



Parapentaneura (Diptera: Chironomidae: Tanypodinae) breeding in phytotelmata and the conflictive phylogenetic relationship with *Pentaneura* and *Hudsonimyia*

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ABSTRACT

A new species of *Parapentaneura* Stur, Fittkau et Serrano 2006 (Diptera: Chironomidae: Tanypodinae) breeding on phytotelmata of the bromeliad *Aechmea distichantha* Lemaire in northwestern of argentinean "Yungas" is described and adults and immatures are figured. The first formal description of a *Parapentaneura* female adult is conducted. Information about bionomics and distribution of the new species is provided. Notes about morphology traits of phytotelm pupae of Tanypodinae are presented. These pupae tend to have thoracic horns relatively long, non-globose, corona absent and the atrium not or slightly lobulated. The phylogenetic relationship between the genera *Parapentaneura*, *Pentaneura* Philippi and *Hudsonimyia* Roback is tested in a phylogenetic analysis in the context of the cladistic setting of Tanypodinae recently proposed and, characters are analyzed and discussed.

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1. Introduction

Structures of terrestrial plants such as modified leaves, leaf axils, flowers, stem holes or depressions, open fruits and fallen leaves that impound water are called phytotelmata. These structures, common in tropical areas, provide a suitable habitat for diverse aquatic invertebrate and vertebrate (Kitching 2004). Phytotelmata ecosystems are highly dynamic because their environment can change rapidly with respect to water volume, temperature, pH, chemistry, detritus, turbidity, and occupants (Jalinsky et al. 2014) yet are highly predictable (Kitching 2004).

Bromeliads (Bromeliaceae) are herbaceous perennials which occur as terrestrial plants or epiphytes in the rainforest canopy, most of them impound water in their leaf axils forming watertight cavities (Kitching 2004). More than 470 species of aquatic animals have been recorded breeding in bromeliads (Sodré et al. 2010). Chironomidae (Diptera) are common inhabitants of phytotelmata, mainly represented by species of the subfamilies Chironominae, Tanypodinae and Orthoclaadiinae. Recently, Siri & Donato (2014) summarized the known phytotelm chironomid fauna from the Americas totaling about 40 species and morphospecies.

Much of the biota breeding in phytotelmata is specific to this type of environment, for which they need morphological and biological adaptations. In the case of insects, adult females must find, distinguish and enter to this type of breeding sites to oviposit. Where the microhabitats are patchy and ephemeral, the organisms that exploit these environments commonly produce extremely high reproductive rates and peculiar modes of reproduction (Price 1997). For example, parthenogenesis has been observed in different phytotelm chironomids, such as in *Polypedilum parthenogeneticum* (Donato & Paggi, 2008) and *Phytotelmatocladus delarosai* (Epler, 2010; Siri & Donato, 2014).

Parapentaneura Stur, Fittkau et Serrano, with *Parapentaneura bentogomensis* Stur, Fittkau et Serrano as the type species, is a Pentaneurini (Tanypodinae) endemic from the Neotropical region (Stur et al. 2006; Oliveira et al. 2010, 2014). Three named species and one pupa with associated larvae have been described. The genus was erected and described by Stur et al. (2006) and expanded and emended by Oliveira et al. (2010, 2014). The genus has been characterized for these authors by the combination of the characters in the adult male: the fore and hind tibia with a single lyrate spur, mid tibia with two lyrate spurs; wing vein R_2 absent; R_{2+3} connecting with C; anal point simple, phallopodeme and lateral sternapodeme well developed; scutal tubercle absent; comb of

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hind tibia distinct, with 5–8 setae. Pupa with alveolar horn, atrium filling most of lumen, tergite I with a prominence on each side of scar. Larva with bifid or trifid paraligula, pecten hypopharyngis with 6–12 teeth, AR about 4, anal tubules long and slender, somewhat shorter than posterior parapods; supraanal setae on small tubercles, stronger and longer than anal setae in the larva. Most of these characters are shared with *Pentaneura* and *Hudsonimyia*, but they are still considered as separate genera, at least until new morphological or molecular differences will be obtained (Silva & Ferrington 2018). At present, there are no valid records of these three genera from outside North and South America (Ashe & O'Connor 2009; Silva et al. 2012; Silva & Ferrington 2018).

The phylogenetic relationships of Tanypodinae based on morphological characters performed by Silva & Ekrem (2015) shows *Parapentaneura* as the sister group of *Pentaneura*. In other study, Silva & Ferrington (2018) stated the monophyly of *Pentaneura* and established *Parapentaneura* as the sister group, although this statement is not clear. The analysis carried out by these authors does not allow establishing the monophyly of the genus and therefore its sister relationships, since only a single *Parapentaneura* terminal was included. In this way, this last genus could well be the sister group, but it could be a basal *Pentaneura*. In order to corroborate the monophyly of *Pentaneura*, at least two species of *Parapentaneura* should have been included. In a recent molecular phylogeny of Tanypodinae (Krosch et al. 2017) *Australopelopia* was the sister group of *Parapentaneura*.

In the present study we describe and illustrate a new species of *Parapentaneura* breeding in the phytotelmata created by the leaf axils of the bromeliad *Aechmea distichantha* Lemaire. The first description and illustration of a *Parapentaneura* female adult are conducted. Morphological traits between the new species and other Tanypodinae species breeding in phytotelmata are presented and discussed. A cladistic analysis was performed in the context of the tribe Pentaneurini to analyze the phylogenetic relationships of the species belonging to *Parapentaneura* and to evaluate possible affinities with *Pentaneura*, *Hudsonimyia* and *Australopelopia*.

2. Material and methods

2.1. Study area

The “Yungas” is a cloud forest extending from the eastern slopes of the Andes and subandean chains from Venezuela to the northwest of Argentina between 500 and 2500–3500 m asl. This cloud forest includes a high biodiversity with high degree of endemism (Cabrera & Willink 1980; Brown et al. 2002). Several authors agree to consider the Yungas as a biogeographic unit even from different biogeographical methods (Cabrera & Willink 1980; Morrone 2001; Brown et al. 2002, 2006). In response to an environmental gradient, the Yungas in Argentina shows different vegetation communities organized in altitudinal levels with well differentiated physiognomic-floristic characteristics: 1) Foothills or piedmont forest between 400 and 700 m asl.; 2) montane forest, between 700 and 1500 m asl., which represents the altitudinal fringe of maximum rainfall; 3) the montane forest between 1500 and 3000 m asl., which represents the “cloud forests”; and 4) the high elevation grassland, above 3000 m asl. (Brown et al. 2002).

2.2. Collection and mounting

Sampled *A. distichantha* plants were usually found growing as epiphytes in the canopy. The impounded fluids of *A. distichantha* plants were extracted using a pipette attached to a vacuum pump. Immatures were transported alive in separate vials and reared in the laboratory following the methods suggested by Epler (2001).

The specimens were cleared and slide-mounted in Canada balsam. General terminology and abbreviations follows Sæther (1980), except cephalic setation which follows Kowalyk (1985). Measurements are in μm and are given as ranges followed by those of the holotype in square brackets. Types are deposited in the collection of the Museo de La Plata, Argentina (MLP) and Instituto de Limnología “Dr. Raúl A. Ringuelet” (ILPLA).

2.3. Cladistic analysis

The phylogenetic relationships of the new species was evaluated in the context of the cladistic setting of the tribe Pentaneurini. Character setting was based on morphological characters obtained from previous phylogenetic studies of Tanypodinae (Siri & Donato 2015; Silva & Ekrem 2015) and the inclusion of additional ones.

Most related taxa of *Parapentaneura* obtained in Silva & Ekrem (2015), Krosch et al. (2017) and Silva & Ferrington (2018) were included, while the Natarsiini genus *Natarsia* was used as outgroup. The analysis was performed with genera as terminal taxa, except for *Parapentaneura*, *Pentaneura* and *Hudsonimyia* which were treated as species. All the named species of *Hudsonimyia* and *Parapentaneura* were included in the analysis with the addition of the new species here described. Concerning *Pentaneura*, only species with known complete life cycle were included.

A male, pupal and larval character data set ($n = 40$) of 34 taxa was extracted from collection materials, from each original species description, as well as from Fittkau & Murray (1986), Murray & Fittkau (1989), Epler (2001), Cranston & Epler (2013), Siri & Donato (2015), Silva & Ekrem (2015) and Silva & Ferrington (2018). The character “*Gonocoxite deeply excavated and extensively membranous on median surface*” which resulted in a synapomorphy of *Pentaneura* in Silva & Ferrington (2018) has some subjectivity and difficulty to compare with other taxa. However, we included this character in the analysis considering it only present in *Pentaneura* species. *Pentaneura cinerea* larval characters was based on *P. nr. cinerea* described in Roback & Coffman (1983). List of characters analyzed is presented below, and data matrix of male, pupal and larval characters and their states is presented in Appendix 1.

2.4. Character list

2.4.1. Adult males

1. Antennal ratio (AR): (0) 0.3–1.3; (1) 1.5–3.00.
2. Clypeus: (0) 0–20 setae; (1) +25 setae.
3. Scutal tubercle: (0) vestigial or absent; (1) present, well developed.
4. Anteprenotals: (0) bare; (1) with few setae.
5. Dorsocentrals: (0) uniserial anteriorly; (1) biserial to multiserial anteriorly.
6. Dorsocentrals: (0) uniserial posteriorly; (1) biserial to multiserial posteriorly.
7. Prealars: (0) uniserial; (1) biserial to multiserial.
8. Preepisternals: (0) absent; (1) present.
9. Wing spots: (0) absent; (1) present.
10. Wing vein R_{2+3} : (0) absent; (1) present.
11. Wing veins R_2 and R_3 : (0) absents; (1) presents.
12. Distance between MCu and RM: (0) absent; (1) present.
13. FCu: (0) some distance before MCu; (1) beyond MCu; (2) joined with MCu.
14. Tarsal claws: (0) spatulate; (1) pointed.
15. Number of tibial spurs on p_1 , p_2 and p_3 respectively: (0) 1-2-2; (1) 1-2-1; (2) 1-1-1; (3) 1-1-0.
16. Tibial spurs shape: (0) lyrate (all the teeth subequals, the inner and outer slightly longer); (1) Outer tooth slightly

longer than the inner one; (2) Outer tooth much longer than the inner tooth; (3) Internal teeth reduced or absents.

17. Hind tibial comb: (0) reduced or absent; (1) well developed.
18. Fore tibial comb: (0) absent; (1) present.
19. Setae on tergite IX: (0) absent; (1) present.
20. Gonocoxite deeply excavated and extensively membranous on median surface: (0) absent; (1) present.
21. Hypopygial complex dorsomedial lobe: (0) reduced or absent; (1) present.
22. Processes or fringes on complex dorsomedial lobe: (0) absent; (1) present.

2.4.2. Pupa

23. Thoracic horn: (0) asymmetrical; (1) symmetrical.
24. Plastron plate: (0) absent; (1) present.
25. Plastron plate: (0) < than $\frac{1}{4}$ of diameter of thoracic horn; (1) > than $\frac{1}{4}$ of diameter of thoracic horn.
26. Corona on thoracic horn: (0) absent; (1) present.
27. Thoracic comb: (0) reduced or absent; (1) present, well developed.
28. Lobe in the base of the thoracic horn: (0) reduced or absent; (1) present, well developed.
29. Shagreen on abdominal tergites: (0) either without shagreen or with a dense covering of normal simple small spinules; (1) longish, upright, mostly multi-branched or bifid spinules.
30. Number of LS on abdominal segment VII: (0) 0; (1) 1; (2) 2; (3) 3; (4) 4; (5) 5.
31. Anal lobe points: (0) straight or slightly curved; (1) strongly divergent; (2) convergent.
32. Apical spines of anal lobe: (0) absent or reduced; (1) present on outer margin; (2) present on inner margin; (3) present on inner and outer margin.
33. Adhesive sheaths on anal macrosetae: (0) absent; (1) present.
34. Genital sac of the male: (0) not reaching apex of anal lobe; (1) reaching somewhat beyond lobe.

2.4.3. Larva

35. Antennal ratio (AR): (0) <3.2; (1) >3.5.
36. Ligula: (0) concave; (1) convex; (2) teeth sub-equal in size.
37. Anal tubules: (0) shorter than posterior parapods; (1) clearly longer than posterior parapods.
38. Supraanal setae. (0) normal; (1) strong and very large.
39. Supraanal setae. (0) not mounted on dark tubercles (1) mounted on dark tubercles.
40. Claws of posterior parapods (0) pale; (1) dark.

Cladistic analysis was conducted in TNT 1.1 (Goloboff et al. 2008). The data matrix was analyzed under implied weighting as optimality criteria. The optimal K value was calculated with the script propk.run performed by Salvador Arias (Instituto Miguel Lillo, Argentina) giving a $k = 7$. Tree searches were performed using a Wagner tree as the starting tree and 1000 random-addition sequences, plus tree bisection and reconnection (TBR), with ten trees saved per replication, followed by TBR branch swapping. Absolute and relative Bremer supports were calculated by saving longer suboptimal trees (up to six steps longer) obtained by branch swapping.

Additional analyses based on a dataset including 13 species with complete life cycle belonging to *Parapentaneura*, *Pentaneura* and *Hudsonimyia* were conducted in order to evaluate the cladistics and

taxonomic importance of the characters used to define these genera. The analyses were made using different approaches such as implied weighting and generalized parsimony as optimality criteria, deleting the homoplastic characters shared by *Parapentaneura* and *Pentaneura*, and assuming the presence of the long anal tubules (Ch.36:1) in all *Pentaneura* species as was described for *Pentaneura* sp. larvae (Epler, 2001; Cranston, 2010; Cranston & Epler, 2013).

3. Results

3.1. Taxonomy

3.1.1. *Parapentaneura* generic emendation

Some pupal characters found in the new species do not match in the *Parapentaneura* diagnosis, so it should be emended as: variable colouration, thoracic horn alveolar or not, corona present or not; thoracic comb consisting of low or high rounded tubercles.

3.1.2. *Parapentaneura* *acoronata* Siri et Donato sp. nov

3.1.2.1. Diagnosis. The new species is distinguished by the combination of the following characters: **Male adult.** Total length 2.95–3.65 mm long, wing length 1.86–1.98 mm, maxillary palp long, thorax setose, anteprenotal setae present; low LR: 0.68–0.76 on p_1 , 0.71–0.74 on p_2 and 0.59–0.68 on p_3 ; lyrate tibial spurs with 6 teeth; tergite IX with 10–12 strong posterior setae. **Female adult.** Gp VIII lobe well developed, projecting posteriorly, Gc IX more or less reduced, without setae, sternite X well developed, with several setae in the proximal area; cercus of normal type; 3 hyaline seminal capsules. **Pupa.** Total length 4.01–4.68 mm long. Thoracic horn with non-alveolar atrium, filling most of the lumen; corona absent; thoracic comb well developed, consisting of low tubercles. **Larva.** Total length 4.00–5.80 mm; paralogula bifid, pecten hypopharyngis with 11–12 teeth; ventral cephalic setation: S5-DP-S7-S8 in row; VP posterior to S9; S10 postero-lateral to S9-VP, SSm posteromedial to S9-VP-S10; dorsal cephalic setation: S5-DP-S7-S8 disposed in a slightly curved row. Anal tubules long, shorter than parapods; supraanal setae very large, mounted on dark tubercles; three shorter claws on posterior parapods.

3.1.2.2. Description male adult ($n = 3-4$, except when stated otherwise). Size. Total length 2.95–3.65 [3.28] mm. Total length/wing length 1.59–1.87 [1.66]. Colouration. Head and thorax pale brownish; vittae marked. Legs and wings pale. Abdominal tergite I pale; II–VII with brown basal band, wider and darker in VII; tergite VIII–IX pale. Gonocoxite pale brown, external area somewhat darker; gonostylus pale brown (Fig. 1A).

Head. (Fig. 1B). Antenna with 13 flagellomeres, AR 1.13–1.28 [1.28]. Temporal uniserials, 10–13 [10]; postorbital 1–2 [1]. Clypeus with 18–20 [18] setae. Palpomeres I–V length: 38–50 [38], 100–110 [110], 155–170 [155], 188–200 [188], 275–280 [275], respectively.

Thorax. Scutal tubercle absent. Anteprenotum with 3–5 [5] lateral setae; dorsocentrals 25–28 [28]; acrostichals 46–52 [52]; prealars 6–8 [8], distributed as 5–7 [7] posteriors and 1 anterior; supraalar 1 [1]; scutellars 20–30 [28].

Wing (Fig. 1C). 1.86–1.98 [1.98] mm long; 0.48–0.54 [0.53] mm wide. L/W 3.61–3.88 [3.77]. VR 0.88–0.92 [0.90]. Brachiolum with 3 [3] distal setae, plus 1 [1] basal seta. Squama with 3–6 [3] setae. Costa not produced beyond R_{4+5} ; R_{2+3} reaching costa; R_2 branch absent; anal lobe weak.

Legs. Tibial spur on p_1 lyrate, 23–25 [25] long, with 6 teeth ($n = 2$); two lyrate spurs on p_2 , subequal, 20–25 [20] long, with 6 teeth ($n = 2$); a single lyrate spur on p_3 , 20–24 [20] long, with 6 teeth ($n = 2$). Tibial comb on p_3 indistinct, but 5–6 straight thin

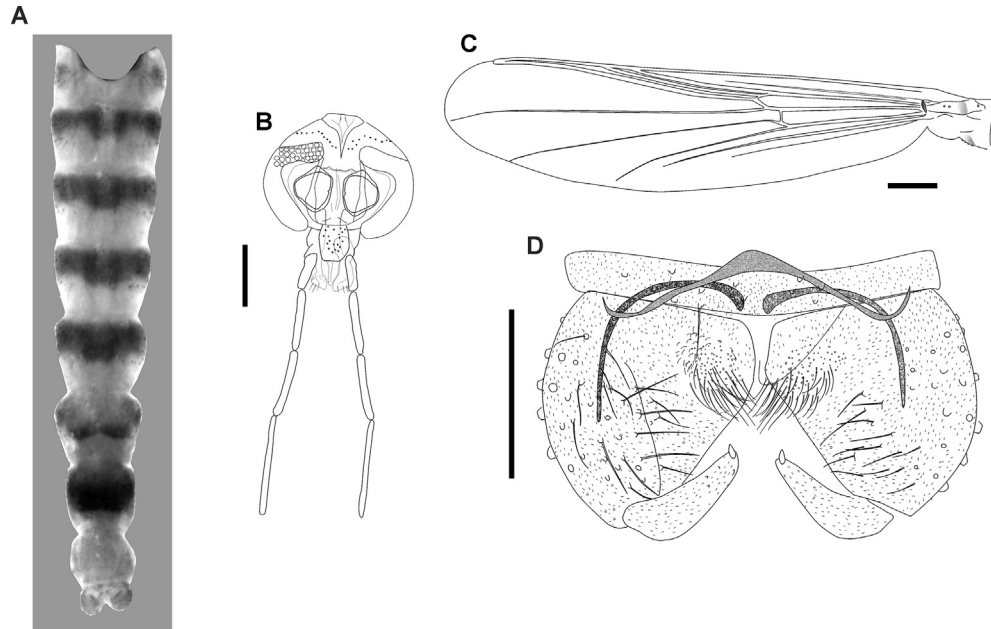


Fig. 1. *Parapentaneura acoronata* sp. nov., male. (A) Abdomen before cleared. (B) Head. (C) Wing. (D) Genitalia, dorsal view. Scale bars: 200 μ m for Figs A–C; 100 μ m for Fig. D.

Table 1

Lengths (μ m) and proportions of male legs of *Parapentaneura acoronata* sp. nov. (n = 3).

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV
p ₁	820–960 [960]	900–990 [990]	670–720 [670]	300–360 [300]	230–280 [230]	170–210 [170]	100 [100]	0.68–0.76 [0.68]	2.55–3.28 [3.28]	2.53–2.91 [2.91]
p ₂	950–960 [950]	1020–1075 [1050]	740–790 [760]	340–365 [350]	240–280 [240]	160–170 [170]	90–100 [90]	0.71–0.73 [0.71]	3.13–3.25 [3.25]	2.56–3.47 [3.47]
p ₃	800–830 [830]	1200–1310 [1310]	770–810 [770]	470–500 [470]	330–380 [330]	180–240 [180]	100 [100]	0.59–0.68 [0.59]	2.37–2.69 [2.69]	2.47–2.78 [2.78]

setae placed in row (n = 1). Tarsal claws apically pointed. Lengths and proportions of legs in Table 1.

Hypopygium (Fig. 1D). Tergite IX with 10–12 [10] setae. Phallopodeme distinct and long, reaching under the anal point anteriorly and the middle of the gonocoxite posteriorly; transverse sternapodeme distinct and pointed anteriorly. Gonocoxite 134–138 [134] long, antero-medially with a group of stouter setae. Gonostylus 67–75 [72] long; megaseta 6–8 [8] long. HR 1.84–1.86 [1.86].

3.1.2.3. Description female adult (n = 2–3, except when stated otherwise). Total length 2.45–2.78 mm. Total length/wing length 1.30–1.53. Colouration. As in male, except abdomen brown.

Head. AR 0.18–0.20; pedicel with 10–12 setae; scape with 4–5 setae. Temporal setae uniserial 8–12; postorbital 1. Clypeus with 22–23 setae. Palpomeres I–V lengths: 40–50, 95–118, 158–174, 174–198, 260–276, respectively.

Thorax. Antepre-notum with 2–4 lateral setae; dorsocentrals 38–47; acrostichals +53–+58; prealars 11–12, distributed in a group of 10 posterior setae plus 1–2 anterior setae; supraalar 1. Scutellum with 24–28 setae.

Wing (Fig. 2A) 1.82–1.92 mm long; 0.57–0.58 mm wide. L/W 3.19–3.31. VR 0.87–0.91. Brachiolum with 2 distal setae, plus 1 basal seta. Squama with 3–5 setae.

Legs. Tibial spur on p₁ lyrate, 25–26 long, with 6 teeth; two subequal lyrate tibial spurs on p₂, 24–28 long with 6 teeth; one lyrate tibial spur on p₃ (length and number of teeth could not be counted). Tibial comb on p₃ indistinct, but 5–6 straight thin setae placed in row (n = 2). Lengths and proportions of legs as in Table 2.

Genitalia (Fig. 2B). Gp VIII lobe well developed, projecting posteriorly. Three hyaline seminal capsules (slightly collapsed in

studied specimens), 58 long (n = 1), notum plus ramus 200–210 long. Gc IX more or less reduced, without setae; sternite X well developed, with +40–+48 setae in the proximal area; cercus of normal type, 50–70 long.

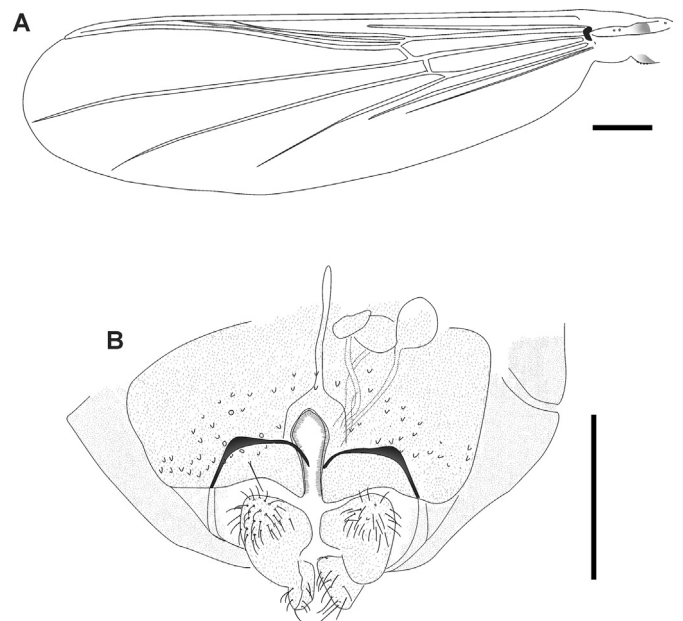


Fig. 2. *Parapentaneura acoronata* sp. nov., female. (A) Wing. (B) Genitalia, ventral view. Scale bars: 200 μ m.

Table 2Lengths (μm) and proportions of female legs of *Parapentaneura acoronata* sp. nov. ($n = 2$, except when otherwise state).

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV
P ₁	800–880 (3)	920–1000 (3)	680	350–360	260	180	100	0.68–0.73	2.75–2.84	2.60–2.76
P ₂	880–1000 (3)	980–1060 (3)	720–740	330–355	240–245	160	100–110	0.70–0.73	3.18–3.22	2.67–2.78
P ₃	840–940	1240–1260	730–805	460–490	250–260	210–220	110	0.59–0.64	2.70–2.81	2.73–2.85

3.1.2.4. *Description pupa* ($n = 6–9$, except when stated otherwise). Total length 4.05–4.68 [4.43] mm. Colouration. Cephalothorax brownish, with no maculation (Fig. 3A); abdomen pale brown to yellowish brown (Fig. 3B).

Cephalothorax (Fig. 3A). Thoracic horn (Fig. 3C), more or less symmetrical. Length 340–380 [340]; width 150–170 [160]; L/W 2.06–2.53 [2.13]; plastron plate encompassing the entire apical width of horn; corona absent; neck 16–24 [20] long, 16–24 [20] wide, connecting the respiratory atrium with the basal portion of the plastron plate. Respiratory atrium almost filling the entire lumen, with only slightly indication of alveolar surface. Plastron plate long/Thoracic horn length 0.44–0.59 [0.47]. Thoracic comb (Fig. 3C) with 11–14 low teeth [12], the longest 16–24 high, plus several tubercle-like close to the base of the TH; basal lobe short. Thoracic setal arrangement as in Fig. 3A; Dc1 30–48 long; Dc2 10–15 long ($n = 3$); Dc3 destroyed (absent?) in all studied specimens.

Abdomen. Scar on tergite I 150–180 long; one prominence on each side of scar. Shagreen on T I–VIII tergites with short, blunt spinules, partially serially arranged in groups of 2 and 3; weak on T IV–VII; shagreen on the base of AL with simple and somewhat longer spines. Segment VII with 3 lateral setae (Fig. 3D), placed at 0.21–0.31 [0.26], 0.37–0.52 [0.43] and 0.48–0.62 [0.61] from the base; segment VIII with 5 lateral setae. Anal lobe (Fig. 3D) 530–580 [540] long; anterior macrosetae located at 0.20–0.26 [0.22] and 0.28–0.36 [0.28] from the anterior margin. Outer margin sclerotized, almost smooth, with few weak spinules; inner margin membranous, without spinules. Anal lobe of female about 1.57–1.63 ($n = 4$) times longer than broad, of male 1.68–1.75 ($n = 5$) times. Male genital sac well sclerotized and pigmented, 200–210 [200] long; Anal lobe length/male genital sac 0.36–0.38 [0.37]. Female genital sac low sclerotized and hyaline; 130–150 long. Anal lobe length/female genital sac 0.20–0.25.

3.1.2.5. *Description fourth instar larva* ($n = 7–11$, except when stated otherwise). Total length 4.20–5.80 mm ($n = 4$). Head capsule 500–590 [590] long. CI (before mounting) 1.40–1.55 ($n = 4$). Colouration. Head and antenna pale, apex of mandible and ligule slightly darker. Cephalic chaetotaxy (Fig. 4A). Ventral: S9 and S10 simple and thin, SSm simple, thicker than S9 and S10; VP posterior to S9; S10 postero-lateral to S9-VP, SSm posteromedial to S9-VP-S10. Dorsal: S5, DP, S7 and S8 forming an almost straight line.

Antenna. AR 4.24–4.76 [4.58]. A₁ 233–252 [252] long, ring organ situated 0.59–0.68 [0.62] from base; blade and accessory blade subequal 48–52 [50] long. A₂ 43–50 [48] long, style 4–6 long [6], peg sensilla 3 long; A₃ 5 [5] long; A₄ 2–3 [3] long.

Maxillary palp. Basal segment 33–42 [38] long, L/W 3.30–4.20 [3.8]; ring organ located 0.60–0.67 [0.6] from base. A₁/PMx 5.67–7.06 [6.63].

Mandible (Fig. 4B) 88–105 [100] long; with a basal tooth 3 long; seta subdentalis 24 long ($n = 1$); 3 lateroventral setae and 1 sensillum campaniforme. A₁/MdL 2.33–2.78 [2.52].

Mentum and M appendage (Fig. 4C). Dorsomentum with 2–3 teeth [2]; pseudoradula uniformly granulate.

Hypopharyngeal complex. Ligula brownish (Fig. 4D) 69–85 [75] long; toothed margin straight; paraligula bifid, 25–33 [25] long. Hypopharyngeal pecten with 11–12 [12] teeth.

Abdomen. Procercus 200–235 [220] long; L/W 7.1–8.3 [8.0]; with 7 anal macrosetae 350–430 long; supraanal seta 450–540 [490] long, placed on small sclerotized tubercles; subbasal seta of posterior parapod 180–215 long [215]. Anal tubules long, 240–255 [240] long ($n = 3$), 40–43 wide ($n = 3$); L/W at the base 5.93–6.13 [6.0] ($n = 3$). Posterior parapods with 17 claws; one with lamellate outer margins; and three basal claws clearly smaller, 8–20 high.

3.1.2.6. *Type material*. Holotype male adult with its pupal and larval exuviae, Argentina, Tucumán Province, San Isidro de Lules

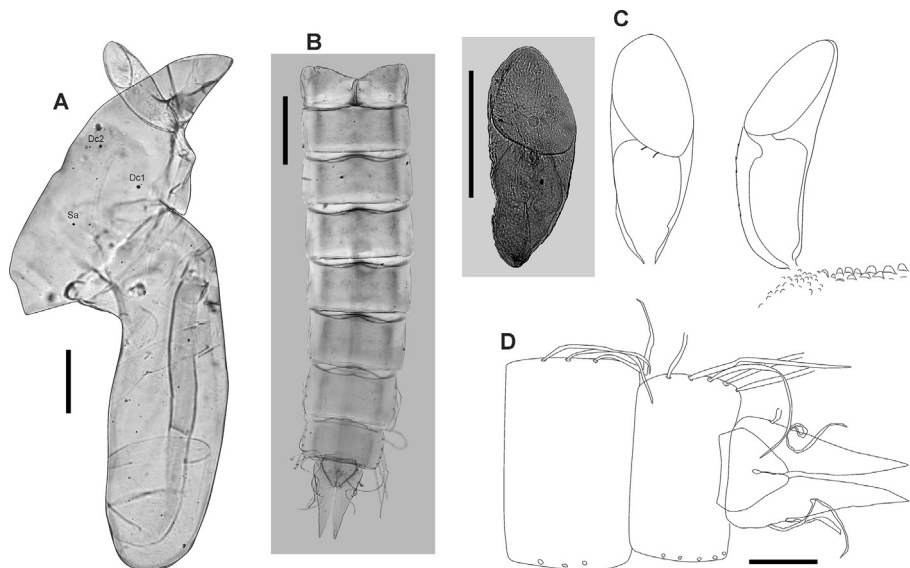


Fig. 3. *Parapentaneura acoronata* sp. nov., pupa. (A) Cephalothorax. (B) Abdomen. (C) Thoracic horn photo, draw in frontal view and, in lateral view from left to right. (D) Segments VII, VIII and anal lobe, dorsal view. Scale bars: 200 μm for Figs A,C,D; 500 μm for Fig. B.

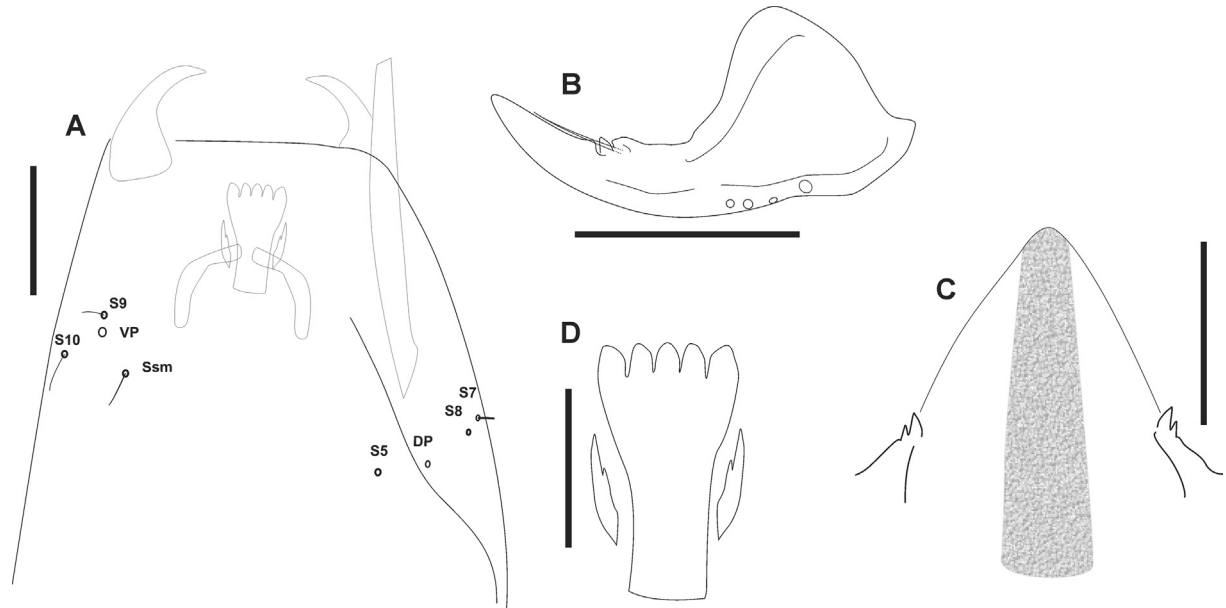


Fig. 4. *Parapentaneura acoronata* sp. nov., fourth instar larva. (A) Cephalic setation, ventral setation in the left side, dorsal setation in the right side. (B) Mandible. (C) Mentum and M appendage. (D) Ligula and paraligula. Scale bars: 50 μ m for Figs B–D; 100 μ m for Fig. A.

Departament, Potrero de las Tablas, epiphytic *A. distichantha* near the border of Río Grande stream, $-26.854833-65.428222$, 700 m asl., collected on 2-x-2016, emerged on 7-x-2016, pipette, Siri, Donato, Mauad (MLP).

Paratypes: 2 larvae, 1 pupa with its larval exuviae, Argentina, Tucumán Province, Tafí del Valle Departament, epiphytic *A. distichantha* at the border of Los Sosa stream, RP 307, $-27.075412-65.663764$, 790 m asl., 9-xi-2014 (MLP); male adult with its pupal and larval exuviae, idem anterior except for collected on 3-x-2016, emerged on 8-x-2016 (ILPLA); female adult with its pupal and larvae exuviae, idem anterior except for collected on 3-x-2016, emerged on 10-x-2016 (ILPLA); 3 larvae, Argentina, Tucumán Province, San Isidro de Lules Departament, epiphytic *A. distichantha* at the border of Duraznillo stream, $-26.80590403, -65.47390646$, 980 m asl., 14-xi-2014 (MLP); male adult with its pupal and larvae exuviae Argentina, Tucumán Province, Montero Departament, Capitán Cáceres, epiphytic *A. distichantha* Lemaire at the border of Río Seco Stream, $-27.194833, -65.616583$, 430 m asl., collected on 4-x-2016, emerged on 23-x-2016 (MLP); female adult with its pupal and larvae exuviae, idem anterior except to emerged on 10-x-2016 (MLP); female adult with its pupal exuviae, idem anterior except to emerged on 5-x-2016 (MLP); 1 pharate male, 1 pupa, 1 larva, idem anterior (MLP). In addition, 5 larvae were deposited in absolute ethylic alcohol to future molecular studies (ILPLA).

3.1.2.7. Etymology. The specific epithet “*acoronata*” refers to the absence of corona in the pupal thoracic horn.

3.1.2.8. Bionomics and distribution. Immature of *Parapentaneura* were previously collected in Brazil from rivers, small ponds and pisciculture tanks, associated with roots of the macrophytes *Eichhornia* sp., *Salvinia* sp., *Mayaca* and, to floating leaf litter. Similar *Parapentaneura* larvae has been found in bromeliad from Costa Rica, and it is possible that references to *Pentaneura* in phytotelms in Central and South America refer instead to this genus (Cranston 2010). *P. acoronata* was collected from the phytotelmata created by the leaf axils of the bromeliad *A. distichantha*. No immature stages of *Parapentaneura* were collected from the surrounding aquatic environments including ponds or streams. We assume a

specificity or, at least a preference of the new species by phytotelmata environment.

P. acoronata was collected from *A. distichantha* growing as epiphytes in the NW of Argentina. Bromeliad plants from 430 to 1700 m asl. and were studied, but *P. acoronata* immatures were not found above 980 m asl. In a previous study conducted on *A. distichantha* in the NE of Argentina (Siri et al. 2015), only *Larsia angusticornis* was present among the Tanypodinae fauna. In contrast to the Yungas, that area is a rainforest with about 50 m asl., in where *A. distichantha* plants are terrestrials. This bromeliad is also distributed in the deciduous, mixed, and evergreen forests in southern Brazil, Bolivia, Paraguay, Uruguay, and northern Argentina (Montero et al. 2010) but the presence of each of these tanypod species are conditioned by the environment in which these bromeliads grow.

The larvae of *P. acoronata* were collected together with a chironomid belonging to genus *Chironomus* (Chironominae), as well as with Ostracoda and immature stages of the insect Order Diptera (Culicidae, Ceratopogonidae, and Psychodidae) and Coleoptera (Scyrtidae). Among the Culicidae, it were collected *Culex* (*Microculex*) *Pleuristriatus* Series (Theobald 1907) and *Toxorhynchites* (*Lynchiella*) *guadeloupensis*. Immatures of the mosquitoes *Aedes* (*Ochlerotatus*) *crinifer*, *Culex imitator* and *Culex fernandezi* were also found breeding in *A. distichantha* plants in the same area (Veggiani Aybar & Rossi 2017). The larva of *P. acoronata* is carnivorous and could prey on small individuals of the biota inhabiting *Aechmea distichantha* phytotelmata, but could also serve as prey for the predator mosquito *T. guadeloupensis*.

3.2. Cladistic analysis

The analysis under implied weights yielded 1 tree (Fig. 5) (Fit = 23.58; CI = 0.32; RI = 0.60). The clade including *Parapentaneura*, *Pentaneura* and *Hudsonimyia* species shares the presence in the male adult of 1, 2, and 1 tibial spurs on fore, middle and hind legs respectively (Ch. 14:1), the presence of 3 lateral setae on the pupal abdominal segment VII (Ch.29:[3]) and a large, dark supraanal setae, longer and stronger than the anal setae (Ch.37:1). The first character reversed to the state (0) in *Pentaneura*, and the

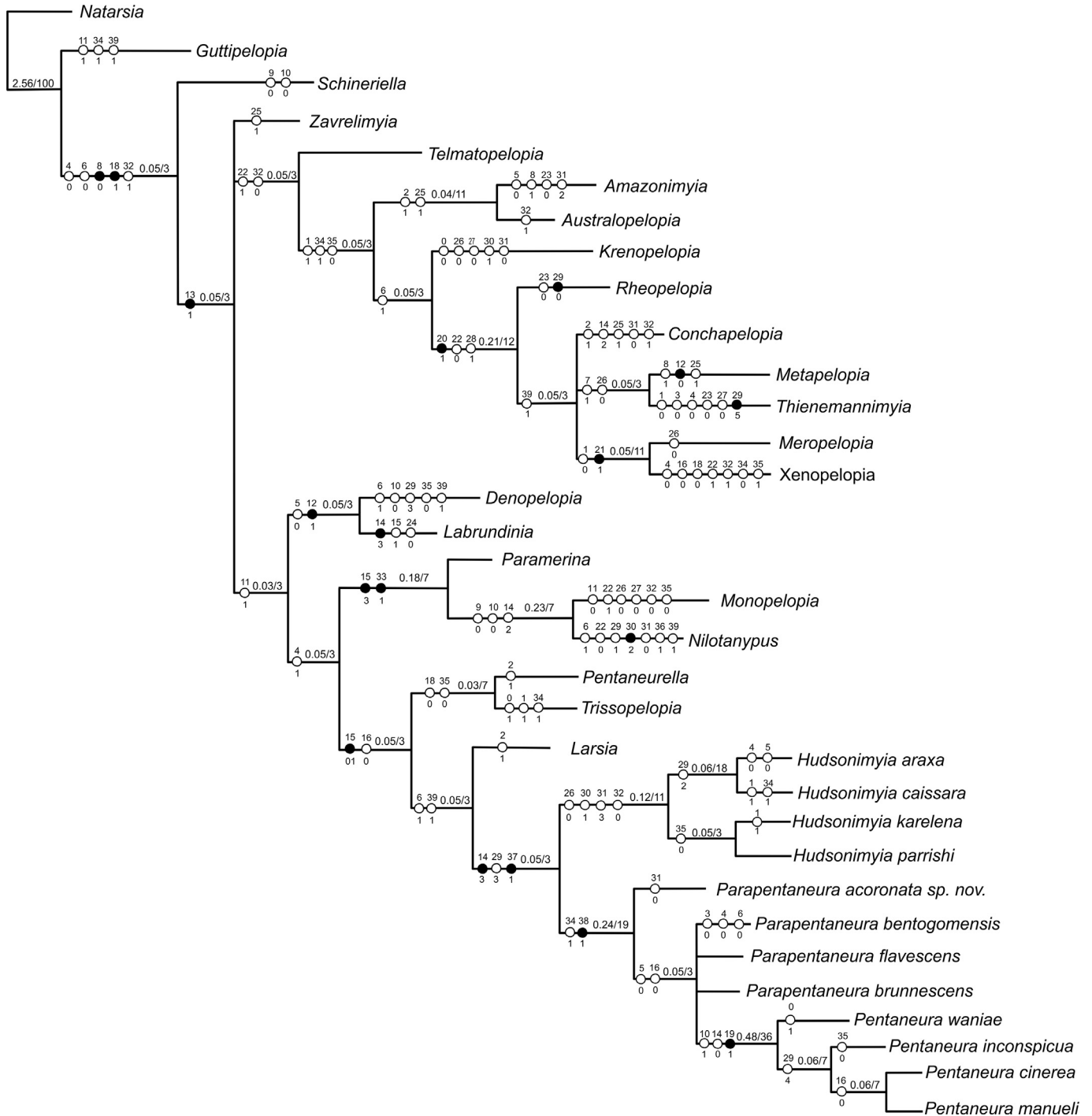


Fig. 5. Cladogram. The numbers above the nodes represent, from left to right the absolute and relative Bremer supports, respectively.

second character also reversed to state (0) in some *Pentaneura* species. *Larsia* resulted sister to this clade.

Hudsonimyia resulted monophyletic, sharing pupal traits such as the absence of thoracic comb (Ch.26:0), strongly divergent anal lobe points (Ch.30:1), presence of apical spines on inner and outer margin of anal lobe (Ch.31:3) and absence of adhesive sheaths on anal macrosetae (Ch.32:0). *Pentaneura* and *Parapentaneura* species, with *P. acoronata* n. sp. as the basal species, share the presence of high AR (Ch. 34:1) and supraanal setae mounted on dark tubercles in the larvae (Ch.38:1). *Pentaneura* species shares the presence of R₂ and R₃ veins (Ch.10:1), the presence of 2 tibial comb on pIII

(Ch.14:0) and gonocoxite deeply excavated and extensively membranous on median surface (Ch.19:1). *Parapentaneura* resulted non monophyletic.

In the analysis including only the closest relatives of *Parapentaneura*, the genus *Hudsonimyia* resulted monophyletic, supported by the same characters mentioned in the previous analysis with the addition of the presence of R₂ and R₃ veins. The clade including *Pentaneura* and *Parapentaneura* species, were supported by the same characters mentioned in the previous analysis with the addition of the absence of distance between RM and MCu cross-veins in the male adult. The internal relationships of this clade

agree with the previous analysis and were supported by the same characters.

In the analysis, in which we deleted the homoplastic characters (Chs.11 and 34) shared by *Parapentaneura* and *Pentaneura* and, in which we assumed the presence of the long anal tubules (Ch.36:1) for all *Pentaneura* species, the consensus tree obtained also showed *Parapentaneura* paraphyletic. *Pentaneura* was supported by the synapomorphies of the gonocoxite deeply excavated and extensively membranous on median surface and by the type of anal tubules (Chs.19:1; 36:1) plus other homoplastic characters (Chs. 5:0, 10:1, 14:0, 30:4), while *Hudsonimyia* was supported by the synapomorphy of the strongly divergent anal lobe points plus other homoplastic characters (Chs. 10:1, 22:1, 26:0, 31:3, 32:0).

4. Discussion

The close relationship between *Parapentaneura* and *Pentaneura* was previously suggested by Silva & Ekrem (2015) and Silva & Ferrington (2018), while in a molecular phylogenetic analysis (Krosch et al. 2017), *Australopelopia* resulted sister to *Parapentaneura* and, *Larsia* sister to *Pentaneura*. In the study of Silva & Ferrington (2018), *Hudsonimyia* resulted sister to [*Pentaneura*-*Parapentaneura*] while in Krosch et al. (2017) this genus was not included in the analysis.

Strong similarities of *Parapentaneura*, *Pentaneura* and *Hudsonimyia* are evident, principally at adult and larval stages. Based on the high similarities between the adult male, Sublette & Sasa (1994) proposed *Hudsonimyia* as a subgenus of *Pentaneura*. However, posterior studies not accepted this status (Caldwell et al. 1997; Epler 2001; Ashe & O'Connor 2009) due to Sublette & Sasa (1994) not considered immature characters. At the male adult stage, these three genera have similar shape of tibial spurs, genitalia and wing venation. In the case of *Parapentaneura* and *Hudsonimyia*, both genera also share the number of tibial spurs, a state unique among the subfamily Tanypodinae. Concerning the wing venation, differences in the placement between RM and M₂ crossveins was suggested, but it seem to be not very significative. Also, the presence or not of R₂₊₃ branches was suggested, but this characters is something difficult to check in the very translucent and highly setose wings. Recently, Silva & Ferrington (2018) proposed the gonocoxite deeply excavated and extensively membranous on median surface as a synapomorphy for the adult male of *Pentaneura*. However, this character has some subjectivity and difficulty to compare it with other taxa.

At larval stages, these genera are also similar with some differences such as the length of molar area, presence or not of tubercles at bases of strong supra-anal setae and differences on ventral and dorsal cephalic setae and pits. However, with respect to the ventral and dorsal cephalic setation, we observed differences even into each the genera. In *Pentaneura*, the most relevant trait is the presence of very long and thin anal tubules in the larvae, but it was not corroborated in all the species of the genus. The major differences among these genera, seem to be present at the pupal stage, mainly in *Hudsonimyia* which has an unusual shape of the anal lobe and 2 or 3 very short LS on the abdominal segment VII.

In our cladistics analyses, the close relationship of *Parapentaneura*, *Pentaneura* and *Hudsonimyia* was also observed, where this clade was supported by the large and strong supraanal setae as the most important character shared, which was not observed in any other Tanypodinae genus.

Hudsonimyia resulted monophyletic supported by pupal characters. Of these, the presence of strongly divergent anal lobe points distinguish this genus from the remaining Tanypodinae. Although the presence of apical spines on inner and outer margin of anal lobe and the absence of adhesive sheaths on anal macrosetae are shared

by *Hudsonimyia* species, these traits are also present in other tanypods.

Pentaneura and *Parapentaneura* species, share the supraanal setae mounted on dark tubercles, a character unique among Tanypodinae. Although a high AR in the larvae is also shared, it is present in other genera. This clade show *Parapentaneura* as paraphyletics since it does not contain to *Pentaneura* species. On the other hand, *Pentaneura* is monophyletics but with low support, and supported by highly homoplastic characters within Tanypodinae, such as the presence of R₂ and R₃ veins and the presence of 2 tibial spur on pIII. In the analysis in which we removed the homoplastic characters shared by these two genera, in which we assumed the presence of the long anal and narrow tubules in all *Pentaneura* species, the consensus tree obtained also showed *Parapentaneura* paraphyletic. Although this result would allow us to suggest the transfer of the species from *Parapentaneura* to *Pentaneura* -following the priority rule-considering the presence of setae mounted on dark tubercles as the synapomorphy of the genus, we still considered *Parapentaneura* and *Pentaneura* as separate genera until more molecular or morphological evidence will be available.

By the other hand, *Australopelopia* resulted sister to *Parapentaneura* in the molecular study of Krosch et al. (2017). However, no close relationship between these genera was found in our analysis. Both genera could be morphologically distinguished due to in *Australopelopia* the adult male has thoracic tubercle, the costa is spinose and extends beyond apex of R₄₊₅, tibial spurs are not lyrate in a number of 1, 2, 2 and there is some evidence of volsella on median base of gonocoxite, while the larva has the 2nd antennal segment annulated and the AR is clearly shorter.

The new species described in the present study shares several traits with these three genera. The male adult shares the unusual number of tibial spurs with *Parapentaneura* and *Hudsonimyia*. In addition, the absence of R₂ and R₃ veins is shared with *Parapentaneura* but differs to *Hudsonimyia*. The female genitalia, with a Gp VIII lobe well developed, projecting posteriorly, Gc IX more or less reduced and bare, the sternite X well developed with several setae in the proximal area, and the cercus shape resembles that of *Pentaneura* and *Hudsonimyia*, while no formal female adult description of *Parapentaneura* was previously made. The pupa is similar to *Parapentaneura* and *Pentaneura*, except for the absence of the corona in the thoracic horn. At the larval stages, the new species resembles to *Parapentaneura* and *Pentaneura* by the presence of a small and dark tubercle in the base of the supraanal seta, but differs with the later genus by the shape and length of the anal tubules.

Having expressed and evaluated the absence of a main synapomorphy to define each of these three genera, we finally include the new species in *Parapentaneura* based on the combination of characters of adults and immatures and, also supported by the results of the phylogenetic analysis. Differing from the described congeners, the male of *P. acoronata* is larger, much more setose, has a longer maxillary palps and shows lower LR I and II values. In addition to the previous differences described for the thoracic horn, the pupa of *P. acoronata* could be easily distinguished by the very short spines of the thoracic comb, the anterior position of the 3 lateral setae on segment VII, and the cephalothoracic and abdominal colouration. Concerning the fourth instar larva, *P. acoronata* shows very low A₁L/Md L ratio. Differences in the larval cephalic setation were observed among *Parapentaneura* species. As in *P. flavescens* and *Parapentaneura* sp., in *P. acoronata* the S10 is posterolateral to VP, while *P. brunnescens* and *P. bentogomensis* have a *Pentaneura*-like setation, in which the S10 is anterolateral to VP. Among the described *Parapentaneura* species, *P. acoronata* shares some similarities with *P. sp* described by Stur et al. (2006) such as the atrium with alveolar area reduced in the thoracic horn of the pupa and, the cephalic setation and bifid paralgula in

the larva. However, differences described before clearly distinguish these species.

4.1. Notes about morphology traits of phytotelmic Tanypodinae

As was previously noted, the new species here described shares most of diagnostic characters with *Parapentaneura*, but differences in the thoracic horn of the pupa are evident. The lack of corona in the thoracic horn is a character not described for *Parapentaneura* and with the lack of lobes in the atrium (shared with other genera in Tanypodinae) could cast doubt in the generic assignment. *L. angusticornis* Siri, Campos et Donato, also breeding in the bromeliad *A. distichantha* of Argentina poses a similar dilemma (Siri et al. 2015). The pupa of *L. angusticornis* has a very long and narrow thoracic horn which lacks corona and lobulations in the atrium, clearly differing from the remaining *Larsia* species. *Monopelopia* is also a common Tanypodinae genus inhabiting phytotelmata, in which the pupa, as well occur in *P. acoronata* and *L. angusticornis*, has a long thoracic horn lacking corona and lobulation in the atrium. The Tanypodinae genera *Ablabesmyia*, *Anatopynia*, *Apsectrotanypus*, *Paramerina* and *Pentaneura* also have been reported but less frequently breeding in phytotelmata. Except for the two last genera, the remainder genera lack corona in the thoracic horn.

Therefore, except for the reports of *Pentaneura* and *Paramerina*, Tanypodinae pupae described from phytotelmata have thoracic horns relatively long, corona absent and the atrium not or slightly lobulated could be interpreted as adaptations of this kind of environments. Considering that *Pentaneura* larvae could actually be *Parapentaneura* (as expressed in Cranston 2010), *Paramerina* would be the only genus cited for phytotelmata whose pupae have a corona. In addition, the thoracic horn tend to be non-globose, except for the report of *Ablabesmyia*. The water–air exposure surface between the bromeliad bracts is low. Therefore, the widened of the plastron plate, thus the absence of a corona, could favor the oxygen uptake by the increase of the surface of capture. On the other hand, the reduction of external spines in the anal lobe of *P. acoronata* pupa could be related to the fact that in this type of environment does not necessary to adhere to the substrate, such as occurs in lotic environments to prevent drift. However, the spines have been observed in other phytotelmic species but could be explained as phylogenetic signal.

5. Conclusions

Despite the diagnostic characters described for *Parapentaneura*, *Pentaneura* and *Hudsonimyia*, the strong similarities between the adult and larval stages generate doubts to consider them as separate genera. However, it is still convenient to consider them as separate genera until more morphological and molecular evidence will be available.

The phytotelmic chironomids shows traits to adapt to this particular environment. As it is detailed in the present study, it has been observed both in the new species described here, as well as in the remaining phytotelmic Tanypodinae. These traits generally differs clearly from their co-generic species from other lentic and lotic environments, which generate doubts about their generic identification.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcz.2018.09.002>.

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