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Clypeoria, a new genus, with clypeate hyphophores separated from *Gyalideopsis* s. lat. (Gomphillaceae, lichenized Ascomycota)

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ABSTRACT. – A new genus *Clypeoria*, including two newly combined species (*C. japonica* and *C. lambinonii*), is proposed, that is segregated from *Gyalideopsis* s. lat. This genus is characterized by the presence of clypeate hyphophores with a central reduced stipe on the underside, moniliform diahyphae with droplet-shaped cells at the terminal segments, and adnate ascomata. Among the polyphyletic assemblage corresponding to *Gyalideopsis* s. lat., *Clypeoria* is distinguished from *G. peruviana*, the type species of the genus, which is characterized by sessile apothecia with a well-developed exciple and the absence of hyphophores. *Gyalideopsis peruviana* is presently included in the *G. africana* group, the other members of which differ from *Clypeoria* by the production of setiform-capitate hyphophores and musicolous habit. Both *C. japonica* and *C. lambinoni*i were previously treated in the *G. palmata* group, due to the presence of flabelliform and corticolous habit. However, in analysis of mtSSU and nuLSU sequence data, *C. japonica* was recovered in a large clade including the ecologically distinct foliicolous genera *Arthotheliopsis*, *Psathyromyces*, *Sipmanidea*, and *Verruciplaca*, which also produce adnate and spot-like apothecia but differ in producing yellow-orange apothecia and/or different hyphophore types. For its part, the *G. palmata* group appears to be heterogeneous, including various types of apothecia and hyphophores. The remaining species in the *G. palmata* group should be revised through future taxonomic research.

KEYWORDS. – Ascomata, conidiomata, corticolous, Japan, nuITS, nuLSU, mtSSU, phylogeny, *RPB2*.

INTRODUCTION

The genus *Gyalideopsis* Vězda, one of the largest genera within Gomphillaceae, contains species primarily with adnate to stipitate, biatorine or lecideine apothecia, and squamiform to stipitate hyphophores (Lücking et al. 2005, 2006). It was originally described by Vězda (1972) to accommodate four species and has grown to include to approximately 100 species, distributed mainly in tropical regions, with some species in temperate to boreal areas (Harada & Kawakami 2011, Holien & Tønsberg 1996, Lendemer & Lücking 2004, Lücking et al. 2007, Xavier-Leite et al. 2018). The genus has a wide ecological amplitude, being found on nearly all the substrata that lichens are known to occur (Lücking et al. 2005, 2006).

The morphological heterogeneity of *Gyalideopsis*, especially regarding the apothecia and hyphophores, has long been recognized, and has led to the establishment of segregate genera, including

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Diploschistella Vain., *Ferraroa* Lücking, Sérus. & Vězda, *Jamesiella* Lücking, Sérus. & Vězda, and *Lithogyalideopsis* Lücking, Sérus. & Vězda (Lücking et al. 2005). The remaining species of *Gyalideopsis* s. lat. have been divided into several groups, suggesting the necessity of additional investigation using molecular techniques (Lücking et al. 2005). In recent molecular studies by Xavier-Leite et al. (2022, 2023), two new genera, *Adelphomyces* Xavier-Leite, M. Cáceres & Lücking and *Vezdamyces* Xavier-Leite, M. Cáceres & Lücking, were separated from *Gyalideopsis* s. lat. These studies support the hypothesis that hyphophore types in Gomphillaceae reflect evolutionary relationships among related species (Xavier-Leite et al. 2022). However, the analysis included only a few species of *Gyalideopsis* s. lat., highlighting the need for a more detailed investigation, focusing particularly on the morphological characteristics of the hyphophores.

Within *Gyalideopsis*, there is one subgroup characterized by a distinctive clypeate (i.e., round shield-shaped) hyphophores, with an extremely reduced central stipe (Harada & Vězda 2000, Lücking et al. 2005, Vězda 1979). There are two species with this distinctive clypeate hyphophore morphology: *G. japonica* H. Harada & Vězda and *G. lambinonii* Vězda (Harada & Vězda 2000, Vězda 1979). Cladistic analyses based on phenotypic data have already suggested that these two species form a monophyletic group (Lücking et al. 2005), emphasizing the importance of re-evaluating their taxonomic placement through molecular phylogenetic analyses.

In this study, we examined the placement of these two species based on morphological and chemical data and DNA sequence data obtained from *G. japonica*. As a result, we propose the establishment of a new genus, *Clypeoria*, to accommodate these species, which are distinct from *Gyalideopsis*.

MATERIALS AND METHODS

MORPHOLOGY AND CHEMISTRY. – To examine the morphological and chemical characters uniting *Gyalideopsis japonica* and *G. lambinonii*, as well as to compare them with the type species of *Gyalideopsis*, *G. peruviana*, morphological observations and chemical analyses were conducted on the following specimens: eight specimens of *G. japonica* collected by us in Japan and exsiccati of *G. lambinonii* (Kalb, *Lich. Neotrop. 64*, TNS) and *G. peruviana* G. Merr. ex Vězda (Kalb, *Lich. Neotrop. 462*, TNS).

Morphological observations and photography were performed using a dissecting microscope (SZX16; Olympus, Tokyo, Japan) and a differential interference contrast microscope (BX51; Olympus) with a digital camera (EOS Kiss X10i; Canon, Tokyo, Japan). Anatomical examinations were performed using hand-cut sections mounted in GAW (glycerin: ethanol: water = 1: 1: 1) solution (Asahina 1936).

Secondary substances were analyzed using a high-performance thin layer chromatography (HPTLC) following Schumm and Elix (2015). The solvent system B′ (n-hexane: methyl tert-butyl ether: formic acid, 140: 72: 18) (Culberson & Johnson 1982) was used for HPTLC. The spot color was checked under 254 and 366 nm wavelength of UV and visible light, before and after spraying with 10% sulfuric acid on the HPTLC plate and charring at 90°C for 20 minutes.

DNA EXTRACTION, PCR AMPLIFICATION AND SEQUENCING. – DNA was extracted from fresh material collected within three years according to a modified method of Izumitsu et al. (2012) (see also Miyazawa et al. 2022) or a modified CTAB protocol (Hosaka 2009). The voucher specimens for DNA extractions are housed in TNS (Table 1).

For PCR amplification, 10 µL of PCR mix contained 1 µL genomic DNA extraction, 0.25 µL of each primer (10 pmol/µL) and 5 µL EmeraldAmp® MAX PCR Master Mix (TaKaRa Bio Inc.). The partial sequences of the internal transcribed spacer and the large subunit of the nuclear ribosomal RNA gene (nuITS and nuLSU), the small subunit of the mitochondrial ribosomal RNA gene (mtSSU), and the RNA polymerase II gene (*RPB2*) were amplified with the primer sets ITS1F (Gardes & Bruns 1993) and LR1 (Vilgalys & Hester 1990) for nuITS, LIC24R (Miadlikowska & Lutzoni 2000) and LR7 or LR3 (Vilgalys & Hester 1990) for nuLSU, mrSSU1 and mrSSU3R (Zoller et al. 1999) for mtSSU, and fRPB2-5F and fRPB2-7cR (Liu et al. 1999) for *RPB2*. The PCR conditions followed the methods of Ohmura et al. (2006) for nuITS, that of Miyazawa and Ohmura (2023a) for nuLSU, and that of Miyazawa and Ohmura (2023b) for mtSSU. The PCR conditions for *RPB2* were as follows: initial denaturation at 94°C for 2 minutes; followed by 35 cycles of denaturation at 94°C for 20 seconds, annealing at 52°C for 1 minute, and extension at 72°C for 2 minutes; and a final extension at 72°C for 15 minutes.

Table 1. Voucher information with GenBank Accession numbers and references in the phylogenetic analysis (Fig. 3). New sequences obtained in this study are in bold.

Taxon	Country; voucher	mtSSU	nuLSU	Reference
Arthotheliopsis trichariodes	Brazil; *A. Xavier-Leite 1924* (ISE)	MZ827202	MZ851505	Xavier-Leite et al. (2022)
Bezerroplaca lucernifera	Costa Rica; *R. Lücking s.n.* (F)	AY341370	AY341356	Lücking et al. (2004)
Clypeoria japonica	Japan; *K. Miyazawa 1036* (TNS)	**LC834121**	**LC834127**	This study
C. japonica	Japan; *M. Chaki 79* (TNS)	**LC834122**	**LC834128**	This study
C. japonica	Japan; *M. Chaki 84* (TNS)	**LC834123**	**LC834129**	This study
C. japonica	Japan; *M. Chaki 191* (TNS)	**LC834124**	**LC834130**	This study
C. japonica	Japan; *M. Chaki 420* (TNS)	**LC834125**	**LC834131**	This study
C. japonica	Japan; *Y. Ohmura 14150* (TNS)	**LC834126**	**LC834132**	This study
Echinoplaca campanulata	Brazil; *M. Cáceres & R. Lücking 167* (B, URM)	MZ827250	MZ851703	Xavier-Leite et al. (2022)
E. epiphylla	Brazil; *M. Cáceres & R. Lücking 165a* (B, URM)	MZ827245	MZ851707	Xavier-Leite et al. (2022)
E. epiphylla	Mexico; *M.A. Herrera-Campos et al. s.n*. (F)	AY341368	AY341354	Lücking et al. (2004)
E. epiphylla	Brazil; *A. Xavier-Leite & M. Cáceres 1736* (ISE)	MZ827191	MZ851604	Xavier-Leite et al. (2022)
E. aff. *epiphylla*-1	Brazil; *A. Xavier-Leite 1933* (ISE)	MZ827267	MZ851509	Xavier-Leite et al. (2022)
Gomphillus americanus	U.S.A.; *J. Lendemer 45954* (NY)	KY353115	KY381580	Unpublished
G. calycioides	U.S.A.; *J. Lendemer 46470* (NY)	MK318271	MH887485	Unpublished, Anderson-Stewart et al. (2018)
G. calycioides	U.K.; *T. Lumbsch 20100d* (F)	KF833341	KF833329	Unpublished

Taxon	Country; voucher	mtSSU	nuLSU	Reference
G. hyalinus (G. ophiosporus)	Costa Rica; *S. Will-Wolf 10006a* (F)	AY341371	AY341357	Lücking et al. (2004)
Gyalideopsis sp.	Costa Rica; *M.P. Nelsen 2066a* (F)	AY341373	AY341359	Lücking et al. (2004)
Psathyromyces heterellus	Brazil; *M. Cáceres & A. Aptroot 28673* (ISE)	MZ827302	MZ851679	Xavier-Leite et al. (2022)
P. heterellus	Brazil; *M. Caceres & A. Aptroot 28675* (ISE)	MZ827285	MZ851680	Xavier-Leite et al. (2022)
P. heterellus	Brazil; *A. Xavier-Leite et al. 2799* (ISE)	MZ827221	MZ851643	Xavier-Leite et al. (2022)
P. heterellus	Brazil; *M. Caceres & A. Aptroot 11953* (ISE)	KF833342	KF833330	Unpublished
P. heterellus	Brazil; *A. Xavier-Leite 1977* (ISE)	MZ827198	MZ851523	Xavier-Leite et al. (2022)
P. heterellus	Brazil; *A. Xavier-Leite 1989* (ISE)	MZ827183	MZ851533	Xavier-Leite et al. (2022)
P. heterellus	Brazil; *A. Xavier-Leite 2533* (ISE)	MZ827206	MZ851561	Xavier-Leite et al. (2022)
Sipmanidea neotropica	Brazil; *A. Xavier-Leite 1927* (ISE)	MZ827201	MZ851507	Xavier-Leite et al. (2022)
Sporocybomyces leucotrichoides	Costa Rica; *R. Lücking s.n.* (F)	AY341369	AY341355	Lücking et al. (2004)
S. leucotrichoides	Brazil; *A. Xavier-Leite et al. 1027* (ISE)	MZ827268	MZ851609	Xavier-Leite et al. (2022)
S. leucotrichoides	Brazil; *A. Xavier-Leite 1989* (ISE)	MZ827184	MZ851534	Xavier-Leite et al. (2022)
Verruciplaca calcarea	Brazil; *A. Xavier-Leite et al. 1262c* (ISE)	MZ827233	MZ851652	Xavier-Leite et al. (2022)
V. verrucifera	Brazil; *A. Xavier-Leite 1955* (ISE)	MZ827169	MZ851517	Xavier-Leite et al. (2022)
V. verrucifera	Brazil; *A. Xavier-Leite 2541* (ISE)	MZ827195	MZ851563	Xavier-Leite et al. (2022)
V. verrucifera	Brazil; *A. Xavier-Leite 2545* (ISE)	MZ827196	MZ851564	Xavier-Leite et al. (2022)

Taxon	Country; voucher	mtSSU	nuLSU	Reference
Verruciplaca sp.	Brazil; *A. Xavier-Leite & M. Cáceres 1718* (ISE)	MZ827271	MZ851602	Xavier-Leite et al. (2022)

The PCR products were purified with the method of Miyazawa and Ohmura (2023a). DNA sequencing was performed on an Applied Biosystems™ 3500xL Genetic Analyzer (Thermo Fisher Scientific) using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) following the manufacturer's instructions.

MOLECULAR PHYLOGENETIC ANALYSES. – The newly obtained mtSSU and nuLSU sequences of *Gyalideopsis japonica* from Japanese material were checked through BLAST searches to make sure they were from the target taxa (not contaminants) and of high quality. The checked sequences were aligned with those of selected taxa in Gomphillaceae from GenBank (Table 1) in MAFFT ver. 7 (Katoh et al. 2019) with default settings. The sequences of taxa included in the alignment, as well as those of *Echinoplaca* spp. as the outgroup (Table 1), were selected according to the BLAST results in GenBank and previous phylogenetic studies (Xavier-Leite et al. 2022). BLAST results suggested the sequences of *Gyalideopsis japonica* had the highest similarity with *Psathyromyces heterellus* (Stirt.) Xavier-Leite, M. Cáceres & Lücking (as *Aderkomyces heterellus* (Stirt.) Lücking, Sérus. & Vězda; approximately 95% for mtSSU and approximately 97% for nuLSU). Additionally, based on the previously published phylogenetic studies using the same loci (Xavier-Leite et al. 2022), our data show that *G. japonica* is included in a clade nested deeper than *Echinoplaca* s. str. and *Sporocybomyces* (as the *E. leucotrichoides* clade). Samples that were analyzed by Xavier-Leite et al. (2022), but which lacked data for both mtSSU and nuLSU were excluded to minimize the data gaps in our analyses.

Due to the limited number of reference sequences for nuITS and *RPB2* in GenBank, our sequences obtained for these regions were not used for molecular phylogenetic analysis. However, we provide them here for future research. Each single locus data set (mtSSU and nuLSU) was separately aligned. After removing sites with gaps, or missing or ambiguous data, initial phylogenetic trees were reconstructed based on each single locus using the Neighbor-Joining method (Saitou & Nei 1987). This initial analysis allowed us to check for any potential conflicts prior to concatenation for a comprehensive phylogenetic analysis. Once it was confirmed that no significant conflicts were observed, the data sets were concatenated. The final alignment of 1099 sites was used for the molecular phylogenetic analyses.

Maximum likelihood (ML) analyses were performed using the General Time Reversible model (Nei & Kumar 2000) plus gamma distribution with invariant sites (G + I), which was selected as the best fitting model based on the lowest Bayesian information criterion score. The bootstrap values (≥70%) of 1,000 replicates for NJ and ML are shown on each branch. All calculations were conducted in MEGA X (Kumar et al. 2018).

RESULTS AND DISCUSSION

MORPHOLOGY AND CHEMISTRY. – Based on the examination of our collections and exsiccati, two closely related taxa, currently recognized as *Gyalideopsis japonica* and *G. lambinonii* (see Harada & Vězda 2000), were confirmed to belong to *Gyalideopsis* s. lat. They share the distinct shape of clypeate hyphophores producing numerous diahyphae (Fig. 1) but these differ from each other in their apothecial morphology. The apothecial margin of *G. lambinonii* was distinct (Fig. 2C), due to the slightly raised excipulum from the thallus (Fig. 2D), whereas that of *G. japonica* was indistinct and widely spread over the thallus (Fig. 2A), due to the excipulum not being raised from the thallus (Fig. 2B). This observation is consistent with Harada and Vězda (2000).

This group, consisting of *G. japonica* and *G. lambinonii* was clearly different from *G. peruviana*. which is the type species of the genus *Gyalideopsis*. The *G. japonica*-*G. lambinonii* group has adnate apothecia (Figs. 1A & B, 2A & C), monosporous asci (in Vězda 1979: fig. 6.16), and clypeate hyphophores (Figs. 1A–D), while *G. peruviana* has sessile apothecia with a well-developed exciple (Figs. 2E & F), 6–8 ascospores per ascus (Fig. 2F), and no hyphophores. Our observations were consistent with previous

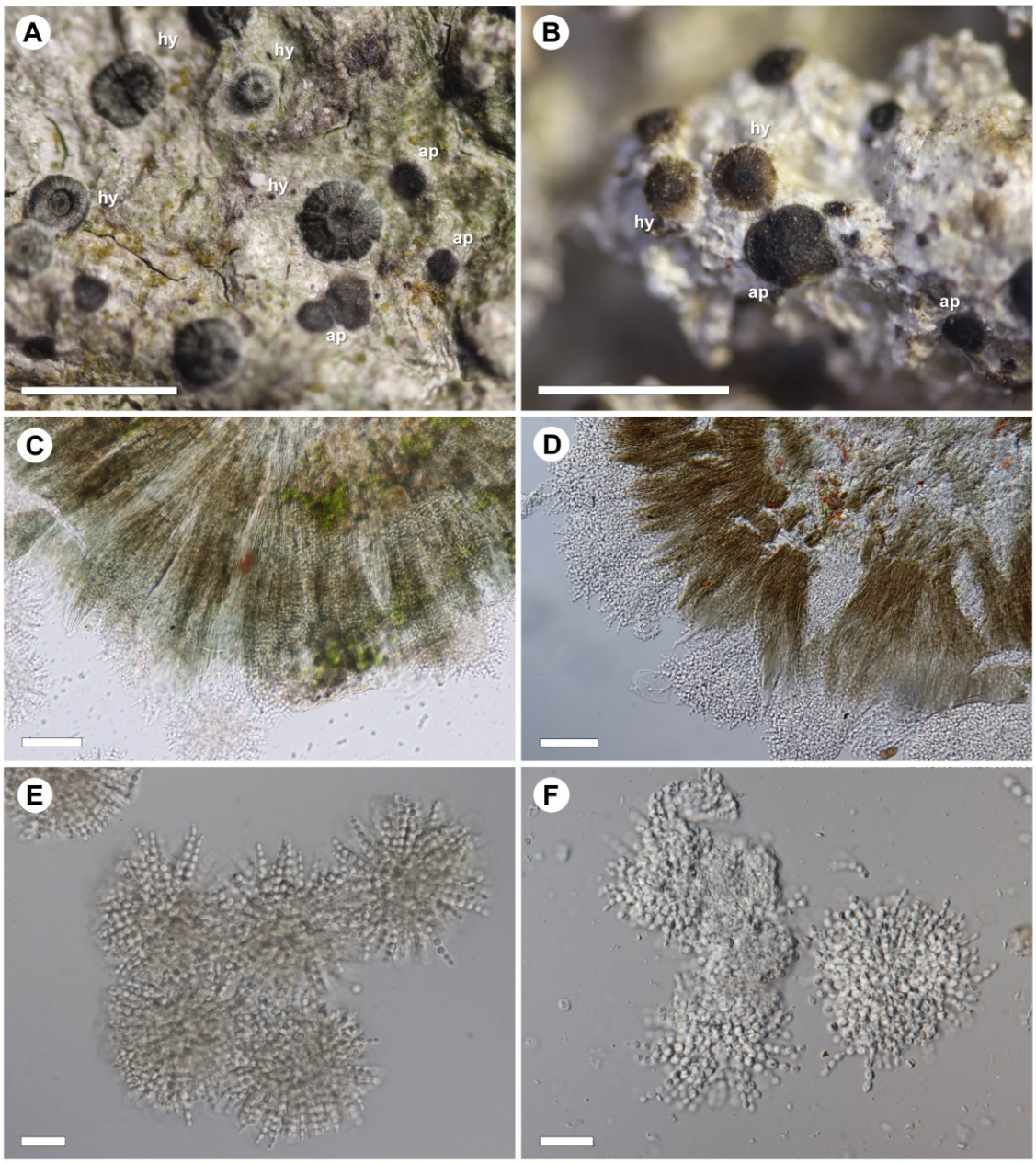

Figure 1. Morphology of *Clypeoria japonica* and *C. lambinonii*. **A**, *C. japonica* (*K. Miyazawa 1036*, TNS); **C & E**, *C. japonica*. (*M. Chaki 79*, TNS); **B, D & F**, *C. lambinonii* (*K. Kalb s.n.* [Kalb, Lich. Neotrop. 64. as *Gyalideopsis lambinonii* Vězda], TNS). A & B, thallus with apothecia (ap) and hyphophores (hy); C & D, a mashed hyphophore with diahyphae from each taxon; E & F, moniliform diahyphae. Scales = 1 mm in A & B, 50 µm in C & D, 20 µm in E & F.

reports of this species (Lücking et al. 2005, Vězda 1972). Additionally, the *G. africana* group, to which *G. peruviana* is considered to belong (Lücking et al. 2005), is characterized by setiform-capitate hyphophores, which clearly differ from those of the *G. japonica*-*G. lambinonii* group. In addition, members of the *G. africana* are typically musicolous (Lücking et al. 2005), while *G. japonica* and *G. lambinonii* are corticolous (Harada & Vězda 2000, Vězda 1979).

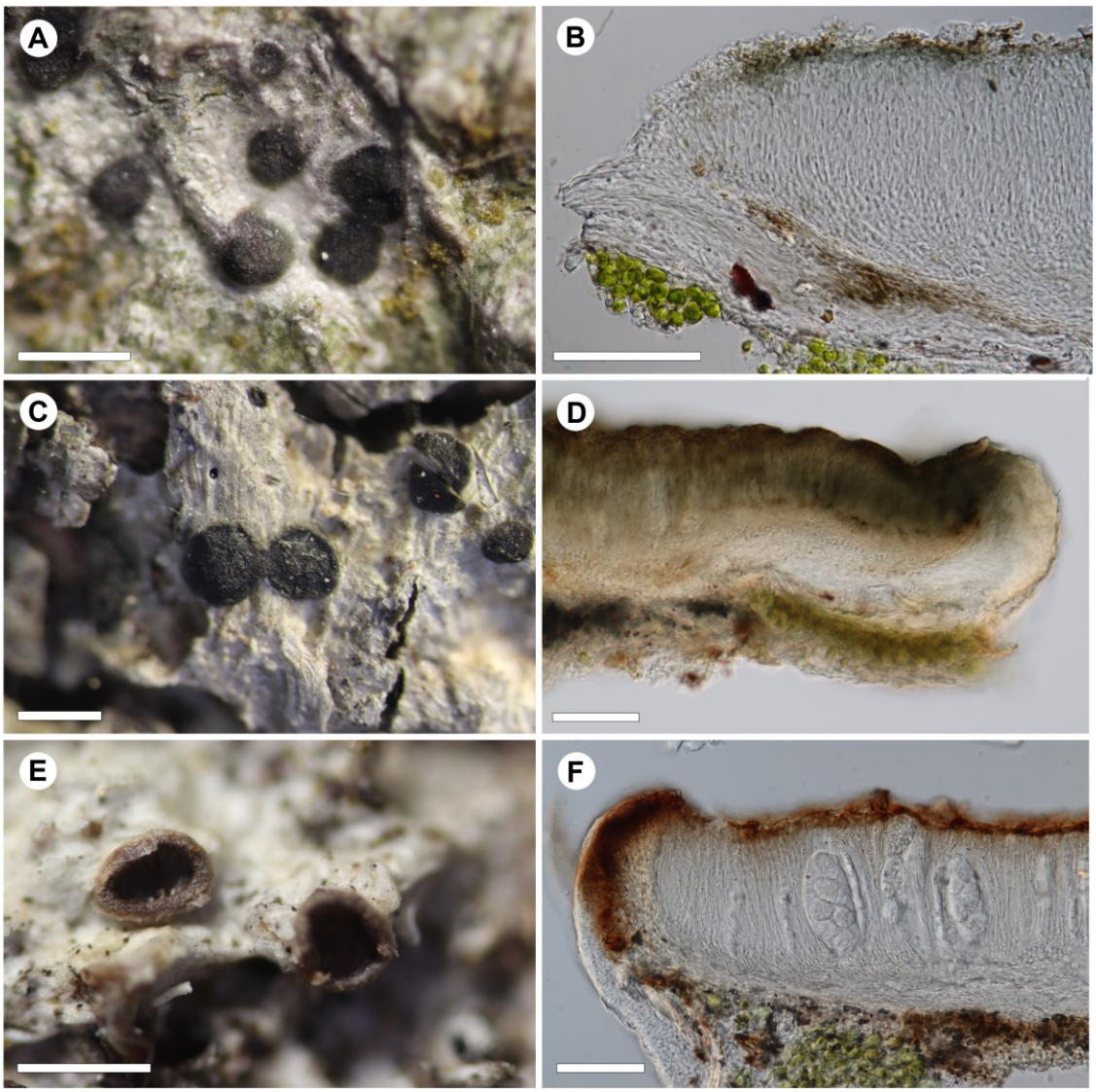

Figure 2. Morphology of *Clypeoria japonica*, *C. lambinonii* and *Gyalideopsis peruviana*. **A** & **B**, *C. japonica* (*K. Miyazawa 1036*, TNS); **C** & **D**, *C. lambinonii* (*K. Kalb s.n.* [Kalb, Lich. Neotrop. 64. as *Gyalideopsis lambinonii*], TNS); **E** & **F**, *Gyalideopsis peruviana* (*K. Kalb & A. Kalb s.n.* [Kalb, Lich. Neotrop. 462. *Gyalideopsis peruviana*], TNS). A, C & E, apothecia; C, D & F, a vertical section of the apothecium in each species. Scales = 0.5 mm in A, C & E, 50 µm in B, D & F.

No secondary substances were detected with HPTLC in any of the specimens examined. In most Gomphillaceae, including *Gyalideopsis*, secondary metabolites have been found to be absent (Lücking 2008, Lücking et al. 2006, Xavier-Leite et al. 2018).

MOLECULAR PHYLOGENETIC ANALYSES. – The tree topologies based on the concatenated alignment between NJ and ML showed no conflicts. Moreover, the topology of this reconstructed ML tree (Fig. 3) was similar to that of Xavier-Leite et al. (2022). However, whereas *Gyalideopsis* sp. (AY341373, AY341359) and *Bezerroplaca lucernifera* (Kalb & Vězda) Xavier-Leite, M. Cáceres & Lücking (as *Echinoplaca lucernifera* Kalb & Vězda: AY341370, AY341356) formed a sister group in fig. 5 of Xavier-Leite et al. (2022), these species do not form sister groups in our analyses (Fig. 3).

The sequences we generated from specimens of *Gyalideopsis japonica* clustered in a single clade with high support values (NJ/ML = 100/100) (Fig. 3). However, the genetic variation among different loci

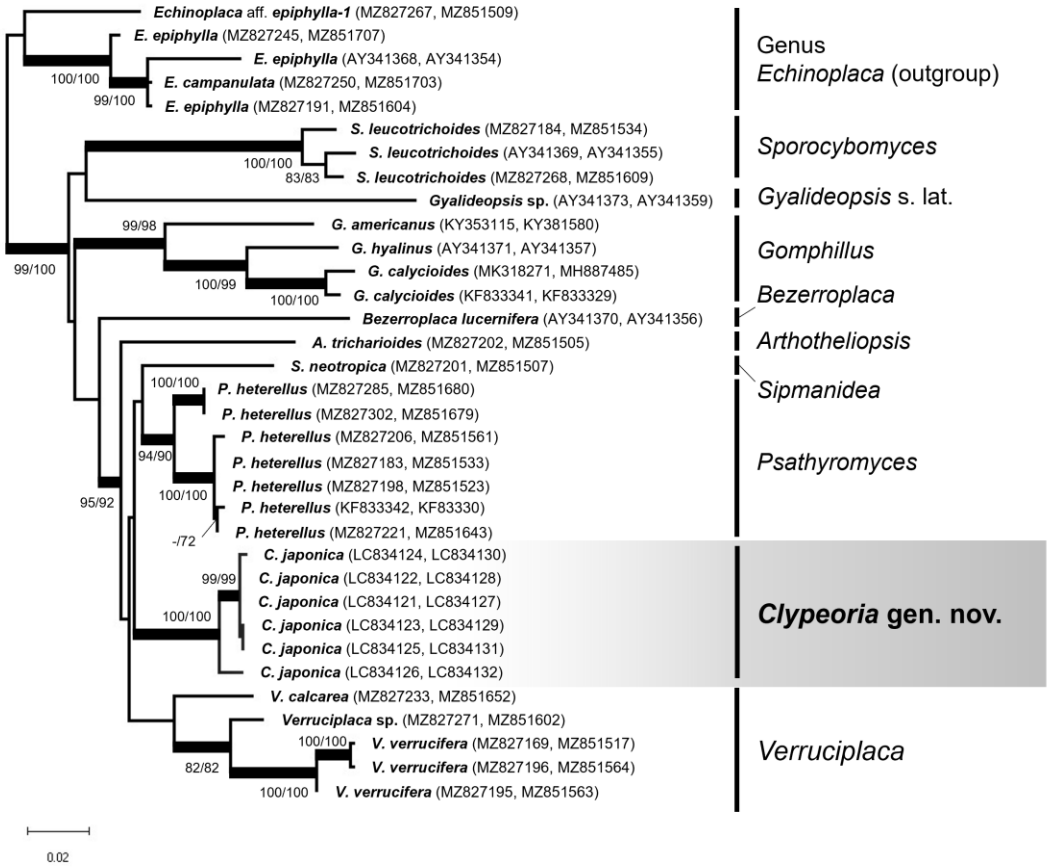

Figure 3. Maximum likelihood tree of selected taxa in *Gomphillaceae* showing the phylogenetic position of *Clypeoria japonica*. The genus *Echinoplaca* is used as an outgroup. ML (first) and NJ (second) support values (≥70) are presented for each node. Branches highly supported (≥90) by both analyses are indicated with bold black lines.

in the six sequenced specimens of *G. japonica* from Japan were as follows: nuITS (623 sites, 51 variables, 93.8–99.3% identity, n = 6), nuLSU (512 sites, 16 variables, 97.2–99.8% identity, n = 6), mtSSU (725 sites, 10 variables, 98.7–100% identity, n = 6), and *RPB2* (519 sites, 13 variables, 97.8–99.8% identity, n = 5). It should be noted that a minimum identity of 93.8% was observed among the specimens examined in the nuITS. Such low identity of nuITS is often recognized differences at the species level in lichens and non-lichen fungi (Kõljalg et al. 2013, Nilsson et al. 2008, Schoch et al. 2012). Because no morphological differences were observed among the specimens, and we consider this to reflect intraspecific variation.

The clade of *G. japonica* sequences was included in a strongly supported clade (NJ/ML = 95/92) containing the mostly foliicolous genera *Arthotheliopsis* Vain., *Psathyromyces* Bat. & Peres, *Sipmanidea* Xavier-Leite, M. Cáceres & Lücking, and *Verruciplaca* Xavier-Leite, M. Cáceres & Lücking (Fig. 3). These genera mostly produce adnate to spot-like apothecia (zeorine in *Sipmanidea*), but except in *Arthotheliopsis*, they are yellow-orange in color. They also differ strongly from both *G. japonica* and *G. lambinonii*, as well as from one another, in their hyphophore types (Xavier-Leite et al. 2022, 2023).

Unfortunately, DNA sequences of *G. peruviana* and other members of the *G. africana* group have not been obtained. However, the dispersed placement of members of *Gyalideopsis* s. lat in previously published phylogenies have already demonstrated the genus is polyphyletic (Xavier-Leite et al. 2022). Additionally, our analysis did not support a sister group relationship between *G. japonica*, and by extension *G. lambinonii*, and other members of *Gyalideopsis* s. lat. (Fig. 3). In light of these results, which are consistent with the findings of Lücking et al. (2005), there is clear evidence supporting the establishment of a new genus for *G. japonica* and *G. lambinonii*. Although it is clear that additional molecular phylogenetic

studies are required to clarify relationships among other members of *Gyalideopsis* s. lat., we propose the name *Clypeoria* for this group.

TAXONOMIC SECTION

Clypeoria K. Miyaz. & Y. Ohmura, **gen. nov.**
Mycobank #855679.

DIAGNOSIS. – *Clypeoria* is recognized by its clypeate hyphophores with a centrally reduced stipe on the underside (Figs. 1A–D), producing moniliform diaphyphae with drop-shaped terminal segments (Figs. 1E & F). It can be distinguished from the type species of *Gyalideopsis*, *G. peruviana*, by its adnate apothecia (Figs. 1A & B, 2A & C) and monosporous asci.

TYPE SPECIES: *Clypeoria japonica* (H. Harada & Vězda) K. Miyaz. & Y. Ohmura.

GENUS DESCRIPTION. – **Thallus** crustose, continuous, very thin, smooth, without sterile setae, greenish grey. **Apothecia** adnate, biatorine, rounded, 0.2–1.0 mm diam; disc brown to blackish brown; excipulum hyphal, colorless; hypothecium prosoplectenchymatous, colorless; epithecium indistinct. **Asci** monosporous, 40–80 × 15–30 µm. **Ascospores** ellipsoid, muriform, 35–60 ×15–30 µm, colorless. **Hyphophores** clypeate, almost adnate to the thallus, attached to the substrate by a central reduced stipe on the underside, 0.3–1.0 mm diam. with dissected margin, brown to black. **Diahyphae** composed of many fascicles, moniliform, with drop-shaped terminal segments. **Photobiont** trebouxioid (cf. Watanabeales). **Habitat** corticolous.

CHEMISTRY. – No secondary substances were detected with HPTLC.

ETYMOLOGY. – The genus name *Clypeoria* is derived from its clypeate (= round shield shaped) hyphophores, a term originated to the Latin *clypeus*. The suffix *-oria* was chosen to emphasize the distinctive clypeate hyphophores characteristic of this genus. In Latin, the suffix *-oria* is commonly used to form nouns that denote objects or places associated with specific features.

NOTES. – *Clypeoria* resembles *Arthotheliopsis* in the applanate to adnate apothecia but can be distinguished by the absence of sterile setae and the production of clypeate, rather than setiform, hyphophores (Lücking et al. 2007). *Sipmanidea*, previously classified as *Echinoplaca* (Xavier-Leite et al. 2023), shares production of monosporous muriform ascospores with *Clypeoria*, but differs in having erumpent to adnate apothecia that appear zeorine to emarginate with yellow-orange color and setiform, branched hyphophores. *Psathyromyces*, formerly included in *Aderkomyces* Bat., shares with *Clypeoria*, the adnate, biatorine apothecia and moniliform diahyphae, but differs in the orange color of the apothecia, the presence of long white setae, and long, setiform, white hyphophores with arrow-like, blackened apex, (Xavier-Leite et al. 2023). *Verruciplaca*, previously treated as a group within *Echinoplaca* s. lat. (Xavier-Leite et al. 2023), can be distinguished from *Clypeoria* by its whitish to bluish pruinose thallus, emarginate, orange apothecia (*Echinoplaca*-type), the thinly branched hyphophores usually formed on a translucent prothallus, and filiform diahyphae with spermatozoid terminal segments.

The hyphophores of *Gomphillus* are somewhat similar to those of *Clypeoria* in their clypeate shape with a central stipe on the underside (Lücking et al. 2005). However, *Gomphillus* has a long and distinct stipe, whereas in *Clypeoria* the stipe is much reduced (Lücking et al. 2005). In addition, *Clypeoria* has adnate biatorine apothecia, muriform ascospores, and moniliform diahyphae, while *Gomphillus* has vertically elongated apothecia, filiform ascospores, and filiform to acicular diahyphae (Lücking et al. 2005). Phylogenetic analyses in this study found support for the independence of these genera from one another. (Fig. 3).

According to the phenotype-based cladistic analysis by Lücking et al. (2005), *Clypeoria japonica* and *C. lambinonii* were treated as members within the *Gyalideopsis palmata* group. This group is characterized mainly by taxa that have flabelliform hyphophores and a corticolous habit (Lücking et al. 2005). However, the *G. palmata* group is apparently heterogeneous, containing species with morphologies ranging from sessile to shortly stalked apothecia, setiform to clypeate hyphophores, and filiform to moniliform diahyphae (Lücking et al. 2005). The molecular study by Xavier-Leite et al. (2022)

demonstrated that thalline, apothecial, and hyphophore characteristics are crucial in defining genus-level lineages within Gomphillaceae. Therefore, further taxonomic studies based on molecular data should address the *G. palmata* group in more detail. At present, the lack of molecular data for this group makes it difficult to conduct a comparative analysis with other taxa and *Clypeoria*.

One taxon '*Gyalideopsis* sp.' reported in Lücking et al. (2006) resembles *Clypeoria lambinonii* and *C. japonica* in having rounded hyphophores, but these differ in their pale color and the presence of a distinct stipe and likely this material does not belong to *Clypeoria*. Due to the lack of DNA sequences and the absence of apothecia and diahyphae, the taxonomic evaluation of '*Gyalideopsis* sp.' could not be conducted.

Clypeoria japonica (H. Harada & Vězda) K. Miyaz. & Y. Ohmura, **comb. nov.**
Mycobank #855680.

≡ *Gyalideopsis japonica* H. Harada & Vězda, Nat. Hist. Res. 6: 5. 2000. **TYPE: JAPAN.** HONSHU. Chiba Pref.: Atago-zawa, Seiwa-kenmin-no-mori, Kimitsu-city, 120 m. elev., on trunk of *Swida controversa* by stream in forest, 10.xi.1997, *H. Harada 18543* (CBM-FL-10573[digital images!], holotype; PRA [n.v.], isotype).

FIGURES 1A, 1C & 1E, 2A & 2B.

NOTES. – *Clypeoria japonica* is characterized by having clypeate, adnate, dark brown to grayish black hyphophores (Figs. 1A & C) attached to the substrate by a central reduced stipe on the underside, with moniliform diahyphae (Fig. 1E), adnate blackish brown to grayish black apothecia with laterally spreading exciple (Figs. 1A, 2A & B), monosporous asci, and lack of representative lichen compounds. For detailed description and illustrations, see Harada and Vězda (2000).

HABITAT AND DISTRIBUTION. – This species is known only from the warm-temperate region of Japan, where it grows on barks of various coniferous and deciduous trees (see also Harada & Vězda 2000, Harada et al. 2017, Sakata & Harada 2012, Takahashi 2009, Yamamoto et al. 2024).

Specimens examined. – **JAPAN.** HONSHU. Chiba Pref.: near Seicho-ji Temple, Kiyosumi, Kamogawa-city (35°09′N, 140°08′E), 300 m. elev., 22.iii.2022., on bark of *Castanopsis sieboldii*, *K. Miyazawa 1036* (TNS; nuITS: LC834115). Hiroshima Pref., Higashihon-machi, Shobara-city (34°51′N, 133°01′E), 280 m. elev., 27.vi.2021., on bark of broad-leaf tree, *M. Chaki 79* (TNS; nuITS: LC834116; *RPB2*: LC834110); Nabara, Kabe-cho, Asakita-ku, Hiroshima-city (34°34′N, 132°30′E), 270 m. elev., 30.xii.2023., on bark of *Cerasus* sp., *M. Chaki 420* (TNS; nuITS: LC834119; *RPB2*: LC834113); campsite, Haji Dam, Yachiyo-cho, Akitakata-city (34°40′N, 132°35′E), about 250 m. elev., 11.vii.2021., on bark of broad-leaf tree, *M. Chaki 84* (TNS; nuITS: LC834117; *RPB2*: LC834111); Haji Dam, Yachiyo-cho, Akitakata-city (34°40′N, 132°36′E), about 260 m. elev., 19.vi.2022., on branch of *Cerasus* sp. *M. Chaki s.n. [= herb. Y. Ohmura 14150]* (TNS; nuITS: LC834120; *RPB2*: LC834114); Hatsukaichi-city, 30 m. elev., 29.viii.2021, on tree bark, *M. Chaki 137* (TNS), 10 m elev., 23.ix.2021., on bark broad-leaf tree, *M. Chaki 191* (TNS; nuITS: LC834118; *RPB2*: LC834112), about 20 m elev., 23.ix.2021., on bark, *M. Chaki 194* (TNS).

Clypeoria lambinonii (Vězda) K. Miyaz. & Y. Ohmura**, comb. nov.**
Mycobank #855681.

≡ *Gyalideopsis lambinonii* Vězda, Folia Geobot. Phytotax. 14: 64. 1979. **TYPE**: **DEMOCRATIC REPUBLIC OF THE CONGO [ZAIRE].** Kivu. Prov: Kahuzi, 2640 m. elev., on bark of *Agarista salicifolia*, 28.xii.1972. *J. Lambinon* 71/1287 (PRA[n.v.], holotype; LG[n.v.], isotype).

FIGURES 1B, 1D & 1F, 2C & 2D.

NOTES. *Clypeoria lambinonii* resembles *C. japonica* in having clypeate adnate hyphophores (Figs. 1B & D) with moniliform diahyphae (Fig. 1F). However, it differs in having more blackish discs with compact exciple (Figs. 1B, 2C & D). See the detailed information on this species in the descriptions by

Vězda (1979) and the line drawings by Vězda (1979) and Kalb & Vězda (1988), as well as the images in Vězda (1979) and Lücking et al. (2007).

HABITAT AND DISTRIBUTION. – *Clypeoria lambinonii* was reported from tree bark at elevations between 100 and 3000 m in Asia (Philippines [Kalb & Vězda 1988), Taiwan [Aptroot & Sparrius 2003], Thailand [Aptroot et al. 2007], Vietnam [Aptroot & Sparrius 2006]), North and South America (Bolivia [Flakus et al. 2015], Brazil [Xavier Leite et al. 2018], Costa Rica [Lücking et al. 2006], U.S.A. (Florida) [Lücking et al. 2007], Venezuela [Komposch & Hafellner 2002]), and Africa (Democratic Republic of the Congo, Rwanda [Vězda 1979]).

Specimen examined. – **BRAZIL:** Mato Grosso. Serra dos Coroados: zwischen Cuiaba und Buriti, in einem dichten Cerrado, 500 m elev., 6.vii.1980., *K. Kalb s.n.* [= Kalb, Lich. Neotrop. 64., distributed as *Gyalideopsis lambinonii* Vězda] (TNS).

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