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Molecular phylogeny of *Cosmetocleithrum* Kritsky, Thatcher & Boeger, 1986 (Monogenoidea: Dactylogyridae), gill parasites of Neotropical catfishes (Siluriformes)

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Research Paper

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Abstract

Cosmetocleithrum Kritsky, Thatcher & Boeger, 1986 (Dactylogyridae) represents one of the most species-rich groups (22 species currently recognized as valid) of all dactylogyrid parasites infecting Neotropical catfishes. Species of *Cosmetocleithrum* exhibit a remarkable affinity towards catfishes of the Doradidae and the Auchenipteridae. However, phylogenetic relationships between members of this genus have not been yet analysed. This study analysed newly obtained partial sequences of the 28S ribosomal RNA gene of seven species of *Cosmetocleithrum*, including its type species *C. gussevi* Kritsky, Thatcher & Boeger, 1986, along with several other dactylogyrids infecting siluriform, gymnotiform, perciform and characiform fishes. *Cosmetocleithrum* appeared as an evolutionary recent group, composed of two well-defined lineages: lineage 1 includes parasites of doradids – namely, *C. bulbocirrus*, *C. confusum*, *C. parvum* and *C. bifurcum* – whereas lineage 2 is composed of species from doradids – that is, *C. rarum*, *C. gussevi*, *C. gigas*, *C. trachydorasi* and *C. falsunilatum* – together with parasites of auchenipterids – namely, *C. laciniatum* and *C. baculum*. The search for synapomorphies to characterize taxonomic groups within *Cosmetocleithrum* appears challenging, since the morphology of their haptoral elements is quite conservative, and that of the copulatory complex is highly variable between species. The results of the present study support the recent synonymization of *Paracosmetocleithrum* Acosta, Scholz, Blasco-Costa, Alves & Silva, 2018 with *Cosmetocleithrum*. Whereas the 28S ribosomal DNA data resolved *Cosmetocleithrum* as monophyletic, the statistical support for the lineage was low, rendering its phylogenetic position between other Neotropical dactylogyrids yet undefined.

Introduction

In the last decade, phylogenetic analyses of partial 28S ribosomal DNA (rDNA) fragments have been used as a fundamental instrument for a better genus allocation, and to assess interrelationships of dactylogyrid parasites infecting mainly Neotropical siluriforms (Mendoza-Palmero et al., 2015, 2020; Acosta et al., 2017, 2019; Franceschini et al., 2018, 2020), as well as other dactylogyrid parasites of characiform, gymnotiform and perciform freshwater fishes (Mendoza-Palmero *et al.*, 2017; Moreira *et al.*, 2019; Zago *et al.*, 2020, 2021).

Cosmetocleithrum Kritsky, Thatcher & Boeger, 1986 (Dactylogyridae) represents a group of gill parasites of Neotropical catfishes strictly associated with fish species of the Doradidae, Auchenipteridae and Pimelodidae (all Siluriformes). Species of this genus are mainly characterized by the presence of a dorsal bar with two submedial ribbon-like projections arising from the anterodorsal surface of the bar (Kritsky *et al.*, 1986). Other morphological elements of the haptor (such as anchors, hooks and ventral bars) are quite conservative between species, whereas the morphology of the copulatory complex (male copulatory organ and accessory piece) is highly variable. To date, 22 species have been described as members of *Cosmetocleithrum* mainly parasitizing doradids (14 species), seven on auchenipterids and only one has been recorded on pimelodids (Cohen *et al.*, 2020; Yamada *et al.*, 2020; Feronato *et al.*, 2022).

Despite the fact that *Cosmetocleithrum* is one of the richest dactylogyrid genera infecting Neotropical siluriforms, only *C. bulbocirrus* Kritsky, Thatcher & Boeger, 1986, *C. bifurcum* Mendoza-Franco, Mendoza-Palmero & Scholz, 2016 and *C. falsinulatum* Feronato, Razzolini, Morey & Boeger, 2022 (all parasites of doradids) have been analysed in recent phylogenetic studies (Mendoza-Palmero *et al.*, 2015, 2020; Acosta *et al.*, 2017, 2019; Franceschini *et al.*, 2018; Zago *et al.*, 2020, 2021; Feronato *et al.*, 2022). In some of these studies, dactylogyrid parasites of Neotropical catfishes have appeared repeatedly in two main clades, even when other parasite groups infecting characids and cichlids were considered

(Zago *et al.*, 2020, 2021). Moreover, *Cosmetocleithrum* has been resolved (with variable nodal support) either as a part of a major clade including parasites of pimelodids (Acosta *et al.*, 2018; Zago *et al.*, 2021), or closely related to loricariids (Zago *et al.*, 2020).

Considering that only three species of *Cosmetocleithrum* spp. (of a total of 22 currently described) have been included in recent phylogenetic studies, the aim of this study is to assess the phylogenetic relationships of species of *Cosmetocleithrum*, parasites of Neotropical doradid and auchenipterid catfishes, using partial sequences of the 28S ribosomal RNA (rRNA) gene, and to evaluate the phylogenetic position of *Cosmetocleithrum* in respect to other dactylogyrid parasites infecting Neotropical freshwater fishes.

Materials and methods

Specimen collection and processing

Specimens of *Oxydoras niger* (Valenciennes) (host field codes: PI 797, PI 1028) (Doradidae) and *Trachelyopterus* sp. (PI 950) (Auchenipteridae) were captured by local fishermen in the surroundings of Iquitos (03°45′51″S, 73°14′50″W), Peru, in 2011 and 2018. Dactylogyrids were removed from the gills, fixed with hot water (*c.* 80°C) and stored in vials with 96% ethanol. For molecular characterization, parasites were cut in half with fine needles; since the haptoral armament in species of *Cosmetocleithrum* is rather similar, the anterior part containing the copulatory complex was used for morphological identification. The posterior part was placed in a sterilized Eppendorf tube and used for molecular characterization. For each isolate, the anterior part was placed on a slide with a drop of Proteinase K (SERVA, Heidelberg, Germany), covered with a coverslip, heated on a hot plate and checked regularly using an optical microscope (Olympus BX51, Tokyo, Japan) until the copulatory complex was clearly visible (more Proteinase K was added when needed). Once the specimens were identified, slides were labelled, and edges of coverslip were sealed with transparent nail polish. Specimens were then photographed, additional nail polish was added and slides were deposited in the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic as molecular vouchers (hologenophores M-766–772) (see Pleijel *et al.*, 2008 for terminology). Fish names follow Froese & Pauly (2022).

Molecular *characterization* and phylogenetic analyses

Procedures of DNA extraction, gene amplification and sequencing followed those of Mendoza-Palmero *et al.* (2015). A total of seven partial sequences of the 28S rRNA gene (1424–1473 bp long) were newly generated in the present study, corresponding to seven species of *Cosmetocleithrum*, including the type species *C. gussevi* Kritsky, Thatcher & Boeger, 1986. Details of all species included in the analyses are provided in table 1.

To the phylogenetiassessc relationships of species of *Cosmetocleithrum* and their position in relation to other dactylogyrid parasites of Neotropical freshwater fishes, the newly generated sequences (present study) along with 57 previously published sequences of the 28S rDNA fragment (918–1575 bp long) representing 48 species of dactylogyrids of siluriforms, and 16 species of non-catfish hosts, were subjected to phylogenetic analyses (see table 1). Sequences of two species of the Diplectanidae were used as outgroup (table 1 and fig. 1).

Sequences were aligned using default parameters of MAFFT implemented in Geneious v.7.1.3 (Kearse *et al.*, 2012). The extremes of the alignment were trimmed, resulting in 914 nucleotide positions for analyses. Phylogenetic analyses were run under maximum likelihood (ML) and Bayesian inference (BI) methods, applying the model of nucleotide evolution GTR+Γ+I, estimated using MEGA 7 (Kumar *et al.*, 2016). ML analyses were performed with RAxML v.8 (Guindon & Gascuel, 2003); model parameters and bootstrap support values (1000 resamples) were estimated with RAxML. BI trees were generated using MrBayes v.3.2 (Ronquist *et al.*, 2012), running two independent Markov Chain Monte Carlo runs of four chains for 10^7 generations and sampling tree topologies every 10^3 generations. 'Burn-in' was set to the first 25,000 generations. MrBayes and RAxML analyses were carried out on the computational platform CIPRES (Miller *et al.*, 2010). Phylogenetic trees were visualized in FigTree v.1.3.1 (Raumbaut, 2009).

Results

A total of 64 species representing 17 genera (along with those identified as Dactylogyridae gen. spp.) of the Dactylogyridae infecting 36 fish host species from the Neotropical region, including two species of the Diplectanidae used as outgroup, were subjected to phylogenetic analyses (see table 1 and fig. 1). In this study, 11 species of *Cosmetocleithrum* (including the type species *C. gussevi*) were included in the analyses. Tree topology from ML and BI analyses was consistent; therefore, only results of the ML analysis are shown (fig. 1). The 28S rDNA data resolved two major lineages of dactylogyrid parasites of Neotropical freshwater fishes labelled as A and B (fig. 1). Clade A is composed of the genus *Characithecium* Mendoza-Franco, Reina & Torchin, 2009 (parasites of characids), forming the earliest branching group of the clade, composing a lineage together with *Unibarra* Suriano & Incorvaia, 1995, *Vancleaveus* Kritsky, Thatcher & Boeger, 1986 and *Ameloblastella* Kritsky, Mendoza-Franco & Scholz, 2000 (parasites of pimelodids, doradids and heptapterids). This group forms a sister clade to the genus *Urocleidoides* Mizelle & Price, 1964 (parasites of gymnotids, anostomids and parodontids), *Cacatuocotyle* Boeger, Domingues & Kritsky, 1997, *Diaphorocleidus* Jogunoori, Kritsky & Venkatanarasaiah, 2004 (both parasites of characids), *Unilatus* Mizelle & Kritsky, 1967, *Heteropriapulus* (Jogunoori, Kritsky & Venkatanarasaiah, 2004) and *Trinigyrus* Hanek, Molnar & Fernando, 1974 (all parasites of loricariids). Clade B is divided in two main subclades, C and D. In the subclade C, *Cosmethocleithrum* appears as an evolutionary recent group, but weakly supported group, sister to a well-supported clade of *Demidospermus* Suriano, 1983 (parasites of loricariids); both genera form the lineage E (fig. 1). These two genus-level lineages are sister to a larger lineage F (all parasites exclusively of pimelodids) consisting of *Demidospermus* sp. 11, *D. mortenthaleri* Mendoza-Palmero, Scholz, Mendoza-Franco & Kuchta, 2012, *Nanayella* Acosta, Mendoza-Palmero, Scholz & Silva, 2019, *Boegeriella* (Mendoza-Palmero, Mendoza-Franco, Acosta & Scholz, 2019) and several unidentified species of the Dactylogyridae. Finally, subclade D is composed of species of *Aphanoblastella* Kritsky, Mendoza-Franco & Scholz, 2000 (parasites of heptapterids), and the sister group formed by *Parasciadicleithrum* Mendoza-Palmero, Blasco-Costa, Hernández-Mena & Pérez-Ponce de León, 2017 and *Sciadicleithrum* Kritsky, Thatcher & Boeger, 1989 (both parasites of cichlids).

Results from both analyses showed that *Cosmetocleithrum* is composed of two well-defined and well-supported lineages

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Table 1. Parasite species used in the phylogenetic analysis. Species newly sequenced in this study are in bold.

Species	Host	Host family	Origin	GenBank ID	Reference
*Ameloblastella chavarriai**	*Rhamdia quelen*	Heptapteridae	Mexico	KP056251	Mendoza-Palmero *et al.* (2015)
Ameloblastella edentensis (referred to as *Ameloblastella* sp. 16 in Mendoza-Palmero *et al.*, 2015)	*Hypophthalmus edentatus*	Pimelodidae	Peru	KP056255	Mendoza-Franco *et al.* (2016)
Ameloblastella martinae	*Sorubim lima*	Pimelodidae	Peru	MT174171	Mendoza-Palmero *et al.* (2020)
Ameloblastella unapioides (referred to as *Ameloblastella* sp. 8 in Mendoza-Palmero *et al.*, 2015)	*Sorubim lima*	Pimelodidae	Peru	KP056254	Mendoza-Franco *et al.* (2016)
Ameloblastella sp. 23	*Hypophthalmus edentatus*	Pimelodidae	Peru	KP056233	Mendoza-Palmero *et al.* (2015)
Aphanoblastella aurorae	*Goeldiella eques*	Heptapteridae	Peru	KP056239	Mendoza-Palmero *et al.* (2015)
Aphanoblastella magna	*Pimelodella avanhandavae*	Heptapteridae	Brazil	MH688484	Yamada *et al.* (2018)
*Aphanoblastella travassosi**	*Rhamdia guatemalensis*	Heptapteridae	Mexico	MK358458	Acosta *et al.* (2019)
Aphanoblastella sp. 3	*Goeldiella eques*	Heptapteridae	Peru	KP056239	Mendoza-Palmero *et al.* (2015)
*Boegeriella conica** (referred to as Dactylogyridae gen. sp. 10 in Mendoza-Palmero *et al.*, 2015)	*Platynematichthys notatus*	Pimelodidae	Peru	KP056226	Mendoza-Palmero *et al.* (2019)
Boegeriella ophiocirrus	*Platystomatichthys sturio*	Pimelodidae	Peru	MK834511	Mendoza-Palmero *et al.* (2019)
Cacatuocotyle papilionis	*Astyanax lacustris*	Characidae	Brazil	MG832889	Zago *et al.* (2018)
Characithecium paranapanemense	*Psalidodon paranae*	Characidae	Brazil	MZ408907	Zago *et al.* (2021)
Cosmetocleithrum baculum	***Trachelyopterus* sp.**	**Auchenipteridae**	**Peru**	**ON982893**	**Present study**
Cosmetocleithrum bifurcum (referred to as *Cosmetocleithrum* sp. 8 in Mendoza-Palmero *et al.*, 2015)	*Hassar orestis*	Doradidae	Peru	KP056216	Mendoza-Palmero *et al.* (2015)
Cosmetocleithrum bulbocirrus	*Pterodoras granulosus*	Doradidae	Brazil	MG001326	Acosta *et al.* (2018)
Cosmetocleithrum confusum	***Oxydoras niger***	**Doradidae**	**Peru**	**ON982791**	**Present study**
Cosmetocleithrum falsunilatum	*Megalodorus uranoscopus*	Doradidae	Peru	OM971057	Feronato *et al.* (2022)
Cosmetocleithrum gigas	***Oxydoras niger***	**Doradidae**	**Peru**	**ON982794**	**Present study**
Cosmetocleithrum gussevi*	***Oxydoras niger***	**Doradidae**	**Peru**	**ON982795**	**Present study**
Cosmetocleithrum laciniatum	***Trachelyopterus* sp.**	**Auchenipteridae**	**Peru**	**ON982796**	**Present study**
Cosmetocleithrum parvum	***Oxydoras niger***	**Doradidae**	**Peru**	**ON982792**	**Present study**
Cosmetocleithrum rarum	***Oxydoras niger***	**Doradidae**	**Peru**	**ON982797**	**Present study**
Cosmetocleithrum trachydorasi	*Trachydoras paraguayensis*	Doradidae	Brazil	MG001323	Acosta *et al.* (2018)
*Demidospermus anus**	*Loricariichthys platymetopon*	Loricariidae	Brazil	KY766957	Franceschini *et al.* (2018)
Demidospermus mortenthaleri	*Brachyplatystoma juruense*	Pimelodidae	Peru	KP056245	Mendoza-Palmero *et al.* (2015)
Demidospermus prolixus	*Loricaria prolixa*	Loricariidae	Brazil	KY796955	Franceschini *et al.* (2018)
Demidospermus rhinelepisi	*Rhinelepis aspera*	Loricariidae	Brazil	MG001324	Acosta *et al.* (2018)
Demidospermus spirophallus	*Loricaria prolixa*	Loricariidae	Brazil	KY766954	Franceschini *et al.* (2018)

(*Continued*)

Table 1. (*Continued.*)

Species	Host	Host family	Origin	GenBank ID	Reference
Demidospermus sp. 11	*Brachyplatystoma vaillantii*	Pimelodidae	Peru	KP056235	Mendoza-Palmero *et al.* (2015)
Diaphorocleidus magnus	*Astyanax lacustris*	Characidae	Brazil	MZ408903	Zago *et al.* (2021)
Diaphorocleidus neotropicalis	*Astyanax lacustris*	Characidae	Brazil	MZ408906	Zago *et al.* (2021)
Heteropriapulus anchoradiatus	*Pterygoplychthys ambrosettii*	Loricariidae	Brazil	MF116371	Acosta *et al.* (2017)
*Heteropriapulus** *heterotylus*	*Pterygoplychthys ambrosettii*	Loricariidae	Brazil	MF116370	Acosta *et al.* (2017)
Heteropriapulus simplex	*Pterygoplychthys ambrosettii*	Loricariidae	Brazil	MF116372	Acosta *et al.* (2017)
Heteropriapulus sp.	*Pterygoplychthys ambrosettii*	Loricariidae	Brazil	MF116373	Acosta *et al.* (2017)
*Nanayella aculeatrium** (referred to as Dactylogyridae gen. sp. 12 in Mendoza-Palmero *et al.*, 2015)	*Sorubim lima*	Pimelodidae	Peru	KP056228	Acosta *et al.* (2019)
Nanayella amplofalcis (referred to as Dactylogyridae gen. sp. 1 in Acosta *et al.*, 2018)	*Hemisorubim platyrhynchos*	Pimelodidae	Brazil	MG001325	Acosta *et al.* (2019)
Nanayella fluctuatrium (referred to as Dactylogyridae gen. sp. 3 in Acosta *et al.*, 2018)	*Sorubim lima*	Pimelodidae	Brazil	MG001327	Acosta *et al.* (2019)
Nanayella megorchis	*Sorubim lima*	Pimelodidae	Peru	MK367407	Acosta *et al.* (2019)
Nanayella processusclavis (referred to as Dactylogyridae gen. sp. 2 in Acosta *et al.*, 2018)	*Hemisorubim platyrhynchos*	Pimelodidae	Brazil	MG001328	Acosta *et al.* (2019)
*Parasciadicleithum octofasciatum**	*Rocio octofasciata*	Cichlidae	Mexico	KY305885	Mendoza-Palmero *et al.* (2017)
Sciadicleithrum meekii	*Thorichthys meeki*	Cichlidae	Mexico	KY305889	Mendoza-Palmero *et al.* (2017)
Sciadicleithrum mexicanum	*Rocio octofasciata*	Cichlidae	Mexico	KY305886	Mendoza-Palmero *et al.* (2017)
Sciadicleithrum splendidae	*Parachromis friedrischsthalii*	Cichlidae	Mexico	KY305890	Mendoza-Palmero *et al.* (2017)
Urocleidoides digitabulum	*Megaleporinus elongatus*	Anostomidae	Brazil	MT556796	Zago *et al.* (2020)
Urocleidoides indianensis	*Parodon nasus*	Parodontidae	Brazil	OK482868	Oliveira *et al.* (2021)
Urocleidoides paradoxus	*Leporinus friderici*	Anostomidae	Brazil	MT556795	Zago *et al.* (2020)
Urocleidoides parodoni	*Parodon nasus*	Parodontidae	Brazil	OK482867	Oliveira *et al.* (2021)
Urocleidoides sinus	*Schizodon nasutus*	Anostomidae	Brazil	MT556799	Zago *et al.* (2020)
Urocleidoides tenuis	*Apareiodon piracicabae*	Parodontidae	Brazil	MT556797	Zago *et al.* (2020)
Urocleidoides tenuis	*Apareiodon piracicabae*	Parodontidae	Brazil	OK465455	Oliveira *et al.* (2021)
Urocleidoides uncinus	*Gymnotus sylvius*	Gymnotidae	Brazil	MT556798	Zago *et al.* (2020)
Trinigyrus carvalhoi	*Hypostomus ancistroides*	Loricariidae	Brazil	MN947608	Franceschini *et al.* (2020)
Trinigyrus anthus	*Hypostomus regani*	Loricariidae	Brazil	MN947622	Franceschini *et al.* (2020)
Trinigyrus peregrinus	*Pterygoplychthys ambrosettii*	Loricariidae	Brazil	MN944890	Franceschini *et al.* (2020)
*Unibarra paranoplatensis**	*Aguarunichthys torosus*	Pimelodidae	Peru	KP056219	Mendoza-Palmero *et al.* (2015)
*Unilatus unilatus**	*Pterygoplychthys ambrosettii*	Loricariidae	Brazil	MF102106	Acosta *et al.* (2017)
*Vancleaveus janaucaensis**	*Pterodoras granulosus*	Doradidae	Peru	KP056247	Mendoza-Palmero *et al.* (2015)

Dactylogyridae gen. sp. 9	*Platynematichthys notatus*	Pimelodidae	Peru	KP056224	Mendoza-Palmero *et al.* (2015)
Dactylogyridae gen. sp. 13	*Hypophthalmus edentatus*	Pimelodidae	Peru	KP056230	Mendoza-Palmero *et al.* (2015)
Dactylogyridae gen. sp. 18	*Pseudoplatystoma fasciatum*	Pimelodidae	Peru	KP056231	Mendoza-Palmero *et al.* (2015)
Dactylogyridae gen. sp. 23	*Brachyplatystoma vaillantii*	Pimelodidae	Peru	KP056232	Mendoza-Palmero *et al.* (2015)
Dactylogyridae gen. sp. 26	*Platynematichthys notatus*	Pimelodidae	Peru	KP056234	Mendoza-Palmero *et al.* (2015)
Outgroup					
Pseudorhabdosynochus epinepheli	*Epinephelus bruneus*	Serranidae	China	AY553622	Wu *et al.* (2006)
Pseudorhabdosynochus lantauensis	*Epinephelus bruneus*	Serranidae	China	AY553624	Wu *et al.* (2006)

*Type species of each genus.

(labelled as 1 and 2 in fig. 1). In lineage 1 (parasites of doradids, fig. 1), *C. bulbocirrus* Kritsky, Thatcher & Boeger, 1986 formed the earliest branching species of the lineage 1, sister to *C. confusum* Kritsky, Thatcher & Boeger, 1986, *C. parvum* Kritsky, Thatcher & Boeger, 1986 and *C. bifurcum* Mendoza-Franco, Mendoza-Palmero & Scholz, 2016. Lineage 2 (fig. 1) is composed of two well-defined groups: the 'doradid group' and the 'auchenipterid group'. The 'doradid group' includes *C. rarum* Kritsky, Thatcher & Boeger, 1986, *C. gussevi* (type species), *C. gigas* Murrieta, Zumaeta & Sánchez, 2019, *C. trachydorasi* (Acosta, Scholz, Blasco-Costa, Alves, & Silva, 2018) and *C. falsunilatum* Feronato, Razzolini, Morey & Boeger, 2022. The 'auchenipterid group' is composed of the species *C. laciniatum* Yamada, Yamada, Silva & Anjos, 2017 and *C. baculum* Yamada, Yamada & Silva, 2020.

Discussion

In this study, interrelationships of species of *Cosmetocleithrum*, for which sequence are available (including its type species), were evaluated based on the analysis of seven novel partial sequences of the 28S rRNA gene, including species of selected genera parasitizing siluriform and non-siluriform hosts. The phylogenetic analyses performed herein suggested that *Cosmetocleithrum* is composed of two well-defined lineages of parasites from doradid and auchenipterid catfishes, which are clustered together with *Demidospermus* spp. parasites of loricariids (clade E), including parasites of pimelodids closely related to the recently erected *Boegeriella* and *Nanayella*, and those unidentified species of Dactylogyridae gen. spp. (clade F). Our results are consistent with previous phylogenetic studies (based on 28S rDNA fragments) where dactylogyrid parasites of Neotropical catfishes formed two well-defined major clades (Acosta *et al.*, 2018; Franceschini *et al.*, 2018; Zago *et al.*, 2021), with *Cosmetocleithrum* comprising a lineage closely related to *Demidospermus* spp.

Recently, Cohen *et al.* (2020, p. 3) mentioned, '[t]he morphological variability of known species of *Cosmetocleithrum* may suggest that the genus contains several subordinate clades'. Our results, based on molecular information, partially confirm that prediction, although only 11 species of the genus were analysed in this study. Cohen *et al.* (2020) also mentioned that known species of *Cosmetocleithrum* could be grouped in two morphological categories: (i) species that resemble *C. gussevi* (type species), having non-articulated bars and accessory piece distally bifid (often resembling a hook) – that is, *C. parvum*, *C. rarum*, *C. sobrinus*, *C. longivaginatum*, *C. striatuli*, *C. laciniatum*, *C. phryctophallus*, *C. gigas*, *C. berecae* and *C. nunani*; and (ii) those species with articulated bars and variably shaped accessory piece – that is, *C. confusum*, *C. bulbocirrus*, *C. tortum* and *C. bifurcum* (see Cohen *et al.*, 2020).

The phylogenetic position of the species analysed in this study only partially corresponds to the morphological categories mentioned above. Most species of the subclade 1 (clade E, fig. 1) correspond to category (ii) of Cohen *et al.* (2020), except for the position of *C. parvum* (category (i) according to Cohen *et al.*, 2020). In contrast, subclade 2 (clade E, fig. 1) partially corresponds to the category (i). However, Cohen *et al.* (2020) did not mention if *C. trachydorasi* belonged to any of these categories, but the morphology of haptoral elements and accessory piece of *C. trachydorasi* indicates that this species corresponds to category (i) (see Acosta *et al.*, 2018 for more details). The position of *C.*

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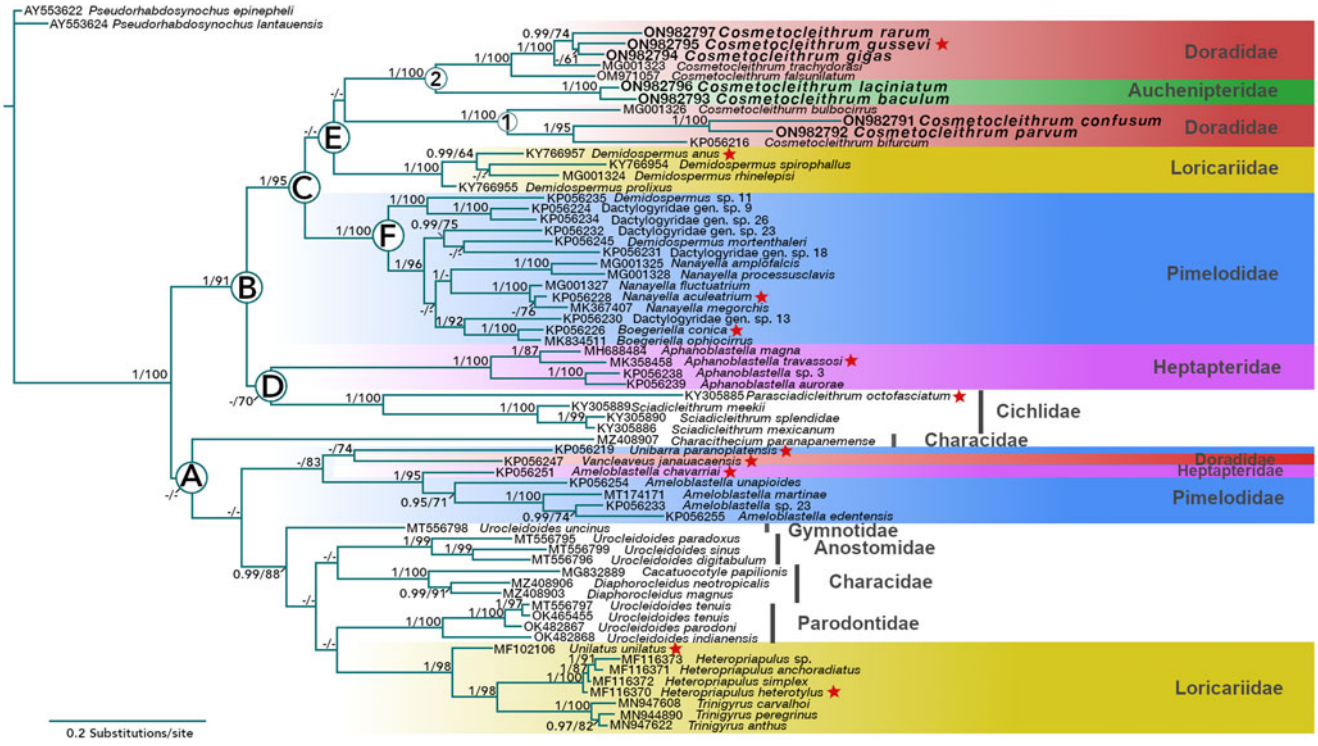

Fig. 1. Phylogenetic relationships of *Cosmetocleithrum* spp. and other dactylogyrid parasites from the Neotropical region, estimated by ML using partial sequences of the 28S rRNA gene. Species of the Diplectanidae were used as outgroup. GenBank sequence ID precedes species name. Newly generated sequences of species are in bold. Type species of selected genera are marked by a red star. Posterior probabilities (BI) and bootstrap values (ML) are given above the nodes (posterior probabilities <0.90 and bootstrap values <60 are not shown).

gussevi within subclade 2, and the morphological characteristics shared by species circumscribed in category (i) – that is, non-articulated bars and accessory piece distally bifid, as defined by Cohen *et al.* (2020) – could be considered to amend the diagnosis of *Cometocleithrum* in order to distinguish this group of species from those of category (ii), which could represent different genera.

In their study, Cohen *et al.* (2020) questioned the validity of *Paracosmetocleithrum* Acosta, Scholz, Blasco-Costa, Alves & Silva, 2018, and based on the morphological evaluation of the type-material, they synonymized *Paracosmetocleithrum* with *Cosmetocleithrum*, transferring its only member to *Cosmetocleithrum* as *C. trachydorasi*. In our study, based on the analysis of partial 28S rDNA fragments, the position of *C. trachydorasi* within the subclade 2 (fig. 1) clearly indicates that the taxonomic action of Cohen *et al.* (2020) is well supported.

Cosmetocleithrum was originally proposed for dactylogyrid parasites of doradid catfishes possessing gonads in tandem, copulatory complex comprising a variably coiled cirrus with counterclockwise rings, elaborate accessory piece, vagina sinistral and dorsal bar with two submedial projections arising from anterodorsal surface of the bar (Kritsky *et al.*, 1986). In the last decade, as many as 15 species of *Cosmetocleithrum* have been described, and some novel morphological characteristics have been added to the generic diagnosis. For instance, *C. bifurcum* (member of the 'doradid group') and *C. baculum* (member of the 'auchenipterid group') possess two types of hooks, whereas all hook pairs are similar in the rest of species. Moreover, *C. tortum* Mendoza-Franco, Mendoza-Palmero & Scholz, 2016 (not analysed in this study) possesses dextral vagina, whereas for all known species of the genus the vaginal aperture is present on the left margin of the body. Regarding the shape of the accessory piece and number of rings of the Male Copulatory Organ (MCO), these characteristics are highly variable between species, with the exception of *C. falsinulatum* (member of the 'doradid' group) having a unique cork-screw-like MCO morphology (see Feronato *et al.*, 2022).

Our study provides two fundamental suggestions for future studies on *Cosmetocleithrum* spp. First, the molecular characterization of 11 species of the genus remains to be carried out – that is, *C. akuanduba* Soares, Neto & Domingues, 2018, *C. berecae* Cohen, Justo, Gen & Boeger, 2020, *C. galeatum* Yamada, Yamada & Silva, 2020, *C. leandroi* Soares, Neto & Domingues, 2018, *C. longivaginatum* Suriano & Incorvaia, 1995 (the only species infecting pimelodids), *C. nunani*, *C. phryctophallus*, *C. sobrinus* Kritsky, Thatcher & Boeger 1986, *C. spathulatum* Yamada, Yamada & de Silva, 2020, *C. striatuli* Abdallah, Azevedo & Luque, 2012 and *C. tortum*, in order to evaluate whether the inclusion of these species may support subclades 1 and 2 obtained in this study. Secondly, the search for synapomorphies to characterize taxonomic groups within *Cosmetocleithrum* is essential, but at the same time this task appears challenging, since the morphology of haptoral elements of *Cosmetocleithrum* spp. is quite conservative, and that of the copulatory complex is highly variable between species as previously mentioned.

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Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of animals.

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