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A Poorly Known Catfish Clade in an Endangered Neotropical Biodiversity Hotspot: Relationships and Distribution Patterns of the *Cambeva variegata* Group (Siluriformes: Trichomycteridae)

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Abstract: The *Cambeva variegata* group (CVG) is endemic to a region situated in the intersection of two endangered biodiversity hotspots, Cerrado and Atlantic Forest, and drained by two important South American river basins, the upper Rio Paraná and upper Rio São Francisco basins. Presently, CVG comprises two nominal species, besides some still undescribed. We first performed a molecular phylogenetic analysis (total of 3368 bp) for five species of the CVG and 30 outgroups, which supported the monophyly of the CVG and its inclusion in *Cambeva*. Most morphological character states distinguishing the CVG from congeners are also present in *Scleronema*, possibly consisting of plesiomorphic features. We also performed the first time-calibrated phylogeny of the group, which supported possible relationships between present geographical distribution patterns and palaeogeographical events. The estimated time of origin of CVG in the Middle Miocene is nearly contemporaneous to a past hydrographical configuration when part of the upper Rio Paraná basin was connected to the Rio São Francisco basin. The first CVG lineage split occurring in the Miocene end corresponds to a major break in that palaeo basin. Species diversification between the Pliocene and early Pleistocene is compatible with final drainage rearrangement. This study highlights the urgent need for more detailed studies on the diversity and phylogenetic relationships of still poorly known organisms in this highly diverse and threatened region.

Keywords: Atlantic Forest; Cerrado; molecular phylogeny; mountain biodiversity; osteology; Rio Paraná; Rio São Francisco

Key Contribution: This paper comprises the first osteological character survey of the CVG, supporting clade diagnoses; the first time-calibrated phylogenetic analysis focusing on Cambeva; and the first attempt to integrate the molecular phylogeny of Cambeva, present distribution patterns, and palaeogeographical scenarios.

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1. Introduction

Fast-flowing rivers and streams draining the mountain ranges of southeastern Brazil in the region comprising the upper Rio Paraná basin and the upper Rio São Francisco basin (hereafter Upper Paraná–São Francisco Region, UPSFR), such as Serra da Canastra, Serra do Espinhaço, and Serra da Mantiqueira, among others, shelter a huge diversity of trichomycterine mountain catfishes, mostly revealed in recent years (e.g., [1] and included

references). The great majority of trichomycterines endemic to this region belong to the genus *Trichomycterus* Valenciennes, 1832, whereas species of *Cambeva* Katz, Barbosa, Mattos, and Costa, 2018, are rare. *Cambeva* presently includes about 50 species occurring between the tropical area drained by the Rio São Francisco basin, about 16°10′ S, and the subtropical area drained by the rivers connected to the Lagoa dos Patos system, about 29°30′ S [2]. However, only two species of *Cambeva* occur and are endemic to UPSFR, *Cambeva concolor* Costa, 1992, and *Cambeva variegata* Costa, 1992, both endemic to the upper Rio São Francisco basin [3]. These species are easily distinguished from all other species of *Cambeva* by the presence of a prominent skin crest on the dorsal margin of the caudal peduncle [3], a condition similar to that occurring in *Scleronema* Eigenmann, 1917 [4–6], and to the adipose fin of the Copionodontinae [7]. Our field studies have shown that this group of *Cambeva*, hereafter the *Cambeva variegata* group (CVG), also includes undescribed species, some of which are endemic to the Rio Grande drainage, upper Rio Paraná basin, consisting of the first records of CVG for UPSFR.

Whereas the great species diversity of *Trichomycterus* from UPSFR has been reported in frequent publications (e.g., [2,8] and included references), little has been published about species of the CVG from the same region. Mentions of this group are restricted to a single taxonomic paper published 32 years ago [3] and a few phylogenetic studies inferring the positioning of *C. variegata* among other congeners (e.g., [2,9,10]). The scarcity of studies on the CVG is probably due to two factors. Firstly, most species of this group are extremely similar when compared only by external morphology, making the identification and recognition of new species inaccessible to biologists not trained in osteological examination or molecular biology. Secondly, there is great difficulty in field collecting, since species in this group have shown to be restricted to small areas and are often rare in their environments.

UPSFR is situated in the intersection of two phytogeographical provinces considered among the main biodiversity hotspots of the world, the savannah-like Cerrado and the Atlantic Forest [11]. On the other hand, rivers and streams of this area are highly impacted by anthropogenic factors, such as large dams for the generation of electricity, destruction of the hydrographic structure for mineral extraction, water pollution by domestic and industrial sewage and by pesticides used in agriculture, and the introduction of exotic species and deforestation of marginal areas (e.g., [1]). The growing process of environmental degradation requires urgency in studies focusing on the diversity of this rare fish group. The objectives of the present study are to present the first phylogenetic analysis involving a large species sample of the CVG, testing the monophyly of the group; to provide a comparative osteological analysis allowing morphological diagnoses of clades supported in molecular analyses; and to present the first time-calibrated phylogeny of the group in order to find evidence of possible relationships between the present geographical distribution patterns and palaeogeographical events.

2. Materials and Methods

2.1. Specimens

Fish collections were performed during daylight with dip nets. Collecting permits were provided by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade; permit numbers: 38553-13 and 64415-5). Methods for fish collection, euthanasia using a buffered solution of tricaine methane sulphonate (MS-222) at a concentration of 250 mg/L (e.g., AVMA Guidelines for the Euthanasia of Animals) [12], and fixation were approved by CEUA-CCS-UFRJ (Ethics Committee for Animal Use of Federal University of Rio de Janeiro; permit numbers: 065/18 and 084/23). For fixation, specimens were kept in formalin for two weeks, or fixed in absolute ethanol in cases of specimens used in the molecular analysis. Formalin-fixed specimens were preserved in 70% ethanol. Among these specimens, between three and five were prepared for osteological examination, using clearing and staining techniques described by Taylor and Van Dyke [13], and later preserved in glycerine. In the list of specimens below, C&S means specimens cleared and stained for osteological examination, and DNA means specimens directly fixed in absolute

ethanol for molecular analysis. Specimens were deposited in the ichthyological collections of Instituto de Biologia, Universidade Federal do Rio de Janeiro (UFRJ), and Centro de Ciências Agrárias e Ambientais, Universidade Federal do Maranhão (CICCAA).

Specimens examined: *Cambeva variegata* (UFRJ 8355, 7 ex.; UFRJ 8314, 1 ex. (DNA); UFRJ 8318, 1 ex. (DNA); UFRJ 8319, 1 ex. (DNA); UFRJ 9346, 2 ex. (C&S): topotypes: 20°15′09″ S 46°24′23″ W; UFRJ 585, 2 paratypes, (C&S); UFRJ 12857, 3 ex.; UFRJ 12833, 1 ex. (DNA): 20°15′50″ S 46°20′56″ W; UFRJ 14072, 9 ex.; UFRJ 12928, 3 ex. (DNA): 20°15′50″ S 46°20′56″ W); *Cambeva* sp. 1 (UFRJ 14061, 12 ex.; UFRJ 14062, 5 ex., C&S; UFRJ 14063, 6 ex., DNA: 22°28′02″ S 45°21′38″ W); *Cambeva* sp. 2 (UFRJ 14059, 5 ex.; UFRJ 14060, 2 ex., C&S: 20°34′47″ S 46°20′56″ W); *Cambeva* sp. 3 (UFRJ 14050, 1 ex.; UFRJ 14051, 11 ex; UFRJ 14052, 4 ex. (C&S); UFRJ 14042, 2 ex. (DNA); CICCAA 08193, 4 ex.: 20°36′27″ S 46°26′09″ W); *Cambeva* sp. 4 (UFRJ 13637, 1 ex.; UFRJ 14054, 1 ex.; UFRJ 13638, 1 ex. (DNA); UFRJ 14055, 9 ex.; UFRJ 14056, 4 ex. (DNA); UFRJ 14057, 5 ex. (C&S): 20°35′19″ S 46°13′41″ W); *Cambeva* sp. 5: (UFRJ 13653, 1 ex.; UFRJ 12967, 6 ex.; UFRJ 12968, 22 ex.; UFRJ 12983, 9 ex.; UFRJ 13654, 4 ex. (C&S); CICCAA 07950, 10 ex.: 19°58′29″ S 43°51′39″ W; UFRJ 12965, 7 ex.; UFRJ 12966, 15 ex.; UFRJ 12982, 3 ex.: 19°58′26″ S 43°47′47″ W). In addition, we accessed data taken from the type material of *C. concolor* and *C. variegata*, deposited in Museu de Zoologia, Universidade de São Paulo, at the time of the original description by one of us (WJEMC). Comparative material appears in Costa et al. [11] and included references.

2.2. Morphological Data

Methods for taking morphological data and terminology followed the methods of our most recent studies on the systematics of eastern South American trichomycterines (e.g., [1]), which were based on Costa [14] and Kubicek [15] for bone nomenclature, Arratia and Huaquin [16] and Bockmann and Sazima [17] for the terminology of pores of the cephalic latero-sensory system, and Bockmann and Sazima [17] for fin-ray formulae. Morphological comparisons were made in the two nominal species, *C. concolor* and *C. variegata*, and in five undescribed species, *Cambeva* sp. 1–5 (see list of specimens above).

2.3. DNA Extraction, Amplification, and Sequencing

Methods for DNA extraction are those described in our recent studies on trichomycterines [1]. Polymerase chain reaction (PCR) was performed with the following primers for mitochondrial encoded genes: Cytb Siluri F and Cytb Siluri R [18] for cytochrome b (CYTB); FISH-F1 and FISH-R1 [19] for cytochrome c oxidase I (COX1); L11935 and H12857 [20] for NADH: ubiquinone oxidoreductase core subunit 4 (ND4), along with t-RNA-His, Ser, and Leu; and for the nuclear gene recombination activating 2 (RAG2), RAG2 TRICHO F and RAG2 TRICHO R [21]. PCR was performed in 45 µL with the following reagent concentrations: 5× GreenGoTaq Reaction Buffer (Promega), 2.0 mM $MgCl_2$, 1 µM of each oligo, 0.2 mM of each dNTP, 1 µL of Promega GoTaq Hot Start polymerase, and 50 ng of total genomic DNA. Negative controls were used to check for contaminants. The thermal profile consisted of initial denaturation at 95 °C for 5 min; followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 45–50 °C for 1 min, and extension at 73 °C for 1–1.5 min; with a final extension at 73 °C for 7 min. PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing reactions were performed in 20 µL reaction volumes containing 4 µL BigDye, 2 µL sequencing buffer 5× (Applied Biosystems), 2 µL of the PCR products (30–40 ng), 2 µL primer, and 10 µL ultrapure water, and the thermal profile was 35 cycles of 30 s at 95 °C, 30 s at 55 °C, and 1.5 min at 72 °C. MEGA 11 [22] was used for analysing sequencing chromatograms and sequence annotation, and for translating DNA sequences into amino acid residues to confirm the absence of premature stop codons or indels. GenBank accessions are provided in Appendix A.

2.4. Phylogenetic Analysis

Terminal ingroup taxa comprised a total of fifteen species, including five species of the CVG and ten species representing other lineages of *Cambeva*. The species of the CVG

analysed were *C. variegata* and four undescribed species. *Cambeva concolor* and *Cambeva* sp. 2, without available material for DNA sequencing, were not included in the phylogenetic analysis, with their possible relationships inferred from an examination of morphological traits (see list of specimens examined above). Outgroups comprised five species of *Scleronema*, the sister group of *Cambeva,* and five species of *Trichomycterus s.s.*, the sister group of *Cambeva* plus *Scleronema* [2], two trichomycterines representing another subfamilial lineage, three species representing other trichomycterid subfamilies, four species representing other catfish families, and one representative of another Ostariophysi lineage, order Characiformes. The gene datasets were aligned using the Clustal W algorithm [23] implemented in MEGA 11, not finding gaps or stop codons. The 3368 bp complete dataset (COX1 732 bp, ND4 693 pb, tRNA His Ser Leu 162 pb, CYTB 993 bp, RAG2 788 bp) was analysed using PartitionFinder2.1.1 [24] for optimal partitioning and evolutionary models, using the Corrected Akaike Information (AICc) selection criteria. Partitions and respective models appear in Appendix B.

Bayesian Inference (BI) and Maximum Likelihood (ML) analyses were utilised as independent approaches for phylogenetic reconstruction, aiming to mitigate methodological biases. BI was conducted using Beast 1.10.4 [25] with the following parameters: Birth–Death process as the tree prior [26], and two independent Markov chain Monte Carlo (MCMC) runs with 10^7 generations with a sampling frequency of 1000 each. The convergence of the MCMC chains and the proper burn-in value were determined by evaluating the achievement of the stationary phase and the effective sample size for all the analysis parameters in both runs using Tracer 1.7.2 [27]. LogCombiner v.1.10.4 and the Tree Annotator version 1.10.4 [25] were employed to combine and calculate the consensus tree, apply the burn-in, and annotate the Bayesian posterior probabilities. The ML analysis utilised IQTREE 2.2.2.6 [28]. Node support was assessed by ultrafast bootstrap (UFBoot) [29] and the Shimodaira–Hasegawa-like approximate likelihood ratio test (SH-aLRT), both with 1000 replicates [30].

2.5. Divergence Time Estimation

The divergence time analysis was conducted in Beast 1.10.4 using the same dataset, partitions, evolution models, tree priors, and parameters as described above. Additionally, the analysis incorporated a lognormal uncorrelated relaxed clock model. Calibration points were established as follows: the origin of Siluriformes Order with a normal prior distribution (mean = 140 MA, SD = 7) following Lundberg et al. [31], the origin of the Trichomycteridae with a normal prior distribution (mean = 106 MA, SD = 5.0) following the estimative of Betancur-R et al. [32]; and the origin of the genus *Corydoras* Lacépède, 1803, with a lognormal prior distribution (mean = 55 MA, SD = 2.5), based on the dating of the oldest known *Corydoras* fossil species, *Corydoras revelatus* Cockerell, 1925. MCMC chains were assessed to verify convergence by evaluating the effective sample size of the runs in Tracer 1.7.1. The time-scaled tree was obtained using Tree Annotator version 1.10.4 to generate the consensus tree.

3. Results

3.1. Phylogenetic Relationships and Comparative Morphology

The CVG was supported as monophyletic with maximum values, whereas relationships among the more inclusive lineages of *Cambeva* were weakly supported (Figure 1). Individual tree loci generated compatible results (Supplementary File S1), with the CVG recovered as monophyletic in both trees, but without any internal resolution in the RAG2 tree. In addition to the presence of an adipose crest on the caudal peduncle (Figure 1A,B), CVG species differ from other congeners by the presence of an interrupted supraorbital canal, with an s4 pore (Figure 2; vs. continuous, s4 absent), a pronounced narrowing at the lateral end of the premaxilla (Figure 3A–E; vs. expansion absent), and a ventral expansion in the preopercle (Figure 4; vs. expansion absent). In addition, only in species of the CVG, the foramen of the parurohyal is rudimentary or absent (Figure 5B–E), but

a broad foramen is present in a new species of this clade described below (Figure 5A), possibly as a result of a reversal due to its apical position in the phylogenetic tree (Figure 1). The CVG group comprises two main lineages, *Cambeva* sp. 1 from tributaries of the Rio Grande drainage at the Serra da Mantiqueira and the *Cambeva variegata* complex, a group of similar species occurring in a broad geographical region encompassing the upper Rio São Francisco basin and an adjacent area of the Rio Grande basin at the Serra da Canastra (Figure 6). A taxonomical revision of the *Cambeva variegata* complex is in progress by the authors and new species here cited will be described elsewhere.

Cambeva sp. 1, previously identified as Trichomycteridae sp. by Thereza and Langeani [8], was strongly supported as a member of the CVG here. Externally, it differs from all other congeners by having a long dorsal fin with twelve or thirteen principal rays (vs. nine or ten) (Figure 1A). Seven osteological features distinguish this species from all other congeners: postero-lateral process of the autopalatine posteriorly directed (Figure 3E; vs. postero-laterally directed, Figure 3A–D); posterior extremity of the lateral process of the vomer sinuous (Figure 3G; vs. convex, Figure 3F); six branchiostegal rays (vs. seven or eight); epibranchial 2 elongated, lacking the postero-distal process (Figure 7B; vs. not elongated, with a postero-distal process, Figure 7A); pharyngobranchial 4 relatively large, occupying more than half the surface of the adjacent dentigerous plate (Figure 7B; vs. one-third or less, Figure 7A); dorsal and ventral hypural plates in close proximity, separated by a short posterior interspace (Figure 8B; vs. completely separated, Figure 8A); and pelvic bone relatively broad, with short anterior processes (Figure 8D; vs. narrower, with longer anterior processes, Figure 8C). In addition, the entire anterior portion of the neurocranium is proportionally slenderer (Figure 3E) than in other congeners (Figure 3A–D). This taxon also differs from all other congeners of the CVG by the absence of a pectoral fin filament and the presence of a lateral expansion of the mesethmoid (Figure 3E; vs. absence); the mesethmoid cornua having a relatively short and sharply pointed cornu (Figure 3E; vs. longer, not sharply pointed, Figure 3A–D); and pharyngobranchial 3 with distinctive lateral constriction (Figure 7B; vs. without lateral constriction, Figure 7A). This species is being formally described by F. Langeani and collaborators (Langeani personal communication to WJEMC, 2 January 2024).

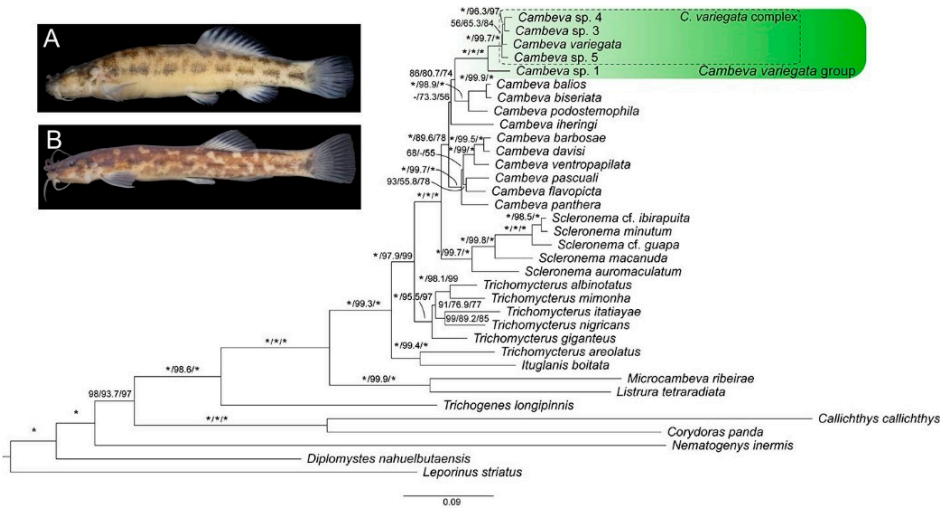

Figure 1. Phylogenetic tree obtained from the Bayesian analysis in BEAST 1.10.4 for 15 species of *Cambeva*, including 5 belonging to the *C. variegata* species group, and 20 outgroup species, using a dataset comprising COI, CYTB, ND4, t-RNA-His, Ser, Leu and RAG2 (total of 3368 bp). Numbers separated by bars (/) above branches indicate posterior probabilities from the Bayesian Inference, followed by ultrafast bootstrap (UFBoot) and the Shimodaira–Hasegawa-like approximate likelihood ratio test (SH-aLRT) from the Maximum Likelihood analysis. Asterisks (*) indicate maximum support values, and dashes (-) indicate support values below 50. (**A**) *Cambeva* sp. 1; (**B**) *Cambeva* sp. 5.

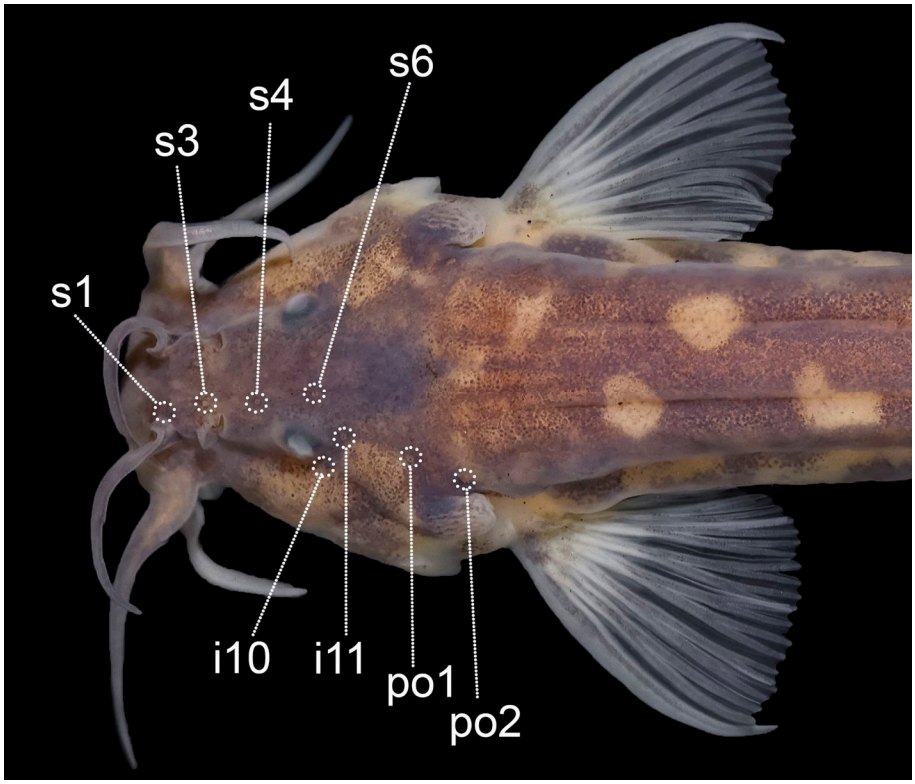

Figure 2. Head, dorsal view, showing cephalic pores of the latero-sensory system of *Cambeva* sp. 5, UFRJ 12967, 46.5 mm SL.

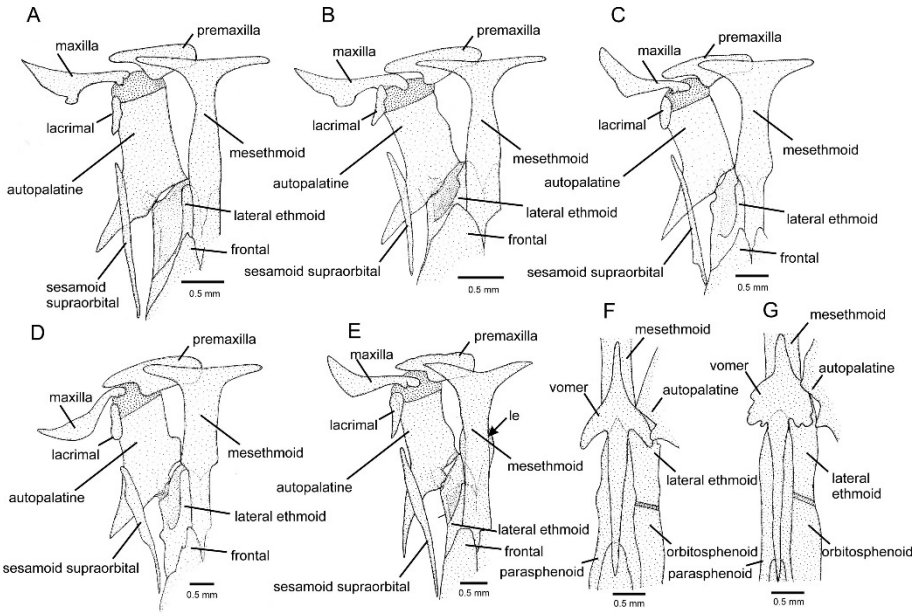

Figure 3. Mesethmoidal region: (**A**–**E**) middle and left portions, dorsal view, (**F**,**G**) middle portion, ventral view: (**A**) *Cambeva* sp. 3; (**B**) *Cambeva* sp. 4; (**C**,**F**) *Cambeva* sp. 5; (**D**) *Cambeva variegata*; (**E**,**G**) *Cambeva* sp. 1. Arrow indicates the lateral expansion of the mesethmoid. Larger stippling represents cartilage.

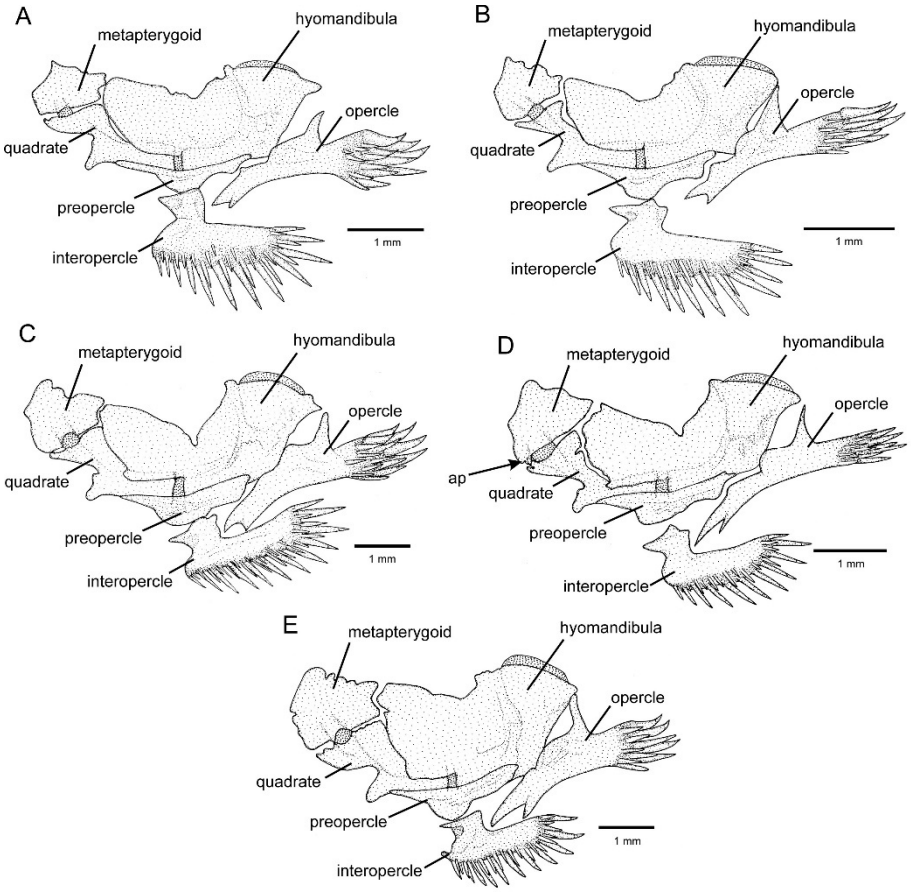

Figure 4. Left jaw suspensorium and opercular series, lateral view: (**A**) *Cambeva* sp. 3; (**B**) *Cambeva* sp. 4; (**C**) *Cambeva* sp. 5; (**D**) *Cambeva variegata*; (**E**) *Cambeva* sp. 1. Arrow indicates the anterior articulatory process. Larger stippling represents cartilage.

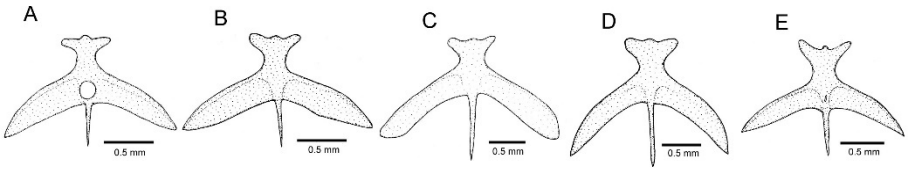

Figure 5. Parurohyal, ventral view: (**A**) *Cambeva* sp. 3; (**B**) *Cambeva* sp. 4; (**C**) *Cambeva* sp. 5; (**D**) *Cambeva variegata*; (**E**) *Cambeva* sp. 1.

The *C. variegata* complex, comprising *Cambeva variegata*, *Cambeva* sp. 3, *Cambeva* sp. 4, and *Cambeva* sp. 5, was corroborated in the phylogenetic analysis with high support values (Figure 1). In addition to the unique character states described for *Cambeva* sp. 1 that are not present in the *C. variegata* complex (see above), species of this complex are distinguished from all congeners by a relatively short posterior process of the vomer, which is about 1.5 times longer than the vomer length excluding the posterior process (Figure 3F; vs. about two times longer or more, Figure 3G). Relationships within the *C. variegata* group were weakly supported, except for the sister group relationships between *Cambeva* sp. 3 and *Cambeva* sp. 4, two species occurring in close localities of the upper Rio Paraná basin at Serra da Canastra (Figure 6).

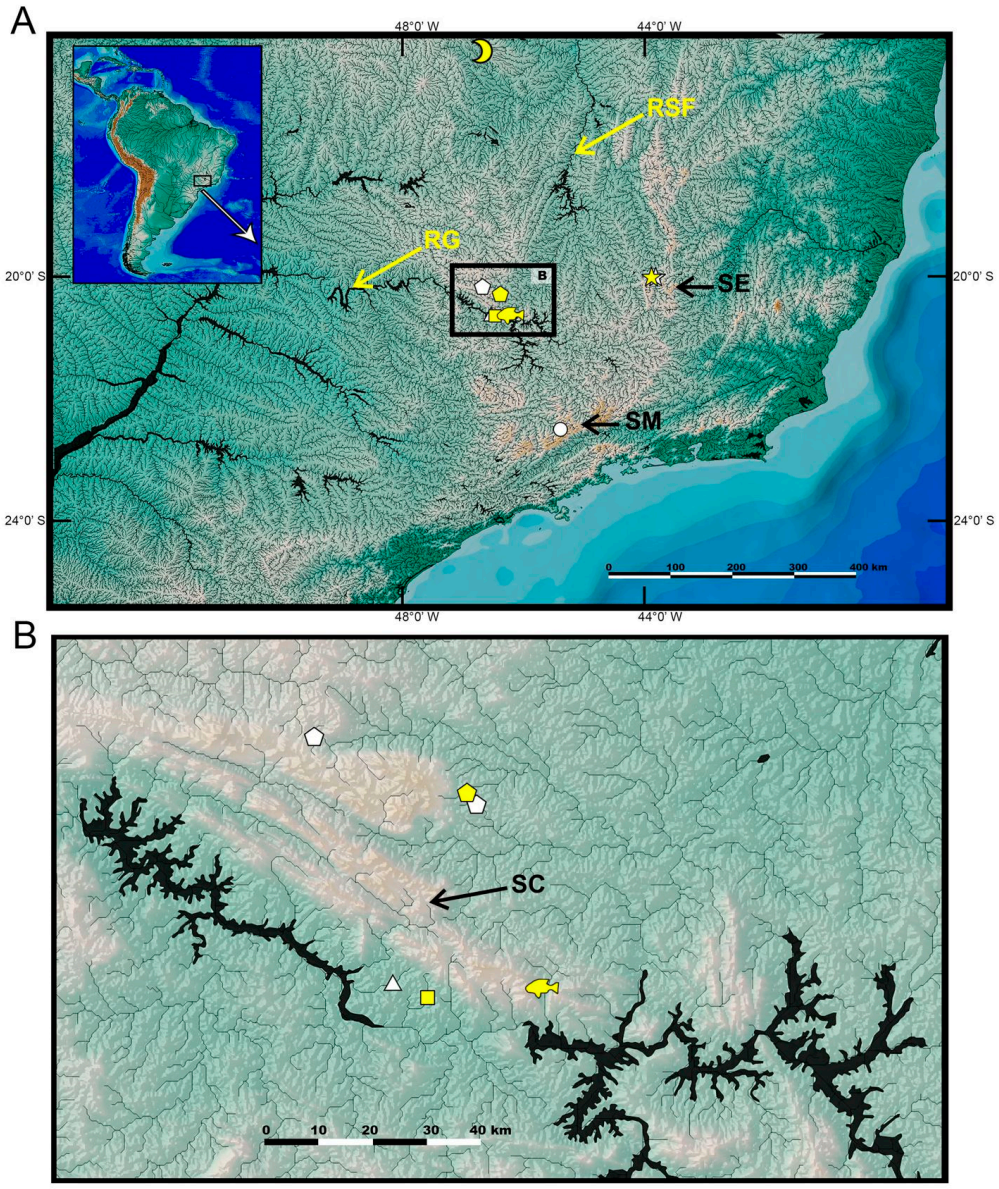

Figure 6. Geographical distribution of the *Cambeva variegata* group ((**A**), general view of the region; (**B**), detailed view of the area in Serra da Canastra): circle, *Cambeva* sp. 1; fish, *Cambeva* sp. 4; half-moon, *Cambeva concolor*; pentagons, *Cambeva variegata*; square, *Cambeva* sp. 3; stars, *Cambeva* sp. 5; triangle, *Cambeva* sp. 2. Black arrows indicate mountain ranges: SC, Serra da Canastra; SE, Serra do Espinhaço; SM, Serra da Mantiqueira. Yellow arrows indicate major rivers: RG, Rio Grande; RSF, Rio São Francisco.

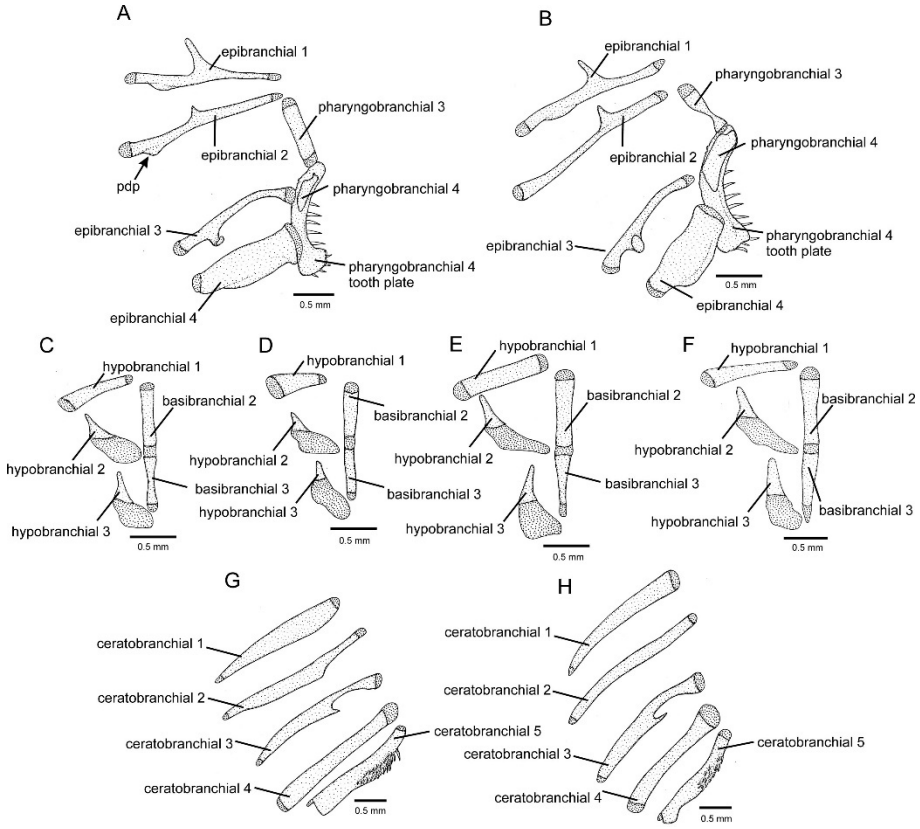

Figure 7. Branchial bones: (**A**,**B**) left dorsal arches, dorsal view, (**C**–**F**) basibranchials and left hypobranchials, dorsal view, (**G**,**H**) left ceratobranchials, dorsal view: (**A**) *Cambeva variegata*; (**B**,**F**,**H**) *Cambeva* sp. 1; (**C**) *Cambeva* sp. 3; (**D**) *Cambeva* sp. 4; (**E**,**G**) *Cambeva* sp. 5. Arrow indicates the poster-distal process of the first epibranchial. Larger stippling represents cartilage.

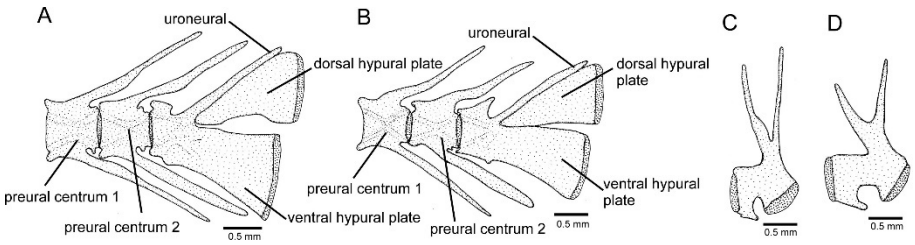

Figure 8. Post-cranial structures: (**A**,**B**) caudal skeleton, left lateral view, (**C**,**D**) pelvic bone: (**A**) *Cambeva variegata*; (**B**,**D**) *Cambeva* sp. 1; (**C**) *Cambeva* sp. 5. Larger stippling represents cartilage.

3.2. Time-Calibrated Phylogeny

According to the time-calibrated analysis, the origin of *Cambeva* would have occurred in the late Oligocene, whereas the origin of CVG occurred in the middle Mioceno, which was followed by the split between *Cambeva* sp. 1 and the *C. variegata* complex in the late Miocene, with the diversification within the *C. variegata* complex occurring between the Pliocene and Pleistocene (Figure 9).

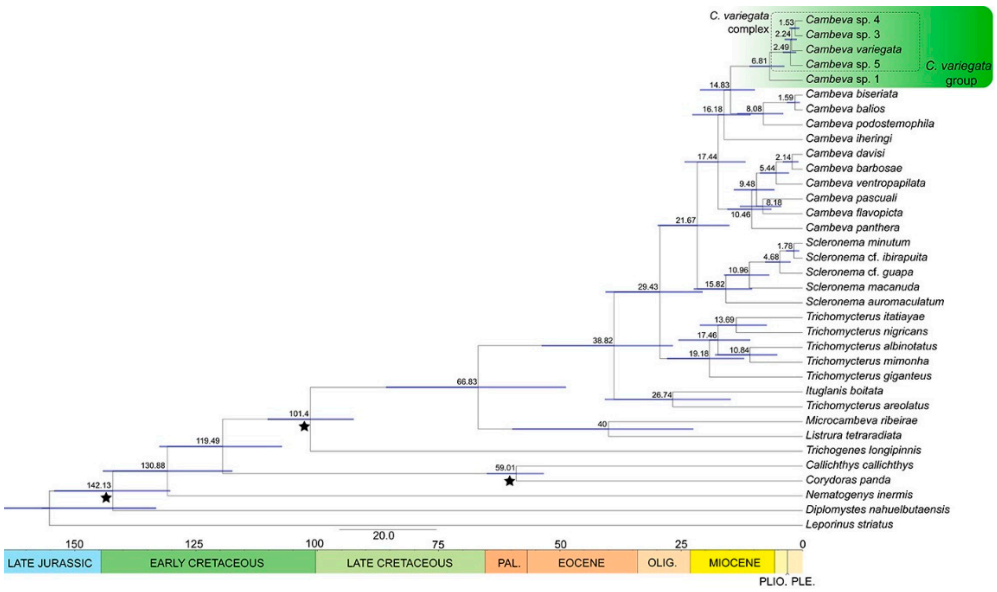

Figure 9. Time-scaled phylogeny obtained from the Bayesian analysis in BEAST 1.10.4 for 15 species of *Cambeva* species, including 5 belonging to the *C. variegata* species group, and 20 outgroup species, using a dataset comprising COI, CYTB, ND4, t-RNA-His, Ser, Leu, and RAG2 (total of 3368 bp). Black stars indicate calibration points, numbers above nodes indicate median age, blue bars on nodes indicate 95% HPD ranges of those ages, and coloured bars below the tree represent geological epochs.

4. Discussion

4.1. Phylogenetic Relationships

Species of the CVG share four morphological character states that are unique among congeners: 1—the presence of an adipose crest on the caudal peduncle; 2—an interrupted supraorbital canal, with an s4 pore (Figure 2); 3—a pronounced narrowing at the lateral end of the premaxilla (Figure 3); 4—a ventral expansion in the preopercle (Figure 4). However, the first three character states are also present in species of the genus *Scleronema* [4–6], which is sister to *Cambeva* [2,33], but not in *Trichomycterus s.s.* [14], sister to the clade comprising *Cambeva* and *Scleronema* [2].

Considering only the distribution of these morphological character states, the most plausible hypothesis would be to interpret *Cambeva* as paraphyletic, with the CVG being more related to *Scleronema* than to the other lineages of *Cambeva*. However, all molecular phylogenetic analyses available to date point to the monophyly of *Cambeva* when including the species of the CVG (e.g., [2,9,10]), although the position of this group within *Cambeva* was different in previous studies (i.e., sister to *Cambeva iheringi* instead of to the *Cambeva* gama-clade as in the present study). It is important to note that the topology found in previous studies that used a smaller sample of genetic markers, where the CVG is sister to *C. iheringi*, is congruent with the unique possession of a long supraorbital sesamoid in these two groups (Figure 3), which does not occur in other species of *Cambeva* and *Scleronema*. The possibility of the three conditions shared by species of the *C. variegata* group and *Scleronema* consisting of primitive conditions present in the most recent common ancestor of the clade comprising *Cambeva* plus *Scleronema* and subsequently lost in other lineages of *Cambeva* cannot be ruled out. An accurate optimisation of these character states depends on a phylogeny where all *Cambeva* lineages are positioned with high support values, which is still not available.

Monophyly of the *C. variegata* group is tentatively supported by the presence of a ventral expansion in the preopercle (character state 4 above), a condition variable within the CST clade. A rudimentary or absent middle foramen of the parurohyal (Figure 5B–E) may be considered further evidence of monophyly if admitting a reversion in *Cambeva* sp.

3, which is the only species of the group with a broad foramen (Figure 5A) and appearing in an apical position within the CVG topology (Figure 1).

4.2. Distribution Patterns

The geographical distribution of the CVG comprises a wide region of southeastern Brazil encompassing the upper Rio São Francisco basin and the Rio Grande drainage, which is a part of the Rio Paraná basin (Figure 6). As in most groups of Trichomycterinae, the distribution of the CVG is concentrated in mountain ranges, such as the Serra da Canastra, Serra do Espinhaço, and Serra da Mantiqueira. Geological data indicate that the Serra da Mantiqueira region, the area of endemism of *Cambeva* sp. 1, which is strongly supported as the sister group to all other CVG species, already acted as a watershed between the basins located south of this mountain range and the Rio Grande drainage at least since the Eocene, which happened synchronously with the installation of the Continental Rift of southeastern Brazil [34]. Evidence indicates that the natural flow path of the Rio Grande drainage course headed towards the São Francisco River basin, with which it was connected in the past [35]. Thus, at least during the Paleogene and part of the Neogene, the Rio Grande drainage acted as an upper tributary of the Rio São Francisco basin, not as a main tributary of the Rio Paraná River as presently. Therefore, the origin of the CVG here estimated to have occurred in the Middle Miocene (Figure 9) portrays a palaeogeographic scenario in which the Rio Grande drainage and the São Francisco basin formed a single hydrographic basin.

Geological data indicate that the disruption of the ancient connections between the current Rio Grande drainage and the São Francisco River basin, and the consequent capture of the Rio Grande drainage by the Rio Paraná basin, began in the Middle Miocene, following a process of widespread uplift in the region [35]. This historical process is compatible with the divergence found between *Cambeva* sp. 1, endemic to the Rio Grande drainage, and the *C. variegata* complex, endemic to the upper Rio São Francisco basin and a small area of the Rio Grande drainage on the periphery of the upper Rio São Francisco, which would have occurred at the end of the Miocene according to our estimates (Figure 9). Therefore, a major rupture in the Grande-São Francisco palaeo basin would be responsible for a vicariance event involving *Cambeva* sp. 1 and the *Cambeva variegata* complex. A similar vicariance event was hypothesised by Vilardo et al. [36] for lineages of the subgenus *Paracambeva* Costa, 2021, of the genus *Trichomycterus* inhabiting the same area.

Additionally, geological data suggest that the drainage rearrangement was a gradual process, reaching the current hydrographic configuration only in the Pliocene or early Pleistocene [35]. Precisely, the area of the Serra da Canastra region that separates the distribution of *C. variegata*, endemic to the São Francisco River basin, from the area inhabited by the clade comprising *Cambeva* sp. 3 and *Cambeva* sp. 4 (Figure 6) is one of the points of altimetric anomalies, characterising an area of probable past connection between the drainage of the Rio Grande and the Rio São Francisco [35]. Therefore, despite the low support values found at internal nodes of the *C. variegata* complex, making the relationship hypothesis weak, the estimated split between those two lineages in the Pliocene may indicate a past rupture event in this particular area, artificially restored with transposition works during construction of the Furnas hydroelectric power dam carried out in the 1960s [37].

5. Conclusions

This study reports the occurrence of the genus *Cambeva* in a vast area of southeastern Brazil, including the first records of the genus in two mountain ranges considered among the most important centres of biodiversity in the world, with high rates of species endemism: the Serra do Espinhaço and Serra da Mantiqueira [38,39]. Over 40 trichomycterines of the genus *Trichomycterus s.s.* have been continuously reported from these two mountain ranges since the nineteenth century [40,41] and early twentieth century [42,43] to the present (e.g., [2] and included references), where they are usually common in every river drainage, contrasting with only two species of the CVG reported here for the first time. Therefore,

this study demonstrates the relative rarity of species of the CVG and its important role in recovering ancient biogeographic patterns, highlighting the urgent need for more detailed studies on the diversity and phylogenetic relationships of still poorly known organisms in this highly diverse and threatened region.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes9040116/s1, Supplementary File S1: Gene loci trees.

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Appendix A

Table A1. Terminal taxa for molecular phylogeny and respective GenBank accession numbers.

	COI	ND4	tRNA Ser His Leu	CYTB	RAG2
Leporinus striatus	JN989019.1	—	—	EU183020.1	AY804096.1
Diplomystes nahuelbutaensis	AP012011.1	NC015823.1	NC015823.1	MN640590	DQ492317
Nematogenys inermis	EU359428	AY307250.1	AY307250.1	—	KY858182.1
Corydoras panda	NC049097.1	GU210065.1	GU210065.1	NC049097.1	KP960362.1
Callichthys callichthys	MZ051783.1	AY307241.1	AY307241.1	KP960058	DQ492324
Trichogenes longipinnis	OQ810037	MN389484	MN389484	MK123704	MF431117
Microcambeva ribeirae	MN385807.1	MN389502.1	MN389502.1	OK334290	MN385832
Listrura tetraradiata	JQ231083	MN389497	MN389497.1	JQ231088.1	MN385826.1
Trichomycterus areolatus	AP012026.1	AP012026.1	AP012026.1	FJ772214	KY858188
Ituglanis boitata	OQ810038	MN389485.1	MN389485.1	MK123706	MK123758
Trichomycterus itatiayae	MW671552	OR948809	—	MW679291	OL779233
Trichomycterus nigricans	MN813005	MN389488.1	MN389488.1	MK123723	MK123765

Table A1. *Cont.*

	COI	ND4	tRNA Ser His Leu	CYTB	RAG2
Trichomycterus albinotatus	MN813007	OM324337.1	OM324337.1	MK123716	MN812990
Trichomycterus mimonha	MW196749	OM324343.1	OM324343.1	MW196758	MW196783
Trichomycterus giganteus	MT470413.1	PP333226	PP336672	MK123720.1	MT446426.1
Scleronema minutum	MK123685	MN389486.1	MN389486.1	MK123707	MK123759.1
Scleronema cf. *guapa*	PP319012	PP333227	PP336673	MK123709.1	MF431118.1
Scleronema cf. *ibirapuita*	MK123688.1	PP333228	PP336674	MK123710.1	MK123761.1
Scleronema macanuda	MK123686.1	PP333229	PP336675	MK123708.1	MK123760.1
Scleronema auromaculatum	OM037445.1	—	—	OM037134.1	OM037136.1
Cambeva barbosae	MK123689.1	MN389487.1	MN389487.1	OQ110808	OQ110815.1
Cambeva balios	OQ810040	PP333230	PP336676	OQ814186	OQ814193
Cambeva pascuali	MF034463	PP333231	PP336677	OQ110811	OQ110820
Cambeva panthera	OQ810041	PP333232	PP336678	OQ814187	OQ814194
Cambeva flavopicta	OQ810042	PP333233	PP336679	OQ814188	OQ814195
Cambeva davisi	PP319014	PP333234	PP336680	MK123714	MK123762
Cambeva biseriata	PP319015	PP333235	PP336681	OQ110806	OQ110817
Camveba ventropapilata	PP319016	PP333236	PP336682	OQ110807	OQ110818
Cambeva iheringi	GU701893	—	—	KY858074	KY858223
Cambeva sp. 1	PP319017	PP333237	PP336683	PP328532	PP333215
Cambeva sp. 5	PP319018	PP333238	PP336684	PP328533	PP333216
Cambeva variegata	PP319019	PP333239	PP336685	PP328534	PP333217
Cambeva sp. 3	PP319020	PP333240	PP336686	PP328535	PP333218
Cambeva sp. 4	PP319021	PP333241	PP336687	PP328536	PP333219
Cambeva podostemophila	OQ810043	—	—	OQ814189	OQ814196

Appendix B

Table A2. Best-fitting partition schemes and evolutive models.

Partition	Base Pairs	Evolutive Model
COI 1st	244	TRN+I+G
COI 2nd	244	HKY+I
COI 3rd	244	TRN+I+G
ND4 1st	231	GTR+I+G
ND4 2nd, CYTB 1nd	562	TRN+I+G
ND4 3rd	231	GTR+G
tRNA His Ser Leu	162	GTR+G
CYTB 1st	331	TRN+I+G
CYTB 3rd	331	GTR+I+G
RAG2 1st	263	HKY+G
RAG2 2nd	263	GTR+I
RAG2 3rd	263	K80+I

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