



## Phylogenetic analysis of the tribe Macropelopiini (Chironomidae: Tanypodinae): adjusting homoplasies

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Received 22 May 2014; revised 22 September 2014; accepted for publication 3 November 2014

Macropelopiini is a widely distributed tribe of Tanypodinae, with immature stages inhabiting cool seeps, springs, and small streams. The present study evaluated the monophyly and the supporting synapomorphies within a phylogenetic context for the first time for Macropelopiini. The monophyly and the intergeneric relationships were tested by morphological evidence in a cladistic framework, and the information gained from each homoplastic character was evaluated. The monophyly of Macropelopiini is corroborated through the objective synapomorphy ‘outer fringe decreasing from base to apex ending in small spines’ in the pupa, and the subjective synapomorphies ‘tibial spurs with main teeth and short lateral tooth’ in males and ‘dorsal setae arising from prominent tubercles’ in the pupa. *Fittkauimyia* Karunakaran, 1969 is excluded from Macropelopiini, *Gressitius* Sublette & Wirth, 1980 is established as a junior synonym of *Alotanypus* Roback, 1971, and the new combination *Alotanypus antarcticus* **comb. nov.** is proposed. Character combination, mainly through the use of the characters with informative taxonomical value, remains an efficient tool to diagnose the Macropelopiini genera. The new genus *Paggipelopia* **gen. nov.** for *Paggipelopia spaccesii* **gen. et sp. nov.** is erected and the emendation of the species diagnosis of *Wuelkerella toncekenensis* Añón Suárez & Sublette, 2012 is conducted.

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doi: 10.1111/zoj.12228

ADDITIONAL KEYWORDS: homoplasy – implied weighting – Macropelopiini – Neotropics – new genus – *Paggipelopia* **gen. nov.** – phylogeny – successive reweighting.

### INTRODUCTION

The chironomid tribe Macropelopiini – erected by Fittkau (1962), based on adults and immature stages – is commonly found in cool seeps, springs, and small streams. In fact, the name Macropelopiini was originally coined by Zavřel (1929), with just the ending being different in the original spelling, which required a small correction according to the International Code of Zoological Nomenclature (ICZN) code (article 29.2). Zavřel used ‘Macropelopidae’ for a family-group taxon, equivalent to a supertribe by today’s standards (Spies, 2005).

From Fittkau (1962) to the present, several studies on the classification of Macropelopiini have been con-

ducted by changing the genera involved and the characters considered to define this tribe (Table 1; Appendix S1). Recently, Cranston, Hardy & Morse (2012) presented a molecular phylogeny for the Chironomidae including 15 genera of Tanypodinae, of which eight belong to Pentaneurini, five to Macropelopiini, one to Procladiini, and one to Tanypodini. As a result of that study, Macropelopiini as defined by Ashe & O’Connor (2009, 2012) was non-monophyletic, as *Fittkauimyia* grouped with *Tanypus* and *Djalmabatista*.

According to the latest world catalogue of chironomids (Ashe & O’Connor, 2009, 2012), the tribe Macropelopiini comprises *Alotanypus* Roback, 1971, *Apsectrotanypus* Fittkau, 1962, *Bethbilbeckia* Fittkau & Murray, 1988, *Bilyjomyia* Niitsuma & Watson, 2009, *Brundiniella* Roback, 1978, *Derotanypus* Roback, 1971, *Fittkauimyia* Karunakaran, 1969, *Gressitius* Sublette & Wirth, 1980,

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**Table 1.** Summary of the taxonomic history of the tribe Macropelopiini

Fittkau, 1962	Roback, 1971	Roback, 1978	Ashe & O'Connor, 2009, 2012
<p><b>MACROPELOPIINI</b></p> <p><i>Apsectrotanypus</i>  <i>Macropelopia</i>  <i>Procladius</i>  <i>Psectrotanypus</i>  <i>Psilotanypus</i></p>	<p><b>MACROPELOPIINI</b></p> <p><i>Alotanypus</i>  <i>Apsectrotanypus</i>  <i>Derotanypus</i>  <i>Macropelopia</i>  <i>Natarsia</i>  <i>Parapelopia</i>  <i>Psectrotanypus</i></p> <p><b>PROCLADINA MACROPELOPIINA</b></p> <p><i>Procladius</i></p>	<p><b>MACROPELOPIINI</b></p> <p><i>Macropelopia (Alotanypus)</i>  <i>M. (Apsectrotanypus)</i>  <i>M. (Macropelopia)</i>  <i>Psectrotanypus (Psectrotanypus)</i>  <i>P. (Derotanypus)</i>  <i>Brundiniella</i></p> <p><b>NATARSINI PROCLADIINI</b></p> <p><i>Procladius</i></p> <p><i>Natarsia</i></p>	<p><b>MACROPELOPIINI</b></p> <p><i>Alotanypus</i>  <i>Apsectrotanypus</i>  <i>Bethbilbechia</i>  <i>Bilyomyia</i>  <i>Brundiniella</i>  <i>Derotanypus</i>  <i>Fittkauimyia</i>  <i>Guassutanypus</i>  <i>Gressitius</i>  <i>Macropelopia</i>  <i>Psectrotanypus</i>  <i>Radotanypus</i>  <i>Wuelkerella</i></p> <p><b>NATARSINI PROCLADIINI</b></p> <p><i>Procladius</i></p> <p><i>Natarsia</i></p>

*Guassutanypus* Roque & Trivinho-Strixino, 2003, *Macropelopia* Thienemann, 1916, *Psectrotanypus* Kieffer, 1909, *Radotanypus* Fittkau & Murray, 1986, and *Wuelkerella* Añón Suárez & Sublette, 2012. Recently, Cranston & Epler (2013) considered *Bethbilbeckia* as a subgenus of *Macropelopia*, and *Guassutanypus* as a junior synonym of *Alotanypus*.

Since its erection and subsequent revisions, the tribe Macropelopiini has been defined by a combination of plesiomorphic and apomorphic characters (Appendix S1). The different classifications and genera composition proposed in the various studies were based on authorship criteria or, as described in Roback & Moss (1978), by applying phenetics. The monophyly of the tribe and the finding of supporting synapomorphies in a phylogenetic context were never assessed.

The purpose of this study was therefore to assess the monophyly of the Macropelopiini and intergeneric relationships by means of morphological evidence within a cladistic framework, with the application of maximum parsimony, followed by successive reweighting and implied weighting, as optimality criteria, and to test the information obtained from the characters used by other authors in order to diagnose this tribe.

## MATERIAL AND METHODS

### PHYLOGENETIC ANALYSIS

The analysis was performed with species as the terminal taxonomical level, with the characters selected being those of common usage in Tanypodinae taxonomy. The character data were extracted from collection materials, from each original species description, as well as from Fittkau & Murray (1986), Murray & Fittkau (1989), Epler (2001), and Cranston & Epler (2013). The material collected of *Wuelkerella toncekensis* Añón Suárez & Sublette, 2012 allowed us to measure and check the characters not included in the original description but used in our analysis. We found variation in some characters with respect to the original description of *W. toncekensis*, which deserves emendation.

Given a number of variables within a single character, two alternatives of character coding are available: an arrangement of all of the variables together within a single multistate character or a treatment of each variable as a binary character. Unfortunately, neither of these two proposed character-coding approaches is problem-free. Pleijel (1995) considered four problems that arise at the time of coding observations: (1) interdependency; (2) hierarchical linkage; (3) missing entries; and (4) information retrieval and testability. The multistate approach minimizes the level of character interdependency, whereas the binary practice evades problems of hierarchical linkage. In our

study, several characters with more than two variables were found during character sampling that were at first interpreted as multistate characters. The phylogenetic trees obtained from analysis of that data matrix indicated the multistate characters to be highly homoplastic, with the clade *Fittkauimyia* lying inside the clade (*Macropelopia*–*Bilyjomyia*) as the result of the common presence of a scutellar tubercle and the basal position of the ring organ on the maxillary palp. To our surprise, this latter character reverted to the apical position in *Fittkauimyia*. After an evaluation of these results we re-coded the multistate characters as a binary, and conducted the analyses presented based on the new matrix. This method represents a simpler and more straightforward approach than the alternative of transforming the observations into a matrix. Each observation here is therefore treated in isolation as a potential apomorphy to be tested against other observations, no matter from what state that character may have developed (Pleijel, 1995).

The characters and character states used in the cladistic analysis of Macropelopiini are shown below. A morphotype with all of its life stages was collected from several field trips in Argentina, and was included in the analysis as an unnamed terminal (= UNA\_GN) because assignment of the morphotype to a known genus of the tribe was not possible. The data matrix consisted of 44 taxa and 79 characters, with 25 characters being coded as polymorphic (Appendix S2).

In order to assess the monophyly of Macropelopiini, species of the following genera belonging to the remaining tribes within the subfamily Tanypodinae were included as an out-group: *Pentaneura* Philippi, 1866 and *Ablabesmyia* Johannsen, 1905 as representatives of the tribe Pentaneurini, *Procladius* Skuse, 1889 and *Djalmabatista* Fittkau, 1968 of Procladiini, *Coelotanypus* Kieffer, 1913 and *Clinotanypus* Kieffer, 1913 of Clinotanypodini, *Natarsia* Fittkau, 1962 of Natarsiini and *Tanypus* Meigen, 1803 of Tanypodini. *Podonomus* Philippi, 1866 (subfamily Podonominae) was used to root and polarize the tree.

The data matrix was analysed under maximum parsimony, followed by successive reweighting and implied weighting as optimality criteria. 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. Analyses with implied weighting were conducted in TNT 1.1 (Goloboff, Farris & Nixon, 2008a) by means of values for the concavity constant  $k = 3-20$ , following Goloboff *et al.* (2008b). The analysis under maximum parsimony with successive reweighting was performed in PAUP 4.0b10 (Swofford, 2002). Character supports were calculated with TNT. Absolute and relative Bremer

supports were calculated by saving longer suboptimal trees (up to six steps longer) obtained by branch swapping.

As part of the analysis, characters with a single step and with consistency (ci) and retention (ri) indices of 100 are differentiated from characters with more steps and with lower ci and ri values. The ri measures the level of synapomorphy expected from a data set that is retained as a synapomorphy on a cladogram. In this way, the evaluation is possible if the homoplasy is informative about the branching pattern of the taxa. Following Sæther (1983), we designated those character states with a ci and ri value of 100 as objective synapomorphy, and those character states with at least a minimum ri value of 75 to be subjective synapomorphies. Unless otherwise stated, characters must be interpreted as subjective synapomorphies.

#### Materials

Immature stages of the 'unassigned terminal' (UNA\_GN), and of *W. toncekensis* were transported live in separate vials and reared in the laboratory following the methods described by Epler (2001). Cleared specimens were slide-mounted in Canada balsam. General terminology follows Sæther (1980), except for the term 'taeniate' (Langton, 1994) and the larval cephalic setation, the terminology of which follows Kowalyk (1985) and Cranston & Epler (2013). Measurements are in  $\mu\text{m}$  (except when otherwise stated) and given as ranges, followed by those of the holotype in brackets. The holotype and paratypes are deposited in the collection of the Museo de La Plata, Argentina (MLP), except for the two paratypes that are deposited in the Museum of Natural History, London, UK (NHM).

#### CHARACTER LIST

##### Male

0. Temporal setae: (0) uniserials; (1) bi- to multiserials.
1. Setigerous lobe between base of pedicel and eye: (0) absent; (1) present.
2. Scutal tubercle: (0) absent; (1) present.
3. Anepisternals: (0) absent; (1) present.
4. Preepisternals: (0) absent; (1) present.
5. Postnotals: (0) absent; (1) present.
6. C vein extension: (0) C vein not produced, or at most produced beyond the R4+5 vein by a distance less than the length of the RM vein; (1) C vein produced beyond the R4+5 vein by a distance at least as long as the RM vein.
7. Relative position of MCu and FCu: (0) MCu after FCu; (1) MCu before FCu.
8. Relation of veins R<sub>2</sub> and R<sub>3</sub>: (0) contacted; (1) not contacted.

9. Veins R<sub>2</sub> and R<sub>3</sub> contacted; R<sub>3</sub> complete: (0) absent; (1) present.
10. Veins R<sub>2</sub> and R<sub>3</sub> contacted; R<sub>3</sub> incomplete: (0) absent; (1) present.
11. Macrotrichia: (0) absent; (1) present.
12. Spots or bands in wings: (0) absent; (1) present.
13. RM: (0) pale; (1) dark.
14. Fourth tarsomere: (0) chordate; (1) cylindrical.
15. Foretibial comb: (0) absent; (1) present.
16. Comb on tibia 3, simple: (0) absent; (1) present.
17. Comb on tibia 3, double: (0) absent; (1) present.
18. Tibial spurs simple, without lateral teeth, and with thin setae: (0) absent; (1) present.
19. Tibial spurs simple, with one or two lateral teeth: (0) absent; (1) present.
20. Tibial spurs with main teeth and short lateral tooth: (0) absent; (1) present.
21. Tibial spurs with main teeth and long lateral tooth: (0) absent; (1) present.
22. Claws: (0) simple; (1) spatulate.
23. Pulvilli: (0) absent or reduced; (1) present.
24. Tergite IX: (0) bare; (1) setose.
25. Inferior volsella: (0) absent; (1) present.
26. Ventral projection of gonostylus: (0) absent; (1) present.

##### Female

27. Seminal capsules: (0) neck placed symmetrically on capsule; (1) neck placed asymmetrically on capsule.

##### Pupa

28. Thoracic comb: (0) absent; (1) present.
29. Horn sac: (0) not filling the entire lumen; (1) filling all or almost all of the entire lumen.
30. Plastron plate: (0) absent or reduced; (1) present.
31. Rods: (0) absent; (1) present.
32. Neck on the thoracic horn: (0) absent; (1) present.
33. Scar on abdominal segment I: (0) absent; (1) present.
34. Shagreen: (0) solitary spines; (1) serially arranged spines.
35. Dorsal setae arising from prominent tubercles: (0) absent; (1) present.
36. Abdominal segment VII with four taeniate lateral setae: (0) absent; (1) present.
37. Abdominal segment VII with five taeniate lateral setae: (0) absent; (1) present.
38. Abdominal segment VII with six taeniate lateral setae: (0) absent; (1) present.
39. Abdominal segment VII with between seven and 11 taeniate lateral setae: (0) absent; (1) present.
40. Abdominal segment fringed: (0) absent; (1) present.
41. Anal lobe asymmetrical, inner margin more or less straight, outer margin convex; anal point in the internal margin of the lobe: (0) absent; (1) present.



42. Anal lobe more or less symmetrical, biconvex, anal point more or less central to each lobe: (0) absent; (1) present.
43. Anal lobe asymmetrical, without anal point; sometimes with the lobes distally overlapped: (0) absent; (1) present.
44. Outer fringe decreasing from base to apex, ending in small spines: (0) absent; (1) present.
45. Outer fringe with setae similar in length from base to apex: (0) absent; (1) present.
46. Inner fringe on anal lobe: (0) absent; (1) present.
47. Ratio of Genital sac length / anal lobe length: (0) greater than 0.5; (1) less than 0.5.

### Larva

48. Cephalic setation: (0) VP lateral to S<sub>9</sub> and S<sub>10</sub>; (1) VP mesial or between S<sub>9</sub> and S<sub>10</sub>.
49. Cephalic setae: S<sub>9</sub> and S<sub>10</sub> branched: (0) absent; (1) present.
50. Cephalic setae: S<sub>9</sub> and S<sub>10</sub> simple, (0) absent; (1) present.
51. Cephalic setae: S<sub>9</sub> simple, S<sub>10</sub> branched, (0) absent; (1) present.
52. Cephalic setae: S<sub>9</sub> branched, S<sub>10</sub> simple, (0) absent; (1) present.
53. Dorsal pore on cephalic capsule: (0) absent; (1) present.
54. Antennal segment 2 (A2), length/width: (0) >10; (1) <6.
55. Labral sclerite: (0) absent; (1) present.
56. Antenna at most one-third of the head: (0) absent; (1) present.
57. Dorsomental teeth in longitudinal rows: (0) absent; (1) present.
58. Dorsomental teeth in two transverse plates (= bipartite dorsomentum): (0) absent; (1) present.
59. Dorsomental teeth in three plates (= tripartite dorsomentum): (0) absent; (1) present.
60. Ligula with four teeth: (0) absent; (1) present.
61. Ligula with five teeth: (0) absent; (1) present.
62. Ligula with six or more teeth: (0) absent; (1) present.
63. Ligula coloration: (0) pale; (1) dark.
64. Inner lateral teeth of ligula, straight: (0) absent; (1) present.
65. Inner lateral teeth of ligula, outcurved: (0) absent; (1) present.
66. Inner lateral teeth of ligula, inner curved: (0) absent; (1) present.
67. Paraligula simple, with very short spines: (0) absent; (1) present.
68. Paraligula, bifid: (0) absent; (1) present.
69. Paraligula, multitoothed: (0) absent; (1) present.
70. Pseudoradula: (0) present; (1) absent.

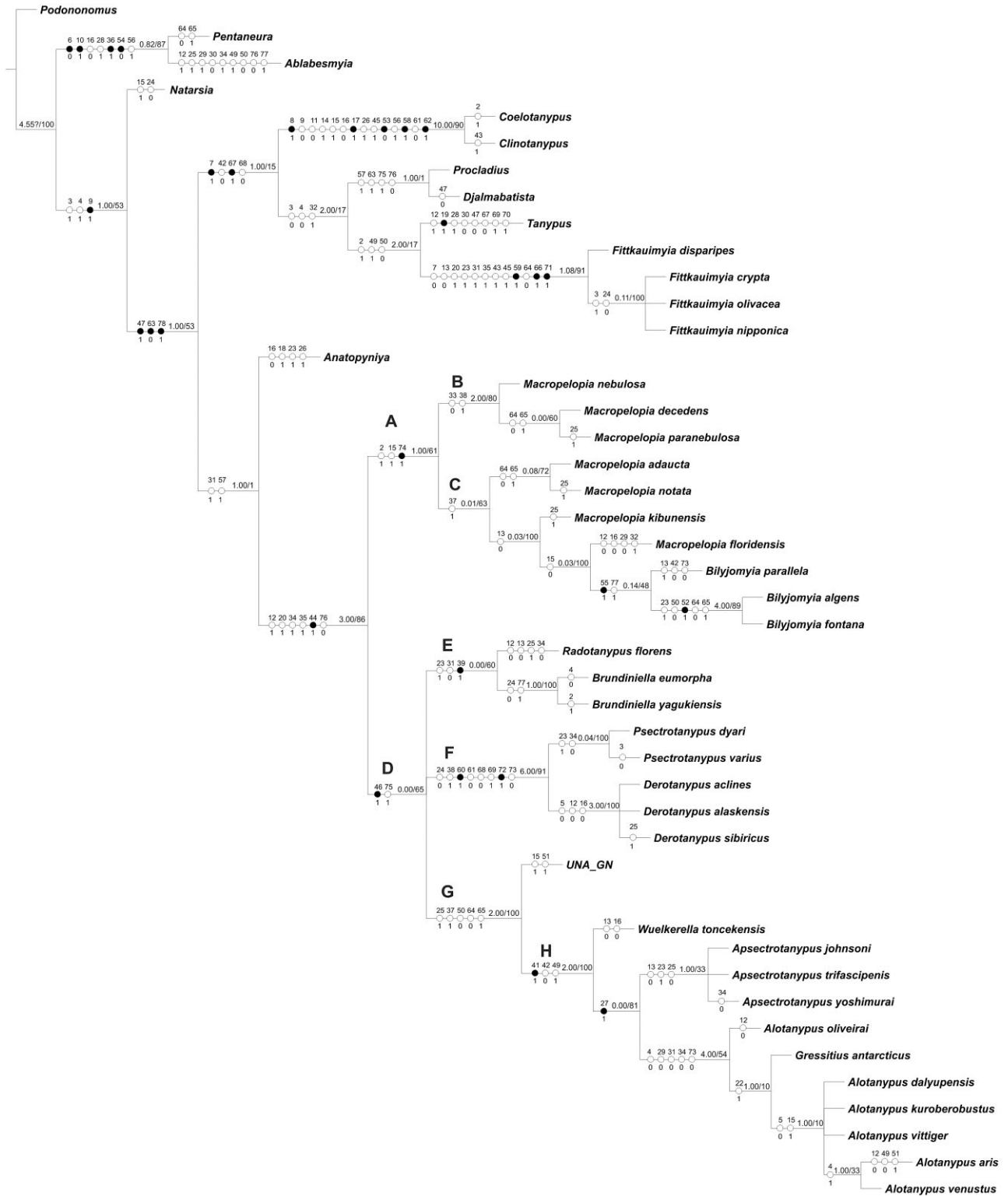
71. Mandible with several rows of small additional teeth, both dorsally and ventrally: (0) absent; (1) present.
72. Row of additional teeth on inner side of the mandible: (0) absent; (1) present.
73. Ventrolateral setae on mandible: (0) all simple; (1) seta 1 simple, setae 2 and/or 3 branched.
74. Ring organ of the maxillary palp: basal, (0) absent; (1) present.
75. Ring organ of the maxillary palp: medial, (0) absent; (1) present.
76. Ring organ of the maxillary palp: apical, (0) absent; (1) present.
77. Base of the small claws on posterior parapods: (0) normal; (1) widened.
78. Body setae on larvae: (0) not fringed; (1) fringed.

## RESULTS

The analysis under implied weights from  $k = 3$  to 20 yielded one tree (fit = 61.6; consistency index, CI = 0.36; retention index, RI = 0.76; Fig. 1). The concavity of  $k = 5$  shows the best Bremer support. The analysis with maximum parsimony followed by successive reweighting yielded the same tree as obtained under implied weighting ( $L = 214$ ; CI = 0.36; ri = 0.77). Table 2 shows the characters with ri values higher than 75.

Macropelopiini is monophyletic only if *Fittkauimyia* is excluded from the tribe. The presence of an anal lobe with the outer fringe decreasing from the base to the apical region, and ending in small spines (character 44: 1), was an objective synapomorphy supporting the tribe. This clade is also supported by the tibial spurs with main teeth and short lateral tooth in the adult male (character 20: 1), dorsal setae arising from prominent tubercles in the abdomen of the pupa (character 35: 1), and the RO of the maxillary palp in the larva never situated apically (character 76: 0). A basal dichotomy is observed in Macropelopiini, with clade A being defined by the presence of a scutal tubercle (character 2: 1) and the RO of the maxillary palp being basally located (character 74: 1), and clade D is defined by the presence of an inner fringe (character 46: 1) and the RO of the maxillary palp being medially located (character 75: 1) (Fig. 1).

Clade A comprises all of the *Macropelopia* and *Bilyjomia* species. Within clade A, clade B with the species of the *nebulosa* group of *Macropelopia* is supported by the presence of six taeniate setae on segment VII (character 38: 1). Clade C consists of species of the *notata* group of *Macropelopia*, *Macropelopia floridensis* (Fittkau & Murray, 1988) and the *Bilyjomia* species. This clade is supported by the presence of five taeniate setae on segment VII (character 37: 1). *Bilyjomia* is supported by the presence of a labral sclerite



**Figure 1.** Cladogram obtained under K = 5. The numbers above the nodes represent, from left to right the absolute and relative Bremer supports, respectively.

**Table 2.** List of characters with a retention index value higher than 75

Character number	L	ci	ri	'Global' character status	Character status considering only the clade Macropelopiini
0	3	33	77	NI	NI
1	1	100	100	NI	NI
2	4	25	80	SS	SS
4	5	20	76	SS	SS
6	1	100	100	NI	NI
7	2	50	75	NI	NI
8	1	100	100	NI	NI
9	2	50	75	NI	NI
10	1	100	100	NI	NI
17	1	100	100	NI	NI
20	2	50	88	SSM	OSM
27	1	100	100	OS	OS
35	2	50	88	SSM	OSM
36	1	100	100	NI	NI
37	2	50	94	SS	SS
38	2	50	85	SS	SS
39	1	100	100	OS	OS
40	2	50	75	NI	NI
41	1	100	100	OS	OS
42	4	25	85	SS	SS
43	2	50	75	NI	NI
44	1	100	100	OSM	OSM
45	2	50	80	NI	NI
47	2	50	95	SS	OS
46	1	100	100	SS	OS
48	1	100	100	NI	NI
49	4	25	75	SS	SS
50	4	25	81	SS	SS
52	1	100	100	OS	A
53	1	100	100	NI	NI
54	1	100	100	NI	NI
55	1	100	100	OS	OS
57	2	50	88	NI	NI
58	1	100	100	NI	NI
59	1	100	100	NI	NI
60	1	100	100	SS	OS
61	2	50	83	NI	NI
62	1	100	100	NI	NI
66	1	100	100	NI	NI
67	2	50	83	NI	NI
68	2	50	92	NI	NI
69	2	50	83	SS	OS
71	1	100	100	NI	NI
72	1	100	100	OS	OS
74	1	100	100	OS	OS
75	2	50	94	SS	OS
76	3	33	77	*	NI
78	1	100	100	NI	NI

Abbreviations: A, autapomorphy; ci, consistency index; L, steps; NI, non-informative for the Macropelopiini; OS, objective synapomorphy; OSM, objective synapomorphy for the tribe Macropelopiini; ri, retention index; SS, subjective synapomorphy; SSM, subjective synapomorphy for the Macropelopiini. \*The absent condition is informative (OS) for Macropelopiini.

(character 55: 1) as an objective synapomorphy and by the small claws on posterior parapods with the bases widened (character 77: 1).

The remaining genera of Macropelopiini (clade D) show a basal trichotomy, consisting of the weakly supported clade E (0.00/60) and the highly supported clades F (6.00/91) and G (2.00/100). Clade E is formed by *Radotanypus florens* (Johannsen, 1908), *Brundiniella eumorpha* (Sublette, 1964), and *Brundiniella yagukiensis* Niitsuma, 2003, all sharing the presence of between seven and 11 taeniate setae on abdominal segment VII (character 39: 1).

Clade F is formed by the species of *Psectrotanypus* and *Derotanypus* analysed, supported by the presence of a row of additional teeth on the inner side of the mandible (character 72: 1) as an objective synapomorphy and the presence of six taeniate setae on abdominal segment VII (character 38: 1), the four-toothed ligula (character 60: 1), and multitoothed paraligula (character 69: 1).

The composition of clade G – with the morphotype UNA\_GN, *Wuelkerella toncekensis* Añón Suárez & Sublette, 2012, *Gressitius antarcticus* (Hudson, 1892), and representatives of *Apsectrotanypus* and *Alotanypus* as members – is supported by the presence of five taeniate setae on abdominal segment VII (character 37: 1) and the absence of the cephalic setal condition of ‘S<sub>9</sub> simple, S<sub>10</sub> simple’ (character 50: 0). Clade H is supported by the presence of an asymmetrical anal lobe, with the inner margin more or less straight, the outer margin curved, and the anal point close to the midline of the abdomen (character 41: 1) as an objective synapomorphy. *Wuelkerella toncekensis* is sister to the clade (*Apsectrotanypus* (*Gressitius*–*Alotanypus*)), which shares the neck of the seminal capsules placed asymmetrically (character 27: 1) as an objective synapomorphy, and by the presence of the setal cephalic condition of ‘S<sub>9</sub> and S<sub>10</sub> branched’ (character 48: 1). *Apsectrotanypus* is not supported by any synapomorphy. *Alotanypus*, which is supported by the absence of pre-episternals (character 4: 0), is monophyletic only if *G. antarcticus* is included. *Gressitius antarcticus* and the remaining *Alotanypus* species share the presence of spatulate claws on the male legs (character 22: 1). The absence of preepisternals in *Alotanypus* and *G. antarcticus* reverted to the present condition in the species *Alotanypus aris* Roback, 1971 and *Alotanypus venustus* (Coquillett, 1902).

## DISCUSSION

### CHARACTER ANALYSIS

As mentioned in the introduction, Macropelopiini has previously been defined as a tribe by character combination (Appendix S1). The results obtained from the

present analyses on the basis of parsimony followed by implied weighting and successive reweighting as optimality criteria allowed us to evaluate the characters considered in diagnosing this tribe and the genera therein through the application of a simple measurement such as ri for assessing the relative level of homoplasy required to form a tree. Nevertheless, not all of the characters with high ri values were informative, nor were all of the characters with low ri values are non-informative, in terms of defining the tribe Macropelopiini (Table 2).

Of the high-ri characters that were not informative for our purpose, an example is the ‘extension of the C vein beyond R<sub>4+5</sub>’. The absence of this character is an objective synapomorphy for the clade Pentaneurini, but the presence of the character has no taxonomic value, as this feature is common to the rest of the Tanypodinae.

In contrast, four characters with low ri values proved highly informative for Macropelopiini. The spatulate claws on the male legs (ri = 63) were highly informative for our purpose, as they were present among Macropelopiini in *G. antarcticus* and the *Alotanypus* species, except for *Alotanypus oliveirai* (Roque & Trivinho-Strixino, 2003). A setose tergite IX in males (ri = 66) was present in all Macropelopiini, except in *Brundiniella* and the (*Psectrotanypus*–*Derotanypus*) clade. The scar on pupal abdominal segment I (ri = 66) showed a high taxonomic value within the context of Macropelopiini, as the absence is shared only with clade B. The condition of the horn sac not filling the entire lumen (ri = 63) occurred in the tribe among *G. antarcticus*, *Alotanypus* species, and *M. floridensis*; however, the presence of this character is only incompletely evident in *M. floridensis* because almost all of the lumen of the horn sac is filled.

From this point on, we will discuss the remaining characters with taxonomic value for Macropelopiini (listed in Table 2): almost all of them were informative within the tribe. In the adult male, the tibial spurs with main teeth and short lateral tooth was a subjective synapomorphy for the clade Macropelopiini and a subjective synapomorphy for *Fittkauimyia*. The presence of a scutal tubercle resulted in a subjective synapomorphy supporting clade A, a character that was shared with *B. yagukiensis* in the Macropelopiini, with some Pentaneurini, and with other non-Pentaneurini genera. As to the thoracic chaetotaxy, the presence of preepisternal setae was the only condition with taxonomical value, as the absence of those setae in the Macropelopiini was observed only in *B. eumorpha* and the *Alotanypus* species, except for *A. aris* and *A. venustus*. In the adult females, the seminal capsule with an asymmetrically placed neck was an objective synapomorphy for the clade (*Apsectrotanypus yoshimurai* (Tokunaga, 1937)–*Alotanypus* species).



With respect to the characters derived from the pupae, the presence of an outer fringe that decrease from the base to the apex and end in small spines was the only objective synapomorphy for Macropelopiini. The presence of dorsal setae arising from prominent tubercles was a subjective synapomorphy for the clade Macropelopiini and for *Fittkauimyia*. The presence of an inner fringe was initially considered to be an objective synapomorphy for clade D, but that feature cannot be regarded as an objective synapomorphy as the character had also been found in both *Fittkauimyia carranquensis* Dantas & Hamada, 2013 and *Fittkauimyia mayumiae* Dantas & Hamada, 2013 (Dantas & Hamada, 2013), species that were not considered in the present analysis because their larvae remain unknown. Each specific number of taeniate setae on abdominal segment VII was treated as an individual character. All of these characters proved taxonomically informative for Macropelopiini, except for the occurrence of four taeniate setae. The presence of five taeniate setae was a subjective synapomorphy for clades C and G, six taeniate setae was a subjective synapomorphy for the *nebulosa* group of *Macropelopia* as well as for the (*Psectrotanypus*–*Derotanypus*) clade, and between seven and 11 taeniate setae was an objective synapomorphy for the (*R. florens*–*Brunдиниella*) clade. Of essential relevance was the feature that all Macropelopiini always have between five and 11 taeniate setae on segment VII, whereas the Pentaneurini have four or fewer, except for *Thienemannimyia*, whose species all possess five. The shape of the anal lobe was taxonomically informative for the tribe. The presence of an asymmetric anal lobe with the inner margins more or less straight, the outer margins convex, and the anal points in the internal margin of the lobes was an objective synapomorphy for clade H; whereas anal lobes that are biconvex, bilaterally symmetrical, or has anal points slightly mesad of the midline are present in the remaining Macropelopiini.

In the analysis of the larval characters, the presence of a pectinate paralgula was taxonomically informative in Macropelopiini, as this feature was a subjective synapomorphy for the clade (*Psectrotanypus*–*Derotanypus*). The presence of a four-toothed ligula was originally considered an objective synapomorphy for the clade (*Psectrotanypus*–*Derotanypus*), but could not be considered as such as this character is in certain species of *Djalmabatista* and *Procladius*. The condition of the simple ventrolateral setae on the mandible was a subjective synapomorphy for the (*Psectrotanypus*–*Derotanypus*) clade and the genus *Alotanypus*. This condition was also observed in some Pentaneurini, Clinotanypodini, and *Natarsia*. The basal location of the RO in the maxillary palp was an objective synapomorphy for clade A, whereas an RO in the medial position is found in the remaining Macropelopiini and

in the Procladiini. A mandible with a row of additional teeth on the inner side was an objective synapomorphy for the clade (*Psectrotanypus*–*Derotanypus*). The cephalic S<sub>9</sub> and S<sub>10</sub> setae were informative for Macropelopiini. Within the tribe, the condition of both multibranching setae is shared by only *Apsectrotanypus* and *Alotanypus*, except for *Alotanypus aris*; whereas the condition of a simple S<sub>9</sub> and multibranching S<sub>10</sub> was an objective synapomorphy for *Bilyjomyia*.

As mentioned above, a tribal diagnosis is confirmed by a single objective synapomorphy and by two subjective synapomorphies. Nevertheless, we observed that although most of the characters analysed had some degree of homoplasy, they were highly informative in the local optimum for Macropelopiini. Therefore, the combination of characters, mainly through the use of characters with informative taxonomical value, still remains an efficient tool for diagnosing Macropelopiini genera.

#### PHYLOGENETIC ANALYSIS

When Fittkau (1962) conducted the phylogenetic analysis of the Tanypodinae, only three genera were known among those currently considered as Macropelopiini. In his phylogenetic analysis, he considered the genus *Anatopynia* as a model of plesiomorphic taxa from which the other Tanypodinae had arisen. Within the tribe Macropelopiini, this author proposed *Macropelopia* as the most basal and plesiomorphic genus, *Psilotanypus* and *Procladius* as the most apomorphic genera, and *Psectrotanypus* as an intermediate form. From this scheme, Fittkau (1962) proposed hypotheses of character evolution in Macropelopiini, such as the evolution from a plesiomorphic five-toothed ligula and bifid paralgula in *Macropelopia* to a four-toothed ligula and multitoothed paralgula in *Psectrotanypus*, *Psilotanypus*, and *Procladius*.

In our analysis, both subjective synapomorphies supporting Macropelopiini are shared with *Fittkauimyia*, but it is excluded from the Macropelopiini and instead exhibits a close relationship with *Tanypus*. This result is similar to that obtained by Cranston *et al.* (2012), as *Fittkauimyia* is the sister group of the clade (*Djalmabatista*–*Tanypus*), even if this relationship has no statistical support. Cranston *et al.* (2012) suggested a ‘non-Pentaneurine’ group with high bootstrap support and Bayesian posterior probability, thus casting doubt on the existing tribal substructure in the Tanypodinae. The findings obtained in our study reinforce Macropelopiini as a monophyletic group defined by an objective synapomorphy and with good support. The inclusion of more genera within this tribe together with more taxa belonging to the rest of the tribes in a molecular *cum* morphological study will no doubt

contribute to an elucidation of the phylogenetic relationships and structure of the 'non-Pentaneurine' group.

The history of the phylogenetic relationship between *Natarsia* and the remaining Tanypodinae is conflictive. This genus was considered as Pentaneurini by Fittkau (1962), as Macropelopiini by Roback (1971), and as Natarsiini by Roback & Moss (1978). Our results have validated *Natarsia* as a separate tribe.

Analysing Macropelopiini, *Macropelopia* was found to be non-monophyletic because of the inclusion of *Bilyjomia* (clade A). This clade was supported by the presence of a scutal tubercle, the absence of an inner fringe in the anal lobe, and the basal position of the RO in the maxillary palp. These character states are in agreement with Fittkau & Murray (1986), Murray & Fittkau (1989), and Cranston & Epler (2013). Clade B indicated the *nebulosa* group of *Macropelopia* to be monophyletic, as supported by the absence of a scar and the presence of six taeniate setae in segment VII. By contrast, clade C comprised the species of the *notata* group, *Macropelopia* (*Bethbilbeckia*) *floridensis* and *Bilyjomia*. According to Niitsuma & Watson (2009), the pupa of *Bilyjomia* keys to *Macropelopia* in Fittkau & Murray (1986), the female keys to *Natarsia* and *Macropelopia* (in part) in Sæther (1977), and if a scutal tubercle is discernible, the male keys to *Bethbilbeckia*, which is currently considered as a subgenus of *Macropelopia*. Nevertheless, the variable development of the scutal tubercle in the adult male, a distinctive labral sclerite, and the plumose S<sub>9</sub> and simple S<sub>10</sub> in the larva would be characteristic of *Bilyjomia*. On the basis of all of these considerations, a revision of *Macropelopia* is needed.

The close relationship between *Radotanypus* and *Brundiniella* previously has been described by Fittkau & Murray (1986), Murray & Fittkau (1989), Siri *et al.* (2011), and Cranston & Epler (2013). This clade was supported by the presence of the objective synapomorphy of between seven and 11 taeniate setae on segment VII of the pupa in our analysis. *Brundiniella* can be distinguished from *Radotanypus* by the absence of the widened base of the small claws on the larvae (Cranston & Epler, 2013) and the absence of setae on the TIX (Murray & Fittkau, 1989).

The close relationship between *Psectrotanypus* and *Derotanypus* has been widely reported (Roback & Moss, 1978; Fittkau & Roback, 1983; Fittkau & Murray, 1986; Murray & Fittkau, 1989; Siri *et al.*, 2011; Cranston & Epler, 2013). As previously discussed in the character-analysis section of our study, the characters supporting this clade were highly informative, principally the four-toothed ligula and the multitoothed paraligula. The clearest differences among these genera are found in the immature stages (Fittkau & Murray, 1986; Cranston & Epler, 2013), such as with respect to the outer teeth of the ligula, the presence or absence of dorsal teeth

in the mandible, and by the number of teeth in the pecten hypopharyngis.

Clade G comprised the morphotype UNA\_GN, *Wuelkerella toncekensis*, *Gressitius antarcticus*, and the species analysed of *Apsectrotanypus* and *Alotanypus*. This clade shared the subjective synapomorphies of five taeniate setae present in segment VII and the absence of the condition 'S<sub>9</sub> and S<sub>10</sub> simple'.

*Alotanypus* was considered monophyletic only if *Gressitius antarcticus* was included. This clade was defined by the absence of pre-episternals and rods, and by the simple setae on the larval mandible. The absence of pre-episternals and rods reverted to the present condition in *Alotanypus aris* and *Alotanypus venustus*. The species *Alotanypus oliveirai*, which was recently transferred to this genus (Cranston & Epler, 2013), lies at the base of the genus, as had been reported by Siri *et al.* (2011), whereas *Gressitius antarcticus* was sister to the remaining *Alotanypus* species. For this reason – and also because the characters used by Sublette & Wirth (1980) to define *Gressitius* were likewise shared with *Alotanypus* – we established that this genus is a junior synonym of *Alotanypus* and the new combination *Alotanypus antarcticus* comb. nov. (Hudson, 1892) is formed.

*Apsectrotanypus* proved to be monophyletic, but with low support. Distinctive characters of *Apsectrotanypus* could be present at immature stages. The pupa of *Apsectrotanypus* resembles *Alotanypus*, but can be distinguished because the horn sac completely fills the horn lumen in *Apsectrotanypus*. According to Cranston & Epler (2013), the larva is distinguished from those of other Macropelopiini in the short antennal segment 2 with an unusually deeply-set style along with the few, unusually large dorsomental teeth. Both characters were not considered in our analysis; the first since we could not check most of the species analysed, the latter due to the high variability and degree of overlap in this feature at the genus level.

Our analysis indicated that the condition of an asymmetrical neck position in the seminal capsules was shared between *Apsectrotanypus* and *Alotanypus*. This character, however, is known from *Apsectrotanypus yoshimurai*, which was described by Niitsuma (2004) to be only slightly asymmetrical. When we considered the condition 'symmetrical neck of the seminal capsules' for *A. yoshimurai*, *Wuelkerella* was sister to *Apsectrotanypus* in our analysis, with the presence of an asymmetrical neck as an objective synapomorphy for *Alotanypus*.

The character combination of the morphotype UNA\_GN is not observed in any known Macropelopiini genus. Therefore, we erect a new genus for this morphotype, which is described and compared with the remaining Macropelopiini in the systematic section below.

Watson (2010), most relevantly, emphasized that current generic concepts are mainly based on Holarctic taxa (Fittkau, 1962; Roback, 1971; Fittkau & Roback, 1983; Fittkau & Murray, 1986; Murray & Fittkau, 1989; Cranston & Epler, 2013). Nonetheless, a large but poorly known Macropelopiini fauna is found in the Southern Hemisphere (Cranston & Martin, 1989; Spies & Reiss, 1996; Ashe & O'Connor, 2009). A knowledge and full description of the austral Macropelopiini will lead to a reassessment of the existing generic boundaries, and could well result not only in the erection of new genera but also in an improvement of our understanding of the evolutionary relationships among the Macropelopiini. Moreover, the use of molecular data may reduce the extensive homoplasy produced by the use of morphological characters in the phylogenetic analysis (Cranston *et al.*, 2012).

#### TAXONOMY

The tribe Macropelopiini is defined by a character in the pupa where the outer anal lobe fringe decreases towards the anal lobe point, ending in small spines. In addition, the tribe Macropelopiini should be emended as sharing the following combination of characters.

*Male:* Temporal setae biserial to multiserial; wing with macrotrichia, usually marked with spots or bands, RM commonly darkened; C produced beyond  $R_{4+5}$  by a distance at least as long as RM; MCu at or slightly beyond FCu;  $R_{2+3}$  fully developed; scutal tubercle present or absent; anteprenotal tubercle present or absent; tibial spurs flattened with lateral short teeth; claws pointed or spatulate.

*Female:* Temporal setae biserial to multiserial; wing and tibial spurs as in male.

*Pupa:* Thoracic comb absent; respiratory atrium commonly filling the entire lumen; with (or rarely without) a scar on abdominal segment I; dorsal setae arising from prominent tubercles; abdominal segment VII with at least five taeniate lateral setae; outer fringe decreasing to the anal lobe point ending in small spines; inner fringe present or absent.

*Larva:* Body segments with fringe of swim setae; CI 75–100%; antenna at most one-third of the head; RO of maxillary palp basal or medial; dorsomentum with row of teeth located on two dorsomentary plates; pseudoradula present; ligula with four or five teeth; paralogula unevenly bifid or pectinate; four relatively short anal tubules. Cephalic setation: VP lateral to  $S_9$  and  $S_{10}$ ; VP in same line as or posterior to  $SS_m$ ;  $SS_m$  anterior, in same line as or posterior to  $S_9$  and  $S_{10}$ .

#### PAGGIPELOPIA GEN. NOV.

*Type species*

*Paggielopodia spaccesii* gen. et sp. nov.

*Etymology*

*Paggi* refers to Dr Analía Constanza Paggi, in recognition of her contributions to our knowledge of the systematics and ecology of the Argentine chironomids; *pelopia* is derived from the suppressed Meigen, 1800 genus name *Pelopia*, being a frequently used suffix within the Tanypodinae.

*Diagnosis*

The new genus can be separated from the remaining genera of Macropelopiini by a combination of the following character states.

*Male:* Scutal tubercle absent; anteprenotal tubercle present, RM and FCu dark; foreleg with tibial comb; legs with pointed claws, inferior volsella present.

*Female:* Elongated terminal flagellomere (as long as flagellomeres 9–13); seminal capsule globose with symmetrical neck position; coxosternapodeme with a bend.

*Pupa:*  $Dc_1$  thin,  $Dc_2$  long and granulated, longer than  $Dc_1$ ; shagreen with short serially arranged spines; anal lobe long, more or less symmetrical, with the apical spine close to the middle, and outer and inner fringe present, both decreasing towards the anal point.

*Larva:* Dorsomentum with three bigger central teeth, plus one basal and two distal and shorter teeth;  $A_2$  with the style arising subapically; prominent membranous area at the junction of  $A_2$  and  $A_3$ ; procercus relatively broad, 2.67–3.03 L/W. *Cephalic setation:*  $S_{10}$  posterior to  $S_9$ ;  $SS_m$  slightly posterior and mesial to  $S_{10}$ , and VP posterolateral to  $S_{10}$ , more or less in same line with  $S_9$  and  $S_{10}$ .

*Remarks:* Distinctive in the adult male of *Paggielopodia* gen. nov. is the presence of the foretibial comb, a feature among the Macropelopiini that is shared with *Alotanypus* and *Macropelopia*. *Paggielopodia* gen. nov. is distinguished in the male adult from these genera by the following characters: from *Macropelopia*, by the absence of a scutal tubercle and from *Alotanypus* by the absence of spatulate claws.

The female of *Paggielopodia* gen. nov. has a long terminal flagellomere and seminal capsules rounded, with the neck placed symmetrically. The coxosternapodeme has a clear bend, a character also observed at least in *Alotanypus aris*, *Alotanypus venustus*, and

*Alotanypus kuroberobustus* (Sasa & Okazawa, 1992).

In the pupal identification key of Fittkau & Murray (1986), *Paggipelopia* gen. nov. keys to *Brundiniella*. *Paggipelopia* gen. nov. differs, however, from this genus because *Brundiniella* has an oval plastron plate, a thin  $Dc_2$ , which is shorter than  $Dc_1$ , and five lateral taeniate setae on segments VI and VII. The pupa of *Paggipelopia* gen. nov. resembles the *notata* group of *Macropelopia* in the five taeniate setae on segment VI, but *Macropelopia* lacks the inner fringe of the anal lobe. The symmetrical anal lobe clearly distinguishes *Paggipelopia* gen. nov. from *Wuelkerella*, *Apsectrotanypus*, and *Alotanypus*. In addition, the serially arranged shagreen, the presence of rods, and the horn sac that fills the entire lumen distinguish *Paggipelopia* gen. nov. from *Alotanypus*.

The larva of *Paggipelopia* gen. nov. keys to *Apsectrotanypus* in Cranston & Epler (2013), sharing a few big dorsomental teeth and antennal segment 2 with a subapical style. The presence of a strong  $S_9$  and a branched  $S_{10}$  cephalic seta in *Paggipelopia* gen. nov., however, distinguishes this genus from *Apsectrotanypus*. This setal arrangement in *Paggipelopia* gen. nov. is shared among Macropelopiini only with *Alotanypus aris*.

#### Generic description

Adult male

*Antenna*: Pedicel, flagellomeres, and plume brown.

*Head*: Temporal bi- to multiserial, postorbitals biserial.

*Thorax*: Dark brown, vittae not distinct. Anteprenotum with anteprenotal lobe and a ventral group of setae. Preepisternals and postnotals always present, anepisternals rarely present. Scutal tubercle absent.

*Wing*: C extended beyond  $R_{4+5}$ . RM and FCu darkened, membrane with dark spots.

*Legs*: Light to dark brown, with apex of femur, base and apex of tibia, and apex of tarsomere 1 darker. Tibial spurs with short lateral teeth; surface of tibial spurs with fine spinules. Tibial comb present on fore- and hindlegs. Claws slender, distally pointed in all legs. Pulvilli small to absent.

*Hypopygium*: Tergite IX with posterior setae distributed in irregular rows. Anal point more or less conical. Gonocoxite with inferior volsella well developed. Gonostylus broad basally, with or without a narrowed apex.

Adult female

*Antenna*: Antenna with 14 flagellomeres; terminal flagellomere long.

*Head*: Temporals bi- to multiserial, postorbitals biserial.

*Thorax*: Coloration as in male. Anteprenotum with anteprenotal lobe and a group of ventral setae. Preepisternals and postnotals always present, anepisternals absent. Scutal tubercle absent.

*Wing*: As in male.

*Legs*: Coloration and tibial spurs as in male. Tibial comb present only on hindlegs. Claws slender, distally pointed in all legs. Pulvilli small to absent.

*Genitalia*: Gonapophysis VIII rounded; coxosternapodeme with a bend; segment X setose; cercus oval; postgenital plate reduced or absent; seminal capsules ovoid with neck placed symmetrically.

Pupa

*Cephalothorax*: Thoracic horn large, horn sac filling almost the entire lumen, with internal supporting rods. Plastron plate well developed, about 0.20 of the total length of the thoracic horn. External surface of the thoracic horn with spines.  $Dc_1$  thin,  $Dc_2$  strong and highly granulated; Sa long, not granulated. Length of dorsocentral setae:  $Dc_1 < Dc_2 < Sa$ .

*Abdomen*: Scar on tergite I well developed. Shagreen with serially arranged spines. Dorsal setae:  $D_1$  spiniform;  $D_2$  and  $D_3$  long, commonly hooked;  $D_4$  short and thin,  $D_5$  almost half as long as  $D_1$ . Segments VII–VIII with five taeniate lateral setae.

Anal lobe symmetrical, with the anal point close to the middle of each lobe; outer and inner border fringed, both decreasing to the apex and ending in small spines. Male genital sac almost half as long as the anal lobe.

Larva

Medium to large larvae, up to 6 mm long.

*Head*: Rounded–oval. Dorsally  $S_7$ ,  $S_8$ , and dorsal pore (DP) forming a right angle;  $S_6$  anterolateral to  $S_7$ . Ventrally  $S_9$  and  $S_{10}$  vertically aligned;  $SS_m$  slightly posterior and mesial to  $S_{10}$ ; ventral pore (VP) posterior and lateral to  $S_{10}$ . Description of cephalic setae:  $S_5$ ,  $S_6$ ,  $S_7$ , and  $SS_m$  multibranching;  $S_{10}$  branching into two or three;  $S_8$  and  $S_9$  simple.

*Antenna*: Somewhat longer than mandible. Antennal ratio about 6.2–7.0. Basal segment with ring organ in the apical third; segment 2 about 3.0–5.0 times as long as wide. Style inserted subapically to segment 2; membranous area at the junction of  $A_2$  and  $A_3$  present.

*Mandible*: Mola with one short distal tooth; seta subdentalis slender.



*Maxilla*: Ring organ of the maxillary palp medially placed.

*Mentum and M appendage*: Dorsomentum with three large and rounded central teeth, plus one short basal and two short distal teeth. Pseudoradula of uniform width, weakly granulose.

*Ligula*: With five teeth, points of the inner lateral teeth distinctly curved outward.

*Paraligula*: Unevenly bifid.

*Body*: With fringe of swim setae. With four rather long, conical anal tubules. Procerus with 13 apical setae. Claws of posterior parapods simple; smallest claws simple, weakly curved.

#### **PAGGIPELOPIA SPACCESI GEN. ET SP. NOV.**

##### *Etymology*

Named in honor of Fernando Spaccesi, our friend and an invaluable collaborator in the fieldwork.

##### *Diagnosis*

See generic diagnosis.

*Type material (all deposited at MLP, except when otherwise stated)*

*Holotype*: Adult ♂ with larval and pupal exuviae, Argentina, Buenos Aires Province, Sierra de la Ventana, Parque Provincial E. Torquinst, unnamed stream at the piedmont of the Bahía Blanca hill, 38°04'06.3"S, 61°58'28.15"W, 468 m a.s.l., 4.X.2013, D-net, M. Donato, A. Siri & F. Spaccesi.

*Paratypes*: One adult ♂, same data as holotype; three adult ♂ and two adult ♀, same data as holotype except for 10.XII.2010; one adult ♂, same data as holotype except for 2–4.XI.2011, light trap; one larva (NHM) and one adult ♂, same data as holotype except for 20.V.2012, light trap. Two larvae and one prepupa, Argentina, Buenos Aires Province, Sierra de la Ventana, Parque Provincial E. Torquinst, Ventana stream, 38°03'42.4"S, 62°01'21.4"W, 518 m a.s.l., 21.V.2011; two larvae and one pupae, 5.XI.2011, D-net, M. Donato, A. Siri & F. Spaccesi. One adult ♂ with its pupal exuviae, Argentina, Buenos Aires Province, Sierra de la Ventana, Parque Provincial E. Torquinst, Cueva del Toro stream, 38°01'19.0"S, 62°01'33.3"W, 672 m a.s.l., 1.VI.2013, D-net, M. Donato, A. Siri & F. Spaccesi. One adult ♂ with the pupal exuviae, Argentina, Chubut Province, Valle Chico stream, 42°55'40.0"S, 71°15'58.0"W, 29.VIII.2012, hand net, D. Anjos Santos & P. Pessaq (NHM). One pharate ♀, Argentina, Chubut Province, Puerto Patriada, 18.X.2012, hand net, D. Anjos Santos & P. Pessaq. Three

pupal exuviae, Argentina, Tierra del Fuego Province, Ruta Complementaria B, 53°54'06.0"S, 67°55'56.3"W, 61 m. a.s.l., 5.XII.2005, drift net, M. Donato. Three adult ♂, one adult male with its pupal exuviae, Argentina, Rio Negro Province, Rincón de Comicó, 41°08'33.8"S, 67°27'36.0"W, 1000 m a.s.l., light trap and hand net, respectively, M. Donato, G. Rossi, and G. Spinelli.

##### *Description*

Male ( $N = 8-12$ , except when otherwise stated in parentheses; Fig. 2A–D)

Total length 4.95–6.58 (4.95) mm. Total length/wing length 1.70–1.82 (1.82).

*Coloration*: Thorax brown to dark brown, without evident vittae. Abdomen (Fig. 2A): tergites II–IV with brown lateromedial bands and an ovoid central spot, tergite V similar to tergites II–IV but generally darker, tergites VI–IX and hypopygium completely brown.

Wing with spots on the apex and on the  $r_{4+5}$  cell membrane. Dark marks present on the base of the squama, on RM, FR, FCu, and on the apex of An (Fig. 2B).

*Head*: Antenna, antennal ratio (AR) 1.6–1.8 (1.73). Temporals bi- to multiserial 40–50 (48); postorbitals biserial, 12–18 (18). Clypeus with 9–20 (13) setae. Tentorium 220–300 (220) long. Palpomere lengths (1–5) 70–75 (70); 110–130 (120); 180–220 (180); 210–280 (210); 350–490 (370).

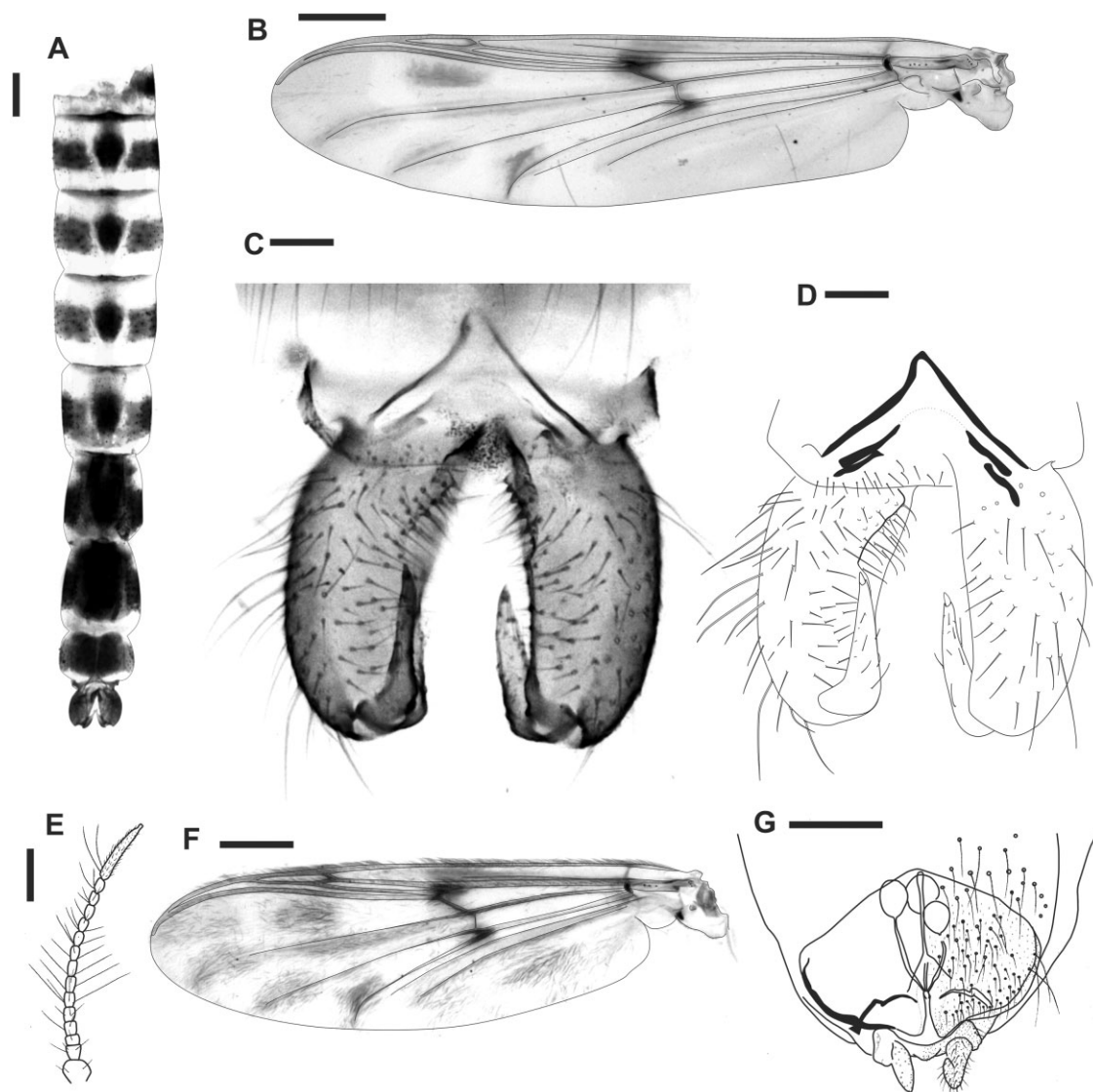
*Thorax*: Anteprenotum with between seven and 11 (10) lateral setae, with a tubercle between anteprenotal setae; acrostichals 40 (1); prescutelar area with three or four setae on each side; dorsocentrals 22–37 (24); prealars 17–29 (17); supraalar 1; scutellars 23–40 (23); preepisternals 3–7 (4); anepisternals 0–1 (1); postnotum with two or three (three) setae.

*Wing*: Length 2.71–3.66 (2.71) mm; width 0.80–1.02 (0.80) mm. L/W 3.39–3.78 (3.39). Costa extended 180–220 (220) beyond  $R_{4+5}$ . Brachiolium with between three and seven (three) distal setae, plus between two and five (three) proximal setae. Squama fringed with 36–54 (38) setae.

*Legs*: Foreleg: tibial spur 75–93 (75) long; comb with at least 12 short spines. Midleg: tibial spurs 80–110 (83) and 63–80 (63) long; four or five (four) sensilla chaetica on tarsomere 1. Hindleg: tibial spurs 83–110 (83) and 63–80 (63) long; comb with 10–14 (12) spines. Lengths and proportions of legs in Table 3.

Hypopygium (Fig. 2C–D). Setae on tergite IX, 28–39 (28). Gonocoxite 220–280 (220) long; inferior volsella well developed. Gonostylus 115–160 (115) long,





**Figure 2.** *Paggipelopia spaccesii* gen. et sp. nov., adults. A–D, Male. A, abdomen in dorsal view; B, wing, borders and veins lined over electronically; C, hypopygium in dorsal view; D, drawing of hypopygium in dorsal (left) and ventral (right) view. E–G, Female. E, antenna; F, wing, borders and veins lined over; G, genitalia. Scale bars = 200  $\mu$ m; except C,D = 50  $\mu$ m.

megaseta 5–20 (10) long. Hypopygium ratio (HR) 1.69–1.96 (1.91); Hypopygium value (HV) 2.21–2.63 (2.25).

Female ( $N = 2$ –4, except when otherwise stated in parentheses; Fig. 2E–G)  
Total length 4.55–4.80 mm. Total length/wing length 1.44–1.55.

*Coloration:* Thorax and wing spots as in male.

*Head:* Antenna (Fig. 2E) with 14 flagellomeres, AR 0.31–0.33. Antenna 880–985 (960) long, terminal flagellomere 210–255 long. Temporals, 38 on each side (1), bi- to multiserial, postorbitals biserial, five on each side (1).

Clypeus with 16–19 setae. Palpomere lengths (1–5): 70–80; 115–155; 170–200; 240–250; and 320–370 long.

*Thorax:* Anteprenotum with between four and ten lateral setae, with a tubercle between the anteprenotal setae; acrostichals 40; prealars 23–28; supraalar 1; prepisternals 3–5; dorsocentrals 45–59; prescutelars 8–12; scutelars 38–43; postnotals 2; anepisternals absent.

*Wing* (Fig. 2F): Length 3.10–3.46 mm; width 1.05–1.15 mm; L/W 2.81–3.01. Costa extended 190–205 beyond  $R_{4+5}$ . Squama fringed with 41–65 setae.

*Legs:* Foreleg: tibial spur 80–90 long, without tibial comb. Midleg: tibial spurs 78–88 and 63–70 long; 13

**Table 3.** Lengths ( $\mu\text{m}$ ) and proportions of male legs of *Paggipelopia spaccesii* gen. et sp. nov.

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>
p <sub>1</sub>	1275–1700 (1275)	1600–2130 (1600)	975–1260 (975)	550–720 (550)	400–520 (400)
p <sub>2</sub>	1425–1775 (1425)	1550–1925 (1550)	790–1050 (790)	420–560 (420)	320–440 (320)
p <sub>3</sub>	1350–1675 (1350)	1725–2225 (1725)	1100–1450 (1100)	590–800 (590)	440–610 (440)
	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
p <sub>1</sub>	250–320 (250)	160–200 (160)	0.59–0.63 (0.61)	2.80–2.92 (2.83)	2.90–3.04 (2.95)
p <sub>2</sub>	200–260 (200)	150–200 (150)	0.51–0.56 (0.51)	3.26–3.45 (3.45)	3.41–3.77 (3.77)
p <sub>3</sub>	270–370 (270)	170–210 (170)	0.64–0.68 (0.64)	2.65–2.88 (2.84)	2.52–2.80 (2.80)

$N = 7$ – $10$ ; value for the holotype given in brackets.

Abbreviations: fe, femur; ti, tibia; ta1-5, tarsomeres 1-5; LR, leg ratio, ratio of metatarsus to tibia; BV, Beinverhältnisse, combined length of femur, tibia, and basitarsus divided by combined length of tarsomeres 2-5; SV, Schenkel-Scheine-Verhältnis, ratio of femur plus tibia to metatarsus.

**Table 4.** Lengths ( $\mu\text{m}$ ) and proportions of female legs of *Paggipelopia spaccesii* gen. et sp. nov.

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>
p <sub>1</sub>	1400–1425	1775–2125	1025–1060	540–650	400–490
p <sub>2</sub>	1500–1550	1640–1975	850–975	450–490	340–350
p <sub>3</sub>	1425–1475	1860–2200	1225–1250	640–750	470–530
	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
p <sub>1</sub>	240–290	180–220	0.55–0.56	2.92–3.09	3.08–3.16
p <sub>2</sub>	210–250	170–200	0.49–0.52	3.45–3.64	3.75–3.82
p <sub>3</sub>	270–330	190–220	0.63–0.64	2.83–2.90	2.72–2.79

$N = 2$  or  $3$ .

Abbreviations: fe, femur; ti, tibia; ta1-5, tarsomeres 1-5; LR, leg ratio, ratio of metatarsus to tibia; BV, Beinverhältnisse, combined length of femur, tibia, and basitarsus divided by combined length of tarsomeres 2-5; SV, Schenkel-Scheine-Verhältnis, ratio of femur plus tibia to metatarsus.

or 14 sensilla chaetica on tarsomere 1. Hindleg: tibial spurs 88–100 and 65–70 long, comb with 14 or 15 spines. Lengths and proportions of legs in Table 4.

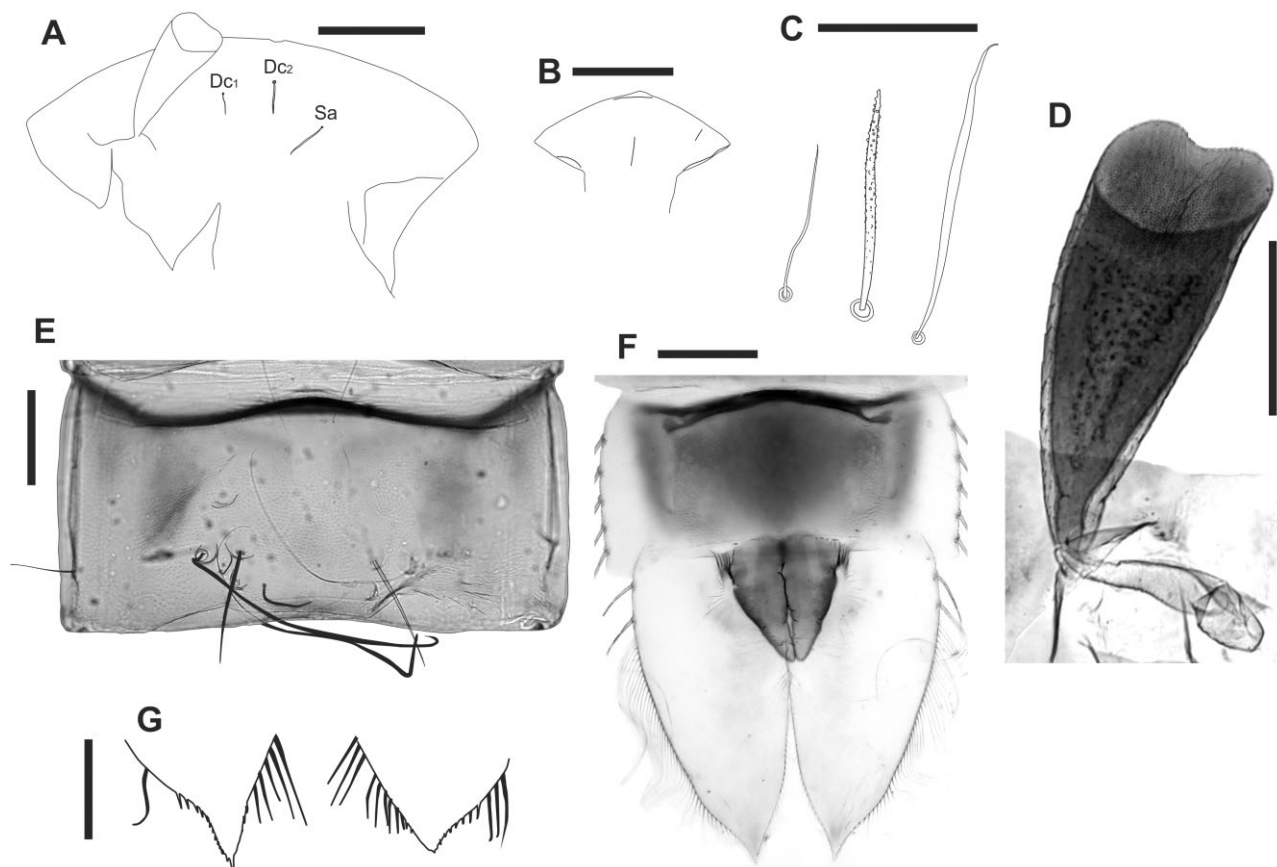
*Genitalia* (Fig. 2G): Cercus 95–108 long. Seminal capsules 75–88 long; notum 220–260 long; segment X with nine setae on each lateral side.

Pupa ( $N = 6$ – $9$ , except when otherwise stated in parentheses; Fig. 3A–G)  
Total length 6.05–7.03 (6.05) mm.

*Cephalothorax* (Fig. 3A): Frontal apotome as in Figure 3B. Thoracic setation (Fig. 3C): Dc<sub>1</sub> (= Mt<sub>2</sub>) 60–113 long (60); Dc<sub>2</sub> (= Mth<sub>1</sub>) strong and granulated, 100–143 (100) long; length of Dc<sub>1</sub>/length of Dc<sub>2</sub> 0.60–0.81 (0.60); Sa (= Mth<sub>3</sub>) 130–180 (130) long. Distance between Dc<sub>1</sub> and Dc<sub>2</sub> 180–230 (180); between Dc<sub>2</sub> and Sa 220–278 (220); one median anteprenotals (MAps)

branched, 55–75 long; two lateral anteprenotals (LAps): one simple, 125–165 long, and one branched into three, 70–95 long. Thoracic horn (Fig. 3D): external membrane with spines; horn sac almost completely filling the lumen of the horn. Length 470–560 (470); width 160–220 (160); L/W 2.50–2.94 (2.94); plastron plate 100–140 (100) long; plastron plate length/thoracic horn length 0.21–0.24 (0.21).

*Abdomen*: Tergite I with scar 120–170 (120) long. Shagreen, with between two and five serially arranged short spines (Fig. 3E). D setae on segment IV (Fig. 3E): D<sub>1</sub> spiniform; D<sub>2</sub> and D<sub>3</sub> long, commonly hooked; D<sub>4</sub> short and thin, D<sub>5</sub> almost half as long as D<sub>1</sub>. Segments VII and VIII with five and anal lobe with two pairs of long and taeniata lateral setae. Position of LS<sub>1</sub>/segment length 0.46–0.60 (0.46) on segment VII; 0.22–0.31 (0.22) on segment VIII. Anal lobe (Fig. 3F,G) 770–920 (770) long; each lobe 360–445 (360) wide; L/W



**Figure 3.** *Paggipeloplia spaccesii* gen. et sp. nov., pupa. A, cephalothorax, general view; B, frontal apotome; C, thoracic setae: Dc1, Dc2 and Sa from left to right; D, thoracic horn; E, abdominal segment IV, dorsal view, borders and left setae lined over; F, abdominal segments VII, VIII and anal lobe; G, detail of apex of anal lobe: male (left) and female (right).

1.91–2.19 (2.14). Position of LS, anal lobe length 0.11–0.16 (0.13) for LS<sub>1</sub> and 0.22–0.24 (0.22) for LS<sub>2</sub>.

Male genital sac 300–350 (300) long (3); length of male genital sac/length of anal lobe 0.38–0.40 (0.39) (3).

Fourth-instar larva ( $N = 4–6$ , except when otherwise stated in parentheses; Fig. 4A–F)

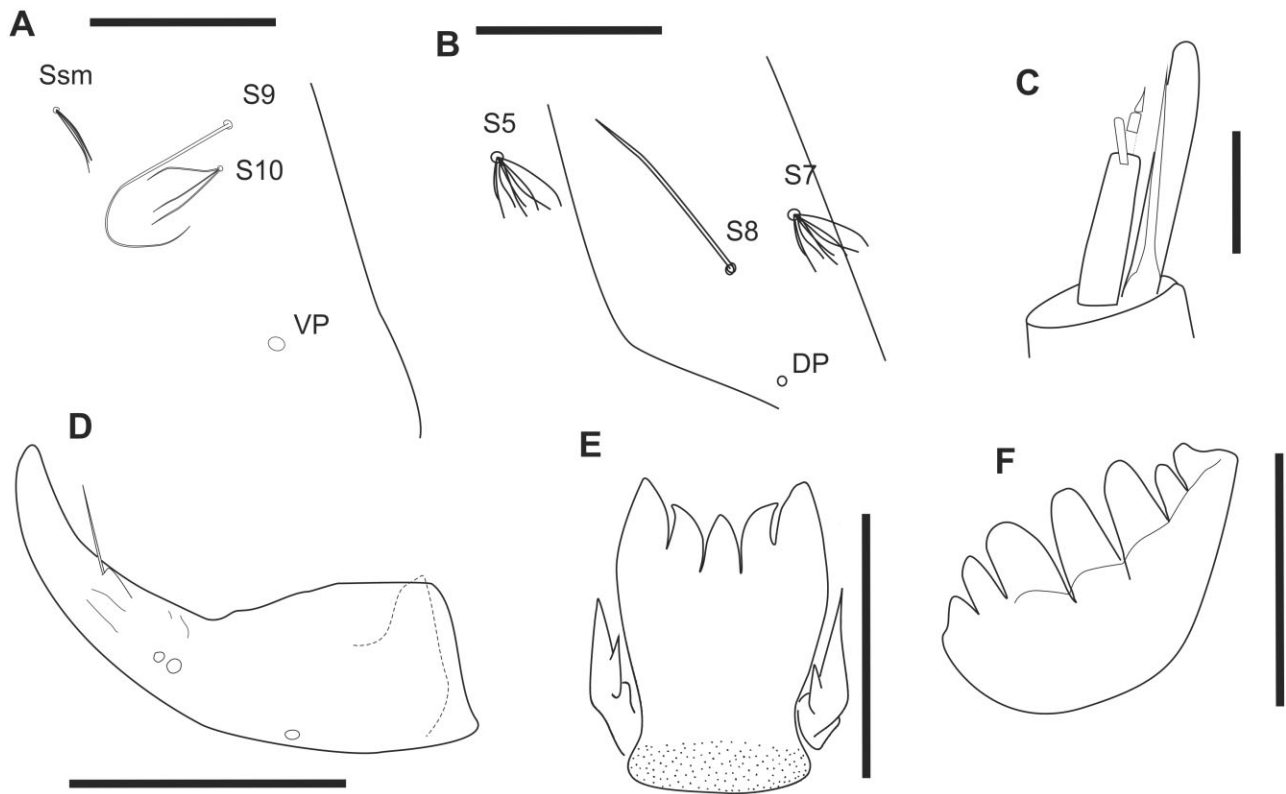
Total length 6.05–10.0 mm. Head: capsule 750–890 (750) long.

**Cephalic setation:** Ventral (Fig. 4A): S<sub>9</sub> and S<sub>10</sub> vertically aligned; SS<sub>m</sub> slightly posterior and mesial to S<sub>10</sub>; VP posterolateral to S<sub>10</sub>. Dorsal (Fig. 4B): S<sub>7</sub>, S<sub>8</sub> and DP forming a right angle; S<sub>6</sub> anterolateral to S<sub>7</sub>. Description of cephalic setae: S<sub>5</sub>, S<sub>6</sub>, S<sub>7</sub>, and SS<sub>m</sub> multi-branched; S<sub>10</sub> branched into two or three; S<sub>8</sub> and S<sub>9</sub> simple.

**Antenna (Fig. 4C):** Antennal ratio 6.25–7.06; A<sub>1</sub> 225–238 (225) long, RO at 0.68–0.76 from base; blade 37–40 long; accessory blade 32–37 (2) long; blade/accessory blade 0.80–0.95 (2); blade/A<sub>2–4</sub> 1.11–1.23 (2); A<sub>2</sub> 20–25 long, A<sub>2</sub> L/W 3.2–5.0; stylus inserted subapically on A<sub>2</sub>, 7–10 long; membranous area between A<sub>2</sub> and A<sub>3</sub> 3–5 long, A<sub>3</sub> 2–4 long, A<sub>3</sub> L/W 0.8–1.3; A<sub>4</sub> 4–5 long.

**Maxillary palp:** Basal segment 50–63 (58) long, L/W 2.58–3.10 (2.58); relative distance of RO 0.38–0.54 (0.43). A<sub>1</sub>/PMx 3.57–4.66 (4.10). **Mandible (Fig. 4D)** 175–205 (185) long. Mandibular setae could not be distinguished, at least S<sub>2</sub> with some branches. A<sub>1</sub>/Md 1.10–1.33 (1.29).

**Hypopharyngeal complex:** Ligula 100–127 (120) long (Fig. 4E), the outermost inner teeth outcurved; unevenly bifid paraligula, 55–70 (65) long; dorsomentum with three large central teeth, plus one short basal and two short distal (Fig. 4F).



**Figure 4.** *Paggipeloplia spaccesii* gen. et sp. nov., A, cephalic setation, ventral; B, cephalic setation, dorsal; C, apex of antenna; D, mandible; E, ligula and paraligula; F, dorsomentral plate. Scale bars = 100  $\mu$ m, except B = 20  $\mu$ m.

*Abdomen:* Procercus 240–253 (250) long; L/W 2.67–3.33 (3.13); with 13 anal setae 700–1020 (850) long. Preanal setae 490–610 (490) long.

#### Remarks

Analyses of water sampled from the Sierra de la Ventana collection sites gave the following values:  $\text{PO}_4/\text{P}$  0.020–0.069 mg P L<sup>-1</sup>;  $\text{NO}_2/\text{N}$  0.001–0.002 mg N L<sup>-1</sup>;  $\text{NO}_3/\text{N}$  0.006–0.192 mg N L<sup>-1</sup>;  $\text{NH}_4/\text{N}$  0.004–0.087 mg N L<sup>-1</sup>; biological oxygen demand <1–5 mg O L<sup>-1</sup>; chemical oxygen demand <1–14 mg O L<sup>-1</sup>; pH 7.67–7.81; conductivity 28–99  $\mu$ S cm<sup>-1</sup>; dissolved oxygen 10.5–118 mg O L<sup>-1</sup> or 80.5–84.1%; temperature 16–19°C.

#### *WUELKERELLA TONCEKENSIS* AÑÓN SUÁREZ & SUBLETTE, 2012

##### Material examined

Two adult ♂ and two adult ♀ with the associated pupal exuviae; eight pupal exuviae, Argentina, Río Negro Province, Parque Nacional Nahuel Huapi, Laguna Schmoll, 41°11'36.7"S, 71°29'51.2"W, 1925 m a.s.l., 20.II.2007, hand net, M. Donato. Two adult ♀, Argentina, Río Negro Province, Parque Nacional Nahuel Huapi, Laguna Tonchek, 41°11'54.2"S, 71°29'12.0"W 1747 m a.s.l., 24.I.2007, sweep net, A. Garré & F. Montes de Oca.

Based on the material above, the generic diagnosis for *W. toncekenensis* male, female, and pupa (Añón Suárez & Sublette, 2012) must be emended as follows.

#### Male

Scutal tubercle absent; anteprenotal tubercle present; Antennal ratio 0.8–1.1, as described in Table 1 and generic description of Añón Suárez & Sublette (2012), but not as described mistakenly in the species description. Legs: tibial spur on p<sub>1</sub> 85–88 long; foretibial comb absent; tibial spurs on p<sub>2</sub> 80–88 and 62–68 long, tibial spurs on p<sub>3</sub> 84–88 and 60–68 long; comb on tibia 3 reduced to absent; simple claws in all legs.

Female (*N* = 3 or 4, except when otherwise stated in parentheses)  
Total length 4.63–4.93 mm. Total length/wing length 1.52–1.68.

*Coloration:* Thorax and wing spots as in male.

*Head:* Antennal ratio 0.25. Terminal flagellomere 130–157 long. Clypeus with 12–20 setae. Palpomere lengths (1–5): 55–60; 90–108; 170–205; 180–215; 300–320 (2).



*Thorax*: Antepnotum with between six and nine lateral setae, with a tubercle between these setae; pre-alars 19–26; supraalar 1; pre-episternals 7–15; dorsocentrals 56–70; prescutelars 4; scutelars 52–60; postnotals 4–7; anepisternals 3–4.

*Wing*: Length 2.75–3.25 mm; width 1.00–1.13 mm; L/W 2.75–2.89. Costa extended 180–200 beyond  $R_{4+5}$ . Squama fringed with 45–72 setae.

*Legs*: Foreleg: tibial spur 85–100 long, foretibial comb absent. Midleg: tibial spurs 92–105 and 70–82 long. Hindleg: tibial spurs 90–100 and 70–82 long; tibial comb absent.

*Genitalia*: Cercus 70–75 long. Seminal capsule 55–70 long; notum 245–255 (2) long; segment X with six (two) setae on each lateral side.

Pupa ( $N = 8–10$ , except when otherwise stated in parentheses)  
Total length 5.6–6.3 mm.

*Cephalothorax*: Thoracic setation:  $Dc_1 (= Mt_2)$  very short, 7–20 long (5);  $Dc_2 (= Mth_1)$  spine shaped, smooth or slightly granulated, 90–120 long; length of  $Dc_1$ /length of  $Dc_2$  0.10–0.17;  $Sa (= Mth_3)$  115–125 (2) long. Distance between  $Dc_1$  and  $Dc_2$  130–170; between  $Dc_2$  and  $Sa$  250–290. One MAs simple, 95–145 long. Two? LAs: one simple, 100–145 (4) long; only the insertions of the other were observed.

*Abdomen*: Scar on segment I present. Shagreen with between two and five serially arranged short spines. Segments VII and VIII with five pairs of lateral taeniate setae; anal lobe with two pairs of long and taeniate lateral setae. Position of  $LS_1$ /segment length 0.48–0.55 on segment VII; 0.18–0.27 on segment VIII. Anal lobe 790–890 long; 350–430 wide; L/W = 2.00–2.29. Position of  $LS$ /anal lobe length 0.14–0.18 for  $LS_1$ ; 0.20–0.28 for  $LS_2$ .

Male genital sac 330 (1) long; length of male genital sac/length of anal lobe 0.39 (1).

*Remarks*: This species closely resembles *Apsectrotanypus* in the presence of postnotals, the absence of a foretibial comb, a reduced comb on p3 and simple claws on the male legs, and the shape of the thoracic horn and shagreen in the pupa. The discovery and description of the larval stage of *W. toncekensis*, or its inclusion in a molecular study, will define the validity of this genus or whether it should be transferred to *Apsectrotanypus*.

## ACKNOWLEDGEMENTS

The authors wish to thank: the Willi Hennig Society for making the TNT software freely available; Anibal Areco and all of the park rangers of Parque Provincial E. Tornquist for helping us with the fieldwork; OPDS for logistic support and for granting authorization to collect; Fernando Spaccesi for his invaluable help in the field collection; Gustavo Rossi, Pablo Pessaq, and Danielle Anjos-Santos for the provision of reared specimens of *Paggipelopia spaccesii* from Esquel; Jorge Donadelli (ILPLA) for the physicochemical analysis of the water; and Mónica Caviglia and Robert Rodriguez for an initial editing of the English. Dr Donald F. Haggerty, a retired career investigator and native English speaker, however, further edited the final version of the article. We extend a special thanks to Susana Trivinho-Strixino for her kindness in re-examining the type of *Alotanypus oliveirai* for us, and to the two anonymous reviewers for their invaluable comments and suggestions. The paper is Scientific Contribution no. 939 of the Institute of Limnology 'Dr. R.A. Ringuelet' (ILPLA, CCT-La Plata, CONICET, UNLP), and is partially supported by a Darwin Initiative 'Capacity Building for Biodiversity Studies in Freshwater Insects' (ref. 15025) and by the Agencia Nacional de Promoción Científica (PICT-1910-2008).

## REFERENCES

- Añón Suárez DA, Sublette JE. 2012.** *Wuelkerella toncekensis* n. gen., n. sp. (Diptera: Chironomidae: Tanypodinae) from Argentina. *Zootaxa* **3177**: 59–65.
- Ashe P, O'Connor JP. 2009.** *A world catalogue of chironomidae (Diptera). Part 1. Buchonomyiinae, Chilenomyiinae, Podonominae, Aphroteniinae, Tanypodinae, Usambaromyiinae, Diamesinae, Prodiamesinae and Telmatogetoninae.* Dublin: Irish Biogeographical Society & National Museum of Ireland.
- Ashe P, O'Connor JP. 2012.** Additions and corrections to Part I of 'a world catalogue of chironomidae (Diptera)'. *Fauna norvegica* **31**: 125–136.
- Cranston PS, Epler JH. 2013.** The larvae of Tanypodinae (Diptera: Chironomidae) of the Holarctic Region – Keys and diagnoses. In: Andersen T, Cranston PS, Epler JH, eds. *Chironomidae of the Holarctic region – Keys and diagnoses. Part 1. Larvae. Insect Systematics and Evolution Supplement* **66**: 39–135.
- Cranston PS, Hardy NB, Morse G. 2012.** A dated molecular phylogeny for the Chironomidae (Diptera). *Systematic Entomology* **37**: 1–17.
- Cranston PS, Martin J. 1989.** Family Chironomidae. In: Evenhuis NL, ed. *Catalog of the Diptera of the Australasian and Oceanian Regions.* Honolulu: Bishop Museum Press & E.J.Brill, 252–274.
- Dantas GPS, Hamada N. 2013.** Two new species of *Fittkauimyia* Karunakaran (Diptera: Chironomidae) from Brazil. *Zootaxa* **3681**: 573–582.



- Epler JH. 2001.** *Identification Manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida.* Special Publication SJ2001-SP13. Raleigh, NC: North Carolina Department of Environment and Natural Resources and Palatka, FL: St. Johns River Water Management District, 526 pp. Available at: <http://home.comcast.net/~johnnepler3/index.html> (accessed 20 May 2014).
- Fittkau EJ. 1962.** Die Tanypodinae (Diptera: Chironomidae) (Die Tribus Anatopyniini, Macropelopiini und Pentaneurini). *Abhandlungen zur Larvalsystematik der Insekten* **6**: 1–453.
- Fittkau EJ, Roback SS. 1983.** The larvae of Tanypodinae (Diptera: Chironomidae) of the Holarctic region – Keys and Diagnoses. In: Wiederholm T, ed. *Chironomidae of the Holarctic region – Keys and diagnoses. Part 1. Larvae. Entomologica Scandinavica, Supplement* **19**: 33–110.
- Fittkau EJ, Murray DA. 1986.** The pupae of Tanypodinae (Diptera: Chironomidae) of the Holarctic region – Keys and Diagnoses. In: Wiederholm T, ed. *Chironomidae of the Holarctic region – Keys and diagnoses. Part 2. Pupae. Entomologica Scandinavica Supplement* **28**: 31–117.
- Goloboff PA, Carpenter JM, Arias JS, Miranda Esquivel DR. 2008b.** Weighting against homoplasy improves phylogenetic analysis of morphological data sets. *Cladistics* **24**: 758–773.
- Goloboff PA, Farris JS, Nixon KC. 2008a.** TNT, a free program for phylogenetic analysis. *Cladistics* **24**: 1–13.
- Kowalyk HE. 1985.** The larval cephalic setae in the Tanypodinae (Diptera: Chironomidae) and their importance in generic determination. *The Canadian Entomologist* **117**: 67–106.
- Langton PH. 1994.** If not ‘filaments’ then what? *Chironomus Newsletter of Chironomidae Research* **6**: 9.
- Murray DA, Fittkau EJ. 1989.** The adult males of Tanypodinae (Diptera: Chironomidae) of the Holarctic region – keys and diagnoses. In: Wiederholm T, ed. *Chironomidae of the Holarctic region – Keys and diagnoses. Part 3. Adult males. Entomologica Scandinavica Supplement* **19**: 37–123.
- Niitsuma H. 2004.** Description of *Apsectrotanypus yoshimurii* (Diptera: Chironomidae), with references to the immature forms. *Species Diversity* **10**: 215–222.
- Niitsuma H, Watson CN Jr. 2009.** *Bilyjomyia*, a new genus of the tribe Macropelopiini from the Holarctic (Diptera: Chironomidae). *Zootaxa* **2166**: 57–68.
- Pleijel F. 1995.** On character coding for phylogeny reconstruction. *Cladistics* **11**: 309–315.
- Roback SS. 1971.** The adults of the subfamily Tanypodinae (= Pelopiinae) in North America (Diptera: Chironomidae). *Monograph of the Academy of Natural Sciences of Philadelphia* **17**: 1–419.
- Roback SS. 1978.** The immature chironomids of the eastern United States III. Tanypodinae-Anatopyniini, Macropelopiini and Natarsiini. *Proceedings of the Academy of Natural Sciences* **129**: 151–202.
- Roback SS, Moss WW. 1978.** Numerical taxonomic studies on the congruence of classifications for the genera and subgenera of Macropelopiini and Anatopyniini (Diptera: Chironomidae: Tanypodinae). *Proceedings of the Academy of Natural Sciences* **129**: 125–150.
- Sæther OA. 1977.** Female genitalia in Chironomidae and other Nematocera: morphology, phylogenies, keys. *Bulletin of the Fisheries Research Board of Canada* **197**: 1–211.
- Sæther OA. 1980.** Glossary of chironomid morphology terminology (Diptera: Chironomidae). *Entomologica Scandinavica, Supplement* **14**: 1–51.
- Sæther OA. 1983.** The canalized evolutionary potential: inconsistencies in phylogenetic reasoning. *Systematic Zoology* **32**: 343–359.
- Siri A, Donato M, Orpella G, Massaferrero J. 2011.** *Alotanypus vittigera* (Edwards) comb. nov.: adult redescription, immature description and a phylogenetic analysis of the genus (Diptera: Chironomidae: Tanypodinae). *Zootaxa* **2795**: 46–64.
- Spies M. 2005.** On selected family-group names in Chironomidae (Insecta, Diptera), and related nomenclature. *Zootaxa* **894**: 1–12.
- Spies M, Reiss F. 1996.** Catalog and bibliography of Neotropical and Mexican Chironomidae (Insecta, Diptera). *Spixiana, Supplement* **22**: 61–119.
- Sublette JE, Wirth WW. 1980.** The Chironomidae and Ceratopogonidae (Diptera) of New Zealand’s subantarctic islands. *New Zealand Journal of Zoology* **7**: 299–378.
- Swofford DL. 2002.** *PAUP\*. Phylogenetic analysis using parsimony (\*and other methods)*. Sunderland, MA: Sinauer Associates.
- Watson CN Jr. 2010.** The Female of *Bethbilbeckia floridensis* Fittkau, 1988 with a review of the genus (Diptera: Chironomidae). In: Ferrington LC Jr, ed. *Proceedings of the XV International Symposium on Chironomidae Research Group*. Saint Paul, MN: University of Minnesota, 334–341.
- Zavřel J. 1929.** Larva a kukly pakomáru. (Chironomidae). *Zprávy komise na přírodovědecký v výzkum Moravy a Slezska. Oddělení zoologické* **18**: 1–52.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Appendix S1.** Characters considered previously to define Macropelopiini.

**Appendix S2.** Data matrix for 44 taxa and 79 morphological characters used in the cladistic analysis.