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Cladistic analysis of *Rheochlus* and related genera, with description of a new species (Diptera: Chironomidae: Podonominae)

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Abstract. A new species, *Rheochlus latisetus* sp. nov., is described from male and female adults from Argentina. Emendations and illustrations of the three previously known species of *Rheochlus* Brundin, 1966 are provided. A cladistic analysis of the genus *Rheochlus* within the tribe Podonomini is presented, and the phylogenetic relationship with congeners is discussed based on adult characters.

Key words. Diptera, Chironomidae, Podonominae, *Rheochlus*, phylogeny, cladistic analysis, new species, Argentina, Neotropical Region

Introduction

The subfamily Podonominae (Diptera: Chironomidae) is mainly present in the southern hemisphere, with species inhabiting cold streams and brooks at high altitudes (Brundin 1966).

The Podonomini genus *Rheochlus* Brundin, 1966 currently contains *R. insignis* Brundin, 1966 and *R. prolongatus* Brundin, 1966 from South America and *R. wirthi* (Freeman, 1961) from Australia (Ashe & O'Connor 2009). Only adults have been described so far. Males are characterized by hairy eyes, maxillary palps more or less reduced, and gonocoxites conspicuously slender with a longitudinal dorsal keel. The gonostylus is basally swollen with longer apical and shorter subapical lobes, and the "p" seta of the gonostylus is exceptionally stout. Female adults are known for *R. insignis* and *R. wirthi*, and characterized by antennae with 13 flagellomeres, wing vein R_1 slightly swollen apically, two seminal capsules, simple cerci and the ninth abdominal segment lacking lateral lobes.

Phylogenetic relationships of Podonominae were suggested by Brundin (1966) who erected the tribes Podonomini and Boreochlini. The Podonomini included the genera *Podonomus*

Philippi, 1866, *Parochlus* Enderlein, 1912, *Zelandochlus* Brundin, 1966, *Podonomopsis* Brundin, 1966, *Podochlu*s Brundin, 1966 and *Rheochlus*. The Boreochlini included *Lasiodamesa* Kieffer, 1924*, Trichotanypus* Kieffer, 1906*, Boreochlus* Edwards, 1938*, Paraboreochlus* Thienemann, 1939*, Afrochlus* Freeman, 1964 and *Archeochlus* Brundin, 1966. Sæther (1977) accepted this division and noted that the females of each tribe could be distinguished by the structure of the tenth segment of the abdomen.

Cranston & Edward (1998) and Boothroyd & Cranston (1999) performed cladistic analyses of Podonominae based on morphological characters, and demonstrated the monophyly of Podonomini and Boreochlini. Unlike Brundin (1966) they placed *Trichotanypus* in Podonomini and *Lasiodiamesa* basal to Boreochlini.

Later, the monophyly of Podonomini and Boreochlini was tested by Cranston et al. (2010, 2011) by means of molecular studies. Both studies showed monophyly of Podonomini with *Trichotanypus* basal to the tribe, but the tribe Boreochlini was paraphyletic. In these studies, *Lasiodiamesa* is basal to all Podonominae.

Phylogenetic relationships within the Podonomini as described by Brundin (1966) were more or less retained in morphological (Cranston & Edward 1998) and molecular studies (Cranston et al. 2010, 2011), with *Parochlus* and *Podonomus* more related to each other than to *Podonomopsis* or *Podochlus*. Morphological analysis of Brundin (1966) and Cranston & Edward (1998) suggested that *Rheochlus* should be grouped with *Podochlus* and *Podonomopsis*, but this has not been tested by any molecular studies.

Brundin (1966) suggested that the genus *Podonomopsis* could be the sister group of *Rheochlus*. This was based on the similar arrangement of the apical and subapical lobe of the gonostylus, conspicuous slenderness of the gonocoxite, and similarities among female adults. Brundin (1966) considered *Podochlus* as the sister group to (*Podonomopsis* + *Rheochlus*) clade, distinguished from these genera by a double gonostylus, which among Podonominae, is shared with *Parochlus latipalpis* (Brundin, 1966) only. In the phylogenetic tree presented by Cranston & Edward (1998), the clade (*Podonomopsis* + *Podochlus* + *Rheochlus*) is unresolved, possibly mainly due to a lack of data for the immature stages of *Rheochlus*.

The present study analyzes the phylogenetic relationships for species of *Rheochlus* and its closest relatives. Monophyly of the tribe Podonomini is tested. Emendation and extensions of the description of the previously known *Rheochlus* species is made, and a new species of the genus is described.

Material and methods

Specimens of cleared *Rheochlus latisetus* sp. nov., the holotype of *R. prolongatus*, paratypes of *R. insignis* and *R. wirthi* were slide mounted in Canada balsam. General terminology follows Sæther (1980). Measurements are in μm, unless otherwise stated, and given as ranges followed by measurements of the holotype or allotype in square brackets. Holotype, allotype and paratypes of *R. latisetus* are deposited in the collection of the Museo de La Plata, Argentina (MLPA). Material of *R. insignis*, *R. prolongatus, R. wirthi*

Table 1. List of characters, character states and coding used in the cladistic analysis of the genus *Rheochlus* Brundin, 1966.

N°	Character	Sex	Character state
1	Eyes	male	0 = bare; 1 = hairy
2	Dorsomedial extension of the eye	male	0 = absent; 1 = present
3	Maxillary palp	male	0 = normal; 1 = slightly reduced; 2 = strongly reduced
4	Ratio of flagellomere 14/13	male	0 = lesser than 1; higher than 1
5	Mcu cross-vein	male	0 = hardly retracted; 1 = not retracted
6	Vein R_{2+3}	male	0 = present; 1 = absent
7	Wing cells r1/r4+5 ratio	male	0 = lesser than 0.33; 1 = 0.33–0.66; 2 = higher than 0.66
8	Distal end of M_{1+2} vein	male	0 = curved; 1 = straight
9	Wing L/W ratio	male	0 = lesser than 3; 1 = higher than 3
10	Wing cells setae	male	0 = bare; 1 = all setose (except r1)
11	Wing anal lobe	male	0 = absent or weakly developed; 1 = clearly developed
12	Keel on postnotum	male	0 = absent; 1 = present
13	Scutal tubercle	male	0 = absent; 1 = present
14	Membrane sole on t_4	male	0 = absent; 1 = present
15	Tibial spurs	male	0=flattened, with lateral teeth; 1 = simple, with no lateral teeth
16	Inner spur of ti_3	male	0 = longer than tibial diameter; 1= shorter or subequal to tibial diameter
17	Ratio of outer/inner spurs of TiIII	male	0 = clearly different in size (ratio lesse than 0.60); Subequals (ratio between 0.7 to 1.0)
18	Tibial comb on p_3	male	0 = weakly developed or absent; 1 = well developed
19	Small and hyaline lateral lobe on TIX	male	0 = absent; 1 = present
20	Anal point on TIX	male	0 = absent; 1 = present
21	Conspicuously slender gonocoxite	male	0 = no; 1 = yes
22	Dorsobasal keel on gonocoxite	male	0 = absent; 1 = present, lesser than 0.5 length of gonocoxite; 2 = present, longer than 0.75 the length of the gonocoxite
23	Gonostylus derivation	male	0 = dominantly ventral; 1 = dominantly dorsal
24	Gonostylus structure	male	0 = simple; 1 = with apical and subapical lobe
25	Gonostylar heel	male	0 = absent; 1 = present
26	"p" seta on gonostylus	male	0 = absent or indistinct; 1 = long and thin, L/W higher than 40; 2 = long and stout, L/W lesser than 25
27	Setae „x" and „y" on gonostylus	male	0 = absents; 1 = near the base of apical lobe; 2 = near the mid-lenght of apical lobe; 3 = near the apex of the apical lobe
28	Strong spines on gonostylus	male	0 = absent; 1 = present
29	Number of flagellomeres	female	0 = 9; 1 = 11; 2 = 13
30	Apical region of R_1 wing vein	female	0 = not thickened; 1 = slightly thickened; 2 = strongly thickened
31	Number of seminal capsules	female	0 = 2 seminal capsules; 1 = 3 seminal capsules
32	Segment X	female	0 = normal, continuous around whole segment; 1 = only sternite present, no tergite or dorsal part present

and one paratype of *R. latisetus* are deposited in the Swedish Museum of Natural History, Stockholm, Sweden (SMNH).

The specimens described by Brundin, from which he made drawings, have probably first been placed in alcohol then transferred to glycerol and finally returned to alcohol. This material, which is deposited at the SMNH, is not in good condition. The problem principally concerns *R. prolongatus* as this species was described from a single specimen which is stored in alcohol and is extremely macerated.

Phylogenetic analysis. The cladistic analysis of four species of *Rheochlus* includes male and female adults of all species except for *R. prolongatus* for which the female is unknown. To root and polarize the trees, *Macropelopia* Thienemann, 1916 of the chironomid subfamily Tanypodinae, was selected as an outgroup. For comparison, *Trichotanypus* Kieffer, 1906, *Lasiodiamesa* Kieffer, 1924 and *Boreochlus* Edwards, 1938 were selected as representatives of the tribe Boreochlini, while *Podonomus tehuelche* Siri & Donato, 2012, *Podochlus gracilistylus* Brundin, 1966, *Podonomopsis illiesi* Brundin, 1966 and *Parochlus squamipalpis* (Edwards, 1931) were chosen as representatives of the tribe Podonomini.

Assessment and coding of characters and character states were, except for *Boreochlus*, *Lasiodiamesa* and *Trichotanypus,* based on material examined by the first author. Characters (n = 32) and character states used in the analysis are self explanatory and listed in Table 1. A matrix is presented in Table 2.

Characters were coded as non-additive and analyzed with the program TNT version 1.1 (Goloboff et al. 2008a) under implied weights as optimality criteria (Goloboff 1993). Analyses with implied weighting were conducted by means of values for the concavity constant k = 5–20 as suggested by Goloboff et al. (2008b). Tree searches were performed by implicit enumeration. Absolute and relative Bremer supports were calculated, saving up to six steps longer suboptimal trees obtained with branch-swapping. Figures of trees were edited with WinClada (Nixon 1999).

Table 2. Character matrix.

	11111111112222222222333 12345678901234567890123456789012
Macropelopia	01000101111110011110000?00000301
Boreoclus	00201000110000100000010000?0201
Lasiodiamesa	01000000?11000111000100001 0?0301
Trichotanypus	10001000110000 11?000001101001301
Parochlus squamipalpis	00000000110000100110000011110120
Podochlus gracilistylus	10210020000000100111000110000211
Podonomopsis illiesi	10000010100000100111010010110210
Podonomus tehuelche	00000020000001100110000010010020
Rheochlus insignis	10100020000000100110012010220210
Rheochlus prolongatus	10200020000000100110011010230???
Rheochlus latisetus sp. nov.	10200020000000100110011010220210
Rheochlus wirthi	10200020000000100110011010210210

Results

Rheochlus latisetus sp. nov.

(Figs 1–9)

Type material. Holotype: adult ♂, "**ARGENTINA / Rio Negro province** / Nahuel Huapi National Park / Blest stream / 41º09'26''S/71º49'20''W / 21.x.2006 / M. Donato leg / sweep net" (MLPA). Allotype: adult ♀, same data as for holotype (MLPA). Paratypes: 1 ♂, same data as for holotype (MLPA); 1 adult ♂ and 1 adult ♀, "**ARGENTINA / Rio Negro province** / Mallín La Cortadera / 41°05'13''S/71°48'26''W, 769 m. a.s.l. / 14-xii-2006–08-i-2007 / Malaise trap / Garré & Montes de Oca cols." (MLPA).

Diagnosis. *Rheochlus latisetus* sp. nov. male adult is characterized by: maxillary palp more or less reduced; wing almost bare with few setae present on veins R, R_1 and R_{4+5}; short dorsal keel on gonocoxite; distinctive wide and stout "p" seta on gonostylus; apical lobe of gonostylus long, subapical lobe short and blunt, both lobes ending in a narrow apical seta ("t" seta"); setae "x" and "y" close to the mid section of the apical lobe. The female adult is characterized by: maxillary palp more or less reduced; flagellomeres 2–12 short and about as long as wide; ultimate flagellomere as long as flagellomeres 8–12 together; wing almost bare; cerci simple.

Description. ***Male imago*** (n = 2–3, except when otherwise stated). (Figs 1–6). Total length 1.68–1.93 [1.93] mm, total length/wing length 1.37–1.58 [1.58].

Head (Figs 1). Eyes hairy. Antenna with 14 flagellomeres (Fig. 2), AR 0.44–0.52 [0.44]. Temporal setae 10–12 [10], postorbitals 4–5 [5] on each side. Clypeus with 2 setae. Palpomere lengths (I–V): 14 [14]; 37–41 [41]; 41–44 [44]; 31–35 [35]; 41–44 [44]. Tentorium 100–106 [106] long.

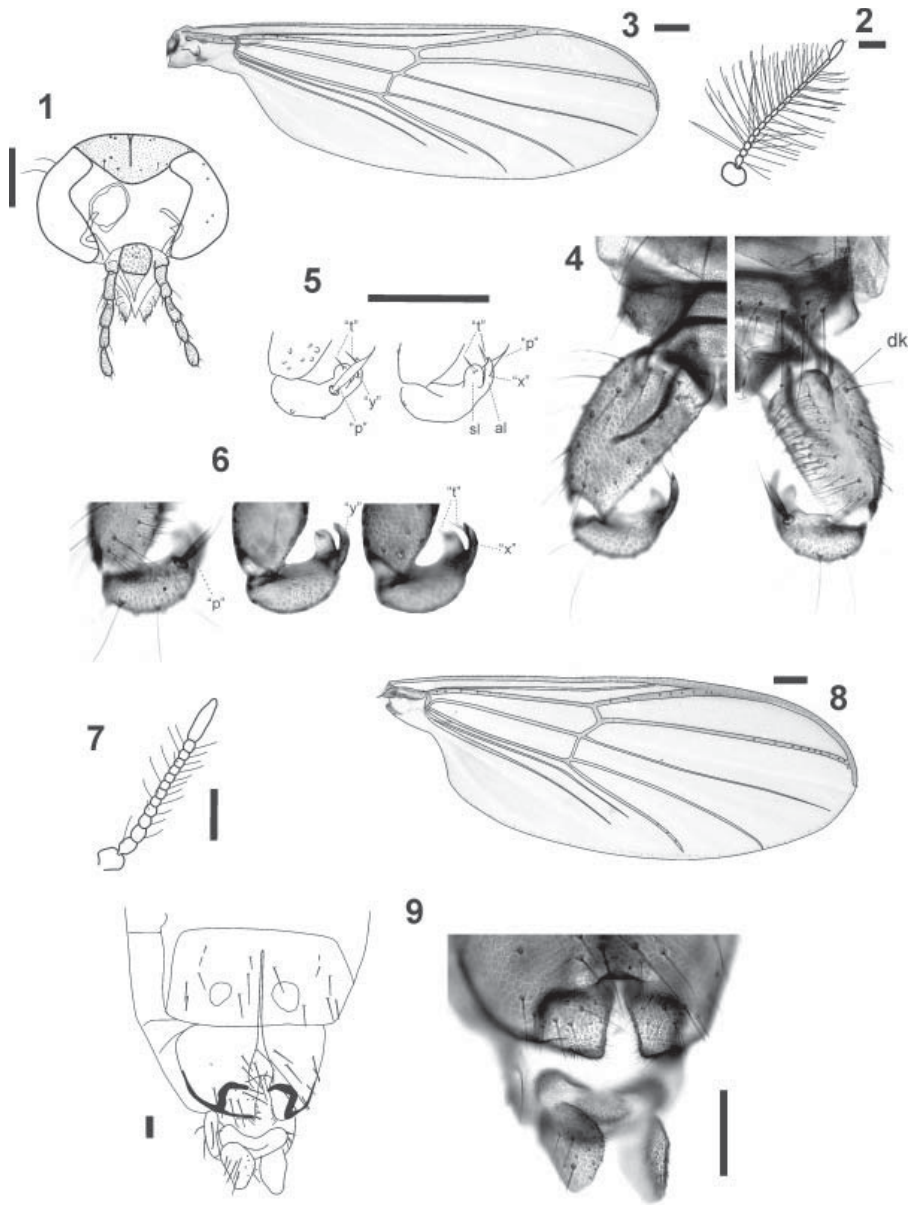
Thorax. Antepronotum with 6 [6] setae (1). Dorsocentrals 11 (1); acrostichals 15–16; prealars 7–8; supraalar 1 (1); scutellars 5–6 [5].

Wing (Fig. 3). 1.22–1.25 [1.22] mm long; 0.46–0.49 [0.46] mm wide; L/W 2.50–2.73 [2.68]. VR 0.92–0.96 [0.96]. Width of cell r_1 at the end of vein R_1 100–102 [100]; maximum width of cell r_5 125–143 [125]; r_1/r_5 0.72–0.80 [0.80]. Brachiolum with 2–3 [2] setae; squama with 2 [2] setae. R with 5–7 [5] setae, R_1 with 5–7 [6] setae, R_{4+5} with 3–7 [3] setae, Cu_1 with 2–3 [2] setae; Sc, Cu, M_{1+2}, M_{3+4} and An without setae.

Legs. Spur of front tibia 35–38 [35] long; spurs of mid tibia 16–22 [22] and 22–24 [24] long; of hind tibia 18–20 [20] and 59–65 [59] long. Apex of ti_1 28–30 [28] wide, of ti_2 26–30 [26] wide, of ti_3 37 [37] wide. Comb with 10–12 [11] spines. Lengths and proportions of legs in Table 3.

Hypopygium (Fig. 4). Tergite IX with 16–20 [20] setae, laterosternite IX with 2–3 [3] setae. Phallapodeme 57–71 [65] long; transverse sternapodeme 55–59 [59] long. Gonocoxite 132–142 [142] long; short basal keel 0.37–0.40 [0.40] times as long as gonocoxite. Gonostylus (Figs 5–6) 73–79 [73] long; apical lobe 33–40 [40] long, apical seta ("t" seta) 12–14 [14] long; subapical lobe 18–20 [18] long, with apical seta ("t" seta) 13–18 [13] long. Seta "p" 41–56 [49] long, 5–7 [7] wide, L/W of seta "p" 7.2–8.0. The "x" and "y" setae discernible, placed close to the mid section of apical lobe. HR 1.79–1.81 [1.81], HV 2.36–2.64 [2.64].

Female imago (n = 1–2) (Figs 7–9). Total length 1.74–1.85 [1.85] mm. Total length / wing length 1.27–1.44 [1.44]. Wing length / profemur length 3.37–4.17 [3.37].



Figs 1–9. *Rheochlus latisetus* sp. nov. 1–6 – male adults: 1 – head; 2 – antenna; 3 – wing; 4 – hypopygium, dorsal view (right) and ventral view (left); 5 – gonostylus: dorsal view (left) and ventral view (right), "p", "x", "y" and "t" setae are drawn and referenced; 6 – Photos of gonostylus: dorsal view (left), subapical lobe (middle) and apical lobe (right); the position of each seta is indicated. 7–9 –female adults: 7 – antenna; 8 – wing; 9 – genitalia ventral view, drawing and photo. Scale bars = 100 μm. Abbreviations: al – apical lobe of gonostylus, dk – dorsal keel on gonocoxite, "p" – seta at the base of subapical lobe of gonostylus, "t" – subapical setae of the apical and subapical lobes, sa – subapical lobe of gonostylus, "x" – ventral seta of the apical lobe, "y" – dorsal seta of the apical lobe.

Head. Eyes hairy. Antenna with 13 flagellomeres (Fig. 7). Last flagellomere 89–93 [89] long, as long as flagellomeres 8–12 together. Flagellomeres 2–12 short, more or less globose. AR 0.34–0.46 [0.34]. Temporal setae 12–26 [26]; postorbitals 4 on each side; clypeals 2. Palpomere lengths (I–V): 18 [18]; 40–42 [42]; 40–42 [42]; 28–35 [28]; 42–50 [42]. Tentorium 77–83 [77] long.

Thorax. Antepronotum with 5 [5] setae. Dorsocentrals 14–17 [17]; acrostichals 21 [21]; prealars 6–9 [6]; supraalar 1 [1]; scutellars 5–8 [5].

Wing (Fig. 8): length 1.29–1.38 [1.29] mm; width 0.58–0.63 [0.58] mm; L/W 2.21–2.22 [2.22]. VR 0.98 [0.98]. Width of cell r_1 at the end of vein R_1 120 [120]; maximum width of cell r_5 150–160 [150]; r_1/r_5 0.75–0.80 [0.80]. Brachiolum with 2 [2] setae; squama with 2 setae. R with 4–6 [6] setae, R_1 with 9 [9] setae, R_{4+5} with 6–16 [16] setae, M_{1+2} with 0–1 [1] setae, Cu_1 with 2–3 [3] setae, An with 0–5 [5] setae. Remaining veins bare. C extension 75–80 [80] long.

Legs. Spur of front tibia 27–30 [27] long; spurs of mid tibia 24–25 [24] and 18–20 [20] long; of hind tibia 22 [22] and 63–75 [75] long. Apex of ti_1 28–33 [28] wide, of ti_2 28–30 [28] wide, of ti_3 35–43 [35] wide. Comb with 11–13 [11] spines. Lengths and proportions of legs in Table 4.

Genitalia (Fig. 9). Cercus 63–70 [70] long; noto plus ramus 132–150 [132] long. Seminal capsule 43–58 [43] long. Sternite VIII with 28–32 [32] setae. Tergite IX with 15 [15] setae, and 3 [3] lateral setae. Segment X with 6 [6] setae on each side.

Etymology. *Latisetus*, from the Latin "*lati*-" meaning broad and "*setus*" meaning hair, referring to the broad and stout "p" seta of the gonostylus.

Distribution. Argentina (Rio Negro Province: Nahuel Huapi National Park).

***Rheochlus insignis* Brundin, 1966**

(Figs 10–14)

Type material examined. Paratypes: 2 adult ♂♂ and 2 ♀♀, "**CHILE / Cautín province /** Río Llancahue at lago Pellaifa / 20-i-1958 / J. Illies col" (SMNH).

Additional material examined. 1 adult ♂ and 1 adult ♀, "**ARGENTINA / Río Negro province** / Valle del Challhuaco / Mallín de los patos / 41°15'48.6"S/71°17'50.3"W, 1020 m. a.s.l. / 10-i-2009 / Siri A. col / sweep net" (MLPA).

Redescription. ***Male imago*** (n = 2–3) (Figs 10–12). Total length 2.38–3.23 mm, total length / wing length 1.30–1.63.

Head. Eyes hairy. Antenna with 14 flagellomeres (Fig. 10). Ultimate flagellomere 150 long, penultimate 80 long. AR 0.43. Temporals 14, postorbitals 8 on each side. Clypeus with 2–4 setae. Palpomere lengths (I–V): 25; 20–25; 68–70; 45–48; 53. Tentorium 125 long.

Thorax. Antepronotum with 3 setae. Dorsocentrals 19; acrostichals 20; prealars 12; supraalar 1; scutellars 8.

Wing (Fig. 11): length 1.63–1.98 mm; width 0.54–0.69 mm; L/W 2.81–2.90. C extension 90 long. VR 0.98–0.99. Width of cell r_1 at the end of vein R_1 115–145; maximum width of cell r_5 150–195; r_1/r_5 0.72–0.79. Brachiolum with 3–7 setae; squama with 9–21 setae. R with 19–44 setae, R_1 with 22–51 setae, R_{4+5} with 27–42 setae, M_{1+2} with 32 (1) setae, Cu_1 with 19 (1) setae, An with 8 setae (1). Cells r_{4+5} and m_{1+2} setose; remaining cells bare.

Legs. Spur of front tibia 48–55 long; spurs of mid tibia 33–45 and 25–28 long; of hind tibia 54–73 and 25–33 long. Apex of ti_1 48–52 wide; of ti_2 50 wide; of ti_3 50–56 wide. Comb with 10–12 spines. Lengths and proportions of legs in Table 3.

Hypopygium (Fig. 12). Tergite IX with 22 setae (1), laterosternite IX with 3 setae (1). Transverse sternapodeme 125 long. Gonocoxite 160–200 long; dorsal keel 0.78–0.82 as long as gonocoxite. Gonostylus 95–120 long; basal portion of gonostylus swollen to nearly spherical; apical lobe 50–63 long, apical seta ("t" seta) 13 long; subapical lobe 19–25 long. Seta "p" 88–90 long, 4–5 wide, L/W of seta "p" 18–22. The "x" and "y" setae discernible, the "x" proximal and the "y" distal to mid section of the apical lobe. HR 1.67, HV 2.03.

Female imago (n = 2–3) (Figs 13–14). Total length 2.24–2.40 mm. Wing length / profemur length 3.29–3.46.

Head. Hairy eyes. Antenna with 13 flagellomeres (Fig. 13). Last flagellomere 83–113 long, broader than the preceding ones; about as long as flagellomeres 10–12. AR 0.18–0.20. Temporal setae 24; postorbitals 8 on each side (1). Palpomere lengths (I–V): 22; 45; 80; 48; 63 (1). Tentorium 88 long.

Thorax. Setation (1): Antepronotum with 4 setae. Dorsocentrals 27; acrostichals 20; prealars 5; supraalar 1; scutellars 10.

Wing (Fig. 14): length 1.78–2.21 mm; width 0.68–0.92 mm; L/W 2.40–2.60. C extension 63–88 long. VR 0.97–1.02. Width of cell r_1 at the end of vein R_1 130–180; maximum width of cell r_5 160–230; r_1/r_5 0.78–0.81. Brachiolum with 3–7 setae; squama with 4–7 setae. R with 25–41 setae, R_1 with 33–70 setae, R_{4+5} with 73–135 setae, M_{1+2} with 39–83 setae, Cu_1 with 24–45 setae, An with 24 (1) setae. Almost all cells setose.

Legs. Spur of front tibia 35–50 long; spurs of mid tibia 23–28 long and 28 long; of hind tibia 60–75 long and 23 long. Apex of ti_1 40–50 wide; of ti_2 40 (1) wide; of ti_3 45–60 wide. Comb with 10–11 spines. Lengths and proportions of legs in Table 4.

Genitalia. Cercus 70–75 long, broadly triangular; noto plus ramus 200 long. Seminal capsule 40–58 long. Sternite VIII with +65 (1) setae. Tergite IX with 34 (1) setae. Segment X with 9 (1) setae on each side.

Distribution. Chile (Cautín province), Argentina (Rio Negro Province: Nahuel Huapi National Park).

Rheochlus prolongatus Brundin, 1966

(Fig. 15)

Type material examined. HOLOTYPE: adult ♂, "**ARGENTINA / Santa Cruz province / Río Gallegos /** 28.iii.58 / Besch W. col." (SMNH).

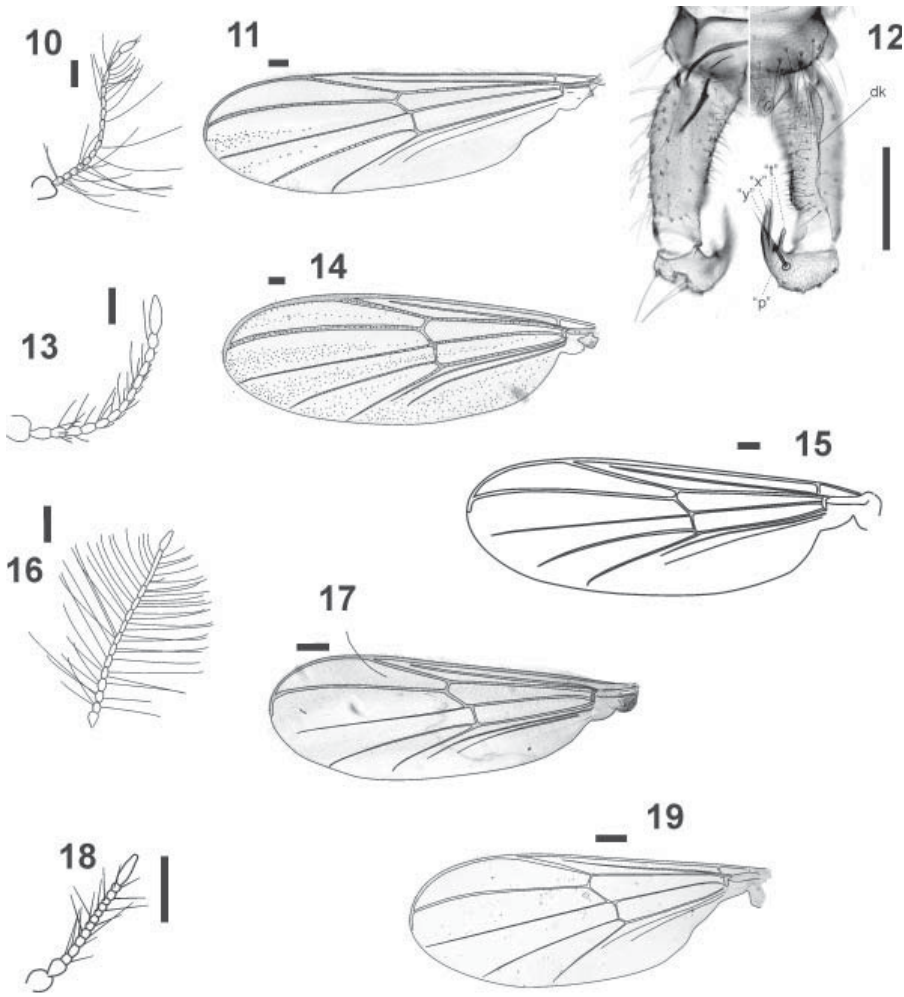
Redescription. ***Male imago*** (n = 1, holotype) (Fig. 15). Total length 1.79 mm, total length /wing length 1.10.

Head. Eyes hairy. Antenna with 14 flagellomeres. AR 0.60. Temporal, postorbital and clypeal setae could not be counted. Palpomere length not measurable.

Thorax. Setae could not be counted.

Wing (Fig. 15): length 1.63 mm; width 0.60 mm; L/W 2.71. VR 0.98. Width of cell r_1 at the end of vein R_1 120; maximum width of cell r_5 165; r_1/r_5 0.73. Brachiolars could not be counted; squama with 7 setae. R with 7 setae, R_1 with 6 setae, R_{4+5} with 6 setae. Remaining veins bare.

Legs. Spur of front tibia 40 long; one spur of mid tibia 33, the other one not observed; of hind tibia 75 and 30 long. Apex of ti_1 40 wide; of ti_3 63 wide. Comb with 15 spines. Lengths and proportions of legs in Table 3.



Figs 10–19. *Rheochlus* species adults. 10–12 – *R. insignis* Brundin, 1966, male: 10 – antenna; 11 – wing; 12 – hypopygium, dorsal view (right) and ventral view (left). 13–14 – *R. insignis* Brundin, 1966, female: 13 – antenna; 14 – wing. 15 – *R. prolongatus* Brundin, 1966, male wing. 16–19 – *R. wirthi* (Freeman, 1961): 16–17 – male (16 – antenna, 17 – wing); 18–19 – female (18 – antenna, 19 – wing). Scale bars = 100 μm.

Hypopygium (see Fig. 357 in Brundin 1966). Setae on tergite and laterosternite IX could not be counted. Transverse sternapodeme 80 long. Gonocoxite 150 long; dorsal basal keel 0.58 as long as gonocoxite. Gonostylus 85 long; apical lobe 37 long, apical seta ("t" seta) 14 long; subapical lobe 21 long, apical seta ("t" seta) 12 long. Seta "p" 65 long, 3 wide, L/W of seta "p" 21.7. The "y" seta situated in the apical third, the "x" seta at mid-length of outer margin. HR 1.76, HV 1.19.

Distribution. Argentina (Santa Cruz province: Rio Gallegos).

Rheochlus wirthi (Freeman, 1961)

(Figs 16–19)

Material examined. 2 adult ♂♂ and 2 adult ♀♀, "**AUSTRALIA** / Narrabeen / Middle Creek / New South Wales / September 1956 / W. W. Wirth leg" (SMNH).

Redescription. ***Male imago*** (n = 1–2) (Figs 16–17). Total length 1.34–1.53 mm, total length / wing length 1.29–1.54.

Head. Eyes hairy. Antenna with 14 flagellomeres (Fig. 16). AR 0.40. Temporals and postorbital setae could not be counted. Clypeus with 2 setae. Palpomere lengths (I–V): 10–12; 13–15; 20; 15–20; 30. Tentorium 70 long.

Thorax. Setae could not be counted.

Wing (Fig. 17): length 0.99–1.35 mm; width 0.37 mm; L/W 2.68–2.80. VR 0.88–0.92. Width of cell r_1 at the end of vein R_1 80–83; maximum width of cell r_5 115–130; r_1/r_5 0.62–0.72. Brachiolum with 2–3 setae; squama with 1–2 setae. R with 0–1 setae, R_1 with 2–3 setae, R_{4+5} with 1 seta. Remaining veins bare.

Legs. Spur of front tibia 25–28 long; spurs of mid tibia 15 and 20 long; of hind tibia 15 and 48–50 long. Apex of ti_1 32 wide; of ti_3 34 wide. Comb with 9–12 spines. Lengths and proportions of legs in Table 3.

Hypopygium. Setae on tergite IX could not be counted, laterosternite IX with 2 setae. Phallapodeme 45 long; transverse sternapodeme 65 long. Gonocoxite 75 long; short basal keel 0.43 (1) as long as gonocoxite. Gonostylus 45 long; apical lobe 17 long, apical seta ("t" seta) 7 long; subapical lobe 10 long, with apical seta ("t" seta) 9 long. Seta "p" 35 long, 3 wide, L/W of seta "p" 11.7. The "x" and "y" setae discernible. The "y" seta close to the base of the apical lobe, clearly proximal to the "x" seta. HR 1.67, HV 2.03.

Female imago (n = 1–2) (Figs 18–19). Total length 1.45 mm. Total length / wing length 1.39. Wing length / profemur length 3.64–4.00.

Head. Eyes hairy. Antenna with 13 flagellomeres (Fig. 18). Last flagellomere 55 long, almost as long as flagellomeres 8–12 together. Flagellomeres 2–12 short, almost as long as wide. AR 0.25. Temporal setae 4; postorbitals 3 on each side; clypeals 2. Palpomere lengths (I–V): 8; 15; 18; 20; 25. Tentorium 63 long.

Thorax. Setae could not be counted.

Wing (Fig. 19): length 1.02–1.04 mm; width 0.41–0.42 mm; L/W 2.4–2.5. VR 0.88–0.91. Width of cell r_1 at the end of vein R_1 90; maximum width of cell r_5 133; r_1/r_5 0.68. Brachiolum with 0? setae; squama with 0? setae. R with 2 setae, R_1 with 4–5 setae, R_{4+5} with 3–7 setae. Remaining veins bare. C extension 50–63 long.

Legs. Spur of front tibia 20–23 long; spurs of mid tibia 20 and 10–13 long; of hind tibia 46–48 and 15 long. Apex of ti_1 24 wide; of ti_3 40 wide. Comb with 9–11 spines. Lengths and proportions of legs in Table 4.

Genitalia. Cercus 30 long; noto plus ramus 150 long. Seminal capsule 40 long. Sternite VIII with 16 setae. Tergite IX with 8 setae, plus 4 lateral setae.

Distribution. Australia (New South Wales: Narrabeen).

Cladistic analysis

Under implied weights with k = 5–20, six trees (Fit = 2.36; CI = 0.73; RI = 0.78) were obtained. The trees under k = 5 showed the best Bremer support. Strict consensus of these

Table 3. Lengths (µm) and proportions of male legs of *Rheochlus* spp. Abbreviations: femur (fe); tibia (ti); tarsomeres 1-5 (ta1-5); 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.

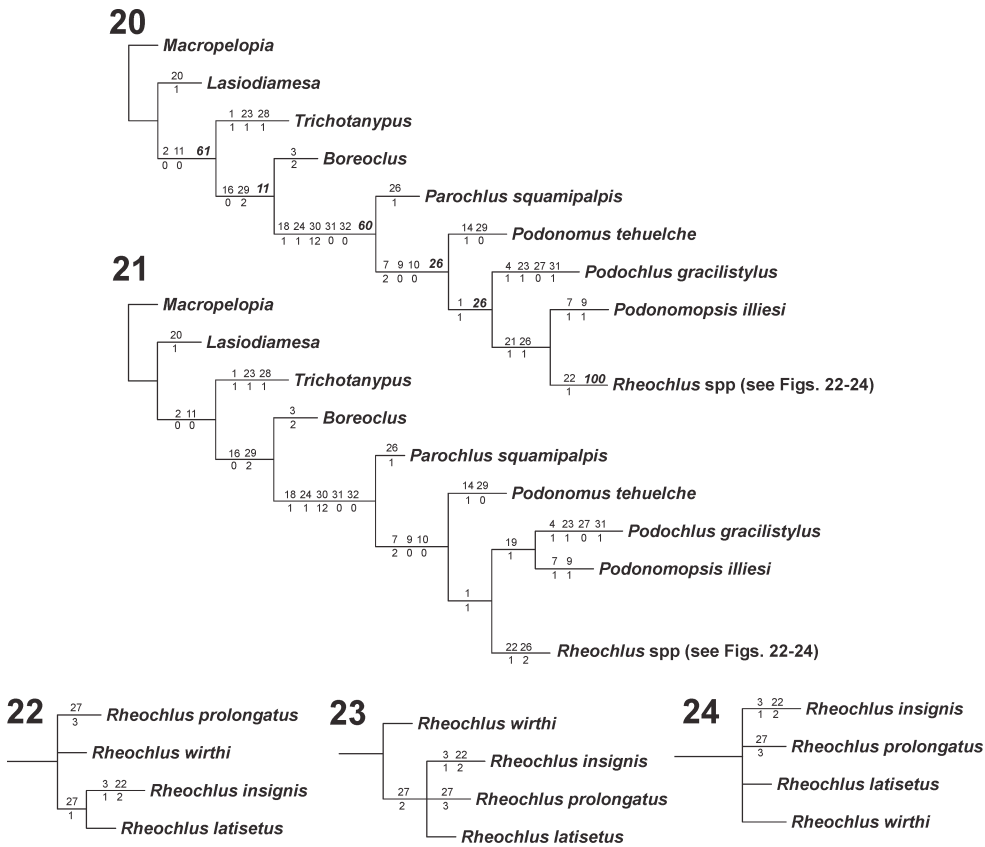
		fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
R. latisetus	p_1	335–378	400–407	199–210	100–116	70–79	40–58	55–66	0.49–0.53	3.08–3.57	3.92–3.94
(n = 2)	p_2	410–440	360–374	166–174	100–108	60–75	45–50	50–58	0.45–0.47	3.34–3.44	4.62–4.85
	p_3	430–457	457–481	241–249	158–166	91–100	55–58	58–60	0.52–0.53	3.09–3.20	3.77–3.79
R. insignis	p_1	530–655	580–750	350–480	190–250	120–140	70	70	0.61–0.64	3.20–3.56	2.93–3.06
(n = 2–3)	p_2	600–740	590–770	310–420	180–220	110–120	60–70	60–70	0.51–0.55	3.61–4.09	4.02–4.09
	p_3	640–800	700–950	410–570	225–300	115–160	70–90	70–80	0.59–0.60	3.68–3.75	3.68–3.75
R. prolongatus	p_1	465	530	245	145	100	70	60	0.46	3.34	4.10
(n = 1)	p_2	550	525	230	150	95	65	55	0.44	3.62	4.67
	p_3	595	660	340	215	130	75	65	0.52	3.33	3.69
R. wirthi	p_1	300–308	330–350	170–175	88–95	50–63	45–48	38–40	0.50–0.52	3.43–3.57	3.71–3.76
(n = 1–2)	p_2	360	310–340	160–175	90–100	45–60	35–40	30–45	0.52	3.57–4.15	4.00–4.19
	p_3	350–370	390–420	180–200	115–133	80	40–45	43–45	0.46–0.48	3.29	3.95–4.11

Table 4. Lengths (µm) and proportions of *Rheochlus* spp. female legs. Abbreviations: femur (fe); tibia (ti); tarsomeres 1-5 (ta1-5); 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	ta_4	ta_5	LR	BV	SV
R. latisetus	p_1	330–360	370–374	180–188	100–105	68–75	50	55–58	0.49–0.50	3.20–3.23	3.89–3.90
(n = 2)	p_2	430	360–370	143–150	90–98	65–68	43–45	50–55	0.40–0.41	3.66–3.67	5.33–5.54
	p_3	450–460	475–490	225–230	145–148	88–95	48–53	58–60	0.47	3.34–3.37	4.09–4.16
R. insignis	p_1	600–635	670–710	350–440	210	120–145	80–85	80–90	0.60–0.62	3.36–3.41	3.06–3.18
(n = 2)	p_2	710–800	720–730	305–370	185–200	100–120	60	70–90	0.48–0.51	4.13–4.18	4.14
	p_3	750–800	870–935	410–510	210–280	150–160	70–95	80–90	0.55	3.59–4.04	3.38–3.40
R. wirthi	p_1	250–280	255–290	140–150	80	50–60	30–40	35–40	0.52–0.55	3.23–3.47	3.61–3.77
(n = 3)	p_2	310–320	270–300	125–130	75	50	35	40	0.43–0.46	3.67–3.78	4.76–4.84
	p_3	325–340	370–375	150–170	100–110	65–70	35–40	40–50	0.41–0.45	3.37–3.43	4.23–4.63

trees (not illustrated) agrees with the selected most parsimonous trees in Figs 20–21 except for unresolved nodes (*Podonomopsis–Podochlus–Rheochlus*) and unresolved topology within *Rheochlus* (as in Fig. 24).

Our results demonstrate that the tribe Boreochlini is paraphyletic. They also show that the tribe Podonomini is monophyletic, and includes *Podonomus, Parochlus, Podonomopsis, Podochlus* and *Rheochlus* (Figs 20–21). Morphological support is gained from the male adult gonostylus with apical and subapical lobes, hind leg comb well developed, and in the female



Figs 20–24. Cladistic analysis of the genus *Rheochlus* Brundin, 1966. 20–21 – selected most parsimonous trees showing two alternative positions of *Rheochlus* revealed in the analysis. 22–24 – three alternative topologies reveled for the species of *Rheochlus*. Numbers in italics above branches of Figure 1 represent the Bremer Support. Synapomorphies are mapped on the branches (characters number above and character state below the branch).

from the slight to strong thickening of the vein R_1 and the lack of any dorsal part of the tenth segment of the abdomen.

Analyzing the six trees obtained, two main tree topologies for Podonomini were recognized (Figs 20–21). One topology included in three trees, placed *Podonomopsis* as the sister group of *Rheochlus*, sharing the conspicuous slenderness of the gonostylus (Fig. 20). In these trees *Podochlus* is the sister group of *Podonomopsis* + *Rheochlus*, sharing hairy eyes and wing vein R_1 being slightly swollen in the female. The second topology (Fig. 21) included in the remaining trees, placed the clade *Podochlus*-*Podonomopsis,* sharing the presence of a small hyaline lateral lobe on tergite IX, as the sister group to *Rheochlus*.

We found the genus *Rheochlus* to be monophyletic, which was supported by the dorsobasal keel of the gonostylus in the first and second topologies, and by the stout "p" seta in

the second topology. Within each main topology described above, phylogenetic relationships among *Rheochlus* species gave rise to three different hypotheses. In one reconstruction (Fig. 22), *R. insignis* and *R. latisetus* share the medial position of the setae "x" and "y" on the apical lobe, while *R. wirthi* and *R. prolongatus* are unresolved. In another reconstruction (Fig. 23), the Australian *R. wirthi* is the sister group to the South American species, distinguished by the relative position of the "x" and "y" setae of the gonostylus. In a third reconstruction (Fig. 24), all *Rheochlus* species are unresolved.

A second analysis was performed in which the character "position of setae x and y" was coded as additive, following the character evolution suggested by Brundin (1966). He stated that the character transformation was from a more plesiomorphic apical to a more apomorphic basal position. This analysis yielded two trees (L = 56; Fit = 3.45; CI = 0.73; RI = 0.76), which coincide with the two main topologies that are described above (Figs 20–21). In both trees, the Australian *R. wirthi* is the sister group to the South American species (Fig. 23).

Discussion

Comments on morphology of *Rheochlus*. The new species *R. latisetus* sp. nov. is rather similar to *R. prolongatus*, but is distinguished by a longer maxillary palp; shorter wings; higher number of setae on squama, higher LRI; gonostylus less globose and with the subapical lobe broader than the apical. The poor status of the holotype of *R. prolongatus* does not allow full comparison with the new species.

Rheochlus latisetus is distinctly separated from *R. insignis* by its smaller size, narrower wings with less dense hair, lower leg ratios, and shorter dorsal keel on the gonocoxite. Brundin (1966) described the basal portion of the gonostylus of *R. insignis* as strongly swollen and almost spherical. The latter character was not evident in the material we studied. The female of *R. latisetus* is distinguished from *R. insignis* by the generally smaller size, almost bare wings and shorter flagellomeres 2–12 of the antenna.

Both *R. latisetus* and the Australian *R. wirthi* have a short dorsal keel of the male gonocoxite, but *R. wirthi* is easily distinguished by its smaller size, slightly narrower r_1 of the wing, and the basal position of the "x" and "y" setae. Wing and female antennae of *R. wirthi* are similar to that of *R. latisetus*, but the former is distinguished by its smaller size.

Cladistic analysis. *Rheochlus* is a monophyletic genus distinguished by the dorsobasal keel on the gonocoxite and the wide and stout "p" seta on the gonostylus. A dorsobasal keel on the gonocoxite is also present in *Boreochlus* and *Trichotanypus*, but the shape of the "dorsal lobes" are clearly different from those of *Rheochlus*. The "p" seta of *Podonomopsis* and *Parochlus* is evident but not as stout as that of *Rheochlus*. A reduced "p" seta is also present within some *Podonomus* species (Brundin 1966).

The phylogenetic relationships of *Rheochlus* are not fully clarified in our analysis. A probable resolution is that the three South American *Rheochlus* species form a monophyletic group, with the Australian species as a sister group. This is in agreement with the phylogenetic and biogeographical analysis of Brundin (1966), who argued that the Podonominae of Tasmania-Australia are always more apomorphic compared to those of South America. In *Rheochlus* a transition has occurred from a plesiomorphic apical setae position on the apical gonostylus lobe of the South American *R. prolongatus* to an apomorphic basal position of the Australian

R. wirthi. The intermediate stage is seen in the South American *R. insignis* and *R. latisetus*.

In our analysis, *Rheochlus* is closely related to *Podonomopsis* and *Podochlus*, which agrees with previous studies of Brundin (1966), Cranston & Edward (1998) and Boothroyd & Cranston (1999). These genera share characters such as hairy eyes, slight thickening of the female wing vein R_1, and a tendency towards reduction of the maxillary palps. Hairy eyes are also present in *Trichotanypus* as well as in a few *Podonomus* and *Parochlus* species. With respect to vein R_1, *Podonomopsis*, *Podochlus* and *Rheochlus* share the slight thickening of the distal end, while in *Parochlus* and *Podonomus* the thickening is very strong. In the remaining Podonominae genera, this vein is not distally thickened. Reduction of the maxillary palp is also found in *Boreochlus* and in a few species of *Parochlus*.

Antennae with 13 flagellomeres in the females of *Podonomopsis*, *Podochlus*, *Rheochlus* and *Boreochlus* is probably an intermediate stage between the plesiomorphic 14-flagellomere antennae of most Boreochlini genera, and the apomorphic reduction to 12 or fewer flagellomeres in *Podonomus* and *Parochlus.*

One of our topologies placed *Podonomopsis* as the sister group of *Rheochlus*, and *Podochlus* basal to these genera with reference to the plesiomorphic retention of the double gonostylus. This is in agreement with the analysis of Brundin (1966). The slenderness of the male gonocoxite is a synapomorphy for *Rheochlus* and *Podonomopsis*, while the slightly thickened wing vein R_1 of the female is also shared with *Podochlus*. We agree with Brundin (1966) that the retraction of the "x" and "y" setae of the apical lobe of *Podonomopsis*, is probably an apomorphic feature.

As also found in previous studies, our analyses confirm that the tribe Podonomini is monophyletic and includes *Podonomus, Parochlus, Podonomopsis, Podochlus* and *Rheochlus*. The synapomorphies shared among Podonomini genera are gonostylus with apical and subapical lobes, slight to strong thickening of the wing vein R_1 in females, and the lack of a dorsal part of the abdominal segment X. The tribe Boreochlini remains paraphyletic as suggested by the studies of Cranston et al. (2010, 2011).

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