Aulaxina* and *Caleniopsis* Vězda & Poelt, as well as *Aplanocalenia* Lücking *et al.* and *Rolueckia* Papong *et al.* (the latter as *Caleniopsis*; Lücking *et al.* 2005). Phenotype-based phylogenetic binning predicted placement of the genus in the early diverging *Calenia tri-septata* Zahlbr. clade, now placed in the new genus *Caleniella* Xavier-Leite *et al.* (Xavier-Leite *et al.* 2024). These results ascribed a greater predictive power to apothecial features than to the presence of sterile, black setae, characteristic of the distantly placed genus *Tricharia* s. lat. in which the species was originally described (now divided into *Batistomyces* Xavier-Leite *et al.*,

Corresponding author: Robert Lücking; Email: r.luecking@bo.berlin

Cite this article: Lücking R, Chaves-Chaves JL and Moncada B (2024) Apothecia trump setae: *Paratricharia* belongs in the *Aulaxina* clade and is distant from *Tricharia* (lichenized *Ascomycota*: *Gomphillaceae*). *Lichenologist* **56**, 371–377. https://doi.org/10.1017/S0024282924000380

Microxyphiomyces Bat. *et al*., *Santricharia* Xavier-Leite *et al*. and *Tricharia*; Xavier-Leite *et al*. 2023), but not known in *Aulaxina* or *Caleniopsis*. Unfortunately, molecular data for *Paratricharia* had not been obtained for the study by Xavier-Leite *et al*. (2022).

Here, we resolve the phylogenetic placement of *Paratricharia* employing newly generated sequences of the mtSSU and nuLSU markers, obtained from material collected in Costa Rica.

Material and Methods

The newly sequenced specimens of *Paratricharia paradoxa* were collected in 1992 and 1997 at La Selva Biological Station in Costa Rica. Since then, the material had been kept in the freezer at −4 to −20 °C after drying and pressing, which apparently helped to preserve the DNA since we had no problems in amplifying the mtSSU and nuLSU markers after DNA extraction. We also included three specimens of *Gyalectidium*, representing *G. filicinum* Müll. Arg. and *G. imperfectum* Vězda, collected by Claudia Hartmann in Costa Rica.

Pieces of thalli from three different specimens were removed and placed in 1.5 ml Eppendorf tubes. Total genomic DNA was extracted at the laboratories of the Botanischer Garten und Botanisches Museum, Freie Universität Berlin, employing the Sigma-Aldrich REDExtract-N-Amp Plant PCR Kit (St Louis, Missouri, USA), following the manufacturer's instructions but using lower amounts of reagents to obtain a lower amount of DNA extract.

Electrophoresis on a 1.5% agarose gel was used to assess the quality of the extracted DNA. In order to amplify the mitochondrial small subunit rRNA (mtSSU), the primer pair mrSSU1 and MSU7 (Zhou & Stanosz 2001) was used, with the following settings for the PCR: initial denaturation for 10 min at 94 °C, 45 s at 94 °C, 45 s at 50 °C, followed by 35 cycles of 45 s at 94 °C, then 10 min at 72 °C. Using the primer pair LR3 and LR0R (Vilgalys & Hester 1990), the nuclear large subunit rRNA (nuLSU) was amplified through PCR as follows: initial denaturation for 3 min at 95 °C, 45 s at 95 °C, 45 s at 54 °C, 1 min at 72 °C, then 35 cycles of 45 s at 95 °C, and a final elongation for 10 min at 72 °C. PCR products were purified with ExoSAP-IT™ (IT PCR Cleanup protocol) and sent for sequencing to Macrogen Europe (Amsterdam, the Netherlands; https://dna.macrogen.com/en).

The new sequences obtained were added to a subset of the concatenated mtSSU-nuLSU alignment provided by Xavier-Leite *et al*. (2022), for a total of 85 terminals representing the main clades of the *Gomphillaceae* (Table 1). We removed one mtSSU sequence previously identified as *Calenia triseptata* (MZ827298), since a recheck revealed it to represent *Aulaxina quadrangula*. Instead of using *Fissurina* (*Graphidaceae*) as an outgroup, we performed ingroup routing with the genus *Gyalidea*, an early diverging clade within the family (Xavier-Leite *et al*. 2022), to reduce alignment ambiguity. The resulting alignment, with a length of 949 bp for the mtSSU region and 660 bp for the nuLSU region, was manually inspected in BioEdit v. 7.2.0 (Hall 1999, 2011). Since only a small number of narrow, ambiguously aligned regions were identified using the HoT scores approach on the Guidance web server (http://guidance.tau.ac.il) (Penn *et al*. 2010*a*, *b*), we did not remove them prior to analysis.

The final concatenated alignment was analyzed with a maximum likelihood (ML) approach using RAxML v. 8.2.0 (Stamatakis 2014) locally, employing the reversible GTR model with 1000 non-parametric bootstrap replicates. To test for potential monophyly of *Paratricharia* with *Tricharia* s. lat., we performed a Shimodaira-Hasegawa (SH) test as implemented in RAxML v. 8.2.0.

Results

The topology in our best-scoring ML tree matched the topology obtained in our previous study (Xavier-Leite *et al*. 2022), with the exception of the *Caleniella triseptata* clade, now positioned as an early diverging clade and no longer part of the *Aulaxina* clade; the previous result was caused by the erroneous mtSSU sequence now removed (MZ827298). Further early diverging clades included the genera *Rolueckia*, *Taitaia* Suija *et al*., and *Corticifraga* D. Hawksw. & R. Sant., the remaining genera forming a large, yet unsupported clade (Fig. 1). Within that clade, the topology largely matched that found in our previous analysis, with the difference that the *Calenia-Echinoplaca* complex formed a late-diverging clade; however, the backbone topology was overall not supported.

The three specimens of *Gyalectidium* clustered within their respective species, *G. filicinum* and *G. imperfectum* (Fig. 1). *Paratricharia paradoxa* was recovered with absolute support as sister to the genus *Caleniopsis*, this clade being strongly supported as sister to a clade containing the genera *Aulaxina* s. str. and *Aulaxinella* (Fig. 1). *Paratricharia* was not found to be closely related to either *Tricharia* s. str. or the *T. vainioi* R. Sant. and *T. santessonii* R. Sant. clades (*Microxyphiomyces*, *Santricharia*), the three groups that produce sterile, black setae. The SH test also rejected the monophyly of *Paratricharia* with *Microxyphiomyces*, *Santricharia* or *Tricharia* s. str. Since *Paratricharia* agrees with the latter three genera in the presence of robust, sterile, black setae, while its apothecial features are more in agreement with those of *Aulaxina* (Fig. 2), our results support the view that apothecial characters are more reliable in predicting the phylogenetic placement of this taxon. *Paratricharia* is now the fifth clade known to produce such large, sterile, black setae.

Discussion

Our study confirmed our previous assessment suggesting that apothecial features are more informative regarding the phylogenetic placement of *Paratricharia paradoxa* than the presence of sterile, black setae, based on which the species had originally been described in the genus *Tricharia* s. lat. (Lücking 1991). Instead, molecular data resolved this taxon close to *Caleniopsis* and *Aulaxina*, notably in the same area of the tree as a previous cladistic analysis based on phenotype data alone, as well as a phenotype-based binning approach, had suggested (Lücking *et al*. 2005; Xavier-Leite *et al*. 2024).

Given that phenotypic characters in *Paratricharia* are conflicting, with the apothecial features most similar to *Aulaxina*, the thallus features most similar to *Tricharia*, plus a columella as unique autapomorphy (Lücking 1997, 2008), the previous placement close to *Aulaxina* based on phenotype characters alone, using two different approaches, and its confirmation with molecular data, seems surprising. However, studies in other lichenized fungi have shown the predictive power of phenotype features when analyzed in the proper context, such as in the *Graphidaceae* or *Arthoniales* (Berger *et al*. 2011; Lücking & Kalb 2018; Perlmutter *et al*. 2020).

https://doi.org/10.1017/S0024282924000380 Published online by Cambridge University Press

Table 1. GenBank Accession numbers of *Gomphillaceae* material used in this study, with voucher information, including collectors for the newly generated sequences (highlighted in bold)

Genus	Species	Country / Voucher	GB mtSSU	GB nuLSU
Actinoplaca	*strigulacea*	Guatemala	MZ827235	—
Actinoplaca	*strigulacea*	Guatemala / Brazil	MZ827235	MZ851583
Asterothyrium	*leucophthalmum*	Brazil	—	MZ851613
Asterothyrium	*leucophthalmum*	Brazil	—	MZ851619
Asterothyrium	*longisporum*	Costa Rica	AY341363	AY341349
Asterothyrium	*microsporum*	Brazil	—	MZ851549
Asterothyrium	*rotuliforme*	Brazil	—	MZ851615
Asterothyrium	*rotuliforme*	Guatemala	—	MZ851740
Aulaxina	*intermedia*	Brazil	MZ827227	MZ851657
Aulaxina	*opegraphina*	Brazil	MZ827294	MZ851672
Aulaxina	*quadrangula*	Brazil	MZ827291	MZ851678
Aulaxina	*quadrangula*	Brazil	MZ827295	MZ851673
Aulaxina	*submuralis*	Brazil	—	MZ851627
Aulaxina	*submuralis*	Brazil	MZ827224	MZ851618
Aulaxinella	*minuta*	Brazil	—	MZ851579
Aulaxinella	*minuta*	Brazil	MZ827230	MZ851578
Calenia	*depressa*	Costa Rica	MZ827256	MZ851711
Calenia	*lueckingii*	Costa Rica	—	MZ851716
Calenia	*lueckingii*	Mexico	—	MZ851741
Calenia	*phyllogena*	Costa Rica	AY341366	AY341352
Calenia	*phyllogena*	Mexico	—	MZ851744
Caleniella	*triseptata*	Brazil	—	MZ851691
Caleniella	*triseptata*	Brazil	—	MZ851663
Caleniopsis	*laevigata*	Brazil	—	MZ851667
Caleniopsis	*laevigata*	Brazil	—	MZ851675
Corticifraga	*peltigerae*	India	KY661684	KY661661
Corticifraga	*peltigerae*	Luxembourg	—	KY462801
Echinoplaca	*diffluens*	Brazil	—	MZ851538
Echinoplaca	*diffluens*	Mexico	AY341367	AY341353
Echinoplaca	*epiphylla*	Brazil	MZ827282	MZ851592
Echinoplaca	*epiphylla*	Mexico	AY341368	AY341354
Echinoplaca	*pellicula*	Brazil	—	MZ851484
Echinoplaca	*pellicula*	Guatemala	—	MZ851736
Gomphillus	*americanus*	USA	KY353115	KY381580
Gomphillus	*calycioides*	USA	MK318271	MH887485
Gomphillus	*ophiosporus*	Costa Rica	AY341371	AY341357
Gyalectidium	*areolatum*	Brazil	—	MZ851596
Gyalectidium	*areolatum*	Brazil	—	MZ851598
Gyalectidium	*filicinum*	Brazil	MZ827171	MZ851521
Gyalectidium	***filicinum***	**Costa Rica, *Hartmann* s. n.**	**PP928950**	**—**
Gyalectidium	***imperfectum***	**Costa Rica, *Hartmann* s. n.**	**PP928951**	**—**
Gyalectidium	***imperfectum***	**Costa Rica, *Hartmann* s. n.**	**PP928952**	**—**

(*Continued*)

https://doi.org/10.1017/S0024282924000380 Published online by Cambridge University Press

Table 1. (*Continued*)

Genus	Species	Country / Voucher	GB mtSSU	GB nuLSU
Gyalectidium	*imperfectum*	Guatemala	MZ827236	MZ851731
Gyalidea	*fritzei*	Sweden	HM244744	HM244767
Gyalidea	*hyalinescens*	Costa Rica	DQ972996	DQ973046
Microxyphiomyces	*lancicarpus*	Brazil	MZ827225	MZ851629
Microxyphiomyces	*lancicarpus*	Brazil	MZ827274	MZ851628
Microxyphiomyces	*similis*	Brazil	MZ827214	—
Microxyphiomyces	*similis*	Brazil	MZ827218	—
Microxyphiomyces	*vainioi*	Brazil	MZ827199	MZ851527
Microxyphiomyces	*vainioi*	Brazil	MZ827280	MZ851585
Monocalenia	*monospora*	Costa Rica	KF833339	KF833325
Monocalenia	*monospora*	Cuba	MZ827264	MZ851728
Paratricharia	***paradoxa***	**Costa Rica, *Lücking* s. n.**	**PP928953**	**PP928947**
Paratricharia	***paradoxa***	**Costa Rica, *Lücking* s. n.**	**PP928954**	**PP928948**
Paratricharia	***paradoxa***	**Costa Rica, *Lücking* s. n.**	**PP928955**	**PP928949**
Psathyromyces	*heterellus*	Brazil	MZ827198	MZ851523
Psathyromyces	*heterellus*	Brazil	MZ827221	MZ851643
Rolueckia	*aggregata*	Brazil	—	MZ851690
Rolueckia	*aggregata*	Brazil	—	MZ851692
Rolueckia	*conspersa*	Brazil	—	MZ851644
Roselviria	*purulhensis*	Brazil	MZ827172	MZ851512
Roselviria	*purulhensis*	Brazil	MZ827283	MZ851595
Rubrotricha	*subhelminthospora*	Brazil	MZ827207	MZ851642
Rubrotricha	*subhelminthospora*	Brazil	MZ827220	MZ851639
Santricharia	*farinosa*	Brazil	—	MZ851515
Santricharia	*farinosa*	Brazil	—	MZ851516
Spinomyces	*aggregatus*	Brazil	—	MZ851588
Spinomyces	*albostrigosus*	Brazil	—	MZ851511
Sporocybomyces	*leucotrichoides*	Brazil	MZ827184	MZ851534
Sporocybomyces	*leucotrichoides*	Costa Rica	AY341369	AY341355
Taitaia	*aurea*	Kenya	—	MF372801
Taitaia	*aurea*	Kenya	MF372798	MF372796
Tricharia	*amazonum*	Brazil	—	MZ851482
Tricharia	*amazonum*	Guatemala	—	MZ851738
Tricharia	*longispora*	Brazil	MZ827219	MZ851638
Tricharia	*longispora*	Costa Rica	AY341374	AY341360
Tricharia	*paraguayensis*	Brazil	MZ827273	MZ851565
Tricharia	*paraguayensis*	Brazil	MZ827276	MZ851495
Tricharia	*urceolata*	Brazil	—	MZ851571
Tricharia	*urceolata*	Brazil	—	MZ851572
Verruciplaca	*verrucifera*	Brazil	MZ827169	MZ851517
Verruciplaca	*verrucifera*	Brazil	MZ827195	MZ851563
Vezdamyces	*vulgaris*	Brazil	—	MZ851481
Vezdamyces	*vulgaris*	Brazil	—	MZ851636

https://doi.org/10.1017/S0024282924000380 Published online by Cambridge University Press

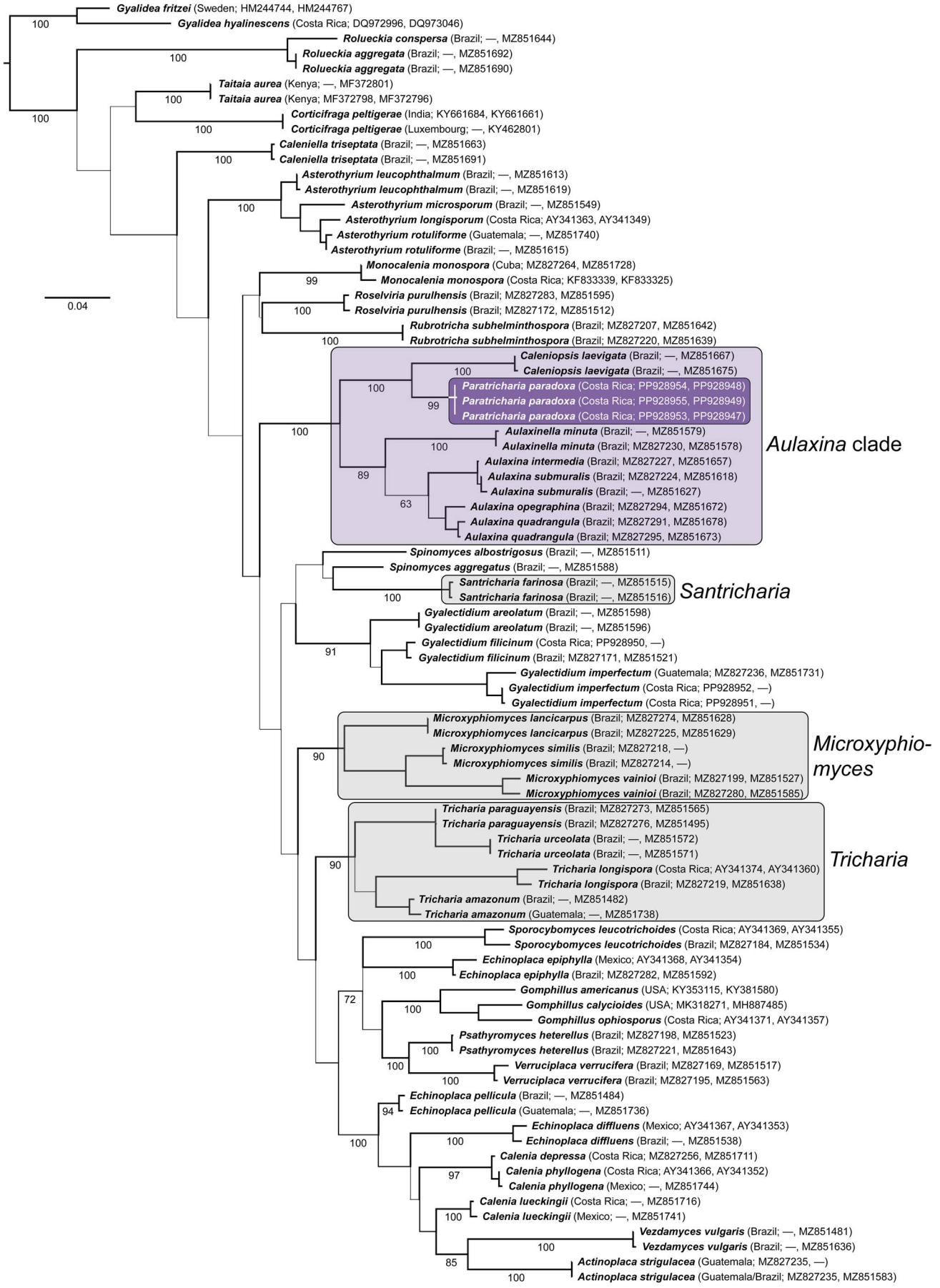

Figure 1. Best-scoring maximum likelihood tree of *Gomphillaceae* using the mtSSU and nuLSU markers, based on selected terminals representing most genus-level clades, showing placement of the genus *Paratricharia*. Bootstrap support values are shown below branches (to genus-level clades only). In colour online.

https://doi.org/10.1017/S0024282924000380 Published online by Cambridge University Press

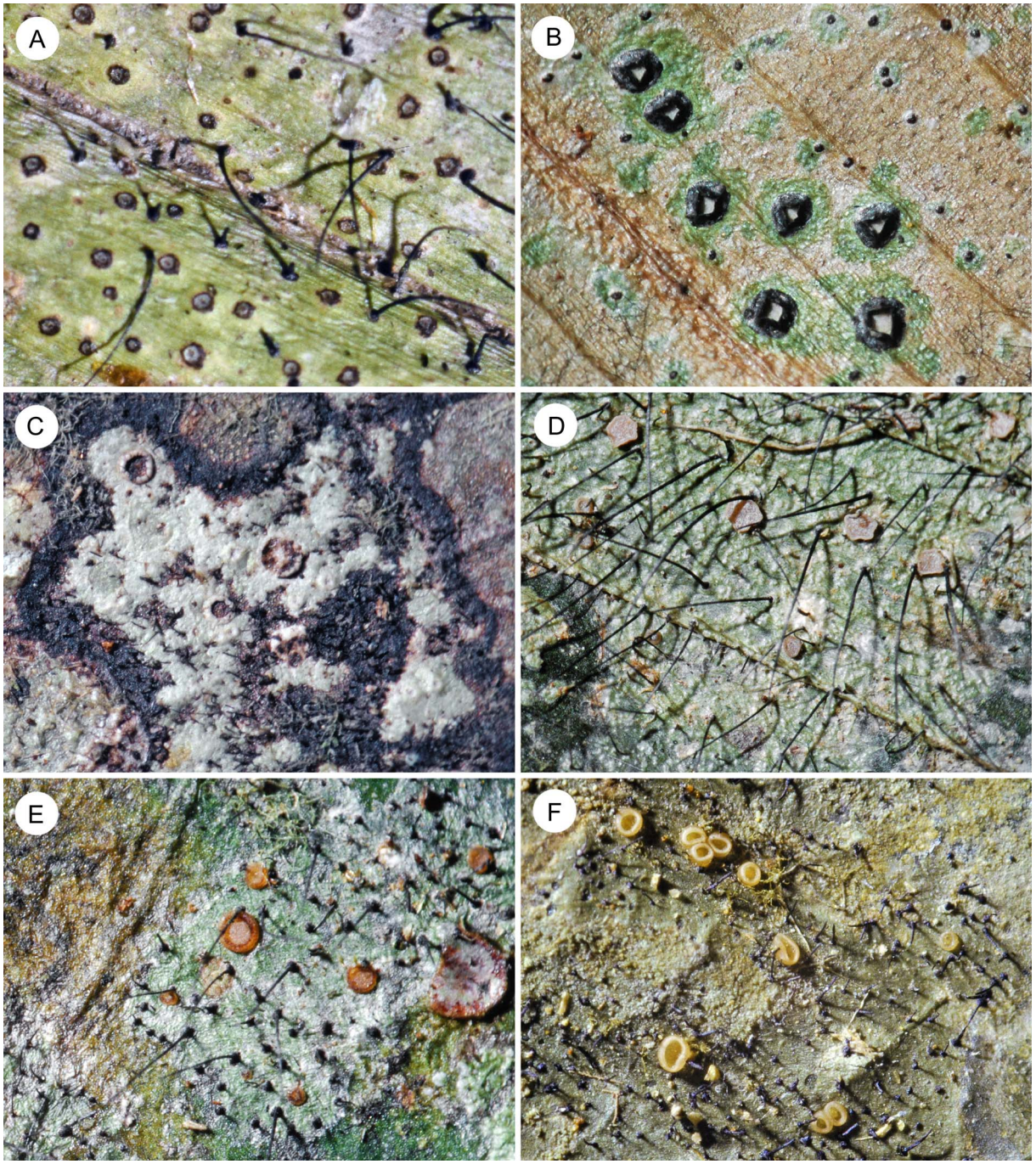

Figure 2. Morphology of selected genera and species of *Gomphillaceae*. A, *Paratricharia paradoxa*. B, *Aulaxina submuralis*. C, *Caleniopsis laevigata*. D, *Tricharia carnea*. E, *Microxyphiomyces vainioi*. F, *Batistomyces pallidus*. In colour online.

Our results support the findings by Xavier-Leite *et al.* (2022, 2024) that apothecial features are more conserved in the *Gomphillaceae*, thus having greater predictive power in the phylogenetic placement of taxa and showing a greater correlation with larger clades within the family. Hyphophore and thallus features are highly informative for genus-level clades, but similar phenotypes also evolved independently in different areas of the tree. Thus, in addition to *Paratricharia*, sterile, black setae are apomorphies for four genus-level clades in the family: *Tricharia* s. str., the *T. vainioi* clade (*Microxyphiomyces*), the *T. santessonii* clade (*Santricharia*) and the *T. pallida* Vězda clade (*Batistomyces*; see Xavier-Leite *et al.* 2023). Similarly, genera with red-brown (*Rolueckia*, *Rubrotricha* Lücking *et al.*) or white setae (e.g. *Aderkomyces* Bat., *Arthotheliopsis* Vain., *Echinoplaca* Fée, *Psathyromyces* Bat. & Peres, *Roselviria* Xavier-Leite *et al.*) are not necessarily closely related (Xavier-Leite *et al.* 2022, 2023).

The function of these sterile setae is not known but for them to have evolved independently in so many clades, one would assume strong environmental pressure. Setiform hyphophores probably evolved to facilitate dispersal of the diahyphae in the semi-aquatic environment that leaf surfaces display during rainfall (Lücking 2001, 2008), but sterile setae do not bear diahyphae. Notably, in

https://doi.org/10.1017/S0024282924000380 Published online by Cambridge University Press

almost all cases, the sterile setae are longer and more robust than the hyphophores in the same species (Vězda 1979; Sérusiaux & De Sloover 1986; Vězda & Poelt 1987; Lücking 1997, 2008).

Acknowledgements. The material of *Paratricharia paradoxa* was collected as part of a research project funded by the DAAD (Kennziffer 320/605/500/1; Jahresstipendium; 1991–1992) and the Deutsche Forschungsgemeinschaft (Kennzeichen LU 597/1-1; 'Neotropical Foliicolous Lichens: Taxonomy, Systematics, Biogeography, Diversity, Ecology, and Applications'; 1995–1997). Claudia Hartmann kindly provided the material of *Gyalectidium*. Colleagues and staff at La Selva Biological Station in Costa Rica are warmly thanked for their hospitality and support. The sequencing work for this study was supported by the Verein der Freunde des Botanischen Gartens Berlin. We thank Elise Lebreton for assisting in rechecking the underlying sequence data used for this study and discovering the problematic mtSSU sequence of *Caleniella triseptata* (MZ827298).

Author ORCIDs. Robert Lücking, 0000-0002-3431-4636; Bibiana Moncada, 0000-0001-9984-2918.

References

Berger SA, Stamatakis A and Lücking R (2011) Morphology-based phylogenetic binning of the lichen genera *Graphis* and *Allographa* (*Ascomycota*: *Graphidaceae*) using molecular site weight calibration. *Taxon* **60**, 1450–1457.

Dennetière B and Péroni J (1998) Approche phylogénétique des *Gomphillaceae*. *Cryptogamie Bryologie Lichénologie* **19**, 105–121.

Diederich P, Lawrey JD and Ertz D (2018) The 2018 classification and checklist of lichenicolous fungi, with 2000 non-lichenized, obligately lichenicolous taxa. *Bryologist* **121**, 340–425.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–98.

Hall TA (2011) BioEdit: an important software for molecular biology. *GERF Bulletin of Biosciences* **2**, 60–61.

Kraichak E, Lücking R and Lumbsch HT (2015) A unique trait associated with increased diversification in a hyperdiverse family of tropical lichen-forming fungi. *International Journal of Plant Sciences* **176**, 597–606.

Lücking R (1991) Neue Arten foliikoler Flechten aus Costa Rica, Zentralamerika. *Nova Hedwigia* **52**, 267–304.

Lücking R (1997) Additions and corrections to the knowledge of the foliicolous lichen flora of Costa Rica. The family *Gomphillaceae*. *Bibliotheca Lichenologica* **65**, 1–109.

Lücking R (2001) Lichens on leaves in tropical rainforests: life in a permanently ephemerous environment. *Dissertationes Botanicae* **346**, 41–77.

Lücking R (2008) Foliicolous lichenized fungi. *Flora Neotropica Monograph* **103**, 1–873.

Lücking R and Kalb K (2018) Formal instatement of *Allographa* (*Graphidaceae*): how to deal with a hyperdiverse genus complex with cryptic differentiation and paucity of molecular data. *Herzogia* **31**, 535–561.

Lücking R, Stuart BL and Lumbsch HT (2004) Phylogenetic relationships of *Gomphillaceae* and *Asterothyriaceae*: evidence from a combined Bayesian analysis of nuclear and mitochondrial sequences. *Mycologia* **96**, 283–294.

Lücking R, Sérusiaux E and Vězda A (2005) Phylogeny and systematics of the lichen family *Gomphillaceae* (*Ostropales*) inferred from cladistic analysis of phenotype data. *Lichenologist* **37**, 123–170.

Lücking R, Hodkinson BP and Leavitt SD (2017) ['2016']. The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – approaching one thousand genera. *Bryologist* **119**, 361–416.

Penn O, Privman E, Ashkenazy H, Landan G, Graur D and Pupko T (2010*a*) GUIDANCE: a web server for assessing alignment confidence scores. *Nucleic Acids Research* **38**, W23–W28.

Penn O, Privman E, Landan G, Graur D and Pupko T (2010*b*) An alignment confidence score capturing robustness to guide tree uncertainty. *Molecular Biology and Evolution* **27**, 1759–1767.

Perlmutter GB, Rivas Plata E, LaGreca S, Aptroot A, Lücking R, Tehler A and Ertz D (2020) *Biatora akompsa* is revealed as a disjunct North American species of *Pentagenella* (*Opegraphaceae*) through molecular phylogenetic analysis and phenotype-based binning. *Bryologist* **123**, 502–516.

Santesson R (1952) Foliicolous lichens I. A revision of the taxonomy of the obligately foliicolous, lichenized fungi. *Symbolae Botanicae Upsalienses* **12**(1), 1–590.

Sérusiaux E and De Sloover JR (1986) Taxonomical and ecological observations on foliicolous lichens in northern Argentina, with notes on the hyphophores of *Asterothyriaceae*. *Veröffentlichungen des Geobotanischen Institutes der Eidgenössischen Technischen Hochschule, Stiftung Rübel, Zürich* **91**, 260–292.

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313.

Vězda A (1979) Flechtensystematische Studien. XI. Beiträge zur Kenntnis der Familie *Asterothyriaceae* (*Discolichenes*). *Folia Geobotanica Phytotaxonomica* **14**, 43–94.

Vězda A and Poelt J (1987) Flechtensystematische Studien. XII. Die Familie *Gomphillaceae* und ihre Gliederung. *Folia Geobotanica Phytotaxonomica* **22**, 179–198.

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

Watson W (1929) The classification of lichens. Part I. *New Phytologist* **28**, 1–36.

Xavier-Leite AB, Cáceres MES, Aptroot A, Moncada B, Lücking R and Goto BT (2022) Phylogenetic revision of the lichenized family *Gomphillaceae* (*Ascomycota*: *Graphidales*) suggests post-K–Pg boundary diversification and phylogenetic signal in asexual reproductive structures. *Molecular Phylogenetics and Evolution* **168**, 107380.

Xavier-Leite AB, Goto BT, Lücking R and Cáceres MES (2023) New genera in the lichenized family *Gomphillaceae* (*Ascomycota*: *Graphidales*) focusing on neotropical taxa. *Mycological Progress* **22**, 88.

Xavier-Leite AB, Goto BT, Cáceres MES and Lücking R (2024) Reclassification of species in the lichenized family *Gomphillaceae* (*Ascomycota*: *Ostropales*) using morphology-based phylogenetic binning. *Cryptogamie Mycologie* **45**, 83–99.

Zhou S and Stanosz GR (2001) Primers for amplification of mt SSU rDNA, and a phylogenetic study of *Botryosphaeria* and associated anamorphic fungi. *Mycological Research* **105**, 1033–1044.

https://doi.org/10.1017/S0024282924000380 Published online by Cambridge University Press