



<http://dx.doi.org/10.11646/zootaxa.3694.5.3>

<http://zoobank.org/urn:lsid:zoobank.org:pub:15D560C8-3DAA-4216-85CE-473D06E4351D>

## New species of *Pseudosmittia* Edwards, 1932 and new records of *Allocladius* Kieffer, 1913 (Diptera: Chironomidae, Orthoclaadiinae) from South America

MELINA MAUAD, AUGUSTO SIRI & MARIANO DONATO<sup>1</sup>

ILPLA (Instituto de Limnología “Dr. Raúl A. Ringuelet”) CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) CCT-La Plata / UNLP. Bv. 120 y 62, La Plata (1900), Argentina

<sup>1</sup>Corresponding author. E-mail: [mdonato@fcnym.unlp.edu.ar](mailto:mdonato@fcnym.unlp.edu.ar)

### Abstract

Three new species of *Pseudosmittia* Edwards from the Neotropical region, *P. sætheri*, *P. larga* and *P. trapezoidea*, are described and figured as males. *Allocladius fortispinatus* (Edwards), *A. globosus* Andersen *et al.* and *A. quadrus* Andersen *et al.* are redescribed on the basis of new material and their geographical distribution updated. A phylogenetic analysis was performed in order to assess the phylogenetic relationships of the new species. As a result, the species *P. sætheri* and *P. larga* are found to be sister species belonging to the *angusta* group. The species *P. trapezoidea* is the sister group of the clade ((*P. propetropis* (*P. tropis* – *P. lamellata*)) and belongs to the *brevifurcata* group sensu lato.

**Key words:** *Pseudosmittia*, *Allocladius*, Orthoclaadiinae, phylogeny, Sierra de la Ventana, Patagonia, Andes, Argentina, Neotropical region

### Introduction

The genus *Pseudosmittia* Edwards was recently reviewed (Ferrington & Sæther, 2011). In that revision, the genus was divided into three genera supported by parsimony analysis. The genera *Pseudosmittia* and *Allocladius* Kieffer are worldwide in distribution and the genus *Hydrosmittia* Ferrington & Sæther is Holarctic and Afrotropical. The cladistic analysis showed that most of the Neotropical and Afrotropical *Allocladius* species together with most of the Holarctic and Afrotropical *Hydrosmittia* species are the sister group of the more widespread *Pseudosmittia*, and this last genus was divided into 13 species groups.

The genus *Pseudosmittia* is one of the representatives of the subfamily Orthoclaadiinae whose larvae are terrestrial or semiterrestrial (Strenzke, 1950) and some species live in the marine intertidal zone. Apparently, there are no fully aquatic species within the genus (Ferrington & Sæther, 2011). The genus *Pseudosmittia*, as a part of the genera within the subfamily with these ecological requirements, is very important since it shows adaptations found both among the more primitive and among the most derived groups of Chironomidae (Andersen *et al.*, 2010). The knowledge of the genus *Pseudosmittia* in the Neotropical region has increased during the last few years; five species were recorded for that time (Spies & Reiss, 1996) and after the revision of the Neotropical species made by Andersen *et al.* (2010) there are nowadays 23 species.

The genus *Allocladius* is represented by six species in the Neotropical region and are found in both fully aquatic environments and in moist earth (Ferrington & Sæther, 2011). Most of these species have restricted distributions except *A. bilobulatus* (Edwards) and *A. neobilobulatus* (Paggi). The former species is found living in North Patagonia in Argentina and Central Chile between 520 m to 1.842 m a.s.l., and in Peru at 1.900 m a.s.l. The latter species is found in North Patagonia and Pampean grasslands in Argentina (Paggi, 1993), a high mountain dam in Bolivia, the Valdivian forest and Central Chile. The last species shows the highest altitude range between 200 to 3.000 m a.s.l. along its distribution.

Three new species of the genus *Pseudosmittia* are here described and their phylogenetic relationships in the context of the cladistic setting proposed by Ferrington & Sæther (2011) are assessed. In addition, new material

obtained during different field trips, allows us to redescribe *Allocladius fortispinatus* (Edwards) and to update the geographical distribution of *A. quadrus* Andersen *et al.* and *A. globosus* Andersen *et al.*

## Material and methods

**Material.** Microscope slides were prepared by clearing specimens with 10% KOH; neutralization with glacial acetic acid; dehydration in 80%, 96% and 100% ethanol and mounting in Canada Balsam. Morphological terminology and measurement standards follow Sæther (1980); all measurements are in  $\mu\text{m}$  except when otherwise stated, the values are rounded off to the nearest 5  $\mu\text{m}$  unless otherwise stated; measurements are given as ranges followed by the measurements of the holotype in square brackets. The holotype measurements of *A. fortispinatus* was taken by the third author and compared with other descriptions (Andersen *et al.*, 2010). The type material is deposited in the collection of the Museo de La Plata, Argentina (MLP).

**Phylogenetic analysis.** Ferrington & Sæther (2011) compiled a data matrix of 128 characters for 130 species of the *Pseudosmittia* complex and for the genera *Camptocladius* v. d. Wulp, *Colosmittia* Andersen & Sæther, *Diplosmittia* Sæther, *Ionthosmittia* Sæther & Andersen, *Lerheimia* Andersen & Sæther, *Prosmittia* Brundin and *Semiocladius* Sublette & Wirth which were used combined as the outgroup. All characters were treated as unordered with the constraint that the *Pseudosmittia* complex is monophyletic with respect to the outgroup and that *Camptocladius* is its sister group. Ferrington & Sæther (2011) carried out several analyses such as using all the outgroups proposed, using *Camptocladius* as outgroup, applying an *a priori* weighting of 44 characters, and a separate analysis of those species where the immatures are known.

In order to assess the phylogenetic relationships of the new *Pseudosmittia* species proposed in this article, a cladistic analysis was performed using the character setting of Ferrington & Sæther (2011). It is well known that when the percentage of missing data increases, the accuracy of the phylogenetic methods decreases (Wiens, 2006; Prevosti & Chemisquy, 2010). The original data matrix of Ferrington & Sæther has 82 % of the species whose immatures are not known and 68 % of the species whose females are not known. For these reasons, together with the fact that the new species are known as males only, only male characters were taken into account. Characters 1 to 75 of Ferrington & Sæther were taken into account except 18 and 24. Character 18 is applicable to females and character 24 was reformulated to be applied in males: R veins: (0) setae present on R, R<sub>1</sub> and usually R<sub>4+5</sub>; (1) setae present on R, absent on R<sub>1</sub> and often R<sub>4+5</sub>; (2) setae absent on R of male, at most 1 apical seta on R<sub>4+5</sub>. The species *Allocladius arenicola* (Strenzke), *A. lusciniolus* Sæther & Andersen, *Hydrosmittia montana* (Strenzke), *H. virgo* (Strenzke), *Pseudosmittia baueri* Strenzke, *P. bicinctura* (Tokunaga), *P. conjuncta* (Edwards), *P. fusivenosa* (Tokunaga), *P. paraconjuncta* (Hardy) and *P. tokunagai* Ferrington & Sæther are known from females only and they were excluded from the analysis. The resulting data matrix included 74 characters and 124 taxa. The selected outgroup was *Camptocladius* as suggested in the analysis of Ferrington & Sæther (2011) and Sæther (1990). New material of *A. fortispinatus* allows us to code several characters analyzed as missing data in the analysis of Ferrington & Sæther (2011). The data matrix of the new species and the new character coding of *A. fortispinatus* added to those of Ferrington & Sæther (2011) is shown in Appendix 1. The data matrix was analyzed with the program TNT, version 1.1 (Goloboff *et al.*, 2008) applying implied weights as optimality criteria. Analyses with implied weighting were carried out by means of values for the concavity constant K = 2–20.

An initial analysis following the same search strategy as that of Ferrington & Sæther (2011) was made using a traditional search of 1000 random addition sequences (RAS) plus tree bisection and reconnection (TBR) with the addition of a swapping of the trees obtained with TBR. The analysis of RAS+TBR under K=2 yielded 10 trees with a fit= 41.136 and the posterior swapping with TBR of these trees gave 567 trees with a similar fit. This result emphasized problems of composite optima (= islands) (Goloboff, 1999). In such cases, it is desirable to reach many of the composite optima present in the tree space of our analysis instead of having a lot of trees from one island. Since the trees found are to be used to create a strict consensus tree, it would be much less wasteful to simply gather the minimum number of trees necessary to produce the same consensus that would be produced by all possible most parsimonious trees (Goloboff, 2002). In order to solve this problem, an analysis using new search algorithms (Goloboff *op. cit.*) in TNT was conducted. A combined analysis of sectorial searches, tree drifting and tree fusing was performed changing initial parameters in order to increase the aggressiveness until the length obtained was stabilized. The selected search strategy was ten initial addition sequences, five findings of the

minimum length and 20 cycles of tree drifting. As a result, the analysis with this search strategy yielded 10 trees with a fit= 41.135 and for this reason this search strategy was applied in the rest of concavities explored. Character support was estimated with absolute and relative Bremer support values saving up to 6 steps longer suboptimal trees obtained with branch swapping. As the amount of support for a group is due to the interaction between characters favouring the group and those that contradict it (Goloboff *et al.*, 2003), the highest group support value was the criterion to choose between the cladograms obtained in each concavity.

## Results

### Taxonomy

#### *Pseudosmittia sætheri* sp. n.

(Figs 1–4)

**Material examined.** ARGENTINA: Holotype male, Buenos Aires, Sierra de La Ventana, A° Naposta, 38° 08' 42.0" S, 62° 05' 35.6" W, 300 m a.s.l., 11.i.2010, sweep net, M. Donato.

**Etymology.** Named in memory of Professor Ole Anton Sæther, leader in chironomid systematics, who passed away during the elaboration of this paper.

**Diagnosis.** Distinguished from all other species of the genus by the combination of the following characters:  $R_{4+5}$  ending proximal to  $M_{3+4}$ ; virga triangular, consisting of a single spine without lateral spinules or additional structures; anal point covered by microtrichia; superior volsella and median volsella separated and about equally long, superior volsella digitiform with a strong anterobasal projection with apical seta; inferior volsella single, more or less triangular;  $Cu_1$  straight.

**Male** ( $n = 1$ ). Total length 1.6 mm. Wing length 0.9 mm. Total length/wing length 1.78. Wing length/length of profemur 2.65. Coloration (specimen preserved in alcohol) uniformly dark brown with legs light brown.

Head. Antennae with 13 flagellomeres, without apical seta; AR 0.97, ultimate flagellomere 280 long. Temporal setae 6, divided in 4 inner verticals, outer verticals lacking, and 2 postorbitals. Clypeus with 5 setae. Tentorium 98 long and 20 wide. Palpomere lengths (1–5): 22; 27; 54; 66; 88.

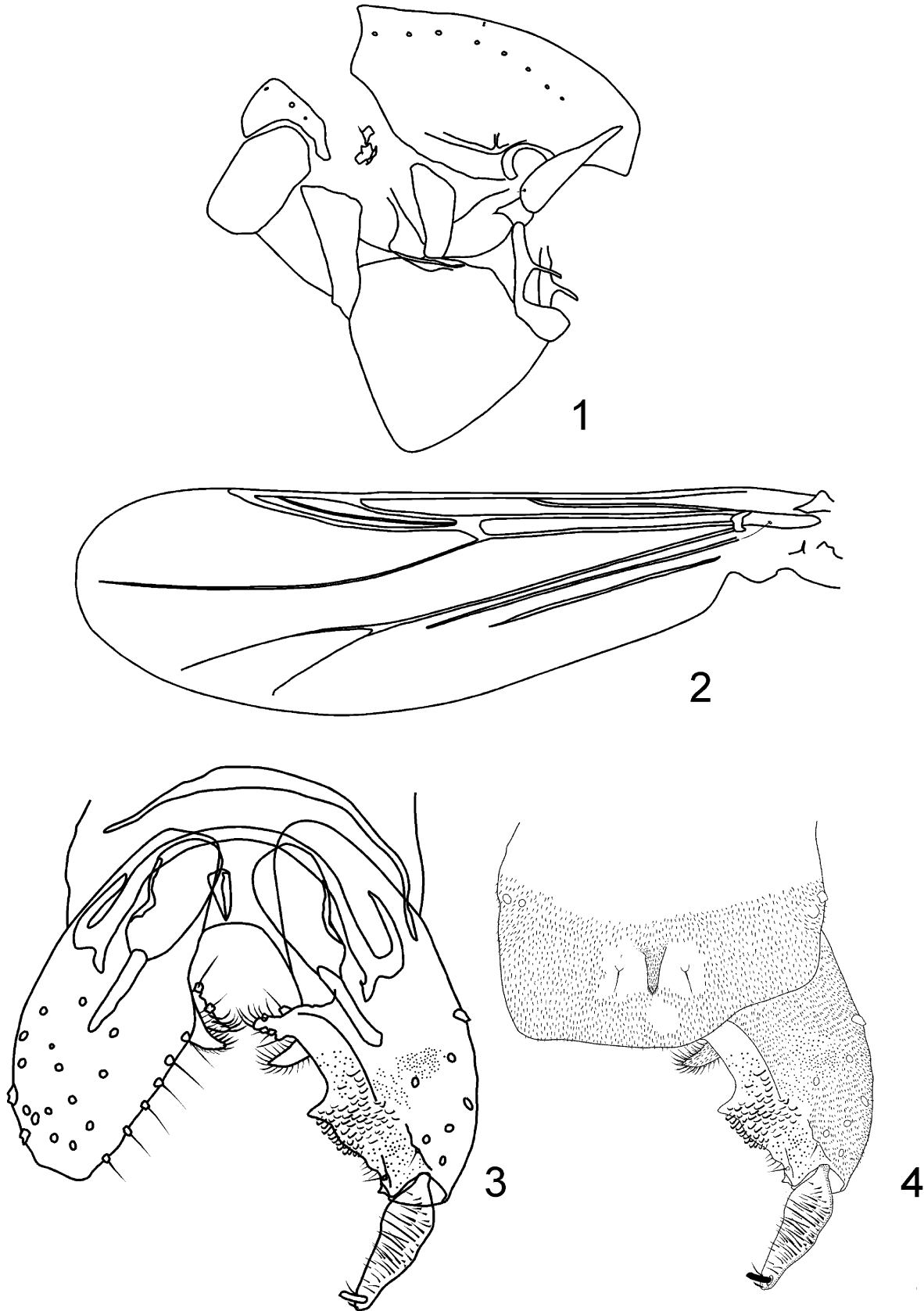
Thorax (Fig. 1). Antepnotum with 1 lateral setae. Dorsocentrals 8; prealars 3; supraalar absent. Scutellum with 4 setae.

Wing (Fig. 2).  $R_{4+5}$  ending proximal to  $M_{3+4}$ . VR 1.4. C extension 16 long. Brachiolum with 1 seta; other veins bare.

Legs. Spur of front tibia 37 long; spurs of mid tibia broken; of hind tibia: 17 and 39 long. Width at apex of front tibia 22; of mid tibia 22; of hind tibia 32. Comb with 11 setae. Lengths and proportions of legs in Table 1.

**TABLE 1.** Lengths (in  $\mu\text{m}$ ) and proportions of legs of *Pseudosmittia sætheri* sp. n. (male,  $n = 1$ ). Abbreviations: Femur (fe); Tibia (ti); Tarsomeres 1-5 ( $Ta_{1-5}$ ); Leg Ratio (LR), ratio of metatarsus to tibia; «Beinverhältnisse» (BV), combined length of femur, tibia, and basitarsus divided by combined length of tarsomeres 2-5; «Schenkel-Scheine-verhältnis» (SV), ratio of femur plus tibia to metatarsus.

	fe	ti	$ta_1$	$ta_2$	$ta_3$
$P_1$	340	350	170	70	50
$P_2$	400	400	190	100	70
$P_3$	400	400	220	110	110
	$ta_4$	$ta_5$	LR	BV	SV
$P_1$	40	40	0.48	3.58	3.90
$P_2$	50	40	0.55	3.19	3.64
$P_3$	50	50	-	-	-



FIGURES 1–4. *Pseudosmittia sætheri* sp. n., male. 1, Thorax; 2, Wing; 3, Hypopygium with tergite IX removed, dorsal aspect to the right, ventral aspect to the left; 4, Hypopygium, dorsal aspect.

Hypopygium (Figs 3–4). Tergum IX with 2 setae, laterosternite IX with 4 setae. Anal point 20 long. Phallapodeme 35 long; transverse sternapodeme 75 long. Virga 20 long. Gonocoxite 132 long; length from base of gonocoxite to apex of inferior volsella/length of gonocoxite 0.16. Gonostylus 54 long; megaseta 10 long. HR 2.44; HV 2.96.

**Remarks.** The species *P. sætheri* keys with *P. magdae* in Andersen *et al.* 2010. The characters gonostylus simple against bilobed gonostylus with lateral lobe adpressed to gonostylus and superior volsella without tubercles against superior volsella with 2–4 large tubercles clearly separates the two species. In the male identification key of Ferrington & Sæther (2011) *P. sætheri* keys in the dilemma 75 but the shape of the inferior volsella is different to those proposed by the authors. The species may be a variety of *P. forcipata*. The main differences consist in the longer median volsella, the anterobasal projection of the superior volsella with one strong seta, and the straight  $Cu_1$ .

**Habitat.** This species was collected in the Naposta stream, one of the most important permanent streams of the Ventania Hill system and drains in the Atlantic Ocean. This stream is originated by several headwaters of short length and small drainage in Naposta Hill at 750 m a.s.l. The flow is variable from low in the dry season (summer and winter) to high in the rainy season (autumn and spring). Naposta stream is characterized by clear water, rocky streambed, low conductivity and nutrient concentrations, neutral to slightly alkaline water and high dissolved-oxygen.

### *Pseudosmittia larga* sp. n.

(Figs 5–8)

**Material examined.** ARGENTINA: Holotype male, Buenos Aires, La Plata, Reserva Natural de Punta Lara, 38° 03' 36.5" S, 62° 01' 18.4" W, 29.xii.2011, sweep net, M. Donato and A. Siri; paratypes: 5 males same data as holotype.

**Etymology.** From Spanish *larga* = long; referring to the long median volsella.

**Diagnosis.** The combination of  $R_{4+5}$  ending proximal to  $M_{3+4}$ ; virga triangular consisting of a single spine without lateral spinules or additional structures; anal point 6–10 long covered by microtrichia; superior volsella and median volsella separated, superior volsella digitiform, median volsella longer than superior volsella; inferior volsella single with an apical triangular tip; distinguishes *P. larga* from all other species of the genus.

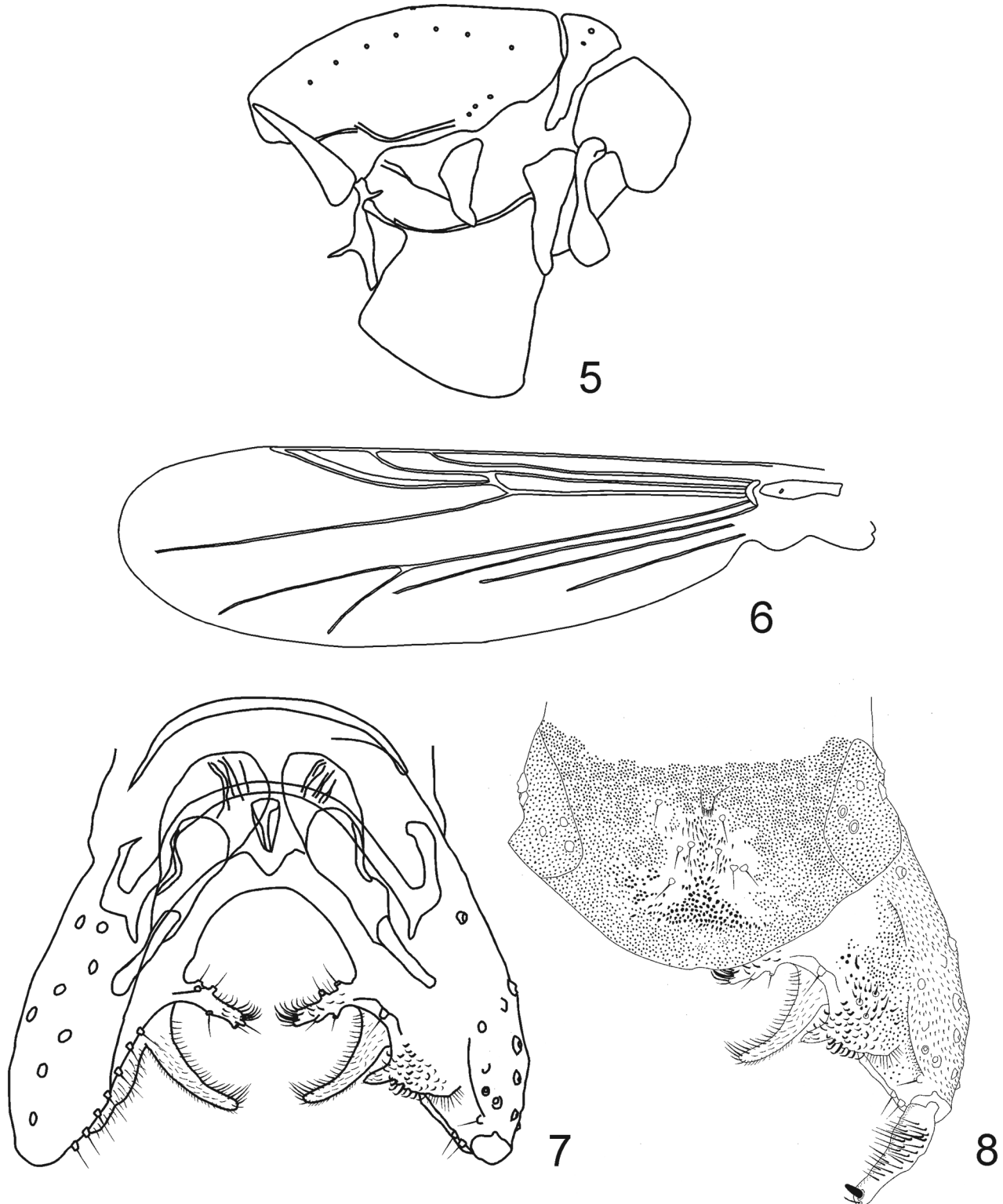
**Male** ( $n = 6$ , except when otherwise stated). Total length 1.6–1.8 (4) mm. Wing length 0.9–1.03 [0.9] mm. Total length/wing length 1.66–1.82 (4). Wing length/length of profemur 2.72–2.96 [2.73]. Coloration (specimens preserved in alcohol) uniformly dark brown with legs light brown.

Head. Antennae with 13 flagellomeres, AR 0.96–1.06 [1.00], ultimate flagellomere length 270–330 [280]. Temporal setae divided in 0–3 [3], inner verticals, 0–2 [0] outer verticals and 0–3 [1] postorbitals. Clypeus with 7–9 [8] setae. Tentorium 98–115 [110] long and 15–17 [15] wide. Stipes 88–98 [90] long and 5–10 [5] wide. Palpomere lengths (1–5): 20–22 [20]; 27–37 [32]; 54–64 [54]; 64–68 [66]; 81–98 [85].

Thorax (Fig. 5). Antepronotum with 1 lateral seta. Dorsocentrals 7–10 [9]; prealars 3–4 [4] (5); supraalars 1 (1). Scutellum with 2 [2] (5) setae.

**TABLE 2.** Lengths (in  $\mu\text{m}$ ) and proportions of legs of *Pseudosmittia larga* sp. n. (male) ( $n = 6$ ). Measurements of the holotype in square brackets. For abbreviation explanations see Table 1.

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>
P <sub>1</sub>	330–370 [330]	350–400 [350]	150–170 [160]	70–80 [80]	60 [60]
P <sub>2</sub>	390–460 [410]	370–430 [390]	180–200 [190]	90–100 [90]	70–80 [70]
P <sub>3</sub>	390–450 [410]	390–450 [400]	220–250 [220]	110–120 [110]	100–120 [110]
	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
P <sub>1</sub>	40 [40]	50 [50]	0.43–0.48 [0.46]	3.65–4.09 [3.65]	4.25–4.53 [4.25]
P <sub>2</sub>	40–50 [40]	40–50 [40]	0.44–0.49 [0.49]	3.62–4.16 [4.13]	4.21–4.69 [4.21]
P <sub>3</sub>	50–60 [50]	40–50 [50]	0.54–0.57 [0.55]	3.17–3.47 [3.22]	3.55–3.72 [3.68]



**FIGURES 5–8.** *Pseudosmittia larga* sp. n., male. 5, Thorax; 6, Wing; 7, Hypopygium with tergite IX removed, dorsal aspect to the right, ventral aspect to the left; 8, Hypopygium, dorsal aspect.

Wing (Fig. 6). VR 1.40–1.45 [1.4]. C extension 10–17 [13] (5). Brachiolum with 1 seta; other veins bare.

Legs. Spur of front tibia 37–42 [37] (4) long; spurs of mid tibia 12–15 [12] (3) and 22 [22] (1); of hind tibia 15–20 [20] (5) and 39 [39] (2) long. Width at apex of front tibia 20–24 [20]; of midtibia 20–24 [20]; of hind tibia 29–34 [29]. Comb with 11–12 [11] setae. Lengths and proportions of legs in Table 2.

Hypopygium (Figs 7–8). Tergum IX with 6–10 [8] setae; laterosternite IX with 4–5 [4] setae. Anal point 6–10 (5) long. Phallapodeme 34–39 [34] long; transverse sternapodeme 68–76 [74] long. Virga 15–24 [17] long. Gonocoxite 124–134 [124] long; length from base of gonocoxite to apex of inferior volsella/ length of gonocoxite 0.24–0.29 [0.26]. Median volsella longer than superior volsella. Gonostylus 49–56 [49] long; megaseta 5–10 [10] long. HR 2.39–2.74 [2.53]; HV 3.18–3.67 (4).

**Remarks.** In the male identification key of Andersen *et al.* (2010), *P. larga* keys in dilemma 21 but its character median volsella longer than superior volsella does not allow to continue in the key. The species *P. magdae* possesses a median volsella nearly as long as the superior volsella and the other option of this dilemma is median volsella shorter than superior volsella. When *P. larga* is keyed in the identification key of Ferrington & Sæther (2011) the same as in *P. saetheri* occurs, since the inferior volsella of this species is different to that proposed by the authors. This species is very close to *P. forcipata* and may be a variation of this species. The key characters consist in the median volsella longer than superior volsella, inferior volsella without accessory lobe, anal point small, and Cu<sub>1</sub> straight.

**Habitat.** The species *Pseudosmittia larga* was collected in the protected area "Reserva natural Punta Lara" (RNPL), declared main area of the Biosphere Reserve "Pereyra Iraola" (UNESCO, Man and the Biosphere Programme, World Network of Biosphere Reserves (<http://www.unesco.org/mab/>)). The RNPL contains wetlands such as marshes formed by the genera *Scirpus*, *Schoenoplectus*, *Typha* and *Zizaniopsis*, pounds and small lagoons. Besides, RNPL also protects the southernmost relict of the Paranean rain forest. This community has a similar specific composition like that of northeastern Argentina, eastern Paraguay and southwestern Brazil and reaches its southernmost distribution using the rivers Parana and Uruguay as biotic corridors. The climate is temperate and wet, between the years 1909 to 2005 the mean annual temperature was 16.2 C with 22.8 C in January and 9.9 C in July (Hurtado *et al.* 2006). The mean annual precipitations from the same period was 1040 mm (Hurtado *op. cit.*).

### *Pseudosmittia trapezoidea* sp. n.

(Figs 9–12)

**Material examined.** Holotype male, ARGENTINA, Buenos Aires, A° s/n RP 30, 34° 54' 30.9" S, 57° 56' 21.8" W, 13.iii.2007, sweep net, M. Donato (MLP).

**Etymology.** The name refers to the trapezoid shape of the inferior volsella.

**Diagnosis.** Distinguished from all other species of the genus by R<sub>4+5</sub> ending proximal to M<sub>3+4</sub>; anal point parallel-sided covered by microtrichia; gonostylus slightly curved; virga consisting of double median spine without lateral spinules or additional structures; superior volsella consisting of rounded and low basal swelling of inner margin of gonocoxite; median volsella absent.

**Male** (*n* = 1). Total length 2.03 mm. Wing length 0.92 mm. Total length/wing length 2.20. Wing length/length of profemur 2.56. Coloration (specimens preserved in alcohol) uniformly dark brown with legs light brown.

Head. Antennae with 13 flagellomeres, AR 1.27, ultimate flagellomere 330 long. Temporal setae divided in 1 outer vertical, inner verticals lacking, and 2 postorbitals. Clypeus with 8 setae. Tentorium 115 long and 22 wide. Stipes 78 long and 7 wide. Palpomere lengths (1–5): 20; 32; 61; 78; 115.

Thorax (Fig. 9). Anteprepronotum without lateral setae. Dorsocentrals 11; prealars 3; supraalar absent. Scutellum with 2 setae.

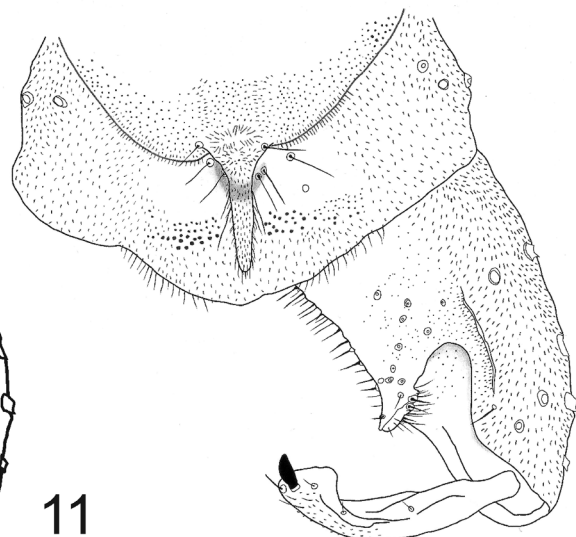
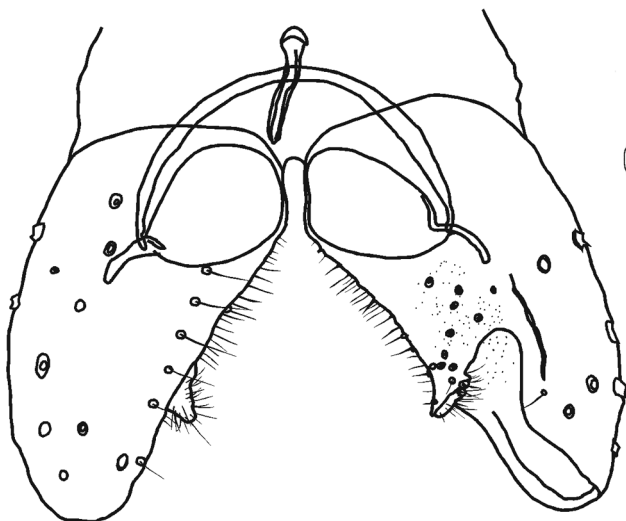
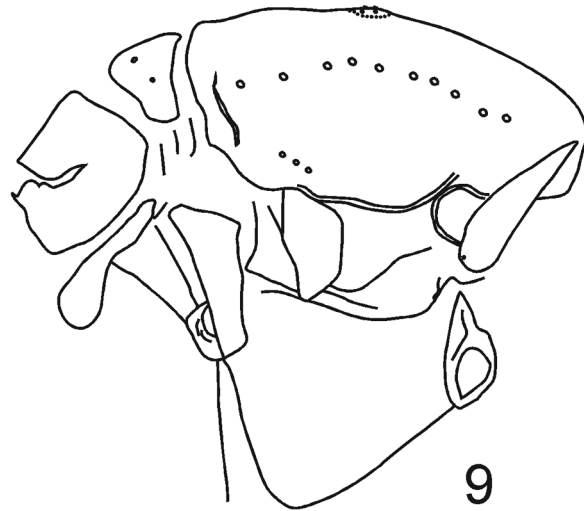
Wing (Fig. 10). VR 1.6. C extension 28 long. Brachiolum with 1 setae; other veins bare.

Legs. Spur of front tibia 39 long; spurs of mid tibia broken; of hind tibia 15 long, the external tibial spur broken. Width at apex of front tibia 24; of mid tibia 24; of hind tibia 34. Comb with 11 setae. Lengths and proportions of legs in Table 3.

Hypopygium (Figs 11–12). Tergum IX with 14 setae, laterosternite IX with 3 setae. Anal point 35 long, parallel-sided. Phallapodeme 20 long; transverse sternapodeme 86 long. Virga 39 long. Gonocoxite 134 long; length from base of gonocoxite to apex of inferior volsella/length of gonocoxite 0.45. Gonostylus 76 long; megaseta 12 long. HR 1.76; HV 2.66.

**Remarks.** *Pseudosmittia trapezoidea* keys to couplet 10 of Andersen *et al.* 2010, the absence of a postcubital fork in this species fits in the first option but differs with the other character proposed since the anal point is very strong with parallel sides. In Ferrington & Sæther (2011) *P. trapezoidea* keys with *P. parifusata* Ferrington &

Sæther but differs from this species in having LR<sub>1</sub> 0.45, superior volsella absent and the shape of inferior volsella without accessory lobe.



**FIGURES 9–12.** *Pseudosmittia trapezoidea* sp. n., male. 9, Thorax; 10, Wing; 11, Hypopygium with tergite IX removed, dorsal aspect to the right, ventral aspect to the left; 12, Hypopygium, dorsal aspect.

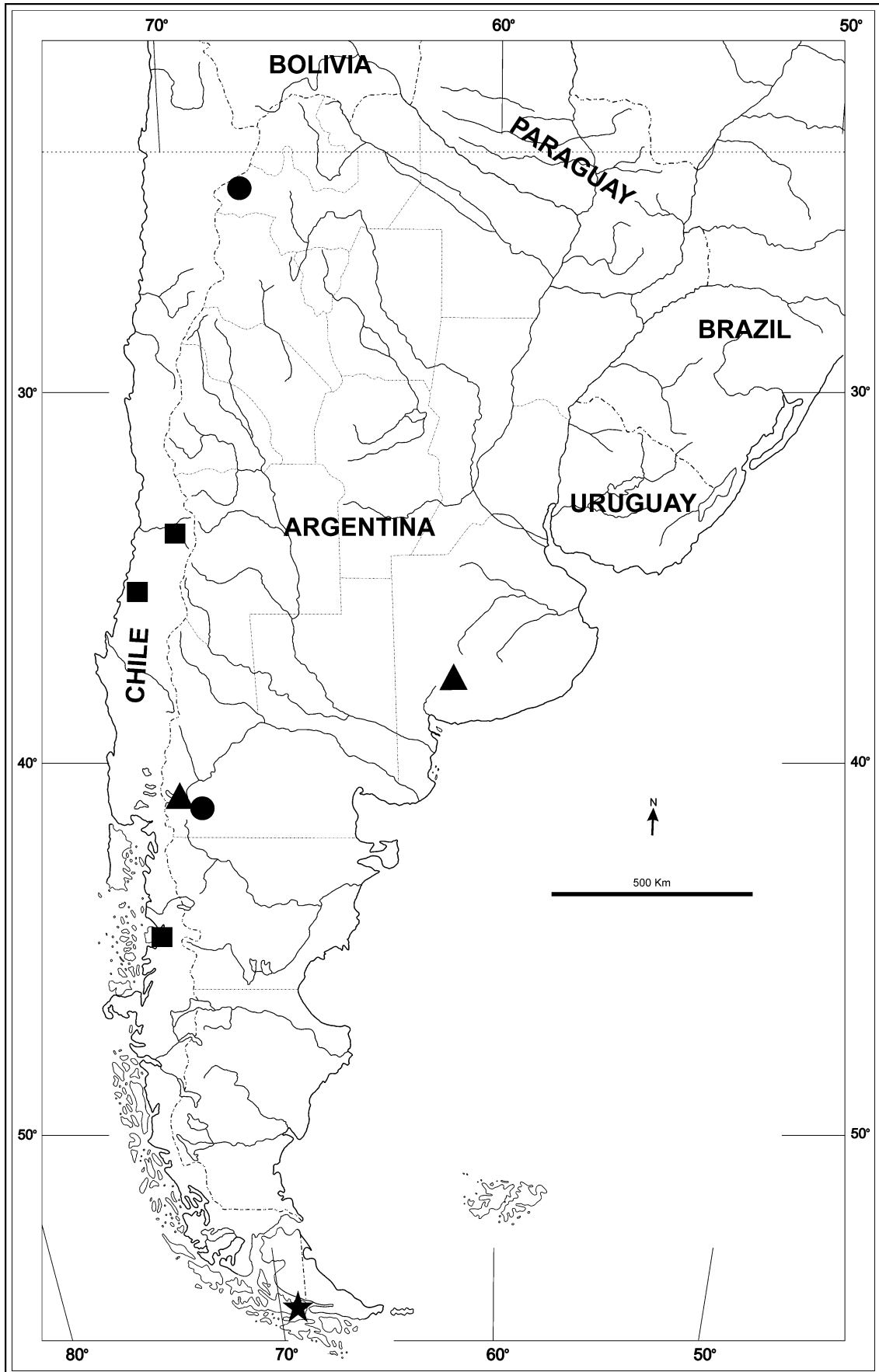


FIGURE 13. Map showing the new records of *Allocladius globosus* and *A. quadrus*. *A. globosus*: square, known records; triangle, new records. *A. quadrus*: star, known records; circle, new records.

**TABLE 3.** Lengths (in  $\mu\text{m}$ ) and proportions of legs of *Pseudosmittia trapezoidea* sp. n. (male,  $n = 1$ ). For abbreviation explanations see Table 1.

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>
P <sub>1</sub>	360	400	180	80	60
P <sub>2</sub>	420	440	190	100	70
P <sub>3</sub>	420	460	240	120	110
	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
P <sub>1</sub>	50	50	0.43	3.67	4.21
P <sub>2</sub>	50	50	0.52	3.39	3.67
P <sub>3</sub>	60	40	-	-	-

**Habitat.** The species *Pseudosmittia trapezoidea* was collected in the shores of one of the first order tributaries of the El Pescado stream. This last stream is a tributary of the Rio de La Plata. It is characterized by a low flow rate with limited transport capacity. This parameter, however, becomes modified as a result of higher precipitation usually occurring about twice a year during autumn and the late spring. El Pescado stream is characterized by neutral to slightly alkaline water with low conductivity, high turbidity and moderated dissolved-oxygen and nutrient concentrations.

### *Allocladius fortispinatus* (Edwards)

*Spaniotoma* (*Smittia*) *fortispinata* Edwards, 1931: 296.

*Bryophaenocladus fortispinata* (Edwards); Ferrington & Sæther (1995: 57).

*Bryophaenocladus fortispinatus* (Edwards); Spies and Reiss (1996: 75).

*Allocladius fortispinatus* (Edwards); Andersen *et al.* (2010: 10); Ferrington & Sæther (2011: 50).

**Material examined.** ARGENTINA: Holotype male, Río Negro, Bariloche, 23–28.x.1926, F.W. Edwards (BM1927–63, BMNH); 2 males, Río Negro, P.N. Nahuel Huapi, Mallín La Heladera, 41° 00' 06.4" S, 71° 48' 40.3" W, 878 m a.s.l., 7–i/4.ii.2007, Malaise trap, A. Garre and F. Montes de Oca (MLP); 5 males, same data except 15.xii.2006, sweep net.

**Diagnosis.** Emended from Andersen *et al.* (2010) and Ferrington & Sæther (2011). The combination of supraalar absent; long costal extension; parallel-sided anal point; superior volsella slightly developed as basal swelling fused with long, narrow and adpressed inferior volsella without accessory lobe; and pars ventralis absent distinguishes *A. fortispinatus* from the rest of the species of the genus.

**Male** ( $n = 8$ , except when otherwise stated). Total length 1.54–2.17 [2.17] mm. Wing length 1.05–1.33 [1.33] mm. Total length/wing length 1.44–1.71 [1.63]. Wing length/length of profemur 2.44–3.00 [3.00]. Coloration uniformly dark brown.

Head. Antennae with 13 flagellomeres, AR 0.43–0.48 [0.48] (3), ultimate flagellomere 180–230 [230] (3) long. Temporal setae 0–2 [not observed] divided in 0–1 [not observed] (6) inner verticals, 1–2 [not observed] outer verticals and 0–2 [2] (6) postorbitals. Clypeus with 9–10 [8] setae. Tentorium 98–120 [120] long and 15–22 [not observed] wide. Stipes 81–100 [100] (6) long and 5–7 [not observed] (6) wide. Palpomere lengths (1–5): 15–22 [20]; 22–34 [34]; 46–64; 51–73; 66–78 (6).

Thorax. Anteprepronotum with 1 lateral setae [not apparent]. Dorsocentrals 6–10 [8]; acrostichals 2–4 [2]; prealars 3 [2]; supraalars absent. Scutellum with 1–2 [2].

Wing. VR 1.4 [1.4]. C extension 88–110 [90]. Brachiolum without setae (5); all veins bare. R<sub>4+5</sub> ending distal to M<sub>3+4</sub>.

Legs. Spur of front tibia 35–42 [not observed] (6) long; spurs of mid tibia: 12–14 [14] (6) and 15–17 [18] (6) long; of hind tibia: 12–15 and 34–42 (6) long. Width at apex of front tibia 24–32 [not observed]; of middle tibia 24–34 [not observed]; of hind tibia 29–42 [not observed]. Comb with 9–11 [not observed] setae. Lengths and proportions of legs in Table 4.

**TABLE 4.** Lengths (in  $\mu\text{m}$ ) and proportions of legs of *Allocladius fortispinatus* (male) ( $n = 8$ , except when otherwise stated). Measurements of the holotype in square brackets. For abbreviation explanations see Table 1.

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>
P <sub>1</sub>	400–490 [443]	420–540 [451]	260–320 (4)	180–220 (4)	120–150 (4)
P <sub>2</sub>	400–500 [451] (6)	390–470 [451]	170–210 [197] (6)	110–120 [123] (6)	70–90 [90] (6)
P <sub>3</sub>	430–520 [467]	440–560 [517]	230–300 [287] (5)	120–140 [164] (5)	110–150 [139] (4)
	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
P <sub>1</sub>	60–80 (4)	50–60 (4)	0.45–0.64 (4)	2.49–3.68 (4)	2.97–4.42 (4)
P <sub>2</sub>	40–50 [49] (6)	40–50 (6)	0.43–0.54 [0.44] (6)	3.17–3.85 (4)	3.44–4.71 (5)
P <sub>3</sub>	50–60 [49] (4)	50–60 (4)	0.46–0.54 [0.56] (3)	3.05–3.54 (3)	4.17–3.75 (3)

Hypopygium. Anal point parallel-sided with rounded apex, 17–28 [19] long, 4–8 [5] wide. Tergum IX with 4–12 [12] setae, laterosternite IX with 3–5 [4–6] setae. Phallapodeme 30–58 [58] long; transverse sternapodeme 46–60 [60] long, with well developed oral projections. Without virga. Gonocoxite 134–154 [146] long; length from base of gonocoxite to apex of inferior volsella/length of gonocoxite 0.15–0.20 [not observed]. Gonostylus 46–54 [54] long; megaseta 6–12 [12] long. HR 2.84–3.14 [2.70]; HV 3.16–4.21 [4.02].

### *Allocladius globosus* Andersen, Sæther & Mendes

*Allocladius globosus* Andersen, Sæther and Mendes, 2010: 11; Ferrington & Sæther (2011: 97).

**Material examined.** ARGENTINA: 2 males, Buenos Aires, Sierra de la Ventana, A° Cueva del toro, spring, 38° 01' 30.2" S, 62° 01' 55.4" W, 932 m a.s.l., 21.v.2012, sweep net, M. Donato, A. Siri and F. Spacessi; 3 males, Río Negro, Río Ñirihuau, near Bariloche's airport, 41° 09' 54.6" S, 71° 07' 54.9" W, 848 m a.s.l., 18.iv.2012, sweep net, M. Donato and A. Siri.

**Male** ( $n = 5$ , except when otherwise stated). The following measurements are different from the original description. Total length 2.13–3.1 mm. Wing length maximum value 1.93 mm. Total length/wing length minimum value 1.61. Wing length/length of profemur 2.45–3.21 (4).

Head. Maximum value for AR 1.31. Maximum length of ultimate flagellomere 550. Temporal setae 8–9, consisting of 0–3 weak inner verticals, 0–4 strong outer verticals, and 1–4 postorbital. Clypeus with 6–14 setae. Tentorium 113–156 long, 25–38 wide. Stipes 109–166 long, 8–15 wide. Palpomere lengths (1–5): 16–34, 35–53, 68–103, 73–105, 85–143.

Thorax. Dorsocentrals 7–13, acrostichals 6–7, prealars 3–4. Scutellum 6–7 (4) setae. Wing. VR minimum value 1.15. R with 2–4 setae, other veins bare.

Legs. Spur of front tibia 38–53 (4) long, spurs of mid tibia 19–23 (2) and 15–18 (3) long, of hind tibia 40–53 (4) and 15–20 (4) long. Width at apex of front tibia 26–48, of mid tibia 25–38, of hind tibia 33–58. Comb of 15–17 setae. Length and proportions of legs as in Table 5.

Hypopygium. Anal point maximum length 45 (3), including 20–28 long sclerotized basal part with 4–5 setae and 10–20 long bare, hyaline distal part. Tergite IX with 13 setae in addition to those on anal point. Laterosternite IX with 2–5 setae. Phallapodeme 35–49 long. Transverse sternapodeme 60–83 long, with weak oral projections. Virga minimum length 6. Gonocoxite maximum length 210. Inferior volsella reaching to 0.43–0.48 gonocoxite length. Gonostylus maximum length 90; megaseta minimum length 9. HR minimum value 2.23. HV minimum value 3.29.

**Remarks.** The new records of *A. globosus* extend its distribution from Chile to Argentina (Fig. 13). The specimens collected in Ñirihuau river show significant differences in several characters from the specimens collected in the spring of Cueva del toro stream. The characters total length, number of clypeal setae, length and width of tentorium, length and width of stipes, length of ultimate flagellomere, AR, wing length, wing length/length of profemur and gonocoxite length of the latter specimens are higher than those of the former specimens.

**TABLE 5.** Lengths (in  $\mu\text{m}$ ) and proportions of legs of *Allocladius globosus* (male) ( $n = 5$ , except when otherwise stated). For abbreviation explanations see Table 1.

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>
P <sub>1</sub>	290–550 (4)	310–770	170–370	90–260	70–190
P <sub>2</sub>	300–780	300–770	135–350	80–190	55–145
P <sub>3</sub>	330–840	330–850	170–450	90–250	70–200
	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
P <sub>1</sub>	40–130	35–85	0.39–0.55	3.28–3.77 (4)	3.53–4.83 (4)
P <sub>2</sub>	40–100	30–80	0.39–0.45	3.36–3.87	4.43–4.96
P <sub>3</sub>	40–110	35–90	0.49–0.54	3.19–3.54	3.64–3.97

The Ventania hill system is located SW of Buenos Aires province, with its highest altitude of 1243 m a.s.l. As a consequence of its orogenic origin and geographic position, Ventania was considered as "an island of biodiversity" since wildlife from the Brazilian region from the north and the Patagonia from the south could occur (Ringuelet, 1961).

The Ventania system is part of the peripampasic orogenic arc defined by Frenguelli (1950) together with other orogenic systems from Argentina and shows disjunct biogeographic distributions of several and different groups of taxa (Ringuelet, 1961). The occurrence of *A. globosus* in this system is an example of this particular distributional pattern and this new record reinforces this pattern also found in other Chironomidae species (Siri & Donato, 2012).

### *Allocladius quadrus* Andersen, Sæther & Mendes

*Allocladius quadrus* Andersen, Sæther and Mendes, 2010: 17; Ferrington & Sæther (2011: 96).

**Material examined.** ARGENTINA: 2 males, Salta, Quebrada del Agua, 24° 30' 33.0" S, 68° 10' 52.6" W, 3678 m a.s.l., 11.i.2005, sweep net, M. Donato; 1 male, Río Negro, P.N. Nahuel Huapi, laguna Los Juncos, 41° 03' 37.9" S, 71° 00' 34.3" W, 906 m a.s.l., 12.xii.2007, sweep net, A. Garre and F. Montes de Oca (MLP).

**Male** ( $n = 3$ , except when otherwise stated). The following measurements are different from the original description. Total length 2.3–2.4 mm. Wing length 1.35–1.55 mm. Total length/wing length extended to 1.7. Wing length/length of profemur 2.70–3.04.

Head. AR 1.2–1.2. Minimum length of ultimate flagellomere 390. Tentorium 115–124 long, 22–34 wide. Stipes 115–120 long, 7–10 wide. Palpomere lengths (2–5) 39–46, 71–83, 88–103, 120–127. Third palpomere with 2 lanceolate sensilla clavata, 19–21 long.

Thorax. Dorsocentrals 7–10, acrostichals 3–5, scutellum with 6–7 setae.

Wing. Minimum value for VR 1.25.

Legs. Width at apex of fore tibia 25–29, mid tibia 24–30, of hind tibia 37–42. Comb of 13–16 setae. Length and proportions of legs as in Table 6.

Hypopygium. Anal point 40–41, including 26 long basal sclerotized part with 8 setae and 14–15 long bare, hyaline distal part. Tergite IX with 6–8 setae in addition to those on anal point. Phallapodeme 44–46 long. Transverse sternapodeme 61–76 long. Virga 5 long, 10–14 wide. Pars ventralis (Fig. 44) single, subquadrangular, 75–93 (2) long, 80–103 (2) wide. Gonocoxite 166–181 long. Inferior volsella reaching to 0.43–0.46 gonocoxite length. Gonostylus 71–73; megaseta 7–10.

**Remarks.** The specimens from Quebrada del agua in Salta province were collected at a Punean spring and the specimen collected at laguna Los Juncos in Rio Negro province at a small temporal lagoon (Fig. 13). This lagoon is located in the Patagonian steppe. These new records define the distributional pattern of *A. quadrus* as Andean Patagonian.

**TABLE 6.** Lengths (in  $\mu\text{m}$ ) and proportions of legs of *Allocladius quadrus* (male) ( $n = 3$ , except when otherwise stated). For abbreviation explanations see Table 1.

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>
P <sub>1</sub>	470–510	570–620	260–270 (2)	160–180 (2)	110 (1)
P <sub>2</sub>	550–600	570–610	220–250	130–140	110 (2)
P <sub>3</sub>	570–610	600–630	350–350 (1)	180–180 (1)	150 (1)
	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
P <sub>1</sub>	70 (1)	70 (1)	0.43–0.44 (2)	3.32–3.32 (1)	4.19–4.23 (2)
P <sub>2</sub>	60–80 (2)	60–70 (2)	0.39–0.41	3.65–3.83 (2)	4.84–5.09
P <sub>3</sub>	80 (1)	70 (1)	0.56–0.56 (1)	3.31–3.31 (1)	3.54–3.54 (1)

### Cladistic analysis

The analyses of the data matrix using only male characters as defined by Ferrington & Sæther (2011) under concavities  $K=2$  to  $K=20$  showed the best group support under  $K=5$ . In this concavity the analysis yielded 7 trees (Fit= 63.62, CI= 0.17, RI= 0.56) and the resulting strict consensus tree is shown in Figure 14. The species *P. larga* and *P. sætheri* conform a clade supported by the characters Cu<sub>1</sub> straight [21 (0)]; superior volsella with strong orally curved microtrichia [57 (1)] and inferior volsella without accessory lobe [63 (1)]. This clade is located in a major clade in the *angusta* group of species.

The species *P. trapezoidea* is the sister group of the clade ((*P. propetropis* (*P. tropis* – *P. lamellata*)) sharing the characters inner verticals of temporal setae absent [5 (1)] and virga 35–57 long [46 (1)]. This species is part of a major clade constituted by all the species of the *brevifurcata* group except *P. brevifurcata* that is located in a clade with several species of the *xanthostola* group.

In the strict consensus tree *Allocladius fortispinatus* is placed at the base of the *Allocladius* clade as the sister species of the rest of the species of the genus except *A. wirthi* sharing the synapomorphies postorbitals present [7 (0)], anal point at least extreme apex without microtrichia or anal point absent [34 (0)], and oral projections of transverse sternapodeme well developed [41 (0)].

### Discussion

The species *P. sætheri* and *P. larga* clearly belong to the *angusta* group fitting all male characters that define the group, this placement is also corroborated in the cladistic analysis. The inclusion of these species in the subgroups proposed by Ferrington & Sæther (2011) is difficult to define, but it seems that they are more closely related to those of the *forcipata* subgroup differing in the absence of a subapical seta in antennae and absence of crista dorsalis.

The species *P. trapezoidea* belongs to the *brevifurcata* group by possessing all characters that define the group. Ferrington & Sæther (2011) stated that the group is monophyletic because it has an objective synapomorphy in the conspicuous microtrichia on the inner margin of the gonocoxite. The cladistic analysis shows *brevifurcata* group as paraphyletic since *P. brevifurcata* is not included and its synapomorphy is not manifested.

The parsimony analysis using male characters performed here recovered the three clades representing the genera *Pseudosmittia*, *Allocladius* and *Hydrosmittia* but with no character support. In Ferrington & Sæther (2011), a few species are outside of the main clades, while our analysis shows the three genera as monophyletic. An extra analysis was performed using all characters in order to test if the use of male characters only biases the results giving the same phylogenetic hypothesis.

The genus *Hydrosmittia* is supported by the synapomorphies virga with lateral groups of triangular spinules in addition to median plate or spine [43 (1)] and superior volsella absent or strongly reduced [55 (1)]. The former character is ambiguous in *H. togadistalis* (Sasa, Watanabe & Arakawa). This character was observed by Ferrington & Sæther (2011) as absent but they hypothesized that it could be present and was coded as missing data in

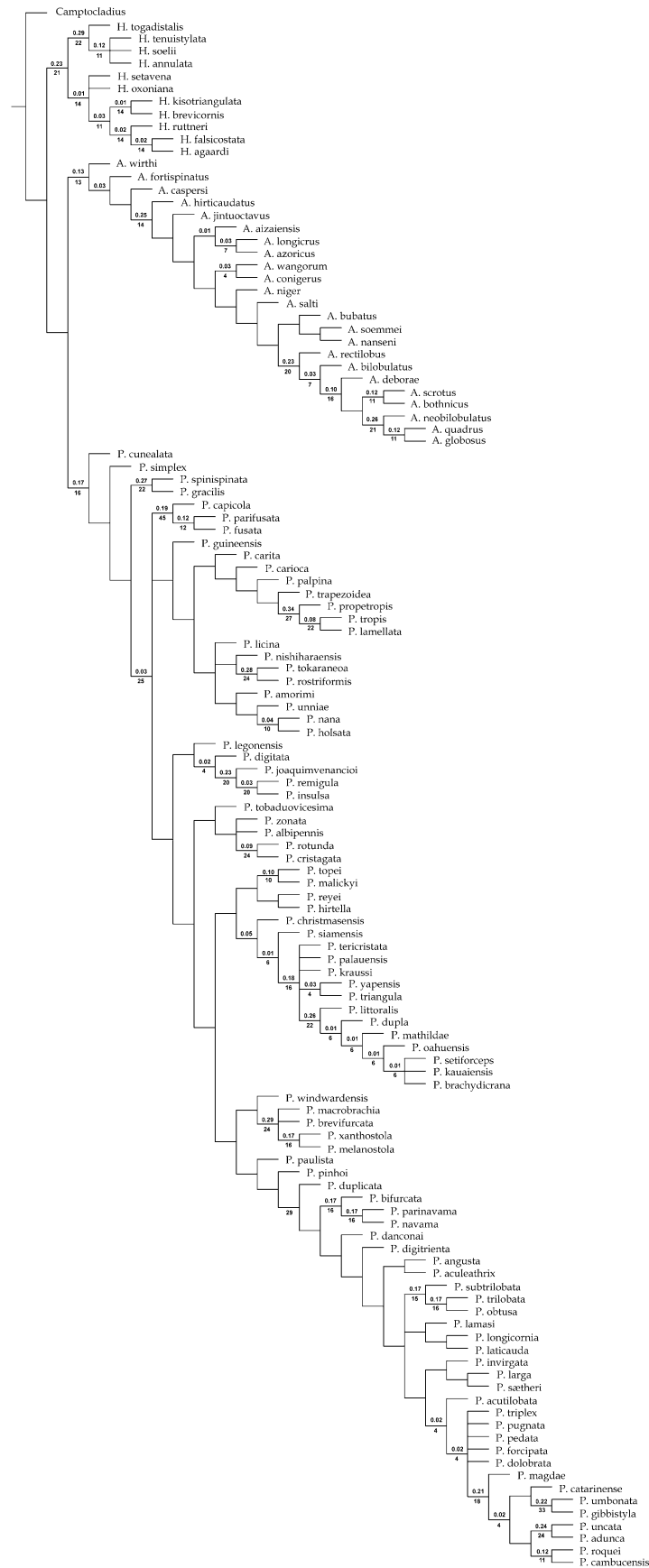


FIGURE 14. Strict consensus tree obtained from the 7 trees yielded under K=5 (Fit= 63.62, CI= 0.17, RI= 0.56). Number above branches indicates absolute Bremer support and number below branches indicates relative Bremer support.

*H. togadistalis*. The latter character is absent in *H. annulata* Ferrington & Sæther. The two characters defining the *Hydrosmittia* clade are present in the diagnosis made by Ferrington & Sæther (2011) and the two main characters used by the authors to separate this genus from *Allocladius* and *Pseudosmittia* are not synapomorphies because the presence of a median anteprenotal lobe well developed is shared with the former genus and the presence of two acrostichals (or when 3, as in some *H. ruttneri* (Strenzke), the third much weaker) is shared with the latter genus. The species groups proposed by Ferrington & Sæther (2011) were not recovered.

The genus *Allocladius* is monophyletic defined by the synapomorphy 4 to 16 acrostichals [12 (1)]. The new data added in this study to *A. fortispinatus* resolved the problem pointed out by Ferrington & Sæther (2011) who considered this species as very different and in their analysis was placed in a trichotomy with *Hydrosmittia* and *Allocladius*. Concerning species groups within this genus, all of them are polyphyletic except the *longicrus* group that is paraphyletic (Fig. 13).

The monophyly of the genus *Pseudosmittia* is supported by the synapomorphies anteprenotal lobes reduced to at least distinctly narrowed medially [9 (1)], anal lobe reduced or wing cuneiform [15 (1)], and transverse sternapodeme distinctly arched [39 (1)]. Ferrington & Sæther (2011) found *Pseudosmittia* as monophyletic in all the analyses except that *P. gracilis* (Goetghebuer), *P. spinispinata* Ferrington & Sæther, *P. holsata* Thienemann & Strenzke and/or *P. cuneolata* Ferrington & Sæther sometimes fall outside the genus. The species groups and their relationships demonstrated by these authors differ from those found in the present analysis. At the base of the *Pseudosmittia* clade, three main clades were recognized (Fig.13). The small clade represented by *P. capicola* Freeman, *P. fusata* Ferrington & Sæther and *P. parifusata* Ferrington & Sæther are the representatives of the *conjuncta* group and it could be as monophyletic since the rest of the species of this group were excluded in our analysis since their males are not known. The synapomorphies supporting this clade are leg ratio (LR) higher than 0.50 [26 (0)] and anal point fusiform [36 (1)]. A second clade contains the paraphyletic *brevifurcata* group and several representatives of different species groups supported by the synapomorphies anteprenotal lobes at most slightly narrowed medially [8 (0)], gonostylus curved [71 (1)] and crista dorsalis not evident or basal [74 (2)]. The third clade is represented by the monophyletic *angusta* group and several species of the remaining groups and supported by the synapomorphies superior volsella absent or strongly reduced [55 (1)] and Cu<sub>1</sub> straight [22 (0)]. From the above mentioned, the only species group recovered was *angusta* group which represents the *Pseudosmittia sensu stricto*.

## Concluding remarks

As pointed out by Ferrington & Sæther (2011) the content of the groups vary. Some species groups are constant, but the relationship between them is highly variable. This is due to the fact that the groups are defined by a mosaic of apomorphies and plesiomorphies. The authors highlighted the usefulness of objective synapomorphies, but in our analysis were not recovered. Undoubtedly, the finding of the females and immatures will improve the understanding of the phylogenetic relationships within the *Pseudosmittia* complex, as would new sources of characters such as molecular data. Molecular data will allow testing proposed morphology-based relationships and synapomorphies and highlight the value of morphological data derived from all stages.

## Acknowledgments

Authors wish to thank the Willi Hennig Society for the free availability of the TNT program, the two reviewers for their comments and suggestions, Anibal Areco and all of the park rangers of Parque Provincial E. Tornquist for helping us in the field work, OPDS for logistic support and for granting authorization to collect, Mónica Caviglia for English proofreading, and Fernando Spaccesi for his invaluable help in the field collection. The paper is a Scientific Contribution N 934 of the Institute of Limnology “Dr. R.A. Ringuelet (ILPLA, CCT-La Plata, CONICET, UNLP). This paper is supported by CONICET, Darwin Initiative “Capacity Building for Biodiversity Studies in Freshwater Insects (Ref. 15025) and Agencia Nacional de Promoción Científica (PICT-2008-1910).

