SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

A New Species of *Metriocnemus* van der Wulp (Diptera: Chironomidae) with a Tentative Phylogeny of the Genus

Mariano Donato[1], Augusto Siri[2]

[1]*Lab de Sistemática y Biología Evolutiva (LASBE), Museo de La Plata, Paseo del Bosque s/n (1900), La Plata, Argentina; mdonato@fcnym.unlp.edu.ar*
[2]*Instituto de Limnología "Dr. Raúl A. Ringuelet" (ILPLA), Av Calchaqui km 23,5 (1888), Florencio Varela, Argentina; augusto@ilpla.edu.ar*

Edited by Roberto A Zucchi – ESALQ/USP

Neotropical Entomology 39(1):050-060 (2010)

ABSTRACT - The male and female of the new species *Metriocnemus puna* sp. n from the Argentinean Puna are described and illustrated. A parsimony analysis including 24 well-described species of the genus plus the new species based on the adult male was conducted in order to access the phylogenetic position of the new species and to provide the first phylogenetic hypothesis for the genus.

KEY WORDS: *Metriocnemus*, Orthocladiinae, Argentina, Puna

The worldwide genus *Metriocnemus* van der Wulp belongs to the subfamily Orthocladiinae (Diptera: Chironomidae). It is currently known from about 60 holarctic species (Cranston *et al* 1989) and five endemic species from the Neotropic (Spies & Reiss 1996, Donato & Paggi 2005). However, the number of species cannot be ascertained until a complete revision of the genus, including the study and redescription of all available types is performed (Sæther 1989).

The genus occurs in one of the widest biotope ranges of any dipteran genus (Sæther 1989), including from mosses and higher vegetation, pitcher plants and hollow trees to margins of springs, ditches, streams, damp soils, and hygropetric biotopes, and occasionally also lakes and rock pools (Cranston & Judd 1987, Sæther 1989, 1995).

The genus *Metriocnemus* is divided into two main groups based on characters derived from the male adult and immature stages (Cranston *et al* 1983, Coffman *et al* 1986, Sæther 1989, 1995), although a third group (*knabi* group) was described based only on pupal characters (Coffman *et al* 1986).

The male adults of the *eurynotus* (=*hygropetricus*) group present the inferior volsella strongly projecting or occupying 0.58-0.80 of the gonocoxite, well developed virga, anal point generally strong and well developed crista dorsalis. On the other hand, the male adults of the *fuscipes* group have a reduced inferior volsella occupying 0.73-0.89 of the gonocoxite, virga absent, weak anal point and low crista dorsalis (Sæther 1989, 1995).

The division of the genus *Metriocnemus* into these groups is problematic since many of the *Metriocnemus* species show characters of both the *eurynotus* and the *fuscipes* groups (Sæther 1995, Donato & Paggi 2005).

New material collected at high altitudes in Northwestern Argentina clearly conforms to Sæther's diagnosis (1989) for the genus *Metriocnemus*. Therefore, the new species *Metriocnemus puna* sp. n is described and a phylogenetic analysis is conducted based on quantitative and discrete characters from the male adult to assess its phylogenetic relationships and to present the first phylogenetic hypothesis for the genus.

Material and Methods

Terminology. General terminology follows Sæther (1980). Measurements are in μm unless otherwise stated. For the counts of setae on wing veins we followed Sæther's (1989) procedure, i.e. both dorsal and ventral setae were counted. Setae located on the margin of a vein are regarded as belonging to that vein.

Depositories. The type material is deposited in the Museo de La Plata (Argentina) (MLP).

Phylogenetic analysis. Given the facts that the coding of the data is extensively based on the literature (Cranston & Judd 1987, Sæther 1989, 1995), that numerous species are in need of re-description (Sæther 1989) and that this analysis is based on adult male characters, the results can only be regarded as tentative. The taxa selected for this analysis were the 24 well-described species of the genus *Metriocnemus* (Cranston & Judd 1987, Sæther 1989, 1995, Sublette & Sasa 1994, Donato & Paggi 2005) and the new species.

The most useful and non-controversial characters are those whose states are exclusive, but other features, such as meristic and continuous data, are also valuable in systematics. Quantitative characters are rarely included in cladistic analyses of morphological data, and the most frequent argument for the exclusion of continuous characters is the difficulty in objectively assigning character states (Pimentel & Riggins 1987, Cranston & Humphries 1988, Stevens 1991). Several methods have been proposed to solve this problem (Mickevich & Johnson 1976, Thorpe 1984, Thiele 1993, Strait *et al*. 1996, Wiens 2001). Quantitative characters have also been criticized because they do not measure homology, but several studies (e.g. Rae 1998, Wiens 2001) have documented the usefulness of continuous characters in phylogenetic studies.

The characters used for this analysis comprise 42 continuous and seven discrete characters. Continuous characters were expressed as means, except in those species whose description is based on one specimen. The characters and character states are self-explanatory and are listed in Table 1. The data matrix is shown in Table 2.

Recently, the program TNT (Goloboff *et al* 2008a) incorporated the analysis of continuous characters by assigning a range to each terminal that goes from the mean minus one standard deviation to the mean plus one standard deviation, given normal distributions (Goloboff *et al* 2006, 2008a). Since continuous or meristic characters are best treated as additive, TNT implemented the optimization

Table 1 List of characters, character states and coding used in the cladistic analysis of the genus *Metriocnemus*.

1	Total lenght/wing length	Mean
2	Wing length/length of profemur	Mean
3	Antennal ratio (AR)	Mean
4	Number of temporal setae	Mean
5	Number of setae on clipeus	Mean
6	Number of antepronotal setae	Mean
7	Number of dorsocentral setae	Mean
8	Number of acrostichal setae	Mean
9	Number of prealar setae	Mean
10	Number of supralar setae	Mean
11	Number of sctutellar setae	Mean
12	Venarum ratio (VR)	Mean
13	C_extension length	Mean
14	Number of setae on Brachiolum	Mean
15	Number of setae in Sc	Mean
16	Number of setae in R	Mean
17	Number of setae in R_1	Mean
18	Number of setae in R_{4+5}	Mean
19	Number of setae in RM	Mean
20	Number of setae in M	Mean
21	Number of setae in M_{1+2}	Mean
22	Number of setae in M_{3+4}	Mean
23	Number of setae in Cu	Mean
24	Number of setae in Cu_1	Mean
25	Number of setae in Pcu	Mean
26	Number of setae in An	Mean
27	Number of setae in Squama	Mean
28	Number of Comb setae	Mean
29	LR1	Mean
30	LR2	Mean
31	LR3	Mean
32	BV1	Mean
33	BV2	Mean
34	BV3	Mean
35	SV1	Mean
36	SV2	Mean
37	SV3	Mean
38	Inferior volsella ending	Mean
39	HR	Mean
40	HV	Mean
41	Number of setae on Tergite IX	Mean
42	Virga length	Mean
43	Eyes	0) Hairy or pubescent; 1) bare
44	Anal point	0) Tapering to sharp point; 1) tapering to blunt apex; 2) parallel sided with blunty rounded apex ; 3) absent
45	Virga	0) Cluster of large spines; 1) cluster of small spines; 2) absent
46	Inferior volsella	0) Well developed lobe-like; 1) well developed trapezoid-like; 2) well developed with apical swelling; 3) weak with apical hump; 4) weak; 5) scarcely indicated
47	Crista dorsalis	0) absent or very low; 1) rounded; 2) bluntly triangular; 3) sharply triangular
48	Number of pseudospurs on ta_1 of mid leg	0) 0; 1) 1; 2) 2
49	Number of pseudospurs on ta_2 of mid leg	0) 0; 1) 1; 2) 2
50	Number of pseudospurs on ta_3 of mid leg	0) 0; 1) 1; 2) 2
51	Number of pseudospurs on ta_1 of hind leg	0) 0; 1) 1; 2) 2
52	Number of pseudospurs on ta_2 of hind leg	0) 0; 1) 1; 2) 2
53	Number of pseudospurs on ta_3 of hind leg	0) 0; 1) 1; 2) 2

Table 2 Data matrix for 29 taxa and 53 quantitative and discrete morphological characters used in the cladistic analysis of the genus *Metriocnemus*. See Table 1 for explanation of coding.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
T. gracilis	1.54	2.68	1.22	7	11	4	19	16	7	1	6	1.2	95	2	0	18	13	31	0	0	33	15	6	11	5	16	3	13	0.52	0.43
T. fulvofasciatus	1.76	2.32	0.44	17	19	6	49	23	15	1	?	1.25	36	4	18	34	34	49	0	6	46	36	24	26	50	31	4	10	0.63	0.42
T. gracei	1.5	2.75	0.47	21	13	6	40	?	18	2	14	1.32	184	3	32	36	50	87	0	6	74	50	30	25	60	28	7	10	0.63	0.46
T. pilinucha	1.46	2.18	0.28	12	16	5	25	18	12	1	8	1.25	165	3	30	37	45	70	0	16	55	42	?	30	33	12	8	10	0.61	0.46
A. fontinalis	1.56	2.78	0.91	12	16	4	16	?	11	2	11	1.22	163	3	13	33	16	35	0	10	43	23	19	15	40	15	8	10	0.54	0.43
A. japonicus	?	2.34	0.16	12	17	6	32	30	16	0	24	1.4	194	8	?	?	?	?	?	?	?	?	?	?	?	?	5	10	0.67	0.51
M. aculeatus	1.63	2.29	1.62	13	12	4	22	20	13	1	12	1.17	128	5	0	26	20	37	0	0	56	38	11	21	0	25	9	13	?	0.57
M. acutus	1.58	2.52	2.2	19	39	7	67	38	43	3	39	1.21	131	?	1	39	22	51	0	0	64	43	20	28	24	26	27	13	0.54	0.4
M. albolineatus	1.62	2.65	1.35	27	20	11	55	30	41	2	31	1.25	97	9	23	47	42	83	4	25	92	39	36	29	70	44	20	12	0.58	0.41
M. atriclava	1.43	2.45	1.61	23	25	6	63	24	41	3	40	1.17	101	11	26	49	51	70	3	23	35	60	24	18	45	37	31	10	0.61	0.38
M. beringiensis	1.8	2.45	1.13	13	17	16	52	28	10	?	33	1.29	92	9	35	55	36	59	5	24	67	45	38	24	45	35	21	?	0.52	0.34
M. brusti	1.8	2.52	1.73	23	26	13	50	25	29	3	35	1.18	88	8	3	54	36	52	1	6	58	42	37	24	7	51	30	10	0.59	0.4
M. calvescens	1.59	2.86	2.6	26	30	9	48	?	41	4	26	1.2	116	6	0	17	5	6	0	0	5	13	1	4	0	9	27	?	0.6	0.37
M. caudigus	1.725	2.48	1.41	24	25	14	58	39	36	3	35	1.22	91	6	31	52	47	78	6	16	80	44	48	30	68	46	21	11	0.515	0.395
M. corticalis	1.74	2.47	1.45	14	16	8	30	17	24	3	28	1.17	128	6	8	32	30	42	2	6	61	38	23	24	25	21	13	12	0.59	0.4
M. costatus	?	?	0.82	31	52	15	88	54	34	4	20	1.25	155	34	?	83	63	138	4	26	0	56	0	37	168	?	34	?	0.85	0.49
M. dentipalpus	?	2.57	1.43	20	29	9	30	15	29	2	34	1.11	94	6	3	29	34	32	4	20	48	33	38	25	8	40	27	10	0.56	0.4
M. eryngiotelmatus	1.89	1.99	1.1	39	32	12	81	48	34	3	16	1.23	159	8	54	76	55	122	6	22	39	58	78	37	139	55	21	10	0.7	0.45
M. exiliaces	1.505	2.43	?	31	17	9	33	38	22	3	25	1.26	173	8	32	60	37	65	2	2	56	45	34	30	52	65	25	13	0.63	0.43
M. fuscipes	1.7	2.25	0.98	31	31	18	77	29	56	3	48	1.23	103	12	41	69	58	99	5	27	79	26	46	26	93	50	36	9	0.57	0.39
M. intergerivus	1.74	2.48	2.17	37	42	18	66	25	62	4	42	1.21	68	13	14	63	50	66	4	23	71	44	40	26	44	44	53	11	0.6	0.38
M. lautus	?	?	0.32	19	17	5	29	28	17	3	28	1.3	155	?	31	47	34	80	0	18	58	34	46	22	83	?	8		0.59	?
M. longipennis	4.6	0.97	0.45	30	33	13	27	0	23	4	32	1.06	40	7	0	35	36	48	1	14	34	40	35	30	0	48	7	15	0.51	0.35
M. obscuripes	1.58	2.35	1.74	27	33	16	57	30	55	4	46	1.18	129	12	38	79	66	122	5	34	92	61	54	41	115	68	29	12	0.63	0.43
M. piscipes	1.7	2.31	2.53	25	34	13	58	32	54	3	48	1.19	93	10	2	52	42	55	3	6	79	48	44	30	42	49	51	12	0.63	0.41
M. puna	1.61	2.16	?	31	14	9	53	37	28	2	24	1.16	58	7	5	38	24	52	2	1	14	12	32	9	2	35	21	10	0.61	0.4
M. sibiricus	2.38	1.53	0.49	32	20	8	35	15	21	2	32	1.12	84	5	11	30	25	31	0	0	9	61	36	33	1	42	7	15	0.55	0.39
M. ursinus	1.515	2.87	2.74	29	50	11	61	31	65	3	50	1.17	124	6	0	19	4	0	0	0	7	0	0	0	0	0	67	12	0.59	0.36
M. virgatus	?	?	0.78	27	35	15	68	0	29	2	17	1.22	140	?	61	82	52	127	1	24	65	41	72	28	0	?	24		0.69	0.45
M. wangi	2.675	2.21	0.565	28	?	10	41	27	29	3	36	1.26	136	16	56	65	52	86	5	24	76	53	43	36	97	54	22	10	0.64	0.415
M. yaquina	2.05	?	0.46	28	32	13	39	20	11	3	20	1.08	?	?	?	?	?	?	?	?	?	?	?	?	?	?	16	?	0.56	0.46

Continue

Table 2 Continuation.

	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
T. gracilis	0.53	3.17	3.81	3.42	3.52	4.55	3.6	0.69	2.01	2.54	10	15	0	0	0	0	0	0	0
T. fulvofasciatus	0.5	3.02	3.94	3.58	3.09	4.82	3.77	0.71	2.19	3.03	17	34	0	1	0	0	2	2	0
T. gracei	0.52	2.8	3.68	3.36	3.02	4.43	3.64	0.75	2.25	3.1	15	33	0	1	0	0	4	2	0
T. pilinucha	0.47	3.71	4.07	3.77	3.16	4.39	3.95	0.83	1.98	2.58	17	19	0	1	0	0	4	2	0
A. fontinalis	0.55	3.01	3.9	3.4	3.29	4.36	3.31	0.83	1.99	2.88	22	0	1	2	3	2	5	0	0
A. japonicus	0.48	3.02	3.9	3.51	2.79	4.03	3.84	0.65	2.13	?	13	0	1	2	3	2	4	0	0
M. aculeatus	0.59	?	3.94	3.59	?	3.57	3.2	0.8	2.11	3.28	15	11	1	2	0	0	0	0	1
M. acutus	?	?	3.83	?	3.39	4.7	?	0.73	2.21	3.25	44	53	1	2	0	0	4	3	1
M. albolineatus	0.47	2.73	3.65	3.2	3.11	4.76	3.75	0.7	1.97	2.74	27	24	1	2	1	0	4	[12]	1
M. atriclava	0.45	2.92	?	3.39	3.03	4.95	3.84	0.82	2.28	2.7	30	0	1	2	0	2	3	0	1
M. beringiensis	0.37	2.72	3.47	3.27	3.48	5.66	4.76	0.76	2.27	2.83	30	0	1	2	[03]	2	4	0	1
M. brusti	0.51	2.77	3.41	3.12	3.11	4.86	3.5	0.71	2.06	3.29	35	43	1	2	1	0	1	2	1
M. calvescens	0.49	2.86	3.65	3.19	3.06	5.12	3.65	0.79	2.12	3.23	39	45	1	2	2	0	5	2	1
M. caudigus	0.44	2.87	3.805	3.48	3.475	4.79	3.9	0.725	1.905	2.455	25	55	1	2	1	0	4	0	1
M. corticalis	0.49	2.84	4.12	3.43	3.09	4.13	3.63	0.75	2.34	2.79	53	45	1	2	0	0	4	1	1
M. costatus	0.5	0.84	3.59	3.05	2.3	4.1	3.63	0.48	1.95	2.43	28	0	1	2	1	2	0	2	1
M. dentipalpus	0.49	2.72	3.49	3.17	3.39	4.95	3.78	0.72	2.13	?	29	49	1	2	1	0	0	2	1
M. eryngiotelmatus	0.48	2.52	3.49	2.98	2.72	4.34	3.83	0.62	2.01	2.76	48	0	1	2	3	2	1	0	1
M. exiliaces	0.45	2.71	3.45	3.2	2.93	4.465	3.98	0.82	2.11	2.86	38	0	1	2	1	2	4	0	1
M. fuscipes	0.38	2.93	3.83	3.5	3.21	4.86	4.68	0.82	2.35	3.1	33	0	1	2	0	2	2	0	1
M. intergerivus	0.44	2.83	3.75	3.54	3.08	5.02	4.07	0.81	2.21	3.31	56	23	1	2	0	0	4	0	1
M. lautus	?	2.92	?	?	3.17	?	?	0.46	1.66	1.88	14	0	1	2	1	2	0	2	1
M. longipennis	0.5	3	3.54	3.12	3.99	5.76	3.83	0.79	2.28	1.95	42	?	1	2	1	1	5	0	1
M. obscuripes	0.51	2.7	3.47	3.2	2.99	4.6	3.58	0.66	1.97	2.79	25	51	1	2	2	0	1	3	1
M. piscipes	0.46	2.86	3.83	3.44	2.95	4.75	3.91	0.77	2.28	3.22	37	0	1	2	1	2	3	0	1
M. puna	0.41	2.73	3.29	3.06	3.21	4.96	4.82	0.505	1.97	2.99	25	0	1	2	3	2	0	0	1
M. sibiricus	0.52	2.89	3.31	3.1	3.59	5.09	3.59	0.77	2.4	2.43	24	?	1	2	1	1	5	0	1
M. ursinus	0.49	2.82	3.82	3.24	3.14	5.43	3.75	0.75	2.24	3.42	40	45	1	2	1	0	4	2	1
M. virgatus	0.51	2.48	3.16	3.23	2.66	4.27	3.56	0.45	1.82	2.38	22	?	1	2	3	0	0	2	1
M. wangi	0.465	2.69	3.485	3.245	2.88	4.79	4.035	0.59	1.95	2.35	22	26	1	2	0	0	0	2	1
M. yaquina	0.5	?	?	?	?	?	?	?	2.12	2.86	?	?	1	2	0	2	5	0	1

algorithms of Farris (1970) and Goloboff (1993a). These algorithms operate with the differences in the numerical values of the variables being optimized, allowing for values between 0 and 65 and up to three decimals (for more details see Farris 1970 and Goloboff *et al* 2006). As TNT requires data with normal distribution, the normal distribution of the quantitative characters used in this analysis was corroborated using the Shapiro-Wilk test implemented in the program PAST ver. 1.82 (Hammer *et al* 2001). Discrete characters were coded as non-additive and quantitative characters were analyzed as additive.

The data matrix was analyzed with TNT version 1.1 (Goloboff *et al* 2008b) under parsimony using equal and implied weights (Goloboff 1993b). In the parsimony analysis under implied weights, character weights are not given a priori but are instead assigned during tree search (the technique is not **iterative**). In this way, this procedure resolves conflict in favor of less homoplastic characters. The level of down-weight of homoplastic characters was calculated according to a concave homoplasy function (Farris 1969, Goloboff 1993b). The strength of this function is controlled by a constant k, with k = 1 being the maximum down-weighting strength. Character weighting has traditionally been a controversial issue in cladistics, but recently homoplasy weighting has been ratified (Goloboff *et al* 2008c and literature cited therein). In this study, analyses with implied weighting were conducted applying concavity k values from one to 15.

All tree searches were performed using a Wagner tree as starting tree and 1000 random addition sequences plus TBR with 100 trees to save per replication.

The presence of characters with weights or costs can lead to wrong conclusions with regard to support using bootstrap and jackknife (Goloboff *et al* 2003). The results obtained in this analysis were estimated as frequency differences with 1000 replicates of symmetrical resampling plus TBR (symmetric resampling is not affected by weighted characters), 10 random addition sequences, and saving 100 trees per replicate.

The species belonging to the genera *Thienemannia* Kieffer and *Apometriocnemus* Sæther were elected as outgroup rooting on *T. gracilis* Kieffer and their characters were taken from the literature (Sæther 1985).

Results

Metriocnemus puna sp. n.

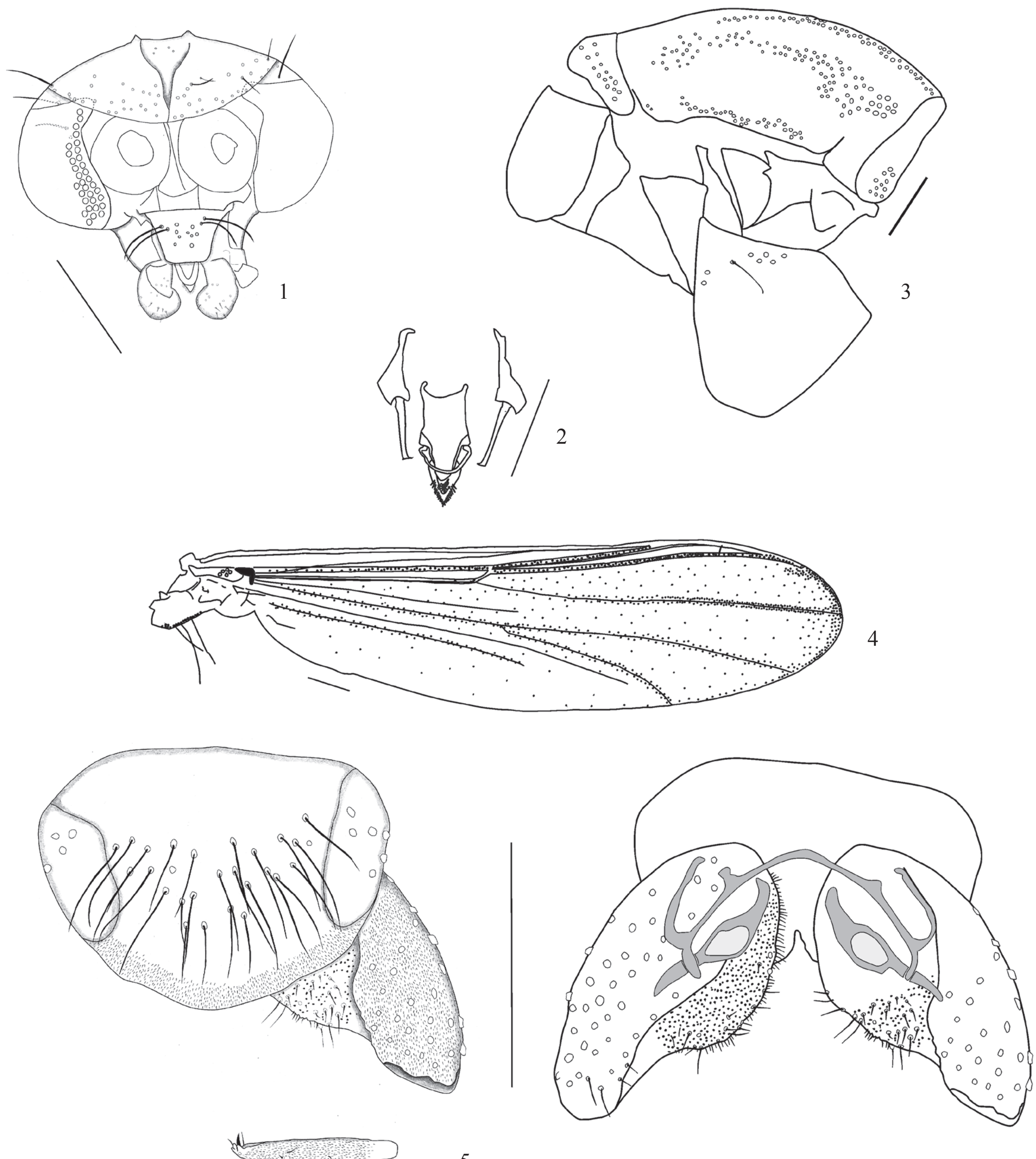
Material examined. Holotype male, ARGENTINA: Salta, Quebrada del Agua, 24º 30' 33''S– 68º 10' 52.6''W, 3678 m, 11-I-2005, Malaise trap, M Donato (MLP); Allotype female (MLP), same data as holotype. Paratypes: two males, one female, (MLP), same data as holotype.

Etymology. The name of the new species refers to the geographic region that this species was collected.

Male imago (n = 4, except when otherwise stated) (Figs 1-6). Total length 3.23-3.47, 3.38 mm (3). Wing length 2.10-2.20, 2.13 mm; width 0.55-0.62, 0.58 mm. Total length/wing length 1.58-1.63 (2). Wing length/length of profemur 2.10-2.20, 2.16 (3). Coloration uniformly dark brown with legs light brown.
Head (Fig 1). Antennae lost. Temporal setae 25-36, 31. Clypeus with 12-17, 14 setae. Cibarial pump, tentorium, and stipes as in Fig 2. Tentorium 140-150 long and 42-47 wide (2). Stipes 78-93, 83 (3); 10-12, 11 (3). Palpi lost.
Thorax (Fig 3). Antepronotum with 7-10, 9 lateral setae. Dorsocentrals 46-57, 53 (3); acrostichals 33-41, 37; prealars 26-32, 28; supraalars 2. Scutellum with 22-27, 24. Preepisternals 9-10, 10.
Wing (Fig 4). VR 1.10-1.20, 1.17. C extension 58 (1). Brachiolum with 6-8, 7 setae; Sc with 2-9, 5; R with 30-46, 38; R_1 with 22-25 (2); R_{4+5} with 52 (1); RM with 1-3, 2; M with 0-2, 1; M_{1+2} with 10-18, 14; M_{3+4} with 8-14, 12; Cu with 27-37, 32; Cu_1 with 7-10, 9; Pcu with 0-8, 2; An with 22-45, 35 setae. Squama with 19-22, 21 setae (3).
Legs. Spur of front tibia 74-75 long (2); spurs of middle tibia: 29-35 and 27-32 (2); of hind tibia: 76-80, 78 and 25-32, 29 long (3). Width at apex of front tibia 49-60, 55; of middle tibia 49-55, 51; of hind tibia 60-71, 64. Comb with 10-11, 10 setae. Tarsomere 1 of middle and hind leg with two pseudospurs; tarsomere 2 of middle and hind leg with one pseudospur. Lengths (in µm) and proportions of legs in Table 3.
Hypopygium (Figs 5-6). Without anal point. Setae on tergum IX 24-25, 25, laterosternite IX with 3-5, 4 setae. Phallapodeme 80-88, 84 long; transverse sternapodeme 110-113, 112 long. Virga absent. Gonocoxite 216-225, 221 long; inferior volsella strongly projecting, ending at 0.49-0.52, 0.51. Gonostylus 109-118, 112 long; crista dorsalis very low; megaseta 10-12, 11 long. HR 1.86-2.07, 1.98; HV 2.93-3.11, 2.99 (3).

Female imago (n = 2, except when otherwise stated) (Figs 7-10). Total length 3.23 mm (1). Wing length 1.93-2.07 mm; width 0.73-0.75 mm. Total length/wing length 1.67 (1). Wing length/length of profemur 1.85-1.95, 1.91. Coloration as in male.
Head. Antennae lost. Temporal setae 57-68. Clypeus with 17-19 setae. Tentorium 184 long and 32 wide (1). Stipes 40 long; 10 wide (1). Palp segment lengths (in µm): 56; 40-42; 150-160; 157-160; 188-220.
Thorax (Fig 7). Antepronotum with 13 lateral setae. Dorsocentrals 91-96; acrostichals 54-60; prealars 33-37; supraalars 3, preepisternals 6-8. Scutellum with 30 setae.
Wing (Fig 8). VR 1.19-1.21. C extension 191 long (1). Brachiolum with 5-7 setae. R with 52-58; R1 with 59 (1); R_{4+5} with 121 (1); M with 11-15; M_{1+2} with 81-61; M_{3+4} with 67-73; Cu with 65-67; Cu_1 with 48-51; Pcu with 29-35; An with 56-68 setae. Wing membrane with setae covering most cells. Squama with 20 setae.
Legs. Spur of front tibia 49 long (1); spurs of middle tibia: 22-27 and 15-17; of hind tibia: 74 and 17-29 long. Width at apex of front tibia 56 (1); of middle tibia 64-66; of hind tibia 76-78. Comb with 11 setae. Lengths (in µm) and proportions of legs in Table 4.
Genitalia (Figs 9-10). Gonocoxite IX with 21-22 setae. Tergite IX subrectangular with 32 setae (1). Cercus 112-120 long. Seminal capsule 98-117. Notum 180-195 long.

Diagnosis. The new species *Metriocnemus puna* agrees



Figs 1-6 *Metriocnemus puna* sp. n. Male adult; 1) Head; 2) Tentorium, stipes and cibarial pump; 3) Thorax; 4) Wing; 5) Hypopygium, dorsal view; 6) Hypopygium with tergite IX removed, left ventral view, right dorsal view. Scale bar = 100µm.

with the diagnosis of the genus provided by Sæther (1989) by having the following characters: presence of bare eyes without dorsomedial extension, hairy wings, numerous setae on squamal fringe, straight Cu_1, numerous body setae and presence of tarsal pseudospurs. The combination of well-developed lobe-like inferior volsella, absence of anal point and virga, two pseudospurs on tarsomere 1 and one pseudospur on tarsomere 2 of middle leg and two pseudospurs on tarsomere 1 and one pseudospur on tarsomere 2 of hind leg separates adult male *Metriocnemus puna* sp. n from all other species of the genus.

Distribution and ecological features. The Puna is a highland plateau (3000 m a.s.l.) extended between the two arms into which the Andes mountain range splits between the 15°S and 17°S parallels. The climate is cold and dry, with striking temperature variations between day and nighttime, and between summer and winter. The Puna is also a biogeographic province (Cabrera & Willink 1973, Morrone 2001) extending to an altitude of 4400 m a.s.l. from Peru to Bolivia and Northwestern Argentina. The Puna is largely a plateau with interspersed mountains and cliffs that delimit internal drainage systems mainly in central and south Puna. The type locality of this species, Quebrada del Agua, is a spring that drains into Socompa saltwater lagoon.

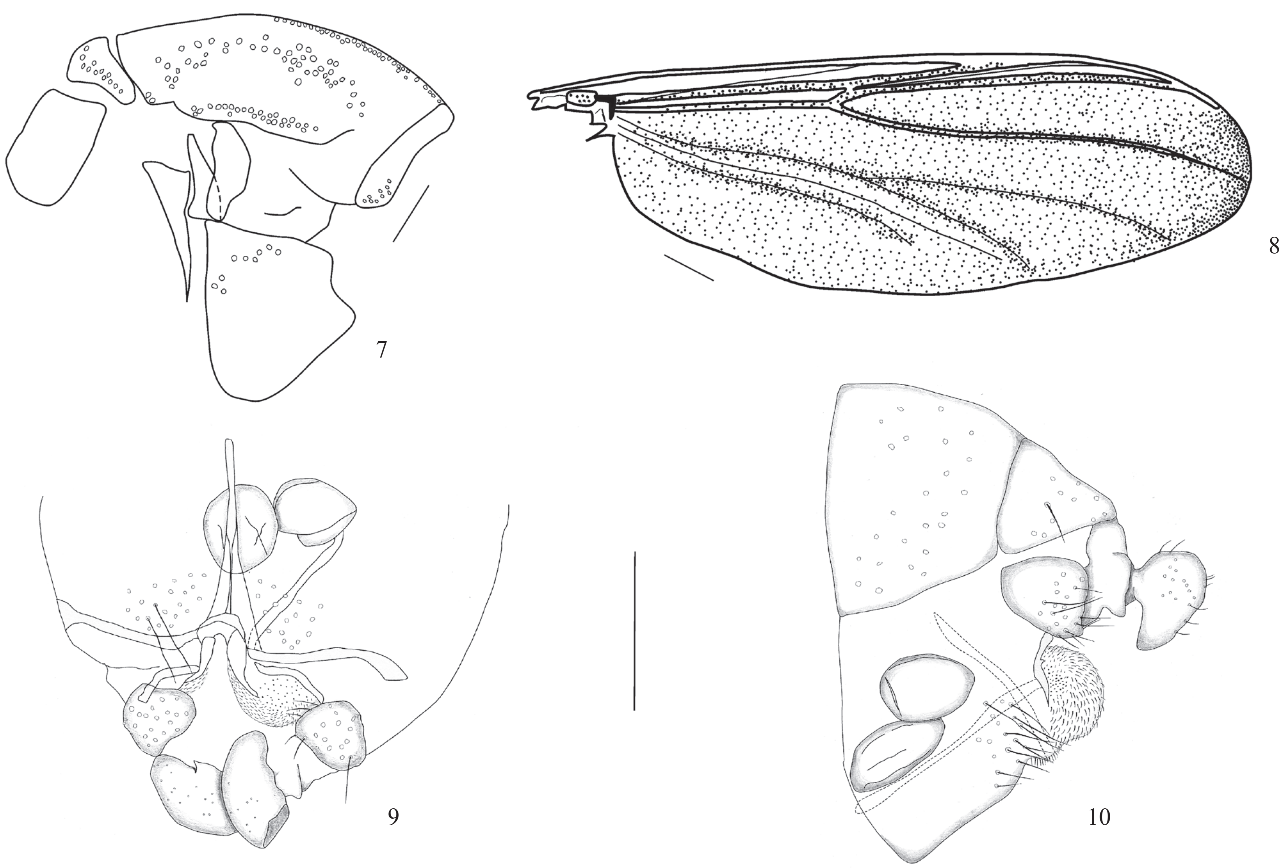
Table 3 Lengths (in µm) and proportions of legs of *Metriocnemus puna* sp. n. (male). Abbreviations: femur (Fe); Tibia (Ti); tarsomeres 1-5 (Ta_{1-5}); leg ratio (LR), ratio of metatarsus to tibia; Beinverhältnisse» (BV), combined length of femur, tibia, and basitarsus divided by combined length of tarsomeres 2-5; «Schenkel-Scheine-verhältnis» (SV), ratio of femur plus tibia to metatarsus.

	Fe	Ti	Ta_1	Ta_2	Ta_3
P_1	900-1000, 965	890-1100, 1008 (3)	580-640 (2)	350-360, 355 (2)	260-270, 265 (2)
P_2	910-1000, 940	880-1000, 945	370-410 (2)	240 (1)	180 (1)
P_3	960-1160, 1054	1100-1275, 1206	350-570, 483 (3)	280-350, 317 (3)	250-280, 267 (3)
	Ta_4	Ta_5	LR	BV	SV
P_1	180-190 (2)	120 (2)	0.58-0.65 (2)	2.60-2.87, 2.74 (2)	3.09-3.22 (2)
P_2	150 (1)	110 (1)	0.39-0.41 (2)	3.29 (1)	4.88-5.05 (2)
P_3	160-180, 179 (3)	130 (3)	0.51-0.59, 0.54	3.01-3.18, 3.07 (3)	3.89-6.41 (3)

The dominant vegetation is xeric shrubland.

Cladistic analyses. The analysis of the data matrix under equal weights yielded one cladogram (Fit = 38.58; length = 102.489; CI = 0.39; RI = 0.57) (Fig 11). The genus *Metriocnemus* is not a monophyletic group since it includes the two species of the genus *Apometriocnemus*, and this clade is supported by the synapomorphy "bare eyes". Although the cladogram shows high resolution, support for the groups is generally very low. Only the clades [*M. longipennis* (Holmgren)+ *M. sibiricus* (Lundström)] and [*M. calvescens* Sæther+ *M. ursinus* (Holmgren)] show a group support value above 50. The former is supported by the combination of character states "squama with seven setae", "anal point tapering to blunt apex" and "virga as a cluster of small spines"; and the latter is defined by the combination of characters "Sc lacking setae", "RM lacking setae", "M lacking setae" and "Pcu lacking setae".

The analysis of the data matrix under implied weights yielded one cladogram per each k value applied. The tree obtained with k = 4 (Fit = 38.97; length = 106.314; CI = 0.38;



Figs 7-10 *Metriocnemus puna* sp. n. Female adult. 7) Thorax; 8) Wing; 9) Female genitalia, ventral view; 10) Female genitalia, lateral view. Scale bar = 100µm.

Table 4 Lengths (in µm) and proportions of legs of *Metriocnemus puna* sp. n. (female). 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	920	930 (1)	–	–	–
P_2	860-930	840-950	–	–	–
P_3	970-1025	1087-1190)	540 (1)	320 (1)	280 (1)

	Ta_4	Ta_5	LR	BV	SV
P_1	–	–	–	–	–
P_2	–	–	–	–	–
P_3	140 (1)	120 (1)	0.50 (1)	3.02 (1)	3.81 (1)

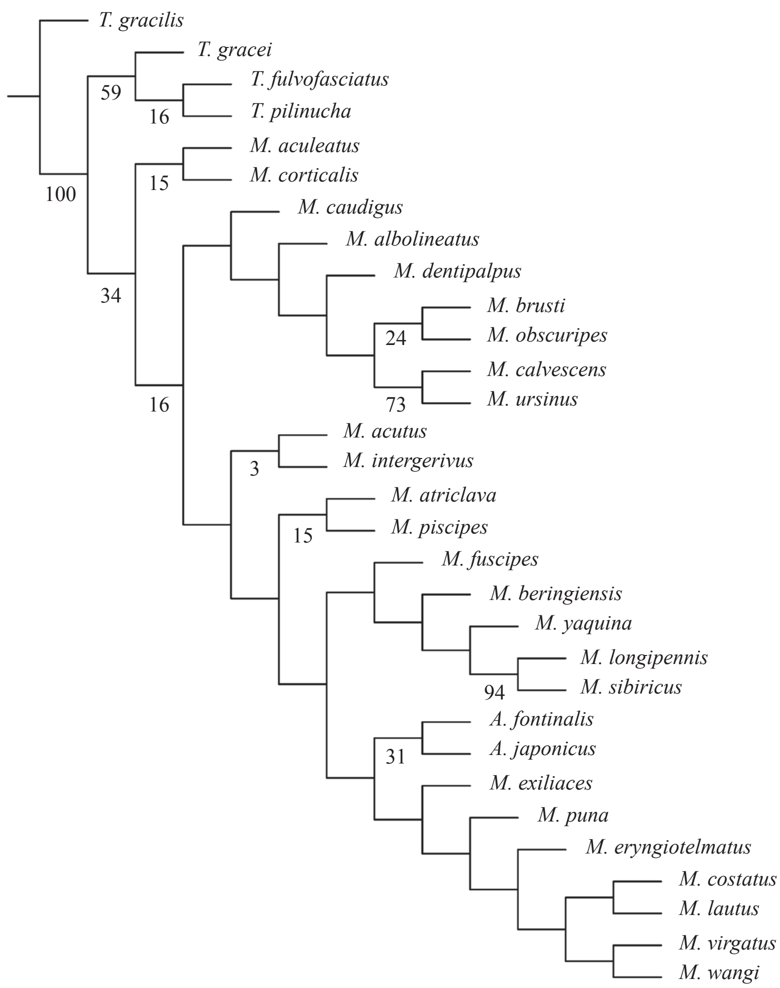


Fig 11 Most parsimonious cladogram obtained under equal weights (Fit = 38.58; length = 102.489; CI = 0.39; RI = 0.57). Numbers below nodes represent the support as frequency differences.

RI = 0.54) shows the highest group support values (Fig 12). The genus *Metriocnemus* is a monophyletic group defined by the synapomorphy "presence of tarsal pseudospurs". The clades with the highest group support values are the same as those found under equal weights, and the clade (*M. longipennis*+ *M. sibiricus*) is supported by the combination of characters "32 scutellar setae", "squama with seven setae" and "virga as cluster of small spines". The clade (*M. calvescens*+ *M. ursinus*) is defined by the combination of characters "Sc lacking setae", "RM lacking setae", "M lacking setae" and "Pcu lacking setae". A strict consensus was calculated in order to summarize the information from

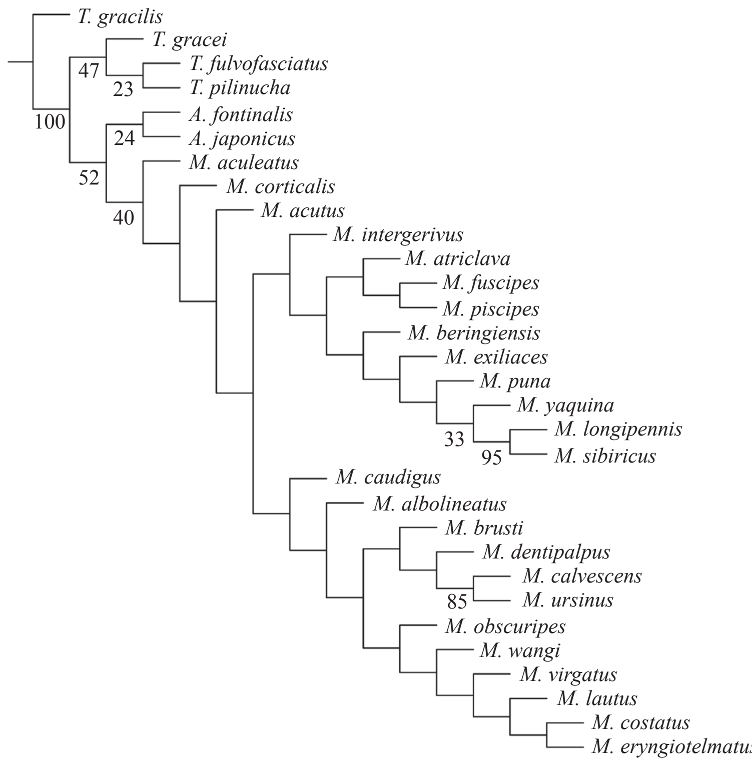


Fig 12 Most parsimonious cladogram obtained under implied weights with k = 4 (Fit = 38.97; length = 106.314; CI = 0.38; RI = 0.54). Numbers below nodes represent the support as frequency differences.

the fifteen trees found under implied weights with different k values (Fig 13).

The position of *Metriocnemus puna* sp. n is uncertain. In the analysis under equal weights, the new species is the sister group of the clade {*M. eryngiotelmatus* Donato & Paggi [(*M. costatus* Sublette & Sasa+ *M. lautus* Sublette & Sasa) (*M. virgatus* Sublette & Sasa + *M. wangi* Sæther)]}, sharing with it the character state "well-developed lobe-like inferior volsella". In the analysis under implied weights with k = 4, *M. puna* sp. n is closely related to the clade [*M. yaquina* Cranston & Jud (*M. longipennis*+ *M. sibiricus*)]. However, as shown in the strict consensus, *M. puna* sp. n, like almost all the *Metriocnemus* species analyzed, is part of a basal polytomy (Fig 13).

Discussion

The use of several tree search strategies allowed identifying which groups were more stable (not in the sense of stability *sensu* Wheeler 1995) and which groups were not. As all trees obtained with equal and implied weights were fully resolved, the highest group support value was the criterion to choose between cladograms. The amount of support for a group is due to the interaction between the characters favoring the group and those that contradict it (Goloboff *et al* 2003). Therefore, using group support as argument, we will discuss the phylogenetic relationships in the tree obtained under implied weights with k = 4.

The genus *Metriocnemus* belongs to the tribe Metriocnemini and is considered as the sister group of

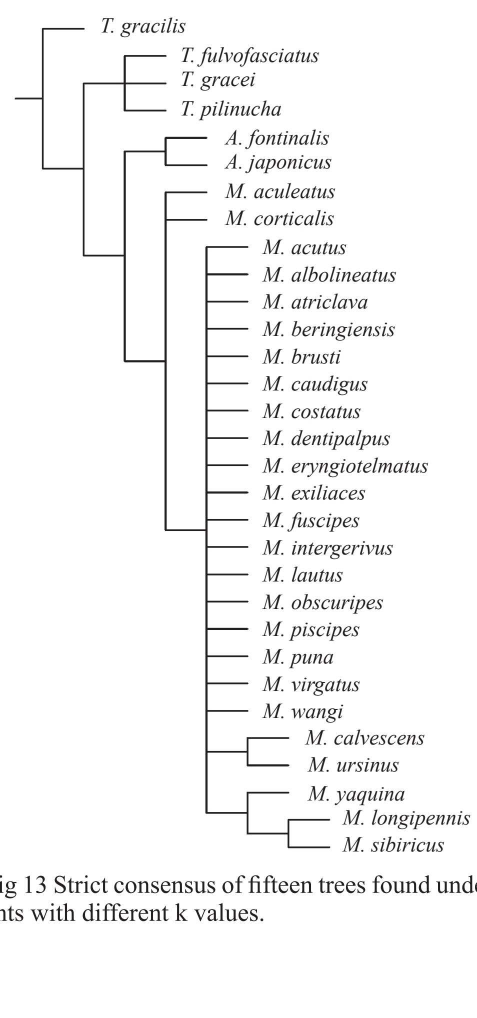


Fig 13 Strict consensus of fifteen trees found under implied weights with different k values.

Thienemannia (Sæther 1977, Sæther & Sublette 1983). Based on pupal characters, Cranston & Judd (1987) defined *Metriocnemus* as a monophyletic group and the position of *Thienemannia* was defined by them as doubtful because the insufficient knowledge of many of its immature stages. These authors suggested that many of the character states that distinguish *Thienemannia* might be interpreted as plesiomorphies.

The genus *Apometriocnemus* keyed between *Parametriocnemus* Goethgebuer and *Paraphaenocladius* Thienemann by having wing venation like the former and eye extension as the latter, within the *Metriocnemus* group *sensu* Brundin (1966) (Sæther 1984). However, Sæther (1984) pointed out that the genitalia of some members of *Thienemannia* and *Metriocnemus* is similar to that of *Apometriocnemus*. As the immature stages of *Apometriocnemus* are unknown, the placement of this genus relative to the above mentioned genera is not possible. Furthermore, this genus could conceivably be incorporated into one of the others as a subgenus (Sæther 1984).

Even though the analysis of the generic relationships of the tribe Metriocnemini was not the goal of this study, the parsimony analysis under equal weights showed the genus *Apometriocnemus* as part of the genus *Metriocnemus* and the parsimony analysis under implied weights showed *Apometriocnemus* as the sister group of *Metriocnemus*. These results reflect the systematic problem pointed out by Sæther (1984) and lead to the conclusion that the only character that clearly separates both genera is the absence/presence of tarsal pseudospurs, since the rest of the characters used by this author and others (Cranston *et al* 1989) are shared. The resolution of this systematic problem will require more data, such as the knowledge of the immature stages of *Apometriocemus*, to allow for a taxonomic decision.

The *eurynotus* and *fuscipes* species groups recognized within *Metriocnemus* were not recovered in this analysis. These results are in agreement with several authors (Cranston & Judd 1987, Sæther 1989, 1995, Donato & Paggi 2005) who have discussed the position of many of the known *Metriocnemus* species and found that some of them showed a combination of characters typical of the *eurynotus* or the *fuscipes* groups.

The close relationship of *M. calvescens* as sister species of *M. ursinus*, and *M. longipennis* as sister species of *M. sibiricus,* as proposed by Sæther (1989, 1995), are confirmed in this analysis, since these clades showed the highest support values. Although the establishment of the phylogenetic relationships of the species of *Thienemannia* were not among the goals of this study, the results show that *T. pilinucha* Sæther and *T. fulvofasciata* (Kieffer) are sister species, while *T. gracei* (Edwards) represents the sister species of the first two combined, as postulated by Sæther (1985).

Future findings of currently unknown immature stages of several species of the genus *Metriocnemus* will improve future research by allowing comparative morphological studies, allowing for the elucidation of their phylogenetic relationships.

Acknowledgments

The authors wish to thank the Willi Hennig Society for the free availability of the TNT program. This research was supported by PIP N° 5535 (CONICET), PICT Nº 26298 (Agencia Nacional de Promoción Científica y Tecnológica) and National Geographic Society Grant 7646-04.

References

Cabrera A L, Willink A (1980) Biogeografía de América Latina. Washington D.C. OEA. Monografía n° 13, Serie Biología, 109p.

Coffman W P, Cranston PS, Oliver D R Sæther O A (1986) The pupae of Orthocladiinae (Diptera: Chironomidae) of the Holarctic region - keys and diagnoses. Entomol Scand Suppl 28: 147-296.

Cranston P S, Humphries C J (1988) Cladistics and computers: a chironomid conundrum? Cladistics 4: 72-92.

Cranston P S, Judd D D (1987) *Metriocnemus* (Diptera: Chironomidae) - An ecological survey and description of a new species. J N Y Entomol Soc 95: 534-546.

Cranston P S, Oliver D R, Sæther O A (1983) The larvae of Orthocladiinae (Diptera: Chironomidae) of the Holarctic region - keys and diagnoses. Entomol Scand Suppl 19: 149-291.

Cranston P S, Oliver D R, Sæther O A (1989) The adult males of Orthocladiinae (Diptera: Chironomidae) of the Holarctic region - keys and diagnoses. Entomol Scand Suppl 34: 165-352.

Donato M, Paggi A C (2005) A new Neotropical species of the genus *Metriocnemus* van der Wulp (Chironomidae: Orthocladiinae) from *Eryngium* L. (Apiaceae) phytotelmata. Zootaxa 1050: 1-14.

Farris J S (1969) A successive approximations approach to character weighting. Syst Zool 18: 374-385.

Farris J S (1970) Methods for computing Wagner trees. Syst Zool 19: 83-92.

Goloboff P A (1993a) Character optimization and calculation of tree lengths. Cladistics 9: 433-436.

Goloboff P A (1993b) Estimating character weights during tree search. Cladistics 9: 83-91.

Goloboff P A, Carpenter J M, Salvador Arias J, Miranda Esquivel D R (2008c) Weighting against homoplasy improves phylogenetic analysis of morphological data sets. Cladistics 24: 1-16.

Goloboff P A, Farris J S, Källersjö M, Oxelmann B, Ramirez M, Szumik C (2003) Improvements to resampling measures of group support. Cladistics 19: 324-332.

Goloboff P A, Farris J S, Nixon K C (2008a) TNT, a free program for phylogenetic analysis. Cladistics 24: 1-13.

Goloboff P A, Farris J S, Nixon K C (2008b) TNT (Tree analysis using new technology) (BETA) ver. 1.1. Published by the authors, Tucumán, Argentina.

Goloboff P A, Mattoni C I, Quinteros A S (2006) Continuous characters analyzed as such. Cladistics 22: 589-601.

Hammer Ø, Harper D A T, Ryan P D (2001) PAST: Paleontological statistics software package for education and data analysis. Palaeo. Electronica 4: 1-9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Mickevich M F, Johnson M F (1976) Congruence between morphological and allozyme data in evolutionary inference and character evolution. Syst Zool 25: 260-270.

Morrone J J (2001) Biogeografía de America Latina y el Caribe. Manuales & Tesis Sociedad Entomológica Aragonesa, Vol 3, Zaragoza, 148p.

Pimentel R A, Riggins R (1987) The nature of cladistic data. Cladistics 3: 201-209.

Rae T C (1998) The logical basis for the use of continuous characters in phylogenetic systematics. Cladistics 14: 221-228.

Sæther O A (1977) Female genitalia in Chironomidae and other Nematocera: morphology, phylogenies, keys. Bull Fish Res Board Can 197: 1-211.

Sæther O A (1980) Glossary of chironomid morphology terminology (Diptera: Chironomidae). Entomol Scand Suppl 14: 1-51.

Sæther O A (1984) *Apometriocnemus fontinalis* gen. n., sp. n. (Diptera: Chironomidae) from Tennessee, USA Entomol. Scand 15: 536-539.

Sæther O A (1985) Redefinition and review of *Thienemannia* Kieffer, 1909 (Diptera: Chironomidae), with the description of *T. pilinucha* sp. n. Aquat Insects 7: 111-131.

Sæther O A (1989) *Metriocnemus* van der Wulp: a new species and a revision of species described by Meigen, Zetterstedt, Staeger, Holmgren, Lundstrom and Strenzke (Diptera: Chironomidae). Entomol Scand 19: 393-430.

Sæther O A (1995) *Metriocnemus* van der Wulp; seven new species, revision of species, and new records (Diptera: Chironomidae). Ann Limnol 31: 35-64.

Sæther O A, Sublette J E (1983) A review of the genera *Doithrix* n. gen., *Georthocladius* Strenzke, *Parachaetocladius* Wulker and *Pseudorthocladius* Goetghebuer (Diptera: Chironomidae, Orthocladiinae). Entomol Scand Suppl 20: 1-100.

Spies M, Reiss F (1996) Catalog and bibliography of Neotropical and Mexican Chironomidae (Insecta, Diptera). Spixiana Suppl 22: 61-119.

Stevens P F (1991) Character states, morphological variation, and phylogenetic analysis: a review. Syst Bot 16: 553-583.

Strait D S, Moniz M A, Strait P T (1996) Finite mixture coding: a new approach to coding continuous characters. Syst Biol 45: 67-78.

Sublette J E, Sasa M (1994) Chironomidae collected in onchocerciasis endemic areas of Guatemala. (Insecta, Diptera). Spixiana Suppl 20: 1-60.

Thiele K (1993) The holy grail of the perfect character: the cladistic treatment of morphometric data. Cladistics 9: 275-304.

Thorpe R S (1984) Coding morphometric characters for constructing distance Wagner networks. Evolution 38: 244-355.

Wheeler W (1995) Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. Syst Biol 44: 321-331.

Wiens J J (2001) Character analysis in morphological phylogenetics: problems and solutions. Syst Biol 50: 689-699.

Received 26/IX/08. Accepted 30/III/09.