A new species of *Petalocladius* Sublette & Wirth, 1972 (Diptera, Chironomidae, Orthocladiinae) from the Dominican Republic, with an emended generic diagnosis

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Abstract

Petalocladius dominiensis Andersen & Baranov sp. n. is described and figured based on an adult male collected in the Ebano Verde Scientific Reserve in the Dominican Republic. The new species can be separated from its only congener, *P. setosus* Sublette & Wirth, 1972 from Jamaica, as it is slightly smaller with about 115 dorsocentrals compared to 56 in *P. setosus*. The inferior volsella is broadly subtriangular, while more lingulate in *P. setosus,* and the gonostylus has a small, but distinct megaseta and a subapical brush of spine-like seta, which apparently lack in *P. setosus*. The generic diagnosis is emended including the new species, and the systematic position is briefly discussed.

Introduction

The genus *Petalocladius* was erected by Sublette and Wirth (1972) based on the male and female of *P. setosus* Sublette & Wirth, 1972. The species was collected at Hardwar Gap in Jamaica in February 1969 and the type material is housed in the Smithsonian National Museum of Natural History (USNM). The locality is situated at an altitude of about 1,200 m in the Blue Mountains in the central, eastern parts of Jamaica. There are apparently no later records of the species.

Phylogenetic position of this genus remains rather ambiguous, with Sæther (1977) noting possible close relations with *Abiskomiya* Edwards, 1937 and *Nasuticladius* Freeman, 1961, based primarily on the combination of the following synapomorphies: females with two seminal capsules; prominent inferior and superior volsella of the gonocoxite absent; female tergite IX is modified (notched, divided or reduced); female gonapophysis VIII without lateral lobe. Still, Sæther (1977) only tentatively placed the genus, due to the severe lack of known characters. He also noted that the study of the "exotic" genera, such as *Petalocladius* will be beneficial for better understanding of the ground pattern of Orthocladiinae and overall evolution of the group (Sæther 1977: 95).

During fieldwork in the Dominican Republic in December 2019, a single male of a new species of *Petalocladius* was collected at a stream in the Ebano Verde Scientific Reserve. The reserve is in the eastern region of the Cordillera Central, a mountain range with peaks above 1,500 m altitude. *Petalocladius dominiensis* Andersen & Baranov sp. n. is described and figured below, the generic diagnosis is emended, and the systematic position of the genus is briefly discussed based on Bayesian phylogenetic analysis and a-posteriori probability mapping (aka "Rouge plots") (Klopfstein and Spasojevic 2019).

Material and methods

The single male of *Petalocladius dominiensis* was collected in a light trap, preserved in ethanol, and later mounted in Euparal following the procedure outlined by Sæther (1969). The morphological nomenclature follows Sæther (1980).

The specimen was collected under the collection permit from the Ministro de Medio Ambiente y Recursos Naturales of the Dominican Republic for the project "Long peace of the Caribbean – have biota of the Dominican Republic really remained virtually unchanged for over 13 million years?" and was exported under export permit # VAPB-07404. The holotype is housed in the collection at the Department of Natural History, University Museum of Bergen, Norway (ZMBN).

The type specimen of *P. setosus* Sublette & Wirth, 1972 is housed in the United States National Museum (USNM). Photos of the holotype were kindly provided by David Pecor.

For the phylogenetic analysis we have used a morphological character matrix, with 46 taxa and 83 characters. Characters were sampled from larvae, pupae, and adult males and females. Fossils were of course underrepresented in terms of characters available for observation (see Supplementary file available at https://github.com/chironomus/Petalocladius/tree/main). The character matrix for the phylogenetic analysis was built using NEXUS Data Editor v.0.5.0 (Page 2001).

First, we conducted a Bayesian analysis of the morphological matrix alone in MrBayes 3.2.2. (Ronquist et al. 2012) using the Bayesian implementation of Lewis' Markov models (Lewis 2001). In Bayesian inference, two Markov chains were run simultaneously for 10,000,000 generations using a discrete Dirichlet distribution with equal state frequencies (Lewis 2001). Substitution model was set to "gamma" (Nylander et al. 2004). The first 25,0000 generations were discarded as a burn-in (number of MrBayes generations of the tree topology before the apparent stationary condition) (Nylander et al. 2004). Consensus trees showing all compatible groups and 50% compatible groups were computed in MrBayes. Ancestral character state analysis was conducted on the allcompat consensus tree based on morphology alone. Ancestral character state analysis was conducted using ancTresh in the phytools package (version 0.7-80) (Revell 2012). R code for this analysis, alongside the data is provided at https://github.com/chironomus/Petalocladius/tree/main.

To deal with the uncertainty of the positions of the genus on the tree, caused by the lack of knowledge of character states, we have applied Klopfstein and Spasojevic RoguePlots approach (Klopfstein and Spasojevic 2019). We have used a morphological allcompat tree from MrBayes (as described above) to place *Petalocladius* into the high posterior probability regions. RoguePlot for every species and accompanying R code are provided at https://github.com/chironomus/Petalocladius/tree/main.

Results

Petalocladius Sublette & Wirth, 1972

The genus *Petalocladius* was erected by Sublette and Wirth (1972: 1) based on the male and female of *P. setosus* Sublette & Wirth, 1972, and described in some detail. Below we give an emended diagnosis to the genus to include characters from the new species.

Included species:

P. setosus Sublette & Wirth, 1972: 2, Jamaica.

P. dominiensis Andersen & Baranov sp. n. Dominican Republic.

Emended generic diagnosis:

Small species, with wing lengths between 1.5 and 2.0 mm.

Antenna. With 13 flagellomeres, strongly setose, groove beginning at flagellomere 3; sensilla chaetica on flagellomeres 2, 3 and 13. Segment 13 slightly club-shaped apically, without strong subapical setae. Antennal ratio about 0.7.

Head. Eye reniform without dorsomedian extension, hairy. Temporal setae few, with inner verticals, outer verticals and postorbitals present. Clypeus trapezoid with 14–18 setae. Palp with five segments; with few subapical sensilla clavata on segment 3.

Thorax. Antepronotum well developed, separated medially by broad notch. Acrostichals short, decumbent, starting close to antepronotum. Dorsocentrals numerous, short, multiserial; alveolus of each seta surrounded by conspicuous pale area. Prealars uniserial. Scutellars numerous, multiserial, weak.

Wing. Membrane without setae, with moderate punctation. Anal lobe well developed, slightly produced. Costa moderately extended; R_{2+3} ending slightly beyond middle between R_1 and R_{4+5}; R_{4+5} ends proximal to M_{1+2} almost directly over M_{3+4}; Cu_1 moderately curved; FCu distal to RM; postcubitus ending well distal to FCu; anal vein ending at FCu. Brachiolum with 1 seta, R with few setae, remaining veins without setae. Squama with less than 10 setae.

Legs. Pseudospurs, sensilla chaetica and pulvilli absent.

Abdomen. Tergites sparsely covered with setae, concentrated in posterior band, most extensive on tergite VIII.

Hypopygium. Tergite IX short and broad, without anal point. Transverse sternapodeme arched, with weak oral projections. Phallapodeme with mesial apex forming a darkened, beak-like structure. Virga absent. Gonocoxite with well developed, triangular or lingulate inferior volsella. Gonostylus short, subrectangular with long, slightly curved heel; megaseta present or apparently absent.

***Petalocladius dominiensis* Andersen & Baranov sp. n.**

Figs 1 A-D, Fig. 2, Figs 3 A-C

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Type material: Holotype ♂, slide mounted in Euparal. Dominican Republic, La Vega Province, Ebano Verde Scientific Reserve, 19°0'31"N 70°5'43"W, 1065 m asl., 08.12.2019, light trap, leg. Amelie Höcherl (ZMBN).

Etymology: Named after the Dominican Republic, using the latin suffix *-ensis* denoting country of origin.

Diagnostic characters

The new species is slightly smaller than *Petalocladius setosus,* with about 115 dorsocentrals compared to 56 in *P. setosus.* The inferior volsella is broadly subtriangular, while lingulate in *P. setosus,* and the gonostylus has a small but distinct megaseta and a subapical brush of spinelike seta, which seems to lack in *P. setosus.*

Description

Male (n = 1). Total length 2.94 mm. Wing length 1.62 mm. Total length / wing length 1.81. Wing length / length of profemur 2.64.

Coloration. Head, thorax and abdomen brown, legs light brown.

Antenna. AR 0.69. Ultimate flagellomere 296 µm long.

Head. Eye hairy. Temporal setae 6 including 2 inner verticals, 2 outer verticals and 2 postorbitals. Clypeus with 17 setae. Tentorium 139 µm long, 30 µm wide; stipes 109 µm long. Palp as in Fig. 1A; palp segment lengths (in µm): 39, 47, 78, 103, 131. Third palpomere with 2 sensilla clavata subapically, longest 13 µm long.

Thorax (Fig. 1B). Antepronotum with 4 ventrolateral setae. Scutum with 13 short, decumbent acrostichals in anterior half. With about 115 weak dorsocentrals in multiple rows, each sitting in circular, pale brown area. Prealars 8 in single line. Scutellum with about 32 weak setae.

Wing (Fig. 2). VR 1.16. Costal extension about 174 µm long. Brachiolum with 1 seta, R with 6 setae, remaining veins and cells bare. Squama with 7 setae. Sc and R_{2+3} could not be discerned, but wing apparently not fully hardened.

Legs. Spur of fore tibia 42 µm long, spurs of mid tibia 28 µm and 21 µm long, spurs of hind tibia 57 µm and 22 µm long. Width at apex of fore tibia 44 µm, of mid tibia 36 µm, of hind tibia 43 µm. Comb apparently with 6 setae, longest 33 µm long, shortest 21 µm long. Lengths and proportions of legs as in Table 1.

Hypopygium (Figs 1C, D, Figs 3 A-C). Tergite IX with 16 setae. Laterosternite IX with 6 setae. Transverse sternapodeme arched with weak oral projections, 117 µm long. Phallapodeme not measurable. Gonocoxite 267 µm long; densely setose, with some strong, wavy setae apico-laterally, longest about 110 µm long. Inferior volsella subtriangular, 51 µm wide basally, 68 µm long, with row of 7 strong spinelike setae along inner margin and additional weaker dorsal and ventral setae. Gonostylus 86 µm long, 66 µm wide, with brush of spine-like seta subapically, the longest slightly lanceolate and curved; megaseta small, 12 µm long, with denticles; heel 166 µm long, 16 µm wide medially, with rounded apex and few setae subapically, longest about 17 µm long. HR = 3.10; HV = 3.42.

Female and immatures. Unknown.

Habitat

The single male was collected in a light trap in the Ebano Verde Scientific Reserve in Cordillera Central. The trap was deployed on the bank of a small (4–6 m wide), rapid, river with rocky and stony substrate close to the Scientific Reserve rangers' station (Fig. 4).

Table 1. Lengths (in µm) and proportions of leg segments in *Petalocladius dominiensis* Andersen & Baranov sp. n., male (n = 1).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
p_1	605	850	523	310	221	163	82	0.615	2.547	2.781	1.20
p_2	621	719	343	188	139	78	57	0.477	3.646	3.905	1.21
p_3	572	776	425	229	172	172	65	0.547	3.239	3.173	1.36

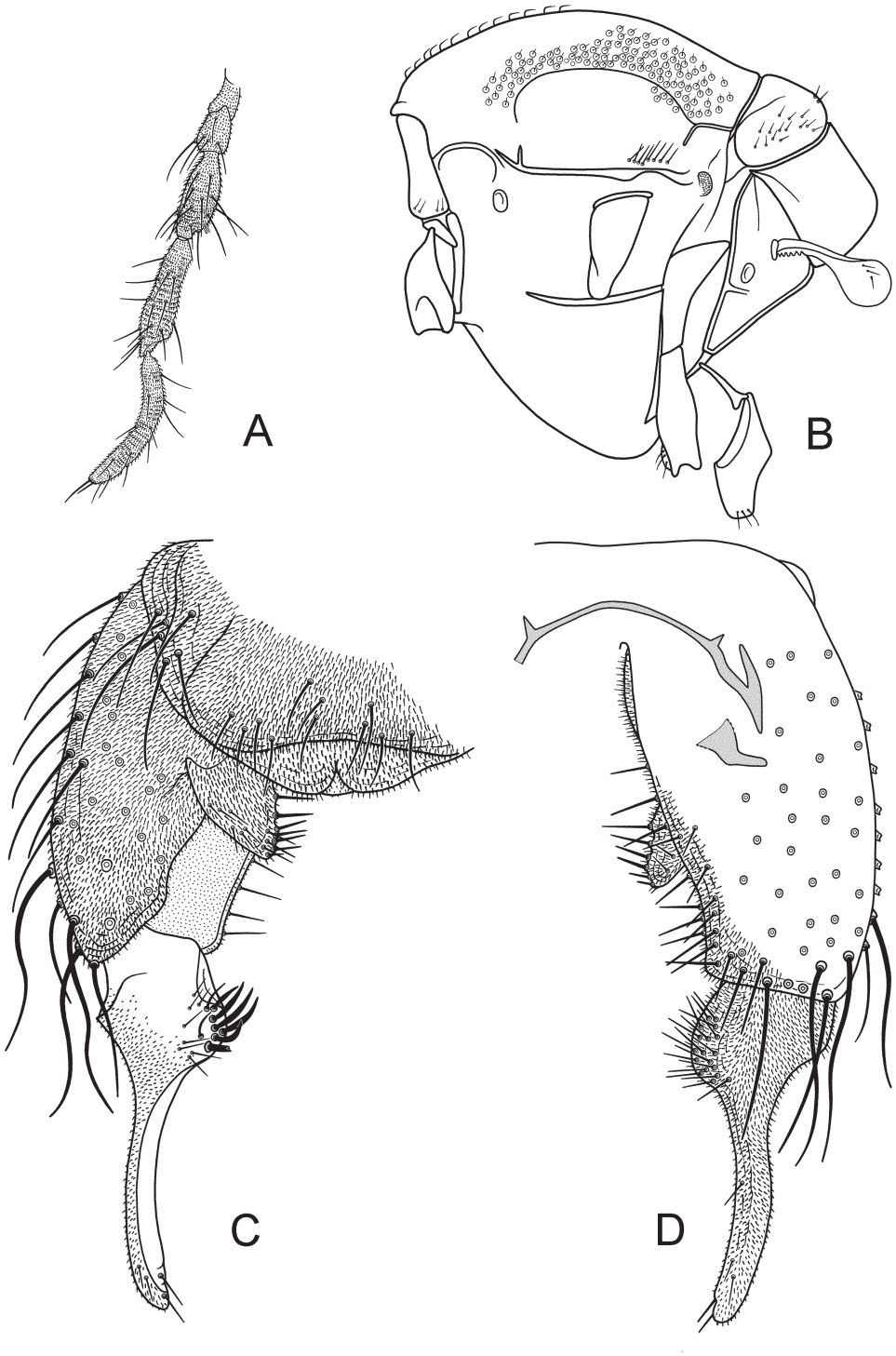

Figure 1. *Petalocladius dominiensis* Andersen & Baranov sp. n., male. A. Palp. B. Thorax. C. Hypopygium, dorsal view. D. Hypopygium, ventral view.

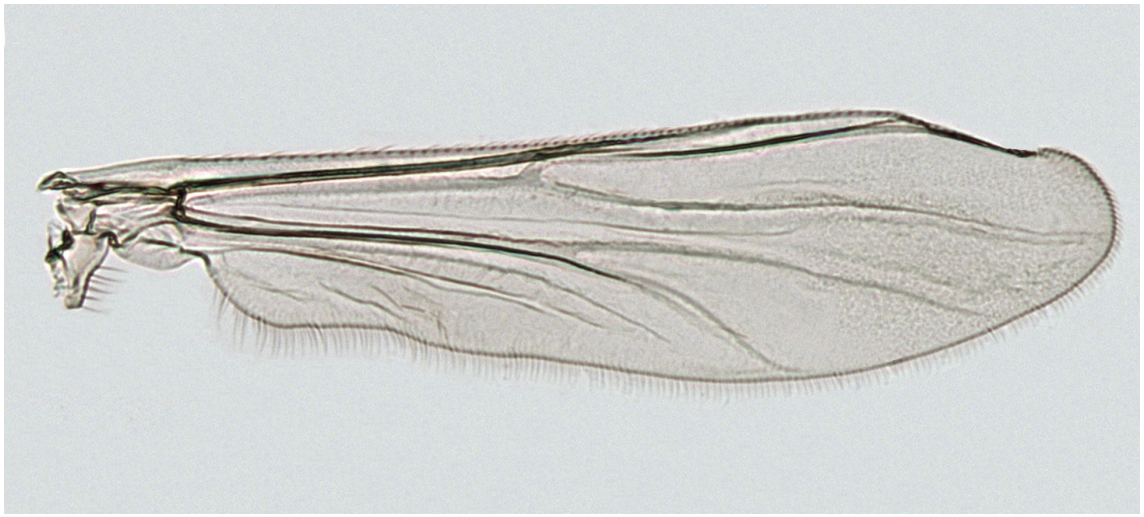

Figure 2. *Petalocladius dominiensis* Andersen & Baranov sp. n., male. Wing.

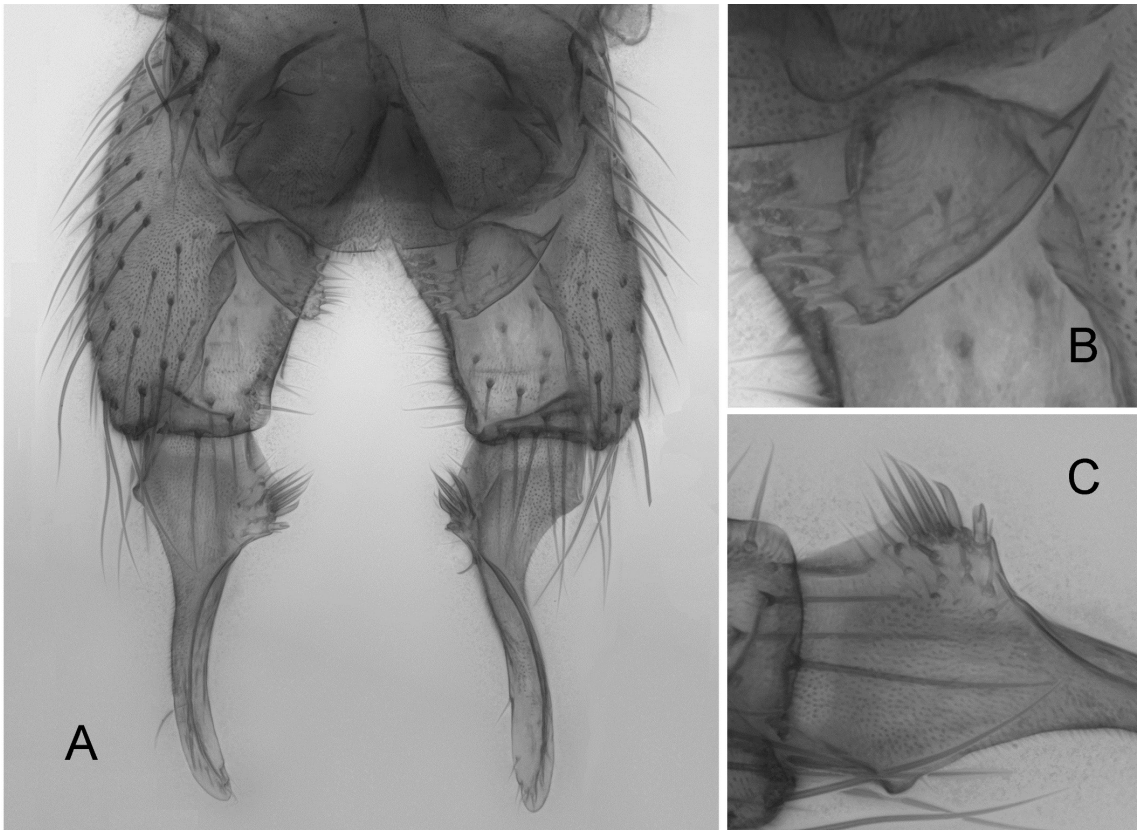

Figure 3. *Petalocladius dominiensis* Andersen & Baranov sp. n., male. A. Hypopygium, dorsal view. B. Inferior volsella. C. Apex of gonostylus, showing the megaseta.

***Petalocladius setosus* Sublette & Wirth, 1972**

Figs 5 A-F

The male and female of *P. setosus* were described in detail by Sublette and Wirth (1972: 2). However, the species is rather sparsely figured by Sublette and Wirth (1972: figs 1-3). David Pecor has therefore kindly sent us photos of the head, thorax, wing and hypopygium of the male (Figs 5 A-F).

Systematics

Phylogenetic analysis using Bayesian inference led to *Petalocladius* being a sister group to the clade *Thalassosmittia* + *Mesosmittia* + *Pseudosmittia* + *Eretmoptera*, with a weak support of 0.16 (Fig. 6), common for morphology-only reconstructions, where some taxa are having many missing character states. In the RougePlot reconstruction, *Pet-*

Figure 4. The type locality of *Petalocladius dominiensis* Andersen & Baranov sp. n.

alocladius was recovered in >30% of trees next to *Abiskomyia* (Fig. 7).

Discussion

According to Sublette and Wirth (1972) *Petalocladius* most closely resembles *Diplocladius* Kieffer, 1908. As mentioned in the introduction, Sæther (1977) hypothesized close relationships with *Abiskomyia* and *Nasuticladius*. In a consensus tree produced in our Bayesian analysis neighboring search it falls out with *Thalassosmittia* Strenzke & Remmert, 1957 and *Eretmoptera* Kellog, 1900 (Figs 6, 7). A position close to *Thalassosmittia* and *Eretmoptera, Pseudosmittia* and *Mesosmittia* is notable. *Thalassosmittia* is a predominately marine genus with 11 named species distributed in the Afrotropical, Nearctic, Neotropical, and Palaearctic Regions (Ashe and O'Connor 2012). With few exceptions, the species are marine shore dwellers (Andersen et al. 2013). The only species described from the Neotropical Region, *T. amazonica* Andersen & Pinho, 2014, was, however, collected in the Amazon rainforest near Manaus (Andersen and Pinho 2014). *Eretmoptera* is a genus with two named species that are distributed in the Nearctic Region and in Antarctica. The adults are wingless. *Eretmoptera browni* Kellog, 1900 is distributed in California, while *E. murphyi* Schaeffer, 1914, was described from South Georgia and is later introduced to the Signy Island in the South Orkney Islands (Ashe and O'Connor 2012). The species is apparently parthenogenetic; the larva has a two-year life cycle and lives in damp moss and peat where they are thought to feed on decaying vegetation (Convey 1992, Cranston 1985).

However, when we have applied posterior position probability mapping ("Rouge plots"), *Petalocladius* was notably plotted in >30% of the cases next to *Abiskomyia* (Fig. 7), corroborating the hypothesis by Sæther (1977). While this was the most frequent position within the generated trees, it plots near *Eretmoptera* in the consensus tree due to cumulatively more frequent occurrence (ca. 40%) in or near this clade (Fig. 7). *Nasuticladius* position in relation to *Petalocladius*, remains highly uncertain, both due to the lack of knowledge of the immature stages of this genus and the probable paraphyletic nature of the "genus" (Hergstrom 1974).

Both *Petalocladius* species have been collected in mountain areas and there might be more species of the genus to be found in streams and rivers in the

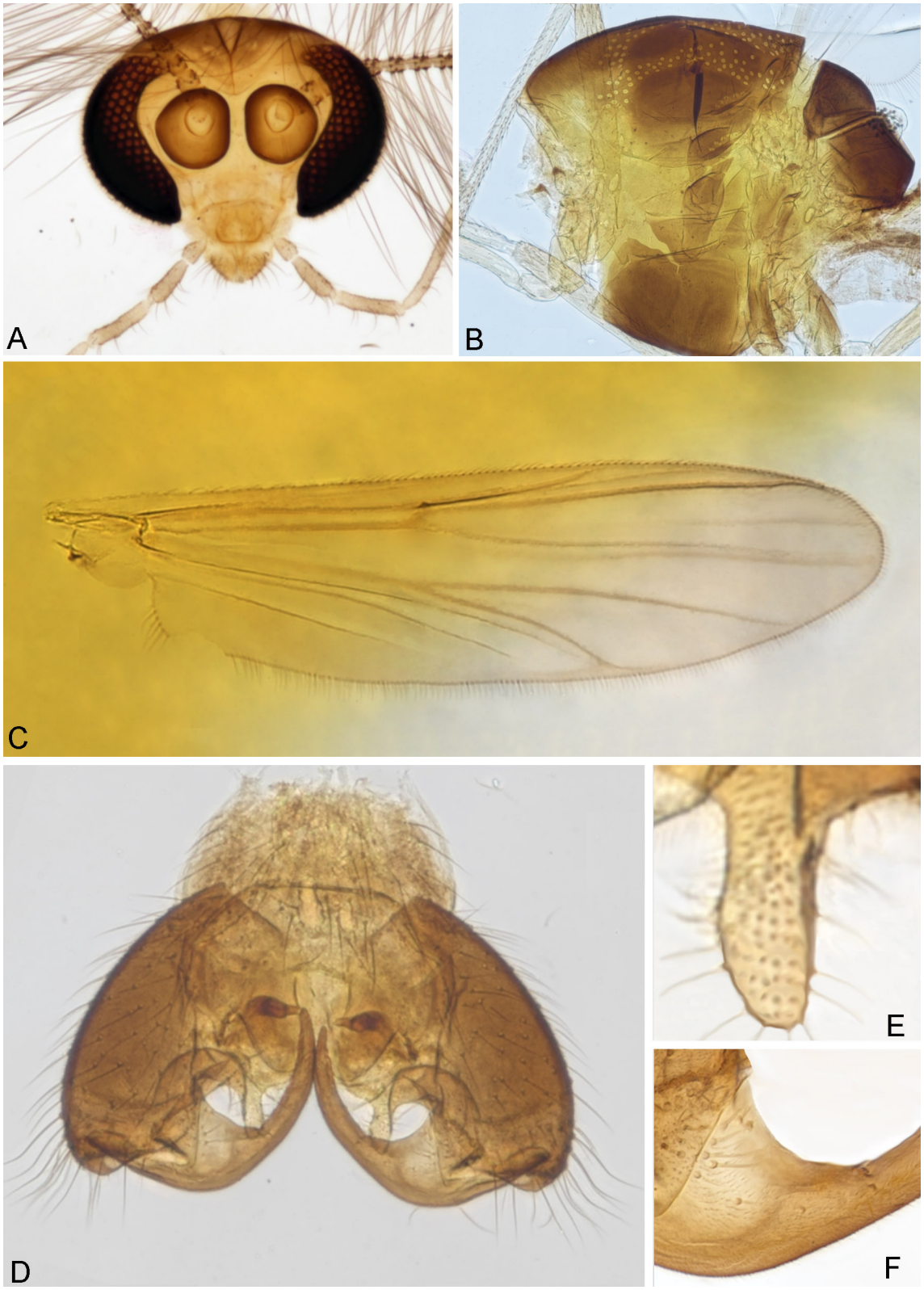

Figure 5. The male of *Petalocladius setosus* Sublette & Wirth, 1972. A. Head. B. Thorax. C. Wing. D. Hypopygium, dorsal view. E. Inferior volsella. F. Apex of gonostylus. Photos: David Pecor, Smithsonian Institution.

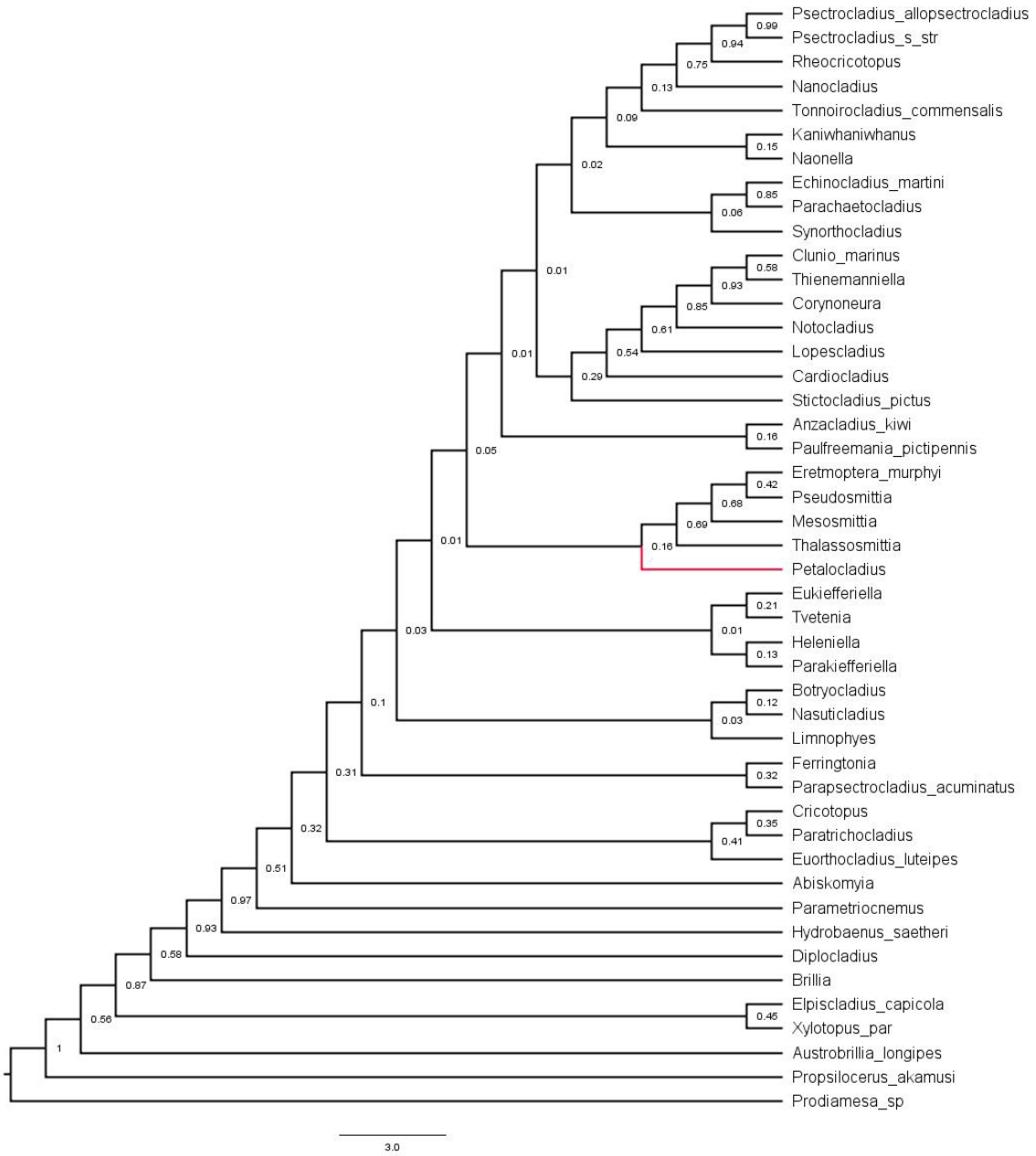

Figure 6. Position of *Petalocladius* (marked in red) in the phylogenetic tree of Orthocladiinae, reconstructed with Bayesian inference (all posterior probabilities of the nodes are displayed, regardless of the value). This is a majority rule consensus tree based on morphology only.

mountains on other islands in the Caribbean. The immatures remain unknown.

Overall, the discovery of *P. dominiensis* has provided the necessary impetus and additional characters required to improve the understanding the place of this enigmatic genus within Orthocladiinae.

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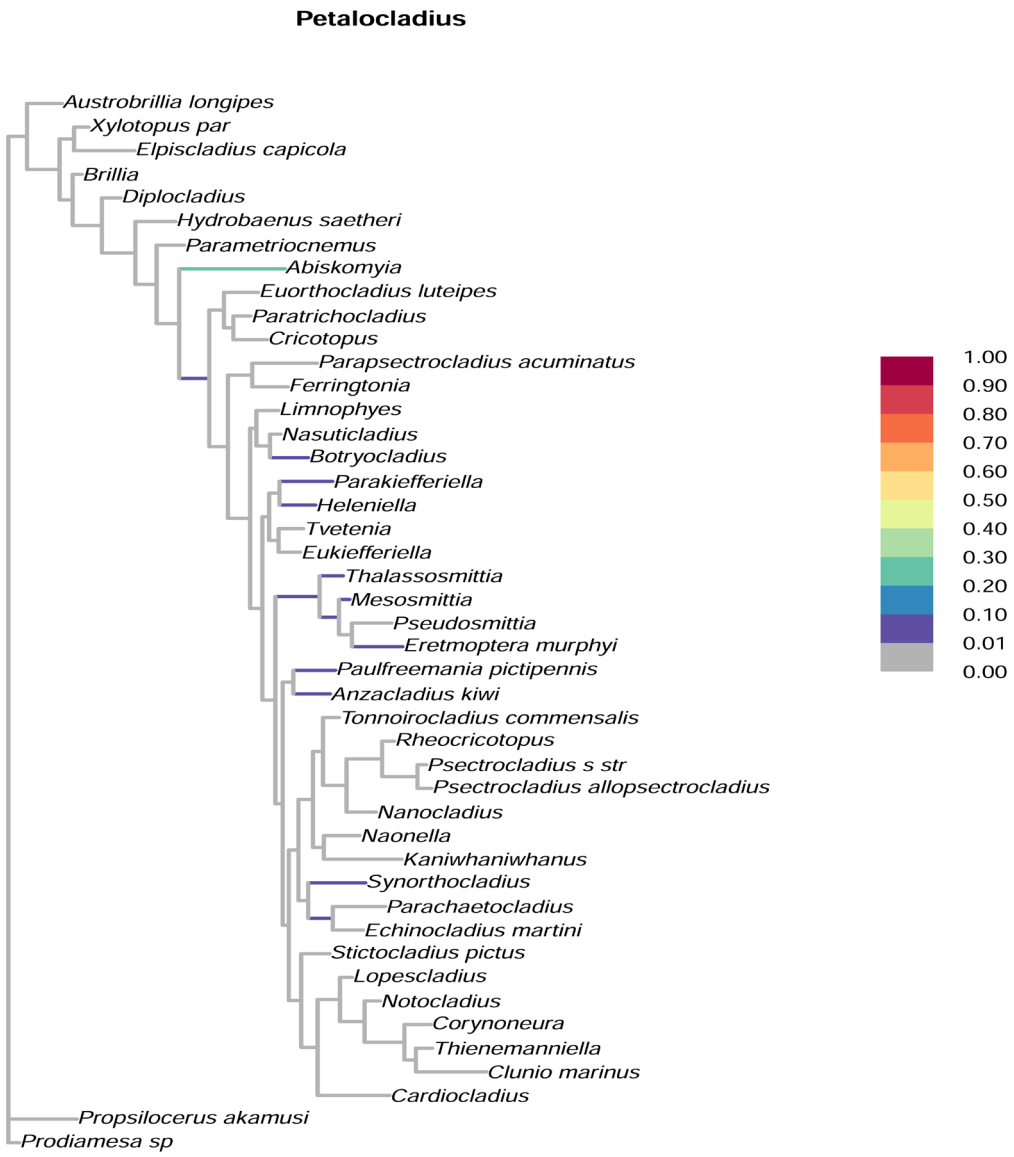

Figure 7. Rouge plot of the frequency of placement of *Petalocladius* on a Bayesian tree from Fig. 6, based on the frequency of occurrence of *Petalocladius* in a given node, based on 50001 most congruent trees from the Bayesian analysis of the genus's morphology.

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