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BIOLOGICAL SCIENCES

The immatures of three Neotropical species of *Palpomyia* Meigen (Diptera, Culicomorpha, Ceratopogonidae)

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Abstract: The first description of the fourth instar larva and pupa of *Palpomyia mapuche* Spinelli, Grogan & Ronderos and the pupa of *P. subfuscula* Ingram & Macfie are provided, as well as the redescription of the pupa of *P. subaspera* (Coquillett). Studied specimens were collected in lotic environments of Argentinian Patagonia, in Neuquén and Chubut Provinces. The described stages were examined and illustrated with a phase-contrast microscope. The larva was examined using a scanning electron microscope. Data on the bionomics for *P. mapuche* and new records for the three species are provided.

Key words: Biting midges, immature stages, larva, Palpomyiini, pupa.

INTRODUCTION

Palpomyia Meigen, a worldwide genus of predaceous midges of the tribe Palpomyiini, includes 283 species. Of these, 46 are recorded for the Neotropical region by Borkent & Spinelli (2007), and three more were recently described for this region: *P. ryszardi* by Spinelli & Ronderos (2013) and *P. amazonensis* and *P. lanceolata* by Feijó (Almeida et al. 2017). They are relatively common inhabitants of streams, lakes and ponds, swamps, marshes and sphagnum bogs (Grogan & Wirth 1975, 1979), and their preimaginales stages are poorly known. So far, immatures are known only for five species that inhabit the Neotropics: *P. guarani* Lane, *P. lacustris* Lane, Forattini & Rabello, *P. ryszardi*, *P. subaspera* (Coquillett) and *P. wirthi* Lane, Forattini & Rabello.

Spinelli et al. (2009) recognized eight species from Patagonia. Two of them, *P. aculeata* Ingram & Macfie and *P. subaspera*, are included in the *tibialis* group, and the remaining six in the *distincta* group: *P. mapuche* Spinelli, Grogan & Ronderos, *P. marinoi* Spinelli, Grogan & Ronderos, *P. patagonica* Ingram & Macfie, *P. septentrionalis* Spinelli, Grogan & Ronderos, *P. subfuscula* Ingram & Macfie and *P. yamana* Spinelli, Grogan & Ronderos. Of these species only the pupa of *P. subaspera* is known, described by Grogan & Wirth (1979) from Nearctic specimens.

During a recent survey carried out in northwestern Argentinean Patagonia, larvae and pupae of *P. mapuche* and pupae of *P. subaspera* and *P. subfuscula* were collected. The purpose of this paper is to describe the immatures of *P. mapuche* and *P. subfuscula* and to redescribe the pupa of *P. subaspera* with modern standards.

MATERIALS AND METHODS

Larvae and pupae were collected from the bordering vegetation of two streams in Chubut Province, one located in the Subantartic forest

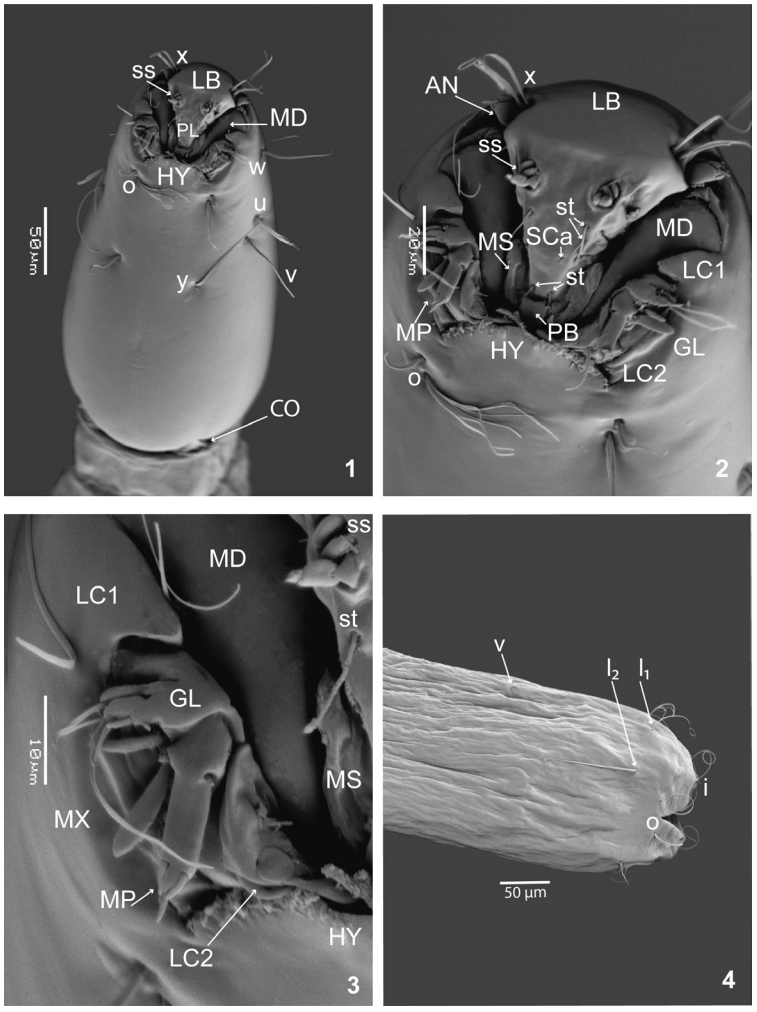
and the other in the Patagonian steppe, and from the bordering mud of a river in the steppe of the Neuquén Province. In all cases, the substrate was removed with the aid of a strainer and transferred to a white tray where immatures were collected with a pipette. Larvae were placed in individual containers with water and substrate from their natural environment. Pupae were isolated in a vial with a drop of water, and observed daily until adult emergence. Adults were allowed to harden for 24 h before being preserved in ethanol to ensure their complete pigmentation. For detailed examination with phase-contrast microscope, larval and pupal exuviae and adults were mounted in Canada balsam following the technique described by Borkent & Spinelli (2007). Photomicrographs were taken with a Leica EC3 digital camera, through a Leica DM 500 microscope. Illustrations were drawn with Adobe illustrator CC®. Larvae were also examined using scanning electron microscopy (SEM) (JOEL 2000) following the technique of Ronderos et al. (2000, 2008). Measurements were taken with a (BCM) Leitz Wetzlar binocular microscope. The temperature of the water and air were measured with an alcohol thermometer in degrees Celsius. For larval terms see Anjos-Santos et al. (2017); for pupal terms, see Borkent (2014). Studied specimens are deposited in the collection of the Museo de La Plata, La Plata, Argentina (MLPA).

RESULTS

distincta group

Palpomyia mapuche Spinelli, Grogan & Ronderos Spinelli et al. 2009: 50 (female, male); Borkent 2016: 170 (in online World catalog).

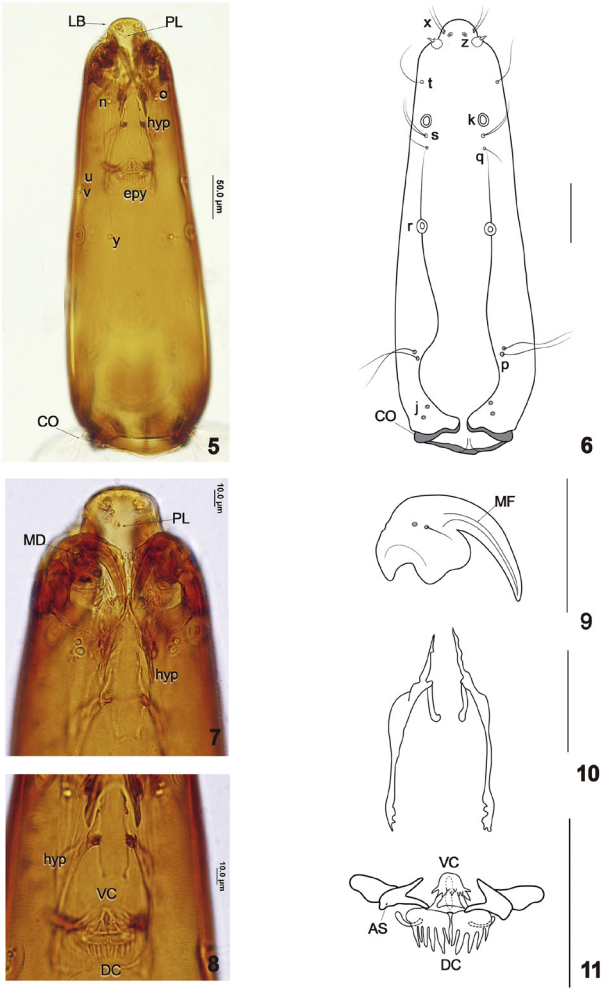
Description of fourth instar larva (Figs. 1–11). Head capsule (Figs. 1–2, 5–6) pale brown, about 2.8 times longer than wide, apex slightly bent ventrally, HL 0.42 (n = 2) mm; HW 0.14–0.19 (0.17, n = 2) mm, HR 2.21–3.00 (2.61, n = 2) mm; SGW 0.104 (n = 2) mm; SGR 1.35–1.83 (1.59, n = 2). Setae simple, medium-sized to long, setae "x", "s", "u" and "o" branched, chaetotaxy as in Figs. 1–2, 5–6. Antenna cylindrical, medium-sized, length 0.02 mm. Labrum (Figs. 1, 2, 5) longer than broad, not extending beyond hypostoma, palatum with three pairs of anterolateral sensilla styloconica (Figs. 1–3), two pairs of sensilla trichoidea (Figs. 2–3) and one pair of campaniform sensillum (Fig. 2); messors (Figs. 2–3) stout, curved, sclerotized, situated away from mandibles; palatal bar (Fig. 2) triangular, situated immediately posterior to messors. Mandible (Figs. 1–3, 7, 9) hooked, strongly sclerotized, apical tooth long, sensory pit and one medium-sized, thin seta present on the aboral surface; with prominent point of articulation, deep fossa mandibularis on ectal surface (Fig. 9); MDL 0.06–0.08 (0.07, n = 2) mm, MDW 0.040–0.044 (0.041, n = 2) mm. Maxilla (Fig. 3) galeolacinia with lacinial sclerite 1 (Figs. 2, 3) with one long, thin seta, lacinial sclerite 2 (Figs. 2, 3) with medium-sized, stout seta; maxillary palpus (Figs. 2, 3) elongated, slightly flattened, with 3 subapical papillae. Hypostoma (Figs. 1–3) smooth on medial margin, flanked by fine tooth. Epipharynx (Figs. 5, 8, 11) gently massive, with 2 combs: ventral comb with 9–10 stout, pointed teeth, dorsal comb with 6 long, pointed teeth on posterior edge; lateral arms elongate; small, curved auxiliary sclerites near lateral arms; LAW 0.048–0.050 (0.049, n = 2) mm, DCW 0.023–0.025 (0.024, n = 2) mm. Hypopharynx (Figs. 5, 7–8, 10) elongate, thin, gently sclerotized, arms slender. Thoracic pigmentation uniformly pale yellowish. Caudal segment (Fig. 4) about 2.3 times as long as wide, with two pairs of long, stout setae "o", two pairs of long, thin setae "i"; two pairs of long, thin setae "l_1", one pair of long, stout setae "l_2", one pair of medium-sized, thin setae "v". CSL 0.65–0.70 (0.68, n = 2) mm, CSW 0.27–0.32 (0.30, n = 2) mm, CSR 2.03–2.60 (2.32, n = 2), OL 0.26–0.30 (0.28, n = 2) mm, OD 0.013–0.015 (0.014, n = 2) mm.



Figures 1-4. ***Palpomyia mapuche*** **Spinelli, Grogan & Ronderos, fourth instar larva (SEM). 1. head capsule, chaetotaxy, frontoventral view; 2. head capsule detail, anterofrontal view; 3. mouthparts, anterofrontal view; 4. caudal segment, lateral view. Scale 0.05 mm. Antennae (AN); collar (CO); galeolacinea (GL); hypostoma (HY); labrum (LB); lacinial sclerite 1 (LC1); lacinial sclerite 2 (LC2); mandible (MD); messors (MS); maxilla (MX); maxillary palpus (MP); palatal bar (PB); palatum (PL); sensilla campaniformia (SCa); sensilla styloconica (ss); sensilla trichoidea (st). Head capsule chaetotaxy: o, parahypostomal setae; u, mesolateral setae; v, posterolateral setae; w, anterolateral setae; x, parantennal setae; y, ventral setae. Caudal segment chaetotaxy: "i", inner seta; "l_1", first lateral seta; "l_2", second lateral seta; "o", outer seta; "v", ventral setae.**

Description of female pupa (Figs. 12–15, 25–28, 38–39). Habitus as in Fig. 14. Exuviae pale brown. Total length 4.12–4.65 (4.34, n = 4) mm. *Head*: Dorsal apotome (Fig. 13) with disc 2 times broader than long, bearing rounded small tubercles, anterior margin covered with stout rounded spinules; posterior margin slightly concave; posterolateral margin with broad raised areas, bearing two dorsal apotomal sensilla; antenna extending posteriorly to midleg (Fig. 14); mouthparts (Fig. 25) with mandible well developed; palpus extending to posterolateral margin of labium; labium separated medially by labrum; apex of labrum slightly rounded; sensilla: dorsal apotomals (Fig. 13): DA-1-H long, thin seta, located on rounded small tubercle, DA-2-H campaniform sensillum; DAL 0.24–0.26 (0.25, n = 5) mm; DAW 0.25–0.27 (0.26, n = 5) mm; DAW/DAL 0.97–1.04 (1.01, n = 5); two dorsolateral cephalic sclerites (Fig. 26): DL-1-H short, stout seta, DL-2-H campaniform sensillum; clypeal/labrals (Fig. 25): CL-1-H medium-sized thin seta, CL-2- H long, thin seta; oculars (Fig. 25): O-1-H short, stout seta, O-2-H campaniform sensillum, O-3-H long, thin seta. Cephalothorax rectangular, surface predominantly smooth with small spinules on mesonotum, between bases of respiratory organs. Length of cephalothorax 1.55–1.67 (1.60, n = 4) mm, width 1.05–1.19 (1.10, n = 3) mm. *Thorax*: Respiratory organ (Figs. 12, 14,

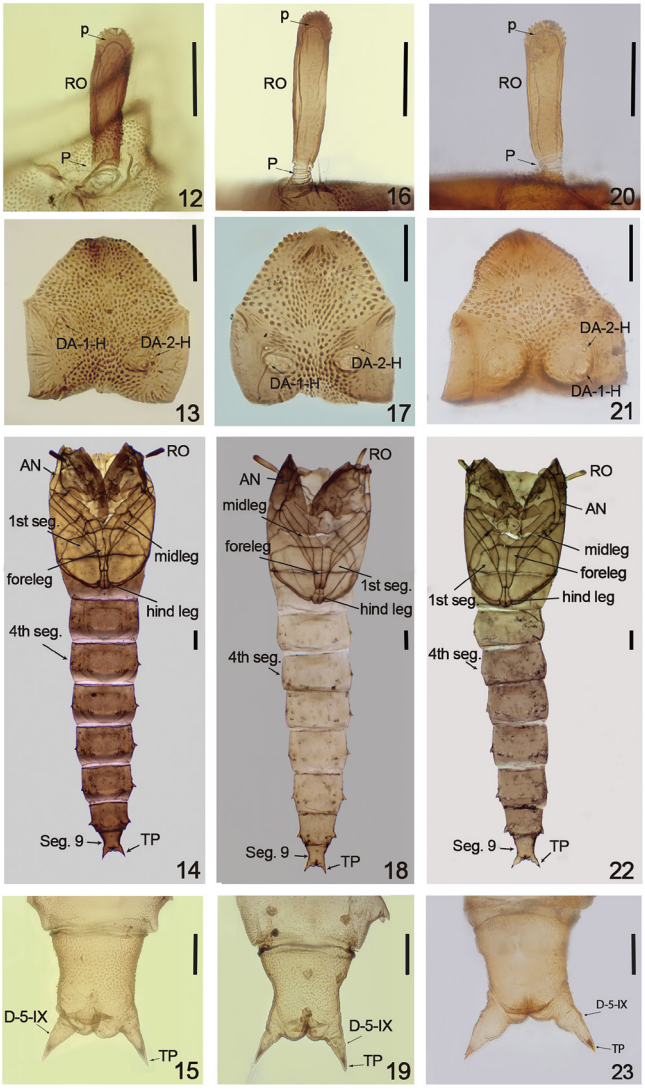
27) smooth, brown, about 3.80–4.04 (3.96, n = 5) times longer than broad, apex rounded, with row of 12–15 pores closely abutting at apex; pedicel (Figs. 12, 27) slender, P 0.020–0.024 (0.021, n = 5) mm; RO length 0.208–0.228 (0.215, n = 5) mm, RO width 0.052–0.060 (0.054, n = 5) mm; P/RO 0.095–0.109 (0.097, n = 5); sensilla: three anteromedials (Fig. 27): AM-1-T, AM-2-T long, stout setae; AM-3-T campaniform sensillum; one anterolateral (Fig. 27): AL-1-T short, stout seta; dorsals (Fig. 28): D-1-T, D-2-T, D-4-T, long, thin setae, D-3-T campaniform sensillum, D-5-T minute seta, all on small rounded tubercle, except D-3-T; supraalar (SA-2-T) campaniform sensillum; metathoracic (Fig. 38): M-3-T campaniform sensillum, near anterior margin of metathorax. *Abdomen*: segments covered with small spicules, segments with simple setae; segment 9 (Figs. 14–15) approximately 1.5 longer than broad, length 0.28–0.33 (0.30, n = 5) mm, width 0.18–0.22 (0.20, n = 5) mm; dorsal and ventral surface covered with pointed spicules; ventral surface with one small, circular mark located on a wide bare, mesal area; terminal process (Figs. 14–15) moderately short, nearly straight, base wide, with few pointed spicules, extreme tip somewhat dark, length 0.09–0.12 (0.10, n = 5) mm, width 0.03–0.05 (0.04, n = 5) mm; sensilla: tergite 1 (Figs. 14, 38) with two anteromesals: D-2-I short, stout seta, D-3-I long, thin seta; 5



Figures 5-11. ***Palpomyia mapuche*** **Spinelli, Grogan & Ronderos, fourth instar larva. 5, 7-8. photomicrographs. 6, 9-11. draw illustrations. 5. head capsule, ventral view; 6. head capsule, dorsal view; 7. detail of anterior portion of head capsule and hypopharynx, ventral view; 8, 11. epipharynx, ventral view; 9. left mandible, ventral view; 10. hypopharynx, ventral view. Scale 0.05 mm.**
Collar (CO); dorsal comb (DC); mandible (MD); fossa mandibularis (MF); labrum (LB); palatum (PL); ventral comb (VC). Head capsule chaetotaxy: j, colar pits; k, pronotal pits; o, parahypostomal setae; p, posterior perifrontal setae; q, postfrontal setae; r, postnotal pits; s, anteroperifrontal setae; t, prefrontal setae; x, parantennal setae; z, frontal pits.

posterior sensilla: D-4-I, D-7-I campaniform sensilla, D-5-I short, stout seta, D-8-I medium-sized, stout seta, D-9-I long, thin seta; 3 lateral sensilla: L-1-I long, thin seta, L-2-I medium-sized, stout setae, L-3-I minute seta; segment 4 (Figs. 14, 39): D-2-IV, D-3-IV medium-sized, thin seta, D-4-IV, D-7-IV campaniform sensilla, D-5-IV short, stout seta, D-8-IV medium-sized, stout seta, D-9-IV long, thin seta; L-1-IV, L-2-IV, L-4-IV medium-sized, stout setae, L-3-IV long, thin seta, all on small or medium-sized tubercles; V-5-IV, V-7-IV medium-sized, stout setae, V-6-IV long, thin seta, all on small tubercles; segment 9 (Figs. 14–15) with D-5-IX, D-6-IX campaniform sensilla.

Description of male pupa (Figs. 24, 40). Similar to female with usual sexual differences: Total length 3.74–3.96 (3.83, n = 4) mm. Dorsal apotome (Fig. 24) with distal margin slightly rounded, DAL 0.24–0.27 (0.26, n = 3) mm; DAW 0.22–0.27 (0.24, n = 3) mm, DAW/DAL 0.85–1.00 (0.93, n = 3). Cephalothorax: length 1.22–1.33 (1.29, n = 4) mm, width 0.92–1.04 (0.99, n = 3) mm. Respiratory organ 3.57–4.08 (3.78, n = 4) times longer than broad, P 0.016–0.020 (0.018, n = 4) mm; RO length 0.18–0.20 (0.19, n = 4) mm, RO

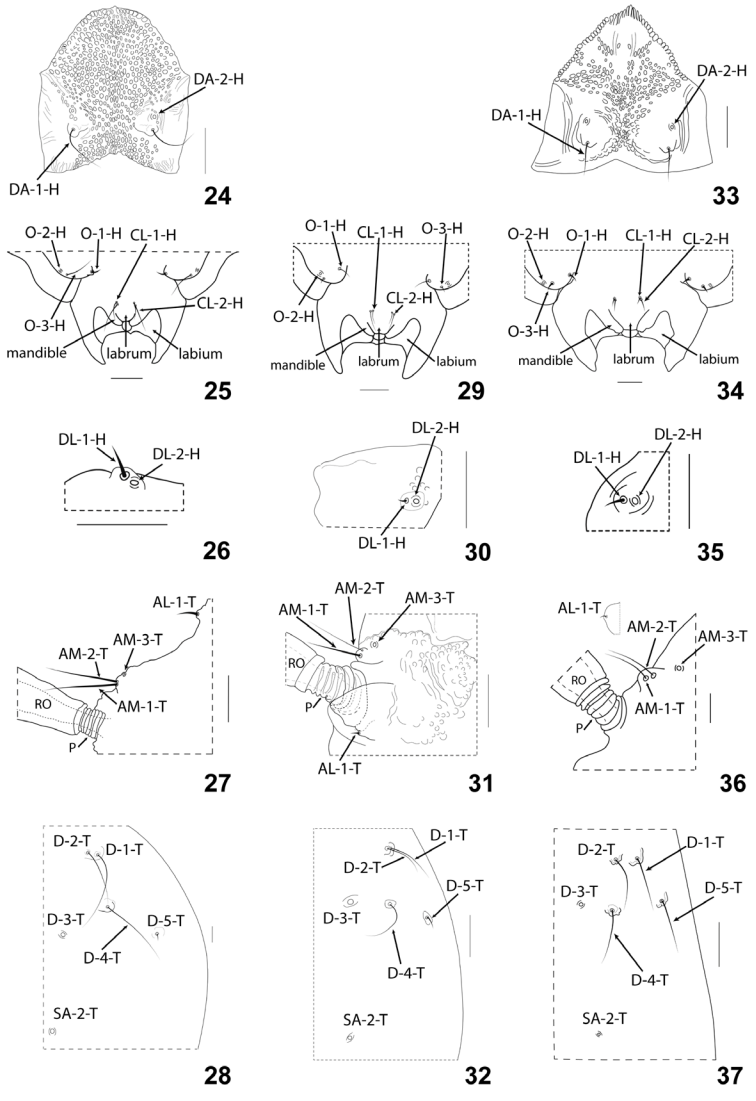


Figures 12-23. Female pupa. 12-15. ***Palpomyia mapuche*** Spinelli, Grogan & Ronderos. 16–19. ***Palpomyia subfuscula*** Ingram & Macfie. 20-23. ***Palpomyia subaspera*** (Coquillett). 12, 16, 20. respiratory organ. 13, 17, 21. dorsal apotome. 14, 18, 22. habitus. 15, 19, 23. Segment 9. Scale 0.05 mm.
Antenna (AN); dorsal apotome sensilla (DA-1-H, DA-2-H); dorsal sensilla of segment 9 (D-5-IX); pedicel (P); pore (p); respiratory organ (RO); segment 1 (1st seg.); segment 4 (4th seg.); segment 9 (Seg. 9); terminal process (TP).

width 0.05–0.06 (0.05, n = 4); P/RO 0.08–0.11 (0.10, n = 4). Segment 9 (Fig. 40) dorsal surface covered anteriorly with pointed spicules, length 0.26–0.29 (0.27, n = 4) mm, width 0.15–0.21 (0.18, n = 4) mm; terminal process (Fig. 40) length 0.09–0.11 (0.10, n = 4) mm, width 0.03–0.06 (0.04, n = 4) mm; genital lobe (Fig. 40) globose, short, not reaching posterior margin of segment, surface smooth.

Distribution. Argentina (western Neuquén, Río Negro and Chubut Provinces); Chile (Valdivia Province).

Material examined. Argentina, Chubut, Parque Nacional Los Alerces, Pucon Pai, unnamed stream, 42°49′33.5′′S; 71°36′′44.1′′W, 547 m, 20-I-2016, adults emerged in laboratory 23-I-2016, D. Anjos-Santos and P. Pessacq, 2 females, 1 male (with pupal exuviae); same data except 29-XII-2016, adult emerged 01-I-2017, D. Anjos-Santos and P. Pessacq, 1 female (with pupal exuvium); same data except adult emerged



Figures 24-37. ***Palpomyia mapuche*** **Spinelli, Grogan & Ronderos;** ***Palpomyia subfuscula*** **Ingram & Macfie;** ***Palpomyia subaspera*** **(Coquillett). 24.** ***Palpomyia mapuche*** **Spinelli, Grogan & Ronderos, male pupa. 25-28.** ***Palpomyia mapuche*** **Spinelli, Grogan & Ronderos, female pupa. 29-32.** ***Palpomyia subfuscula*** **Ingram & Macfie, female pupa. 33.** ***Palpomyia subaspera*** **(Coquillett), male pupa. 34-37.** ***Palpomyia subaspera*** **(Coquillett), female pupa. 24, 33. dorsal apotome, dorsal view; 25, 29, 34. mouthparts, ventral view; 26, 30, 35. dorsolateral cephalic sclerites, dorsolateral view; 27, 31, 36. anterolateral sensillum and anteromedial sensilla, dorsolateral view; 28, 32, 37. dorsal sensilla and supraalar sensillum, dorsal view. Scale 0.05 mm. Anterolateral sensillum (AL-1-T); anteromedial sensilla (AM-1-T, AM-2-T, AM-3-T); clypeal/labral sensilla (CL-1-H, CL-2-H); dorsal apotome sensilla (DA-1-H, DA-2-H); dorsal sensilla (D-1-T, D-2-T, D-3-T, D-4-T, D-5-T); dorsolateral cephalic sclerite sensilla (DL-1-H, DL-2-H); respiratory organ (RO); pedicel (P); ocular sensilla (O-1-H, O-2-H, O-3-H); supraalar sensillum (SA-2-T).**

04-I-2017, D. Anjos-Santos and P. Pessacq, 1 male (with pupal exuvium); same data except adult emerged 06-I-2017, D. Anjos-Santos and P. Pessacq, 1 male (with pupal exuvium); same data except pupa emerged in laboratory 01-I-2017, adult emerged 09-I-2017, D. Anjos-Santos and P. Pessacq, 1 female (with larval and pupal exuviae); same data except pupa emerged in laboratory 02-I-2017, adult emerged 10-I-2017, D. Anjos-Santos and P. Pessacq, 1 female (with larval and pupal exuviae).

Bionomics. Larvae and pupae were collected in an unnamed stream located in the Parque Nacional Los Alerces in western Chubut Province. The stream flows into the Futalaufquen lake and is surrounded by Subantarctic *Nothofagus* forest. Immatures were collected in a puddle of water with muddy bottom, decomposing organic matter and filamentous algae formed among the roots of a tree, on the bank of the stream. The water temperature ranged between 13–14 °C, and the air temperature between 28–32 °C. Under laboratory conditions, the larvae took around 5 days to reach the pupal stage, and 9 days to complete its development to the adult stage. Specimens collected as pupae completed their development in 4–9 days in the laboratory.

Palpomyia subfuscula Ingram & Macfie (Figs.16–19, 29–32, 41–42)

Palpomyia subfuscula Ingram & Macfie 1931: 216 (female; Argentina); Wirth 1974: 55 (in catalog of species south of USA); Borkent & Wirth 1997: 134 (in World catalog); Spinelli 1998: 326 (in list of Argentinean species); Borkent & Spinelli 2000: 64 (in catalog of species south of USA); Borkent & Spinelli 2007: 97 (in Neotropical synopsis); Spinelli & Marino 2009: 205 (in list of Patagonian species); Spinelli et al. 2009: 60 (in revision of Patagonian species, diagnosis, description, key, distribution); Borkent 2016: 172 (in online World catalog).

Description of female pupa. Habitus as in Fig. 18. Exuviae brownish. Total length 3.74 mm. *Head:* Dorsal apotome (Fig. 17) with disc 1.9 times broader than long, bearing rounded small tubercle, anterior margin covered with stout rounded spinules; posterior margin slightly concave; posterolateral margin with broad raised areas, bearing two dorsal apotomal sensilla; antenna extending posteriorly to midleg; mouthparts (Fig. 29) with mandible well developed; palpus extending to posterolateral margin of labium; labium separated medially by labrum; apex of labrum nearly straight; sensilla: dorsal apotomals (Fig. 17): DA-1-H long, stout seta, located on rounded small tubercle, DA-2-H campaniform sensillum; DAL 0. 22 mm; DAW 0.21 mm; DAW/DAL 0.95; two dorsolateral cephalic sclerites (Fig. 30): DL-1-H short, stout seta, DL-2-H campaniform sensillum; clypeal/labrals (Fig. 29): CL-1-H medium-sized, thin seta CL-2- H long, thin seta; oculars (Fig. 29): O-1-H medium-sized, thin seta, O-2-H campaniform sensillum, O-3-H short, thin seta. Cephalothorax rectangular, surface of mesonotum with small spinules between bases of respiratory organs. Length of cephalothorax 1.56 mm, width 1.11 mm. *Thorax:* Respiratory organ (Figs. 16, 18, 31) smooth, pale brown except distal 1/4 darker, about 4.42 times longer than broad, rounded apex, with simple row of 18–20 pores closely abutting at apex; pedicel (Figs. 16, 31) slender, P 0.02 mm; RO length 0.23 mm, RO width 0.05 mm; P/RO 0.10; sensilla: three anteromedials (Fig. 31): AM-1-T long, stout seta, AM-2-T long, thin seta; AM-3-T campaniform sensillum; anterolateral (Fig. 31): AL-1-T short, stout seta; dorsals (Fig. 32): D-1-T, D-2-T, D-4-T, long, thin setae, D-3-T campaniform sensillum, D-5-T short, thin seta, all on small rounded tubercle except D-3-T; supraalar (SA-2-T) campaniform sensillum; metathoracic (Fig. 41): M-3-T campaniform sensillum, near anterior margin of metathorax. *Abdomen:*

segments covered with small spicules, segments with simple setae; segment 9 (Figs. 18–19) approximately 1.3 longer than width, length 0.31 mm, width 0.24 mm; dorsal and ventral surface covered with pointed spicules; ventral surface with one small, circular mark located on a wide bare, mesal area; terminal process (Figs. 18–19) moderately short, nearly straight, base wide, smooth, extreme tips somewhat dark, length 0.11 mm, width 0.05 mm; sensilla: tergite 1 (Figs. 18, 41) with two anteromesals: D-2-I short, stout seta, D-3-I long, thin seta; 5 posterior sensilla: D-4-I, D-7-I campaniform sensilla, D-5-I short, stout seta, D-8-I short, thin seta, D-9-I long, thin seta; 3 lateral sensilla: L-1-I long, thin seta, L-2-I, L-3-I short, stout setae; segment 4 (Figs. 18, 42): D-2-IV short, stout seta, D-3-IV long, thin seta, D-4-IV, D-7-IV campaniform sensilla, D-5-IV short, stout seta, D-8-IV medium-sized, stout seta, D-9-IV long, thin seta; L-1-IV, L-2-IV, L-4-IV medium-sized, stout setae, L-3-IV long, thin seta, all on small rectangular tubercles; V-5-IV, V-7-IV short, stout setae, V-6-IV long, thin seta, all on small subquadrangular tubercles; segment 9 (Figs. 18–19) with D-5-IX, D-6-IX campaniform sensilla.

Distribution. Argentina (Neuquén, Río Negro and Chubut Provinces); Chile (Ñuble, Concepción, Valdivia, Casa Pangue, Chiloe and Mechuque Islands).

Material examined. Argentina, Neuquén, Parque Nacional Nahuel Huapi, Río Cuyín Manzano, 40°44′13′′S; 71°09′17′′W, 760 m, 06-II-2009, A. Siri, 1 female (with pupal exuvium).

tibialis group

Palpomyia subaspera (Coquillett)

(Figs. 20–23, 33–37, 43–45)

Ceratopogon subasper Coquillett 1901: 606 (female; USA).

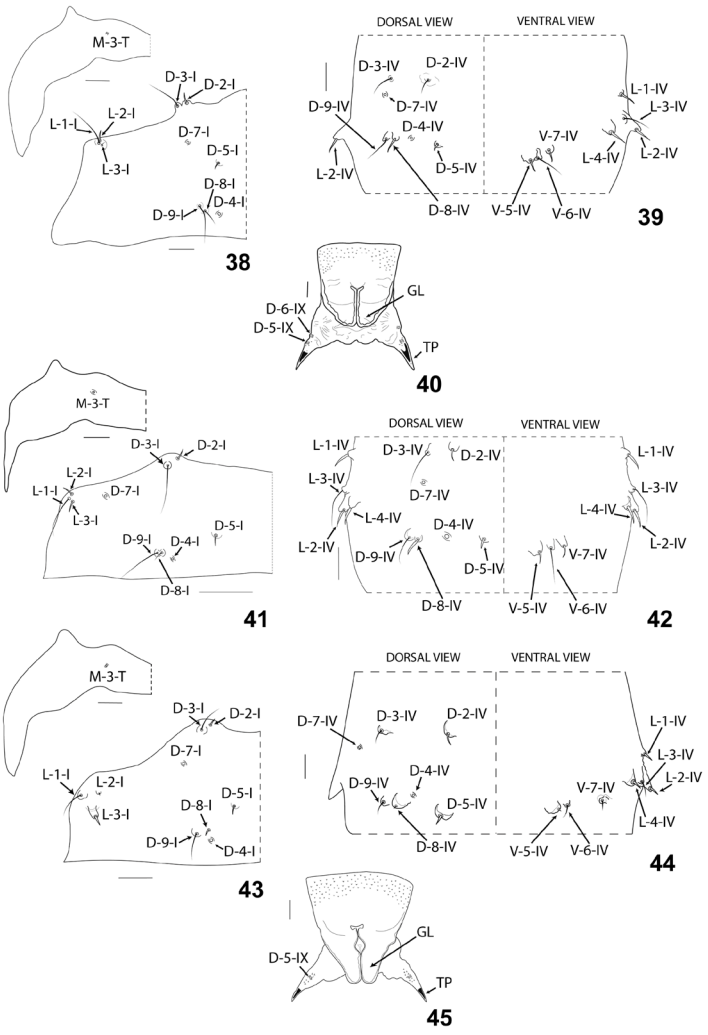
Palpomyia subasper: Malloch 1914: 22 (combination); Johannsen 1943: 784 (in list of USA species); Johannsen 1952: 166 (in key); Snow et al. 1957: 34 (habitat notes); Wirth 1965: 140 (distribution).

Palpomyia subaspera: Grogan & Wirth 1975: 10 (lectotype designation; redescription); Grogan & Wirth 1979: 23 (description, adults, pupa; distribution; *P. essigi* as synonym); Spinelli & Wirth 1993: 68 (*P. maculicrus* as synonym); Borkent & Wirth 1997: 134 (in World catalog); Spinelli 1998: 326 (in list of Argentinean species); Borkent & Spinelli 2000: 64 (in catalog of species south of USA); Borkent & Spinelli 2007: 96 (in Neotropical synopsis); Spinelli et al. 2009: 46 (revision of patagonian species, diagnosis, description, key, distribution); Borkent 2016: 172 (in online World catalog); Cazorla et al. 2018: 8 (in list of Punta Lara Reserve).

Palpomyia essigi Wirth 1952: 225 (female, male; California); Wirth 1965: 140 (distribution).

Palpomyia maculicrus Ingram & Macfie 1931: 230 (female; Argentina); Wirth 1974: 55 (in catalog of species south of USA).

Redescription of female pupa (Figs. 20–23, 33–37, 43–44). Habitus as in Fig. 22. Exuviae brownish. Total length 4.02 mm. *Head:* Dorsal apotome (Fig. 21) with disc 2 times broader than long, covered with stout spines, anterior margin triangular, posterior margin concave, posterolateral margin with broad raised areas, bearing two dorsal apotomal sensilla; antenna extending posteriorly to midleg; mouthparts (Fig. 34) with mandible well developed; palpus extending to posterolateral margin of labium; labium separated medially by labrum; apex of labrum truncated; sensilla: dorsal apotomals (Fig. 21): DA-1-H elongate, thin seta, located on rounded small tubercle, DA-2-H campaniform sensillum; DAL 0.23 mm; DAW 0.24 mm; DAW/DAL 1.04; two dorsolateral cephalic sclerites (Fig. 35): DL-1-H short, stout seta, DL-2-H campaniform sensillum; clypeal/labrals (Fig. 34): CL-1-H short, thin seta, CL-2-H medium-sized, thin seta;



Figures 38-45. 38-39. *Palpomyia mapuche* Spinelli, Grogan & Ronderos, female pupa. 40. *Palpomyia mapuche* Spinelli, Grogan & Ronderos, male pupa. 41-42. *Palpomyia subfuscula* Ingram & Macfie, female pupa. 43-44. *Palpomyia subaspera* (Coquillett), female pupa. 45. *Palpomyia subaspera* (Coquillett), male pupa. 38, 41, 43. metathoracics and tergite 1 chaetotaxy, dorsal view; 39, 42, 44. segment 4 chaetotaxy, dorsal and ventral view. 40, 45. segment 9, ventral view. Scale 0.05 mm. Dorsal sensilla of segment 1 (D-2-I, D-3-I, D-4-I, D-5-I, D-7-I, D-8-I, D-9-I); dorsal sensilla of segment 4 (D-2-IV, D-3-IV, D-4-IV, D-5-IV, D-7-IV, D-8-IV, D-9-IV); dorsal sensilla of segment 9 (D-5-IX, D-6-IX); genital lobe (GL); lateral sensilla of segment 1 (L-1-I, L-2-I, L-3-I); lateral sensilla of segment 4 (L-1-IV, L-2-IV, L-3-IV, L-4-IV); metathoracic sensillum (M-3-T); terminal process (TP); ventral sensilla of segment 4 (V-5-IV, V-6-IV, V-7-IV).

oculars (Fig. 34): O-1-H, O-3-H short, stout setae, O-2-H campaniform sensillum. Cephalothorax rectangular, surface predominantly smooth with small spinules on mesonotum, between bases of respiratory organs. Length of cephalothorax 1.50 mm, width 1.01 mm. *Thorax:* Respiratory organ (Figs. 20, 22, 36) smooth, pale brown, about 3.77 times longer than broad, with rounded apex, with simple row of 14 pores closely abutting at apex; pedicel slender (Figs. 20, 36), P 0.02 mm; RO length 0.20 mm, RO width 0.05 mm; P/RO 0.10; sensilla: three anteromedials (Fig. 36): AM-1-T, AM-2-T long, stout setae, AM-3-T campaniform sensillum; one anterolateral (Fig. 36): AL-1-T short, curved, stout seta; dorsals (Fig. 37): D-1-T, D-2-T, D-4-T, D-5-T long, thin setae, D-3-T campaniform sensillum, all on small rounded tubercle; metathoracic (Fig. 43): M-3-T campaniform sensillum, near anterior margin of metathorax. *Abdomen:* segments with anterior and posterior bands bearing small spicules; with simple setae; segment 9 (Figs. 22–23) approximately 1.6 longer than broad, length 0.31 mm, width 0.20 mm; dorsal and ventral surface bearing band of pointed spicules on anterior margin; dorsal and ventral surface smooth; ventral surface with one small, ventral surface with one small, star-shaped mark located on a

wide bare, mesal area; terminal process (Figs. 22–23) moderately short, nearly straight, base wide, smooth, extreme tips somewhat dark, slightly curved; length 0.13 mm, width 0.06 mm; sensilla: tergite 1 (Figs. 22, 43) with two anteromesals: D-2-I short, stout seta, D-3-I long, thin seta; 5 posterior sensilla: D-5-I short, stout seta, D-4-I, D-7-I campaniform sensilla, D-8-I short, stout seta, D-9-I long, thin seta; 3 lateral sensilla: L-1-I long, thin seta, on rounded small tubercle, L-2-I minute seta, L-3-I short, stout seta on cylindrical tubercle; segment 4 (Figs. 22, 44): D-2-IV short, stout seta, D-3-IV short, thin seta, D-4-IV, D-7-IV campaniform sensilla, D-5-IV short, stout seta, D-8-IV minute seta, D-9-IV short, thin seta, all on small rounded tubercles; L-1-IV, L-2-IV, L-4-IV short, stout setae, L-3-IV short, thin seta, all on small triangular tubercles; V-5-IV minute seta, V-6-IV, V-7-IV short, thin setae, all on small tubercles; segment 9 (Figs. 22–23) with D-5-IX, D-6-IX campaniform sensilla.

Redescription of male pupa (Figs. 33, 45). Similar to female with usual sexual differences: Total length 3.11–3.95 (3.57, n = 4) mm. Dorsal apotome (Fig. 33) pointed, ventral line of weakness; DAL 0.21–0.24 (0.23, n = 2) mm; DAW 0.24–0.25 (0.25, n = 2) mm, DAW/DAL 1.11–1.62 (1.37, n = 2). Cephalothorax: length 1.26–1.44 (1.35, n = 4) mm, width 0.86–0.95 (0.91, n = 2) mm. Respiratory organ 4.5–5.0 (4.8, n = 4) times longer than broad, P 0.020–0.024 (0.021, n = 4) mm; RO length 0.18–0.20 (0.19, n = 4) mm, RO width 0.04 (n = 3); P/RO 0.10–0.12 (0.11, n = 4). Segment 9 (Fig. 45) ventral surface with anterior band bearing few pointed spicules, length 0.30–0.33 (0.32, n = 4) mm, width 0.17–0.24 (0.21, n = 3) mm; terminal process length 0.10–0.14 (0.13, n = 4) mm, width 0.06–0.07 (0.06, n = 3) mm; genital lobe (Fig. 45), longer than broad, surpassing base of terminal process.

Distribution. Canada (Alberta to Ontario), United States of America (south to California, Florida), Mexico, Haiti, Cuba, Paraguay, Chile and Argentina (Misiones, Buenos Aires, Río Negro and Chubut Provinces).

Material examined. Argentina, Chubut, Ruta Provincial 35, 33 km SW Cushamen, unnamed stream, 42°26'10.5"S; 70°30'31"W, 520 m, 10-XII-2006, G. Rossi, 1 female, 4 males (with pupal exuviae).

TAXONOMIC DISCUSSION

Palpomyia mapuche and *P. subfuscula* belong to the *distincta* group and *P. subaspera* to the *tibialis* group, as they were defined by Grogan & Wirth (1975). The first two species are herein compared to each other and to their congeners *P. guarani*, and *P. subaspera* with *P. ryszardi*.

The larva of *P. mapuche* is very similar to *P. guarani* by virtue of the labrum longer than broad, the maxillary palpus long and cylindrical with three apical papillae, the hypostoma finely toothed, the epipharynx with curved auxiliary sclerites, and the elongate and thin hypopharynx lacking fringe. However, *P. guarani* differs by the following combination of characters: the head capsule is smaller (HL 0.30–0.32 mm) and its setae are simple, the mandible has one short and strong tooth and bears stout seta on the aboral surface, the dorsal comb of the epipharynx is armed with 2 lanceolate and stout teeth interrupted by 1–2 shorter ones while the ventral comb is unarmed, and the caudal segment bears 6 pairs of setae (4 long, 2 thinner ones). Ronderos et al. (2004) in the description of the larva of *P. guarani*, incorrectly mentioned that the messors are thin and omitted to describe the palatar bar. A detailed revision of the larva and pupa of *P. guarani* revealed that the messors are stout and the palatal bar is triangular, as they are herein described for *P. mapuche*.

The pupa of *P. subfuscula* differs from that of *P. mapuche* by the longer and stouter DA-1-H, the medium-sized and thinner O-1-H and the shorter and thinner O-3-H; the respiratory organ is pale brown except its distal 1/4 darker, with 18 pores. Besides, in the thorax the AM-2-T is longer and thinner, in the first segment the D-5-I and D-8-I are shorter and stouter, and in the fourth segment the V-5-IV, V-6-IV and V-7-IV setae are located on subquadrangular tubercles.

In the redescription of the pupa of *P. guarani* by Ronderos et al. (2004), the authors used the terms proposed by Huerta et al. (2001). In this study we homologate this terminology with the one proposed by Borkent (2014). The pupa of *P. guarani* is distinguished from *P. mapuche* by the smaller dorsal apotome (DAL 0.08–0.11 mm), the longer and stouter pedicel of the respiratory organ which bears 10-12 pores, the presence of two long and subequal dorsolateral setae, one clypeal labral sensilla and two oculars, the D-5-IV of the fourth segment represented by a minute seta, and the straight and parallel terminal processes of the caudal segment.

Finally, the pupa of *P. ryszardi* differs from *P. subaspera* by the shorter dorsal apotomal sensilla D-1-H, the presence of one anteromedial sensillum as pore, the medium-sized clypeal/labrals, the presence of two oculars, the medium-sized D-2-I and D-3-I on the first abdominal tergite, the fourth segment with D-5-IV represented by a minute seta, and the genital lobe not reaching the posterior margin of segment. We would like to remark that in the original description, Spinelli & Ronderos (2013), incorrectly indicate the anterolateral sensilla (DL-1-H, DL-2-H) as dorsolateral sensilla (AL-1-T, AL-2-T) and the sensilla DL-1-H, DL-2-H mentioned are, in fact, the antennal sensilla. Besides, the D-7-IV described as a seta was omitted in Fig. 23 of the mentioned publication.

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DAS performed the fieldwork, mounted part of the material, made all the drawings, worked the digital images, made the MEB and drawing plates; DAS and FD performed most of the morphological studies, wrote the manuscript and manuscript revision; GRS identified the species; MMR made photomicrographs and plates; MMR and GRS review the manuscript.