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## Revision of *Protonephrocerus* Collin (Diptera: Pipunculidae)

Revisión de *Protonephrocerus* Collin (Diptera: Pipunculidae)

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**Abstract:** Two new species of *Protonephrocerus* Collin, *P. flavipilus* Skevington, Marques & Rafael sp. nov. and *P. misionensis* Skevington sp. nov. are described. The only other currently recognized species in the genus, *P. chiloensis* Collin, is redescribed and genetic data ascribed to this species are transferred to *P. flavipilus* sp. nov. All previously published genetic data refer to *P. flavipilus* sp. nov. The first records of *Protonephrocerus* in Argentina are documented.

**Keywords:** Argentina, Chile, DNA barcoding, New species.

**Resumen:** Se describen dos nuevas especies de *Protonephrocerus* Collin, *P. flavipilus* Skevington, Marques & Rafael sp. nov. y *P. misionensis* Skevington sp. nov. La única especie actualmente reconocida en el género, *P. chiloensis* Collin, se redescribe y los datos genéticos atribuidos a dicha especie se transfieren a *P. flavipilus* sp. nov. Todos los datos genéticos publicados anteriormente se refieren a *P. flavipilus* sp. nov. Se documentan los primeros registros de *Protonephrocerus* en Argentina.

**Palabras clave:** Argentina, Chile, Código de barras de ADN, Nuevas especies.

## INTRODUCTION

*Protonephrocerus* Collin is a key lineage of big-headed flies (Diptera, Pipunculidae). Along with species in the extinct genus *Metanephrocerus* Aczél, they are currently placed in the subfamily Protonephrocerinae and considered to be sister to the diverse subfamily Pipunculinae (Collin, 1931; Aczél, 1948; Skevington & Yeates, 2000; Kehlmaier et al., 2014). *Protonephrocerus chiloensis* Collin is the only currently recognized extant member of the subfamily Protonephrocerinae and all previously published work on the genus, including sequences, are ascribed to this species. As it turns out, *P. chiloensis* is very local in its distribution and a much more widespread, undescribed species is what has been referred

to in the literature. All published molecular data actually refer to this undescribed species. We describe this species below and clarify which of the two species have been cited in previous literature.

A third species for the genus was also discovered while preparing this manuscript. It is known from a single specimen collected in a protected area near the Antonia Ramos Research Center (CIAR) in the Atlantic Forest ecoregion of northeastern Argentina. DNA barcoding efforts in the region brought the specimen to light.

*Protonephrocerus* has also been considered to be sister to *Nephrocerus* Zetterstedt in the subfamily Nephrocerinae (Rafael, 1988; Rafael & De Meyer, 1992). They possess characters of both Pipunculinae and Nephrocerinae and decisions about which characters are plesiomorphic and which are apomorphic have led to the differing hypotheses. Fossil data and molecular data suggest that this hypothesis is incorrect, but more data are needed to test this. The discovery of two additional extant species of *Protonephrocerus* will help with such future analyses.

Knowing the sister taxon of the diverse subfamily Pipunculinae may help us better understand what led to the radiation of this group. One thousand three hundred and forty-six of the 1462 currently recognized species of pipunculids are in the subfamily Pipunculinae (De Meyer, 1996; De Meyer & Skevington, 2000; Evenhuis & Pape, 2020; Skevington, 2020).

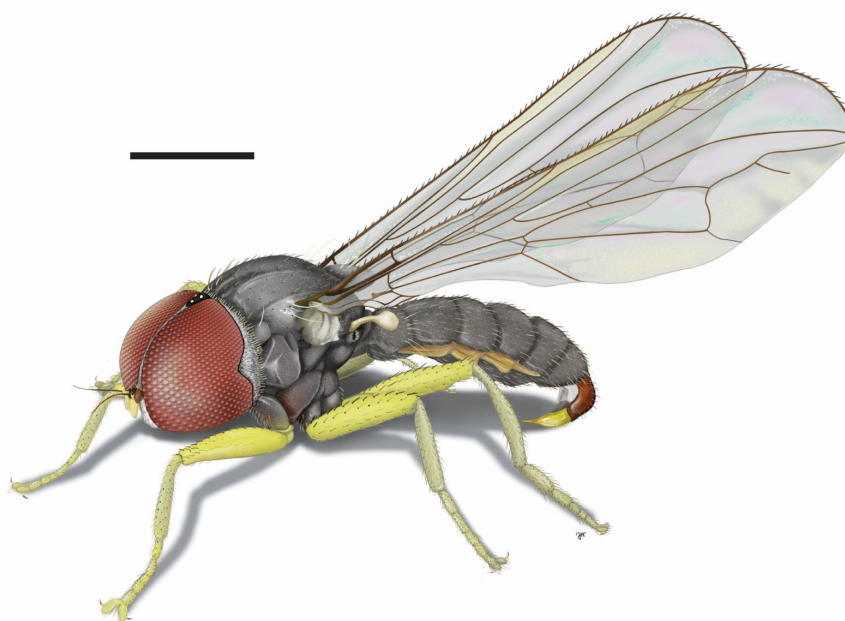


Fig. 1. *Protonephrocerus flavipilus* sp. nov., lateral oblique habitus, female.  
Scale bar = 1 mm.

## MATERIAL AND METHODS

Specimens examined in this study were obtained from the following collections (abbreviations follow Evenhuis (2020)): The Biodiversity

Institute of Ontario (BIO), Canadian National Collection of Insects, Ottawa, ON, Canada (CNC), University of Guelph Insect Collection, Guelph, ON, Canada (DEBU), Field Museum of Natural History, Chicago, USA (FMNH), Instituto Fundación Miguel Lillo, Tucuman, Argentina (IFML), Illinois Natural History Survey, Champaign, Illinois, USA (INHS), Instituto Nacional de Pesquisas da Amazonia, Coleção Sistemática da Entomologia, Manaus, Amazonas, Brazil (INPA), Institut Royal des Sciences Naturelles de Belgique, Brussels, Belgium (IRSNB), Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", MACN-CONICET (MACN), Universidad Nacional de La Plata, Museo de la Plata, La Plata, Argentina (MLPA), Museo Nacional de Historia Natural, Santiago, Chile (MNNC), Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZSP), The Natural History Museum, London, United Kingdom (NHMUK) and the National Museum of Natural History, Washington, DC, USA (USNM).

Genitalia were excised by removing the whole abdomen, placing them in standard 1.5 mL Eppendorf tubes (Eppendorf Canada, Mississauga, Ontario, Canada) and immersing them in 85% lactic acid for no less than four hours on a 95 °C dry bath incubator (Fisher Scientific, Toronto, Ontario, Canada). Macerated abdomens were then placed on a glass depression slide and immersed in glycerin for dissection. The genitalia were then separated from the abdomen for comparison under a dissecting scope and were ultimately stored in genitalia vials with the specimens. Drawings were made of the genitalia of exemplar males and females. Genitalic terminology nomenclature follows Cumming & Wood (2017) and is discussed by Skevington (2001) with specific reference to Pipunculidae.

Exemplar specimens were photographed with a Canon 50D EOS Digital Camera with an MP-E 65mm macro lens (Canon Canada Inc., Mississauga, Ontario, Canada) and images were then stacked using Zerene Stacker Version 1.04 (Zerene Systems, Richland, Washington, United States of America). These images were further manipulated using Photoshop CS6 software (Adobe Systems Incorporated, San Jose, California, United States of America). Figure plates were prepared in Adobe Illustrator CS6 (Adobe Systems Incorporated). Illustrations of specimens were made by T.O.B. The microscope used for preparing illustrations was a Nikon SMZ1500 with a HI-150 High Intensity Illuminator (Nikon Canada Inc., Mississauga, Ontario, Canada). Measurements were made using a graticule following methods outlined in Skevington (2001).

All specimens are labeled with a unique reference number, typically in the format J. Skevington Specimen # *n*, CNC Diptera # *n* or CNC*n*. These have been shortened to follow the format JSS*n*, CNCD*n*, and CNC*n* respectively throughout the text. These numbers are used in public specimen database maintained at the CNC (Skevington, 2020) and are cited in the material examined sections and figure captions for illustrations to allow for repeatability of the research.

Molecular methods and analysis follow Motamedinia et al. (2020). Genetic work was carried out at the molecular laboratory of the CNC in Ottawa, Ontario, Canada and at the Biodiversity Institute of Ontario in Guelph, Ontario, Canada. Uncorrected pairwise genetic distances (p-distance) were calculated with Mega X (Kumar et al., 2018). Sequence accession numbers issued by GenBank (GB) are provided for each specimen in Table I.

GenBank Number		BIOUG25021-F05	JSS16399	CNC1065350	DW0351	DW0354	JSS15436	15444	CNC1078304	JSS16840	CNC1078305	DW0353
MW201180	<i>P. missionensis</i> BIOUG25021-F05											
MW201189	<i>P. flavipilus</i> B JSS16399	0.10914										
MW201183	<i>P. flavipilus</i> B CNC1065350	0.10914	0.00154									
MW201186	<i>P. flavipilus</i> B DW0351	0.10914	0.00000	0.00154								
MW201184	<i>P. flavipilus</i> A DW0354	0.10570	0.03135	0.02976	0.03135							
MF124088	<i>P. flavipilus</i> A JSS15436	0.10742	0.03294	0.03135	0.03294	0.00464						
MF124087	<i>P. flavipilus</i> A JSS15444	0.10570	0.03135	0.02976	0.03135	0.00154	0.00309					
MW201181	<i>P. flavipilus</i> A CNC1078304	0.10914	0.03135	0.02976	0.03135	0.00464	0.00619	0.00309				
MF124086	<i>P. flavipilus</i> A JSS16840	0.10932	0.03459	0.03300	0.03459	0.00776	0.00932	0.00620	0.00310			
MW201187	<i>P. flavipilus</i> A CNC1078305	0.10354	0.02396	0.02210	0.02396	0.00915	0.01283	0.00915	0.01099	0.01471		
MW201188	<i>P. flavipilus</i> A DW0353	0.10556	0.02583	0.02396	0.02583	0.01099	0.01468	0.01099	0.01283	0.01656	0.00548	
MW201185	<i>P. flavipilus</i> A DW0350	0.10118	0.01901	0.01688	0.019008	0.00000	0.00000	0.00000	0.00209	0.00631	0.00630	0.00840

Table I. Pairwise differences for all known DNA barcoded *Protonephrocera* specimens.



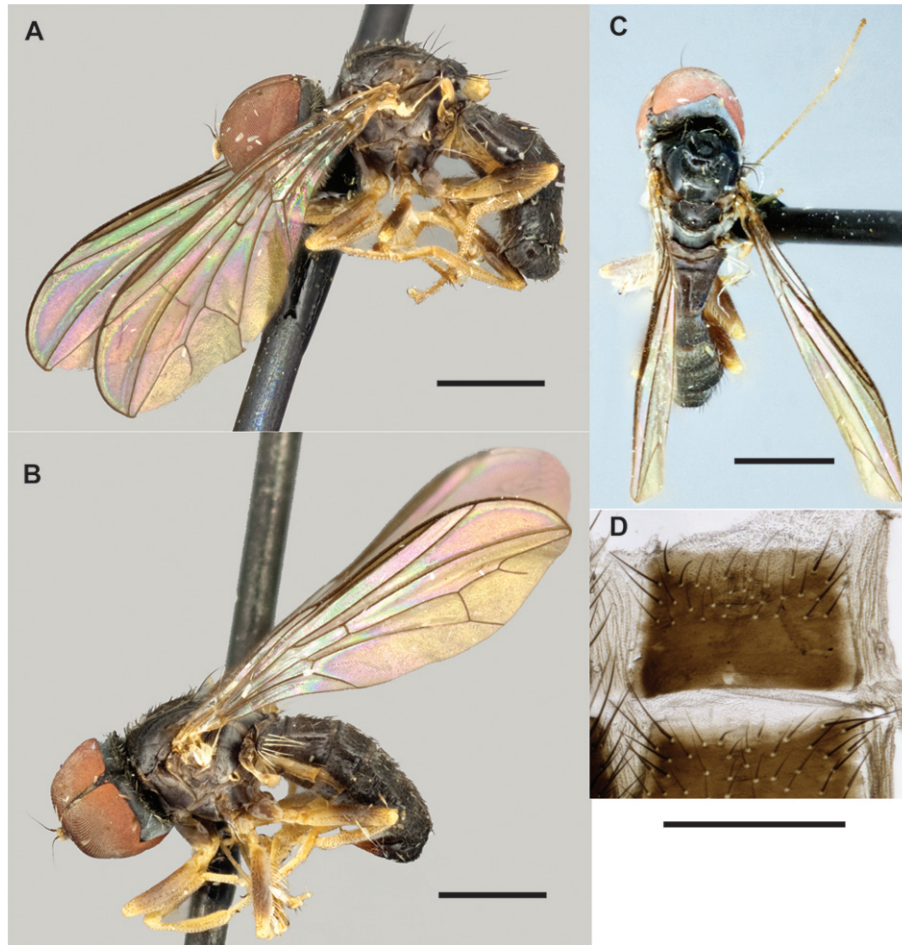


Fig. 2. *Protonephrocerus chiloensis*. A. lateral *habitus*, male, CNCD190035. B. lateral *habitus*, female, CNCD190032. C. dorsal *habitus*, female, CNCD190032. D. male sternite 5.

Scale bars A-C = 1 mm; D = 0.25 mm.

## RESULTS AND DISCUSSION

*Protonephrocerus* Collin, 1931: 52. Type species: *Protonephrocerus chiloensis* Collin, 1931, by original designation; Rafael, 1988: 465; Rafael & De Meyer, 1992: 652; Skevington & Yeates, 2001: 440.

The most recent key to genera that includes *Protonephrocerus* can be found in Skevington & Yeates (2001).

**Description** (modified from Rafael, 1988; Rafael & De Meyer, 1992; Kehlmaier et al., 2014): **Head** (cf. Figs. 1, 2A-B) hemispherical, holoptic in males, dichoptic in females. Ocellar triangle bare, slightly raised. Frons and face narrow, the latter slightly protruding, both with silvery white pubescence which are longer and denser on face. Posterior eye margin notched in middle. Posterior head margin running down straight. Occiput moderately wide, silver-pubescent laterally, sparsely brown-pubescent dorsally; covered with numerous short yellow hairs. Arista dark brown with yellow base. Postpedicel yellow, roughly ovate, slightly pointed ventrally. Pedicel and scape brown. Labellum and palpi yellow. **Thorax** (cf. Figs. 1, 2A-B, 4A). Proepisternum without propleural

fan. Postpronotal lobe light brown to dark brown with yellow edges. Scutum dark brown to black dorsally, yellow to light brown on postalar callus. Pleuron entirely brownish black, only occasionally yellow around posterior spiracle and meron. Chaetotaxy: dorsocentral row of short bristles and terminating in 1 strong seta; intra-alar bristles small; 1 posterior supra-alar; 2 postalars; 2 pairs of scutellars; 2 notopleurals, posterior one longer; proepimeron with some short proepimeral setae and anepimeron with one moderately strong bristle. **Legs** mostly yellow with brown marks; long and slender; with dense pubescence and bristles; front and mid coxae with numerous long setae on anteroapical half; hind coxae with numerous shorter hairs along anteroapical and outer lateral margin. Front femur with posteroventral row of longer hairs in basal half, and several rows of shorter bristles; mid femur with posterior row of long hairs from base to apex (about as long as width of femur), and several rows of shorter bristly hairs; hind femur at least anterodorsally with 2 or more outstanding long bristles near apex (longer than width of femur), and posterior as well as antero-/posteroventral rows of longer bristly hairs, and several rows of shorter bristly hairs. First and second tarsal segments very long. **Wings** (cf. Fig. 1) long, slightly more than three times as long as broad; hyaline. Pterostigma present, brownish. Third costal section about four times as long as fourth. Cross-vein r-m placed near basal third of discal medial cell (dm); vein M. present in most species (absent in *P. misionensis*), long but never reaching wing margin. Anal lobe absent. Halter yellow. **Abdomen** (cf. Figs. 1, 4A) slightly narrower than and about twice as long as thorax; entirely dark brown to black, hairs longest along lateral and posterior margins of tergites; tergites 1-7 and syntergosternite 8 visible from dorsal; tergite 1 with a tuft of long yellow bristles anterolaterally; sternites 1-5 light brown. Sternite 6 bulbous, protruding beyond sternite 7, with apical thickening. Syntergosternite 8 large, dark brown, without membranous area. **Male genitalia** (cf. Figs. 3A-E). Epandrium short, narrower medially, partly concealed basally by syntergosternite 8. Surstyli simple and symmetric. Subepandrial sclerite conspicuous, yellow, elongated and narrow. Hypandrium symmetrical; small, about half length of simple-shaped phallic guide complex; gonopods reduced and symmetric. Phallus simple, short, single. Ejaculatory apodeme elongate, narrow, horn-shaped distally. **Female** (cf. Fig. 1) as male except: Eyes dichoptic. Frons widest medially. Frontal ommatidial facets enlarged. Tergites 1-6 but not 7 visible from dorsal. Ovipositor short, piercer strong, yellow, distinctly curved upwards.

KEY TO *PROTONEPHROCERCUS* SPECIES

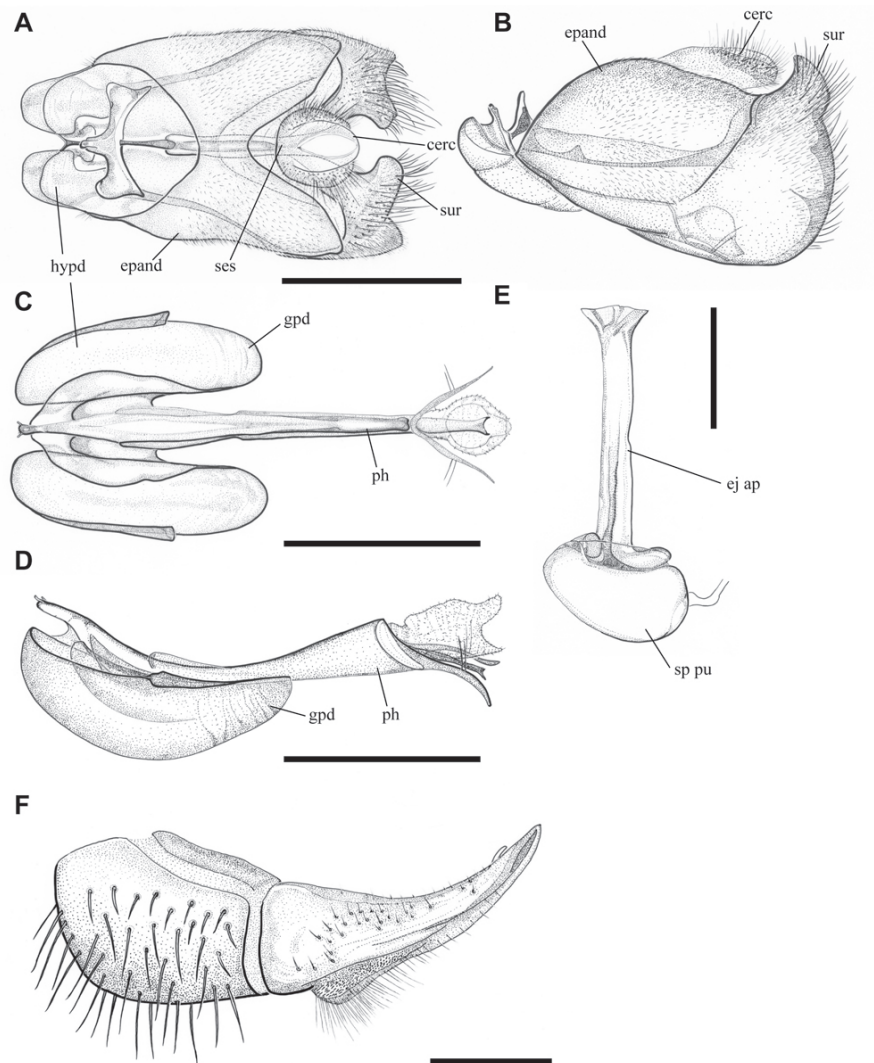


Fig. 3. *Protonephrocercus chiloensis*. A. dorsal, male terminalia, JSS22401. B. left lateral, male terminalia, CNCD190030. C. ventral, male phallus and gonopods, CNCD190030.

D. lateral, male phallus and gonopods, CNCD190030. E. male ejaculatory apodeme and sperm pump, CNCD190030. F. right lateral of female ovipositor, CNCD190034.

Scale bars = 0.25 mm. Abbreviations: cerc = cerci; ej ap = ejaculatory apodeme; epand = epandrium; gpd = gonopod; hypd = hypandrium; sp pu = sperm pump; ses = subepandrial sclerite; sur = surstylus; ph = phallus. Scale bars = 0.25 mm.

1. Wing with M. vein absent (Fig. 6C). Female with ovipositor short, curved out away from sternites (Fig. 6A) ..... *P. misionensis* Skevington sp. nov.

—.... Wing with M. vein present. Female with ovipositor short, straight-edged dorsally, curved towards sternites (Fig. 3F) ..... 2

2. Legs mostly yellow, sometimes with light brown marks (Figs. 1, 4A). Female with ovipositor short, curved towards sternites, with piercer about same length as base (Fig. 5F). Male surstyli with elongate, pointed tips (Figs. 5A-B). Phallic guide with 2 small bristles near tip (Figs. 5C-D).



Gonopods pointed (Figs. 5C-D). Sternite 5 with two invaginations on posterior margin (Fig. 4C) ..... *P. flavipilus* Skevington, Marques & Rafael sp. nov.

—.... Legs yellow with brownish black areas on all femora; brown on basal third to half of hind femur (Figs. 2A-B, 6). Female with ovipositor short, curved towards sternites, with piercer longer than base (Fig. 3F). Male surstyli with short, blunt tips (Figs. 3A-B). Phallic guide with 2 small bristles medially (Figs. 3C-D). Gonopods rounded (Figs. 3C-D). Sternite 5 rectangular (Fig. 2D) ..... *P. chiloensis* Collin, 1931

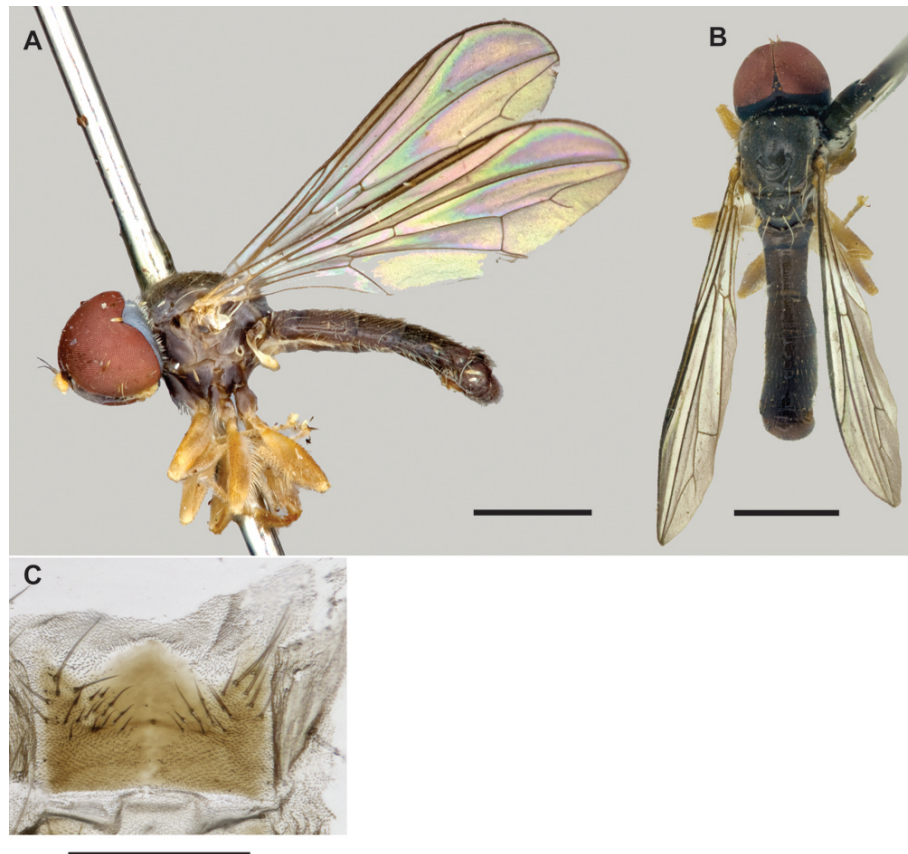


Fig. 4. *Protonephrocerus flavipilus* sp. nov. A. lateral *habitus*, male, JSS22401. B. dorsal *habitus*, male, CNCD190036. C. male sternite 5.

Scale bars A-B = 1 mm; C = 0.25 mm.

## SPECIES ACCOUNTS

### *Protonephrocerus chiloensis* Collin, 1931

(Figs. 2, 3, 7)

*Protonephrocerus chiloensis* Collin, 1931: 52, figs. 15a-b. Holotype ♀ : CHILE, Ilha Chiloé (NHMUK); Aczél, 1952: 239 (cat.); Hardy, 1966: 2 (cat.); Rafael, 1988: 469 (partim).

**Diagnosis. Both sexes:** Scutum mostly black with black bristles. Hind femur anterodorsally with two outstanding long bristles near apex. **Male:** Sternite 5 rectangular. Surstyli simple and symmetrical, stubby (Figs. 3A-

B), swollen basally in lateral view. Gonopods with rounded apex (Fig. 3C). **Female:** Ovipositor short, upcurved, not sinuous dorsally, in lateral view (Fig. 3F).

**Description.** Body length 4.1-4.2 mm; wing length 4.5-4.6 mm.

**Male. Head** (Figs. 2A-C). Eyes touching on frons for about 2.4 times as long as frontal triangle. Pedicel brown with 4 short dorsal bristles and 3 ventral bristles, two of them short and one long. **Thorax** (Figs. 2A-C). Postpronotal lobe dark brown with yellow edges, with 3-4 yellow hairs on posterior edge. Scutum mostly dark brown to black with black bristles. Scutellum black with 2 pairs of strong black marginal setae and numerous small black bristles over entire surface. **Wing** with M. vein present. **Legs** mostly yellow with brown marks medially on fore and mid femora, and distal half of hind femur; long and slender; with dense pubescence and hairs. Small hairs on trochanters, femora, tibiae and tarsi mostly black; front femur with posteroventral row of longer hairs in basal half, and several rows of shorter bristles; mid femur with posterior row of about 20 long hairs from base to apex, and several rows of shorter bristly hairs; hind femur anterodorsally with 2 outstanding long bristles near apex and several rows of shorter bristly hairs. **Abdomen** (Figs. 2A-D) dark brown to black with black bristles. Tergite 1 with 13 yellow lateral hairs anterolaterally. Tergites 6 and 7 large and shining, densely covered with pubescence and bristles; tergite 7 scarcely visible from above. Sternites 1-5 light brown. Sternite 5 rectangular (Fig. 2D). Sternites 6 and 7 yellow, not visible dorsally; sternite 6 dark brown basally, markedly desclerotized in apical half, bulbous, protruding beyond sternite 7. Syntergosternite 8 dark brown, large, without membranous area. **Male genitalia** (Figs. 3A-E) large, grayish pruinose. Epandrium brownish yellow, shorter medially; symmetrical. Surstyli stubby, symmetric, with cluster of long bristles on dorsomedial surface. Hypandrium small, about half length of simple-shaped phallic guide complex. Gonopods reduced and symmetrical, with rounded apex. Phallic guide V-shaped, with two setae medially. Phallus short, bifid. **Female.** As male except: Ovipositor yellow, short, piercer distinctly curved upwards, not sinuous dorsally, in lateral view, 0.8-0.9 mm (Fig. 3F).

**Material examined.** Holotype ♀, South Chile, Chiloé Island, 43°7'17.85"S, 74°1'48.05"W, 30m, 17-19.xii.1926, B.M. 1927-63, F. & M. Edwards, JSS26913 (NHMUK). **Other Material Examined:** **CHILE.** Carelmapu, Llanquihue, 41°44'29.94"S, 73°41'54.94"W, 6m, *Nothofagus* forest, 24.xi.1957, L.E. Peña, CNC190030-5 (2 ♂ 4 ♀, CNC).

**Remarks.** Only seven specimens of this species have been collected so any inferences about behavior or phenology are tentative. The flight period appears to be predominantly between late November and late December in lowland, coastal *Nothofagus* forests. We were unable to sequence any of the known specimens of this species (presumably due to degradation of the tissues over time).

**Distribution.** Known only from Los Lagos Province, Chile (Fig. 7).

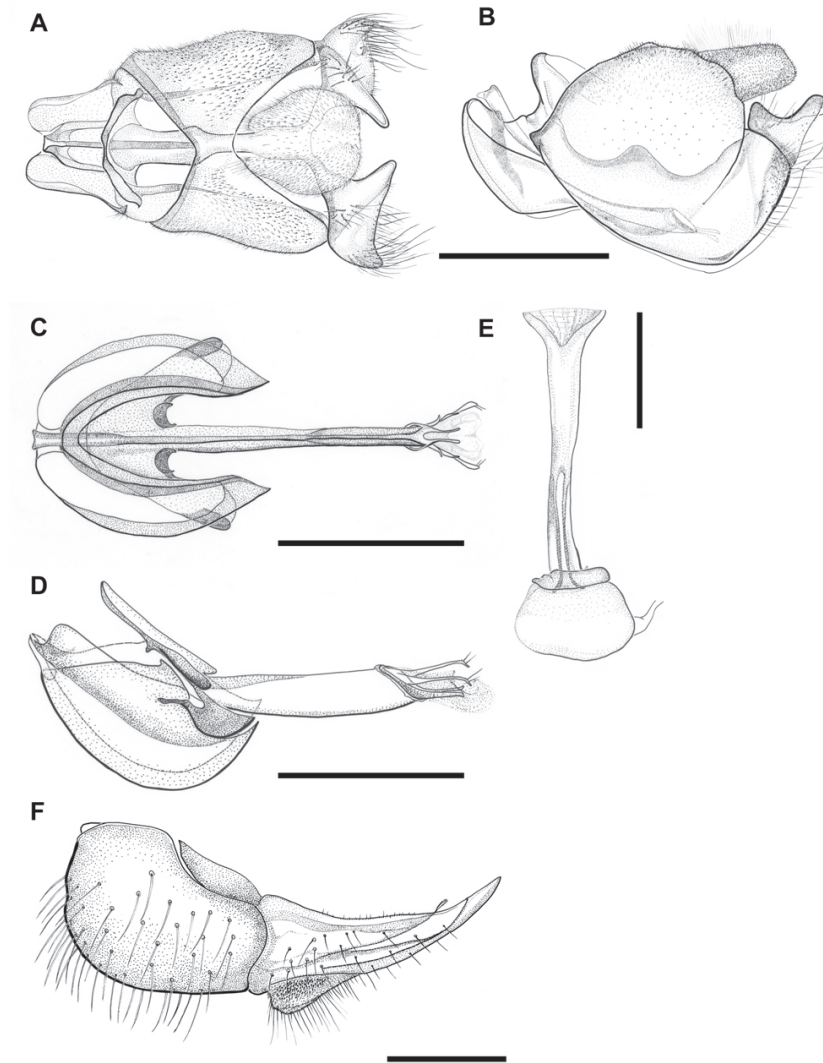


Fig. 5. *Protonephrocerus flavipilus* sp. nov. A. dorsal, male terminalia, CNCD190028. B. left lateral, male terminalia, CNCD190028. C. ventral, male phallus and gonopods, CNCD190028. D. lateral, male phallus and gonopods, CNCD190028. E. male ejaculatory apodeme and sperm pump, CNCD190028. F. right lateral of female ovipositor, JSS15436. Scale bars = 0.25 mm.

*Protonephrocerus flavipilus* Skevington, Marques & Rafael n. sp.

(Figs. 1, 4, 5, 7)

*Protonephrocerus chiloensis* sensu Rafael, 1988: 465. Figs. 3-9.

**Diagnosis. Both sexes:** Scutum mostly black with yellow bristles. Hind femur anterodorsally with 6-8 outstanding long bristles near apex. **Male:** Sternite 5 with two invaginations on posterior margin. Surstyli simple and symmetrical, tapering distally, with pointed apex (Figs. 5A-B). Gonopods with pointed apex (Fig. 5C). **Female:** Ovipositor short, upcurved, sinuous dorsally (Fig. 5F).

**Description.** Body length 3.9-5.3 mm; Wing length 4.0-5.4 mm.

**Male. Head.** Eyes touching on frons for about 2 times as long as frontal triangle. Pedicel brown with 4 short dorsal bristles and 3 ventral bristles, two of them short and one long. **Thorax** (Figs. 4A-B). Postpronotal lobe brownish yellow, with 5 yellow hairs on posterior edge. Scutum mostly dark brown to black with yellow bristles. Scutellum black with 2 pairs of strong yellow marginal setae and numerous small yellow bristles over entire surface. **Wing** with M. vein present. **Legs** mostly yellow with light brown on basal half of fore femur, medially on mid femur and distal half of hind femur; long and slender; with dense pubescence and bristles. Small hairs on trochanters, femora, tibiae and tarsi mostly black; front femur with posteroventral row of longer hairs in basal half, and several rows of shorter bristles; mid femur with posterior row of about 20 long hairs from base to apex, and several rows of shorter bristly hairs; hind femur anterodorsally with 6-8 outstanding long bristles near apex, and several rows of shorter bristly hairs. **Abdomen** (Figs. 4A-B) dark brown to black with yellow bristles. Tergite 1 with 10 long yellow lateral hairs anterolaterally. Tergites 6 and 7 large and shining, densely covered with pubescence and bristles; tergite 7 scarcely visible from above. Sternites 1-5 brownish yellow. Sternite 5 with two invaginations on posterior margin (Fig. 4C). Sternite 6 dark brown basally, markedly desclerotized in apical half, bulbous, protruding beyond sternite 7. Syntergosternite 8 dark brown, large, without membranous area. **Male genitalia** (Figs. 5A-E) large, grayish pruinose. Epandrium brownish yellow, slightly wider than long, narrower medially; symmetrical. Surstyli symmetric, tapering at apex, with pointed apex; almost parallel sides in lateral view (Fig. 5A-B); varies from having short to long points at the apex (as long as the base of the surstylus). Hypandrium small, about half length of simple-shaped phallic guide complex. Gonopods reduced and symmetrical, with pointed apex; phallic guide V-shaped, with two small setae apically in each ramification (Figs. 5C-D). Phallus bifid, short (Figs. 5C-D). **Female.** As male except: Ovipositor yellow, short, piercer distinctly curved upwards, not sinuous dorsally, in lateral view, 0.7-0.8 mm (Fig. 5F).

**Material examined.** Holotype ♂, Chile, Osorno, Pq. Nac. Puyehue Antillanca, límite de la vegetación, 40.774444°S, 72.211389°W, 1054m, D.S. Amorim & V.C. Silva cols., 14.i.-3.ii.2017, Malaise trap, DW0350 (MNNC). **Paratypes: ARGENTINA. Chubut:** 2 km SE entrance to Alerces Nat. Park near stream in *Nothofagus* forest, 42.993333°S, 71.5775°W, 590m, M.E. Irwin, 23-31.xii.2005, Malaise trap CNC267261, CNC267266 (1 ♂ 1 ♀, CNC); Parque Nacional Alerces, Villa Futalaufquen, meadow, 42.8935°S, 71.618167°W, 340m, M.E. Irwin, 23-31.xii.2005, Malaise trap, CNCD137704, CNCD137716, CNCD137718, CNCD137719, CNCD137726, CNCD137729 (2 ♂ 4 ♀, MLPA), CNCD137730, CNCD137736 (2 ♀, INPA), CNCD137745, CNCD137751, CNCD137753 (1 ♂ 2 ♀, CNC); Alerces National Park, Lago Fataaufquen nr Quime Quipan, *Nothofagus* Forest, 42.861167°S, 71.602667°W, 530m, M.E. Irwin, 23-31.xii.2005, Malaise trap, CNC267651, CNC267652, CNC267655, CNC267658, CNC267663, CNC267664, CNC267666,



CNC267671, CNC267672, CNC267681, CNC267688, CNC267689, CNC267693, CNC267694, CNC267696, CNC267698, CNC267702, CNC267703, CNC267710, CNC267715, CNC267719, CNC267720, CNC267730, CNC267736 (10 ♂ 14 ♀, MLPA), CNC267740, CNC267749, CNC267750, CNC267752, CNC267754, CNC267755 (5 ♂ 1 ♀, INPA), CNC267757, CNC267761, CNC267767, CNC267770, CNC267771, CNC267773, CNC267775, CNC267789, CNC267790, CNC267792, CNC267798, CNC267801, CNC267807, CNC267818, CNC267823, CNC267829, CNC267832 (10 ♂ 7 ♀, CNC), CNC267644-46 (1 ♂ 2 ♀, USNM); Neuquén: Chapelco, 40°10'0.02"S 71°19'59.99"W, 1400m, Ecoregion Monte de llanuras y Mesetas, Malaise trap, JSS1441-31464 (4 ♂ 20 ♀, IML); Río Negro: Río Manso, Nahuel Huapi National Park, 41°36'5.8"S 71°36'24.8"W, 466m, Montes de Oca & Garre coll., Malaise trap, CNC1766523-6 (1 ♂ 3 ♀, MACN); CHILE. Santiago Province, la Plata, 5 km west La Rinconada de Maipú, 33°50'24"S 71°30'42"W, 400m, in canyon bottom, 24.ix-7.xii.1997, G. Barrie, M.I. Irwin, Malaise trap, JSS4485-6, (2 ♀, INHS); Santiago Province, Queb la Plata, 5 km west La Rinconada de Maipú, 33.5°S 70.9°W, 400m, 24.xi.-7.xii.1997, G. Barrie, M.I. Irwin, Malaise trap, CNCD233559 (1 ♀, CNC); 20 km East of Potrero Grande, El Relvo, 35°11'0.81"S 70°55'57.50"W, 990m, 14.i.2004, leg. J.E. Barriga, JSS19037 (1 ♀, CNC); Chillán Las Trancas, 36°37'S, 72°5'W, iii.1984, L.E. Peña, Malaise trap, FMNH 1986 L.E. Peña Colln. Acc. # 17.422, JSS50720 (1 ♂, FMNH); Nuble, Las Trancas, -36.605708 -72.073483, 1.ii.1983, L.E. Peña, CNC1766812-13 (2 ♂, INPA); Chillán, Shangri-la, 36°37'S 72°5'W, 19-30.xii.1988, L.E. Peña, Malaise trap, FMNH 1986 L.E. Peña Colln. Acc. # 17.422, JSS50722-50728 (6 ♀, FMNH; 1 ♂, INPA); Chillán, Shangrilá, -36.891458 -71.473092, 19.xi-30.xii.1983, L.E. Peña, CNC1766814-18 (3 ♂, 2 ♀, INPA); Cord. Nahuelbuta Cabreria, -37.827500 -73.009008, 1100m, 9-15.1977 [sic], L.E. Peña, CNC1766819; CNC1766820 (2 ♀, INPA); RN Nahuelbuta, 37°48'58"S 73°0'36"W, 1320m, *Nothofagus* forest, 9-10.ii.2005, L. Masner, yellow pan trap, CNCD190029, CNCD190090-8, (5 ♂ 5 ♀, CNC); Region IX, Parque Nacional De Nahuelbuta, 37°49'0"S 73°1'0"W, 1122m, 6-9.i.2000, D. Webb, D. Yeates, JSS15436, JSS15444 (2 ♀, CNC); Region IX, PN Nahuelbuta, 37°49'30"S 72°58'24"W, 1168m, in meadow and *Nothofagus*, 8-24.ii.2005, UCR AtoL, Malaise trap, JSS16840 (1 ♂, CNC); Region IX, Malleco, P.N. Nahuelbuta, Pichinahuel, 38°1'0"S 73°13'0"W, 90m, 16-20.xii.1993, G.&M. Wood, JSS3651 (1 ♂, DEBU); Mulchen, Caledonia, -37.7211079 -72.2426007, 709m, 6-10.ii.1981, L.E. Peña, CNC1766821 (1 ♀, INPA); Malleco, Termas Tolhuaca, 38°14'4.77"S 71°43'41.88"W, 1160m, 1959, Peña, CNCD190028 (1 ♂, CNC); same data as holotype, CNCD190026, CNCD190042-44, CNC190046-88, JSS22401 (20 ♂ 29 ♀, CNC); Río Blanco, Curacautin, Malleco, 38°26'0"S 71°53'0"W, 1100m, ii.1964, Peña, CNCD190036, CNCD190039-41 (2 ♂ 2 ♀, CNC), CNCD190037-38 (1 ♂ 1 ♀, USNM); Río Blanco, Malleco, 38°26'0"S



71°53'0"W, 1100-2000m, ii.1964, Peña, CNCD190045 (1 ♂, CNC); Malleco, Las Raíces, 38°33'S, 71°30'W, 1100m, 21.xii.1976, L.E. Peña, Malaise trap, FMNH 1986 L.E. Peña Collection, Acc. # 17.422, JSS50721 (1 ♂, FMNH); Los Lagos, MN Alerce Costero, W of La Union, 40.193889°S, 73.433889°W, 938m, UCR ATOL C05-026, 20.ii.2005, mature *Fitzroyia* forest, JSS16399 (CNC); Osorno, Pq. Nac. Puyehue Antillanca, Termas Aguas Calientes, 40.733333°S, 72.316667°W, 440m, V.C. Silva & D.S. Amorim cols., 14.i.-3.ii.2017, Malaise trap, DW0349 (1 ♂, CNC), CNC1078320-22 (3 ♀, MNNC); Osorno, Pq. Nac. Puyehue Antillanca, límite de la vegetación, 40.774444°S, 72.211389°W, 1054m, D.S. Amorim & V.C. Silva cols., 14.i.-3.ii.2017, Malaise trap, DW0351-52 (2 ♂ MNNC), DW0353-54 (2 ♂ MZSP), CNC1078304-11 (8 ♀, MNNC), CNC1078312-19 (8 ♀, MZSP), CNC1078323 (1 ♂, MNNC), CNC1078324 (1 ♂, MZSP), CNC1065345-54 (5 ♂ 5 ♀, CNC); Osorno Province, P.N. Puyehue, Ag. Calientes to 2 km South, 40°46'48"S 72°15'36"W, 600m, 10-22.ii.1979, D. & M. Davis, B. Akerbergs, CNCD190027, CNCD190089 (1 ♂ 1 ♀, CNC); Bariloche, Río Negro, Arg., 41°4'40.25"S 71°10'19.03"W, 785m, xi.1926, R.&E. Shannon, CNCD190099 (1 ♂ 1 ♀, USNM).

**Etymology.** From the Latin *flavus* for yellow and *pilus* for hair, in reference to the extensive yellow pile on the thorax and abdomen.

**Remarks.** This is the most common species of *Protonephrocercus* and appears throughout the literature as *P. chiloensis* (Kehlmaier et al., 2014, Fig. 32, page 33; Rafael, 1988, Figs 3-9, page 468 and male description on page 469 based on *P. flavipilus* sp. nov.; Skevington & Yeates, 2000, specimens coded in matrix and used in phylogeny based on *P. flavipilus* sp. nov.). All previously sequenced specimens of *Protonephrocercus* are of this new species (JSS4485 - GenBank numbers AF154736 and AF154811 - 12S and 16S ribosomal DNA sequenced and were identified as *P. chiloensis* in Skevington & Yeates (2000); JSS3651 - GenBank numbers AF154737 and AF154812 - 12S and 16S ribosomal DNA sequenced and were identified as *P. chiloensis* and incorrectly listed as JSS945 in Skevington & Yeates (2000)). Specimens have been collected between mid-September and early March, mostly between 340 and 1400m elevation (with one specimen, JSS3651, as low as 90m). Specimens have been collected in *Nothofagus* and *Fitzroyia* forests as well as meadows.

The holotype specimen has been DNA barcoded (Table I). This species differs from *P. misionensis* by 10.1 to 10.9% (uncorrected pairwise distance). Intraspecific variation is 0-3.5%. There are two genetic clusters of specimens with 0-1.7% pairwise divergence within one cluster (n = 8 - *flavipilus* A) and 0-0.2% within the other (n = 3 - *flavipilus* B) (Table I). Pairwise divergence between these clusters is 1.7 to 3.5%. Although this is suggestive that two species may be involved, we do not find any morphological support for this. Specimens from the same location and date occur in each cluster. The only variable morphological character, the shape of the surstylus tip (long vs. short), is also suggestive of multiple

species, but examination of many specimens shows that this character varies continuously and individuals with short-tipped surstyli occur in both genetic clusters.

**Distribution.** Occurs from Santiago south to Los Ríos Province in Chile and in adjacent Argentina (Chubut and Neuquén Provinces) (Fig. 7).

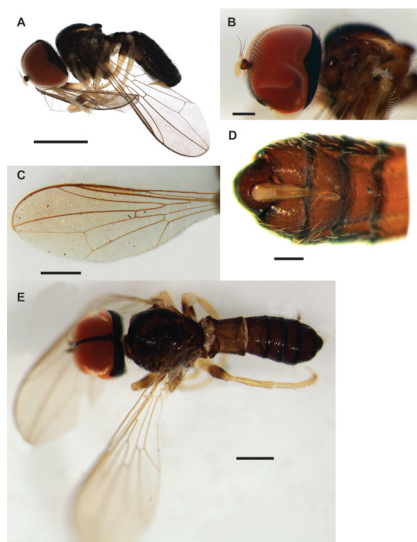


Fig. 6. *Protonephrocerus misionensis* sp. nov. BIOUG25021-F05; holotype female. A. lateral habitus. B. lateral close up of head and thorax. C. wing. D. ventral abdomen. E. dorsal habitus.

Scale bar A = 1 mm, B, D - 200  $\mu$ m, C, E - 500  $\mu$ m.

*Protonephrocerus misionensis* Skevington, n. sp.

(Figs. 6, 7)

**Diagnosis. Female (male unknown):** Scutum mostly black with yellow bristles. Hind femur anterodorsally with 6-8 outstanding long bristles near apex. Wing with M. vein absent. Ovipositor short, downcurved (Fig. 6).

**Description.** Female: Body length 3.10 mm; Wing length 2.92 mm.

**Female. Head** (Fig. 6). Pedicel brown with 2 short dorsal bristles and 2 ventral bristles. **Thorax** (Fig. 6). Postpronotal lobe dark blackish brown. Scutum mostly dark brown to black with yellow bristles. Scutellum black (bristles missing, rubbed off). **Wing** with M. vein absent. **Legs** mostly yellow with dark brown on basal half of femora; long and slender; with dense pubescence and bristles. Small hairs on trochanters, femora, tibiae and tarsi mostly black; mid femur with posterior row of about 15 long yellow hairs from base to apex, and several rows of shorter bristly hairs; hind femur anterodorsally with 6-8 outstanding long yellow hairs near apex. **Abdomen** (Fig. 6) dark brown to black with yellow bristles. Tergite 1 with long yellow lateral hairs anterolaterally. Sternites brown. Ovipositor yellow, short, piercer distinctly curved downwards in lateral view, 0.58 mm.

**Material examined.** Holotype ♀, Argentina, Misiones, Oberá, CIAR, 27.4447°S 54.9403°W, 147m, 3-17.x.2017, Pablo Tubaro, Malaise trap, BIOUG25021-F05 (MACN).

**Etymology.** Named after the Misiones region where the only known specimen was collected.

**Remarks.** The Misiones region where this species occurs is the southern extent of the Atlantic Forest ecozone that supports the highest biodiversity in Argentina. This species distribution is disjunct from that of other *Protonephrocercus* species, lying 1650 km NE of the closest collecting locality in central Chile (near Santiago).

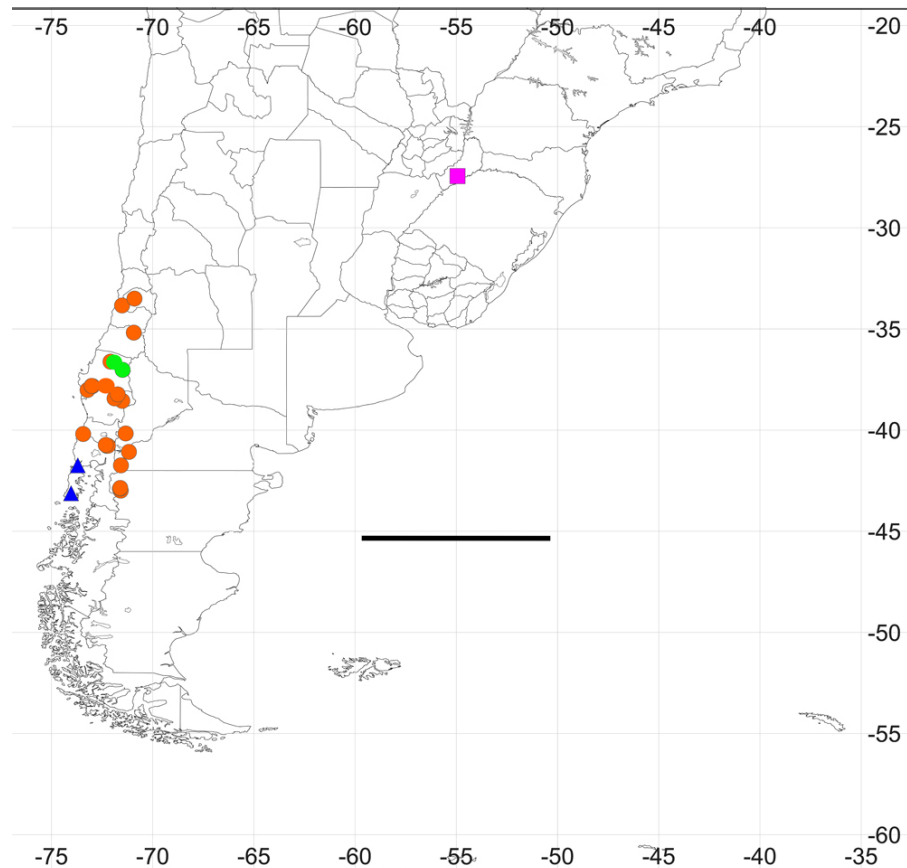


Fig. 7. Distributions. A. *Protonephrocercus chiloensis* (blue triangle). B. *Protonephrocercus flavipilus* sp. nov. (orange circle). C. *Protonephrocercus misionensis* sp. nov. (purple square). D. *Protonephrocercus* sp. 1988-01 (green circle). Scale bar = 1000 km.

We are generally opposed to describing pipunculids from females as the males are more character rich. However, we feel that the DNA barcode, the diagnostic missing M. wing vein, the short, narrow ovipositor, as well as the disjunct distribution makes it extremely unlikely that this species will become a *nomen dubium* like so many pipunculids described solely from females. We also feel that it is important to name this species to make it visible for conservation reasons. The Atlantic Forest has been decimated and much of the flora and fauna has disappeared or their distributions have become severely fragmented. This is thus the first stage

of getting to know this species. Hopefully awareness of it will spur further research.

The holotype specimen has been DNA barcoded (Table I). This species differs from its closest relative (*P. flavipilus* sp. nov.) by 10.1-10.9% (uncorrected pairwise distance). This considerable genetic difference from its nearest neighbour along with the missing M. wing vein suggest that this species has had a long independent history apart from other *Protonephrocerus* species, and as such is a significant piece of the puzzle needed to unravel the relationships of the family. Hopefully more specimens will be found now that we are aware of its existence.

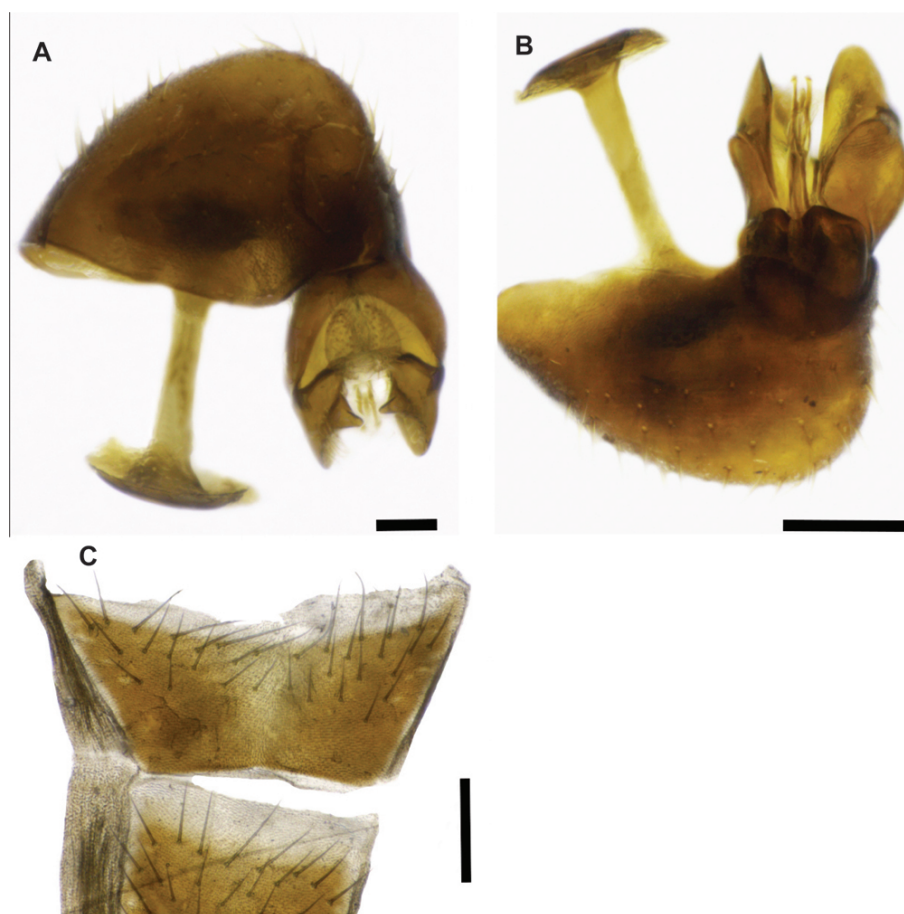


Fig. 8. *Protonephrocerus* sp. 1988-01. A. dorsal, male terminalia, CNC1766811. B. ventral, male terminalia, CNC1766811. C. male sternite 5, CNC1766810. Scale bars A = 0.1 mm; B-C = 0.2 mm.

**Distribution.** Known only from the Antonia Ramos Research Center (CIAR) near Oberá (Misiones province, Argentina) (Fig. 7).

*Protonephrocerus* sp. 1988-01 (Figs 7, 8)

Additionally, one putative undescribed species was mentioned by Rafael (1988) based on one male in poor condition (crushed), characterized by vein r-m more basal, placed at level of the end of vein Sc (versus slightly beyond the vein Sc in *P. chiloensis*, *P. flavipilus* sp. nov. and *P. misionensis* sp. nov.), male sternite 5 concave on posterior border (versus rectangular in *P. chiloensis* and with two invaginations on posterior border in *P. flavipilus* sp. nov.), surstyli bigger, as long as wide

in dorsal view (versus wider than long in *P. chiloensis* and *P. flavipilus* sp. nov.) and ejaculatory apodeme umbeliform (versus horn-shaped in *P. chiloensis* and *P. flavipilus* sp. nov.). The male specimen is from Chile, Ñuble, Las Trancas [-36.605707, -72.073482; CNC1766810], collected 1.ii.1983 by L.E. Peña and belongs to INPA. The undescribed male specimen does not fit the species described here and is another indication that the genus is more speciose than expected. A second specimen, also in very poor condition, appears to be this species and was collected 19-30.xii.1983 by L.E. Peña at Chile, Chillán, Shangrilá [-36.891458, -71.47309; CNC1766811] (also in INPA).

This article has been registered in the Official Register of Zoological Nomenclature (ZooBank) as [urn:lsid:zoobank.org:pub:989E813B-51E6-46E5-ACFEA7A3857943A9].

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