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New species and phylogenetic relationships of the spider genus *Coptoprepes* using morphological and sequence data (Araneae: Anyphaenidae)

MARIANA L. BARONE[1,3], VICTORIA WERENKRAUT[2] & MARTÍN J. RAMÍREZ[1]

[1] *Division of Arachnology, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Av. Ángel Gallardo 470 (C1405DJR), Ciudad Autónoma de Buenos Aires, Argentina*

[2] *INIBIOMA, UNCOMA, CONICET, Laboratorio Ecotono, Centro Regional Universitario Bariloche, Quintral 1250, 8400 Bariloche, Río Negro, Argentina*

[3] *Corresponding author. E-mail: marianlbarone@gmail.com*

Abstract

We present evidence from the standard cytochrome c oxidase subunit I (COI) barcoding marker and from new collections, showing that the males and females of *C. ecotono* Werenkraut & Ramírez were mismatched, and describe the female of that species for the first time. An undescribed male from Chile is assigned to the new species *Coptoprepes laudani*, together with the female that was previously thought as *C. ecotono*. The matching of sexes is justified after a dual cladistics analysis of morphological and sequence data in combination. New locality data and barcoding sequences are provided for other species of *Coptoprepes*, all endemic of the temperate forests of Chile and adjacent Argentina. Although morphology and sequences are not conclusive on the relationships of *Coptoprepes* species, the sequence data suggests that the species without a retrolateral tibial apophysis may belong to an independent lineage.

Key words: Amaurobioidinae, genetic barcoding, integrative taxonomy, Neotropical Region

Introduction

The species of the spider genus *Coptoprepes* Simon are endemic of the temperate forests of Chile and adjacent Argentina (Ramírez 2003). Their taxonomy has been recently revised by Ramírez (2003) and Werenkraut & Ramírez (2009), who also studied their phylogeny using morphological characters. According to those studies, *Coptoprepes* belongs to the tribe Amaurobioidini, and is atypical by presenting variability in characters that are usually constant in entelegyne genera; for example, *Coptoprepes* species have intrageneric variation in the presence and shape of a retrolateral tibial apophysis (RTA) in the male palp, the wrapping of the copulatory ducts along an axis, or the spermathecae being spherical. Because all those characters are important as potential synapomorphies in clades of the tribe Amaurobioidini, it is not surprising that this variation erodes support in the neighboring branches of the phylogenetic tree (Werenkraut & Ramírez 2009). The relationships and monophyly of *Coptoprepes* were further tested in a total evidence analysis by Labarque *et al.* (2015), who added to the previous morphological data two nuclear genes, the ribosomal 28S rRNA and the protein-coding histone H3, and two mitochondrial genes, the ribosomal 16S rRNA and the protein-coding cytochrome c oxidase subunit I (COI). They found that the support for the monophyly of *Coptoprepes* came mainly from the morphological data; the sequence data by itself segregated *Coptoprepes campanensis* Ramírez, precisely one of the species that lacked the RTA. There is another species in the genus, *C. ecotono* Werenkraut & Ramírez, which has only a small remain of a RTA. However, in that study they lacked sequences for *C. ecotono*. Here we include COI sequence for this species, which led us to conclude that the males and females were mismatched. In addition, we present a new species of *Coptoprepes*, based on a male from Chile. In the light of this new evidence, we believe it is necessary to re-assess the relationships of *Coptoprepes*, particularly with regards to *C. ecotono*.

The identification of males and females as conspecifics is indeed a challenge in *Coptoprepes*. Such a matching is sometimes difficult in spiders, whenever the species lack diagnostic characters shared by both sexes (Edwards

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2013). *Coptoprepes* species pose this difficulty because they have a conservative somatic morphology, several species may occur in sympatry, and for same rare species, males and females were not collected together in the same locality. It has been shown that the standard COI barcoding marker has a good performance in delimiting species in spiders (Barrett & Hebert 2005; Robinson *et al.* 2009; Blagoev *et al.* 2013; Hamilton *et al.* 2013; Castallanelli *et al.* 2014), thus we applied the technique here to validate, when possible, the matching of sexes in *Coptoprepes*.

Material and methods

Female genitalia were dissected and placed in clove oil for further observation on a compound microscope Olympus BH-2. Male palps were separated from the body and observed on a stereoscopic microscope Leica M165C with a digital camera Leica DFC 290. Both structures were illustrated using camera lucida and images were obtained with focal planes composed with Helicon Focus 4.62 Pro software. The format of descriptions follows Ramírez (2003) and Werenkraut and Ramírez (2009); measurements are given in millimeters. Images and illustrations are referred to through their museum or voucher numbers or using our preparation codes: 3 letters ("MLB" for Mariana L. Barone, "VIW" for Victoria Werenkraut) followed by a number.

The scorings of the morphological dataset were added to those of two recent published datasets by Labarque *et al.* (2015) and Aisen & Ramírez (2015). We also added cytochrome c oxidase subunit I (COI) sequences of *Negayan coccinea*, *Coptoprepes ecotono* and *Coptoprepes flavopilosus*, produced by Laboratory of Molecular Techniques in the Museo Argentino de Ciencias Naturales (MACN) and deposited in the Barcode of Life Data System (BOLD System) (Ratnasingham & Hebert 2007). These sequences were appended to the molecular dataset of Labarque *et al.* (2015), with sequences of two mitochondrial (COI, 16S) and two nuclear markers (28S, H3). Following the same procedures described there, the total evidence matrix (morphological and molecular datasets) was analyzed under parsimony using TNT version 1.1-June 2015 (Goloboff *et al.* 2008) with equal and implied weights. As support measures we used jackknifing frequencies expressed as absolute frequency (Goloboff *et al.* 2003). In order to allow comparisons with previous results, the constant of concavity for the weighting function was $k = 100$, as determined in Labarque *et al.* (2015) for the molecular and combined datasets, and $k = 6$ for the morphology-only dataset, as in Werenkraut & Ramirez (2009). The total evidence matrix was also analyzed under Bayesian analysis using Mr.Bayes 3.2.1 (Ronquist & Huelsenbeck 2003) running on the CIPRES Science Gateway (Miller *et al.* 2010). To select partitions and the best-fitting model of evolution for each partition we used Partition Finder v.1.1.1 (Lanfear *et al.* 2012). Supports respond to Bayesian posterior probability. COI Barcode fragments were amplified with primers C_LepFolF/C_LepFolR in MACN Barcoding laboratory or in the Canadian Centre for DNA Barcoding (CCDB), Ontario, sequenced in the CCDB, and analyzed with the default values of BOLD Systems Public Data Portal (http://www.boldsystems.org/).

To assist with the matching of sexes in somatically similar species of *Coptoprepes* and in the absence of sequence data, we performed a dual cladistic analysis as proposed by Platnick & Shadab (1978), scoring the male and female as separate terminals in the morphological phylogenetic dataset (named split matrix). If they were the same species, the expectation is that they are grouped together or nearby in the tree. Once the sexes were matched, the final matrix was composed merging the scorings of male and female (named *merge* matrix). The datasets are publicly available in https://dx.doi.org/10.6084/m9.figshare.3473777.v1.

The material examined is deposited in the following collections (acronyms and curators in parentheses): California Academy of Sciences, San Francisco (CAS/CASENT, Charles E. Griswold); Collection of Arachnids, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires (MACN-Ar, Cristina Scioscia); Museo Nacional de Historia Natural de Santiago, Santiago (MHNS, Mario Elgueta).

Abbreviations: AB, accessory bulb; ap, apical; bas, basal; C2, secondary conductor; CD, copulatory duct; CO, copulatory opening; d, dorsal; E, embolus; EBP, embolus basal process; FD, fertilization duct; MA, median apophysis; p, prolateral; RTA, retrolateral tibial apophysis; r, retrolateral; S, spermathecae; T, tegulum, v, ventral.

Taxonomy

Coptoprepes Simon, 1884

Coptoprepes Simon, 1884: 130, 136 (type species *Coptoprepes flavopilosus* Simon, 1884); Ramírez, 2003: 79; Werenkraut & Ramírez, 2009: 4.

Coptoprepes laudani, new species

Figs 1–2

Coptoprepes ecotono Werenkraut & Ramírez 2009: 9 (only female paratype, MACN-Ar 20320, misidentification, examined).

Note. The female paratype of *Coptoprepes ecotono* (MACN-Ar 20320, from Lago Espejo, Argentina) was collected from a locality about 54 km apart from the type locality of that species (Bariloche). The dual cladistic analysis of the split matrix resulted in a tree in which the Lago Espejo female and an undescribed male from Nahuelbuta National Park in Chile (unfortunately not well preserved for DNA extraction) arise together in a polytomy with *Coptoprepes bellavista* (Fig. 6B). Males and females of this last species can be considered as adequately matched, as they were abundantly collected in pitfall traps near Bellavista, a low elevation locality at the north shore of Lake Villarrica. Despite of intensive sampling by entomologists Alfred Newton, Margaret Thayer, Stewart Blaine Peck and Jarmila Kukalova-Peck, only *C. bellavista* was found there. After this result we decided to match tentatively the undescribed male and the female once regarded as belonging to *C. ecotono*, as the new species *C. laudani;* we do this with some hesitation, as they were collected in localities separated by 400 km, but other *Coptoprepes* species also show wide distributions.

Type material. Holotype. Male from Chile, Región IX (Araucanía), Prov. Malleco, Nahuelbuta National Park, S37.78464°, W72.95329°, March 2001, J.E. Barriga, pitfall trap (MHNS, ex MACN-Ar 31144, temporary preparations MLB 04086, MLB 00578).

Etymology. The specific epithet is a noun in apposition, dedicated to M. Barone's parents; "laudani" is a combination of their first names, Laura and Daniel.

Diagnosis. Females are similar to those of *C. valdiviensis* Ramírez by having a flat unsclerotized median field (Werenkraut & Ramírez 2009: fig. 7G) but differs in the first portion of the copulatory ducts, which are longitudinally orientated (Werenkraut & Ramírez 2009: figs 24A–C). Males are distinguished from those of *C. valdiviensis* by having a median apophysis with wide base and two processes, one conic and short and the other long, curved and thin, less sclerotized (Figs 1C–D, 2).

Description. *Male* (holotype). Total length 4.52 (including pedicel, stretched). Carapace length 1.83, width 1.23. Tibia of the palp shorter than patella, width/length 1.00. Length of tibia/metatarsus I—1.27/1.10; II—0.93/0.83; III—0.70/missing; IV—1.05/1.30. Chelicerae with four (left) or five (right) promarginal teeth and six (left) or five (right) retromarginal teeth. Sternum length 0.97, width 0.75. Abdomen length 2.20, width 1.13. Tracheal spiracle 0.30 from spinnerets, 1.23 from epigastric furrow. *Spines*: leg I—femur d 1-1-1, p d1ap; patella d 1 bristle bas, r d1; tibia v 2-2-p1 (all displaced to prolateral side); metatarsus v 2bas. II = I, except tibia d (r1-0-1) bristles, v r1-r1-2. III—femur d 1-1-1, p and r 0-d1-d1; patella d 1bas bristle, r d1; tibia d r1bas, p and r 1-d1-1-0, v p1-p1-2; metatarsus missing (right legs II and III missing). IV—femur d 1-1-1, p 0-d1-d1, r d1ap; patella d 1bas bristle, r d1; tibia d r1bas, p and r 1-d1-1-0, v p1-2-2; metatarsus d 0-p1-2, p and r d1-1-1, v 2-2-comb. Color in ethanol: dark brown, carapace reddish brown, sternum and legs lighter than carapace. Abdomen brown, with small light spots. Ventral abdomen lighter than dorsal, with lighter lateral lines (Figs 1A–B). RTA slightly curved, with truncated tip. Embolus with basal process ample, flattened. Primary conductor with canal where embolus fits, apical tip rounded, unsclerotized. Apical end of secondary conductor straight and laminar, with apical edge thicker and more sclerotized. Median apophysis with wide base and two medium sized processes, one conic and shorter and the other long, curved and lightly sclerotized (Figs 1C–D, 2).

Female (Paratype of *Coptoprepes ecotono*). Described in Werenkraut & Ramírez (2009: 14, figs 6, 7G–H, 22A, 24A–C).

Distribution. Known from Nahuelbuta National Park in Región IX (Araucanía) in Chile, and Río Negro and Neuquén provinces in Argentina.

Other material examined. ARGENTINA: Río Negro: Bariloche: Nahuel Huapi Natl. Park, Bariloche, S41.15415° W71.29899°, March 1947, A.G. Giai, 1♂ (MACN-Ar 2227). **Neuquén: Los Lagos:** Nahuel Huapi Natl. Park, Lago Espejo, S40.61322° W71.74867°, 21.I.1985, M.J. Ramírez 1♀ (MACN-Ar 20320, paratype of *Coptoprepes ecotono*).

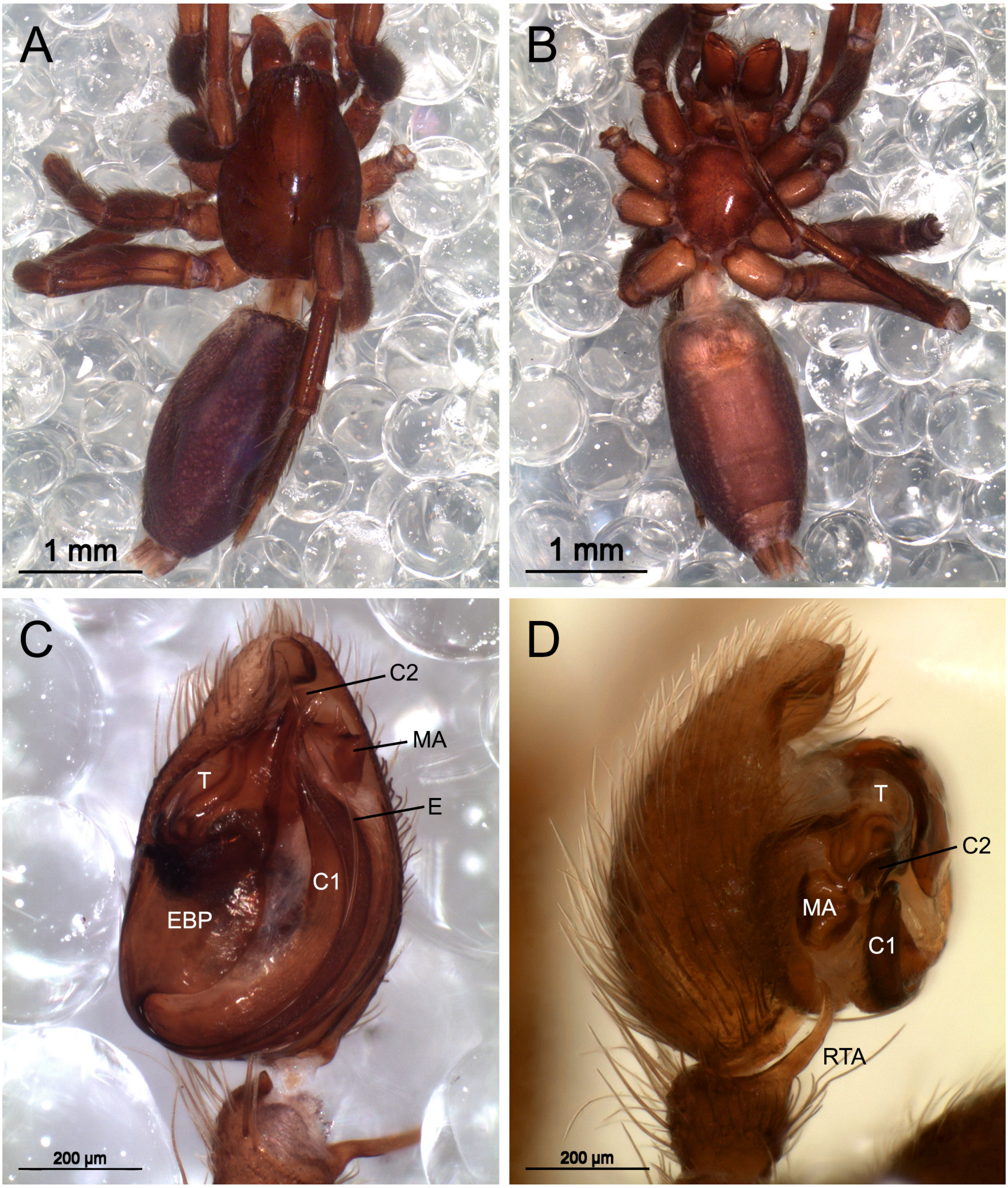

FIGURE 1. *Coptoprepes laudani* new species (MHNS, holotype, temporary preparation MLB 00578), male: **A**–**B** habitus; **A** dorsal; **B** ventral; **C**–**D** palp; **C** left palp, ventral view; **D** right palp, expanded, bulb in apical view. Abbreviations: C1, primary conductor; C2, secondary conductor; E, embolus; EBP, embolus basal process; MA, median apophysis; RTA, retrolateral tibial apophysis; T, tegulum.

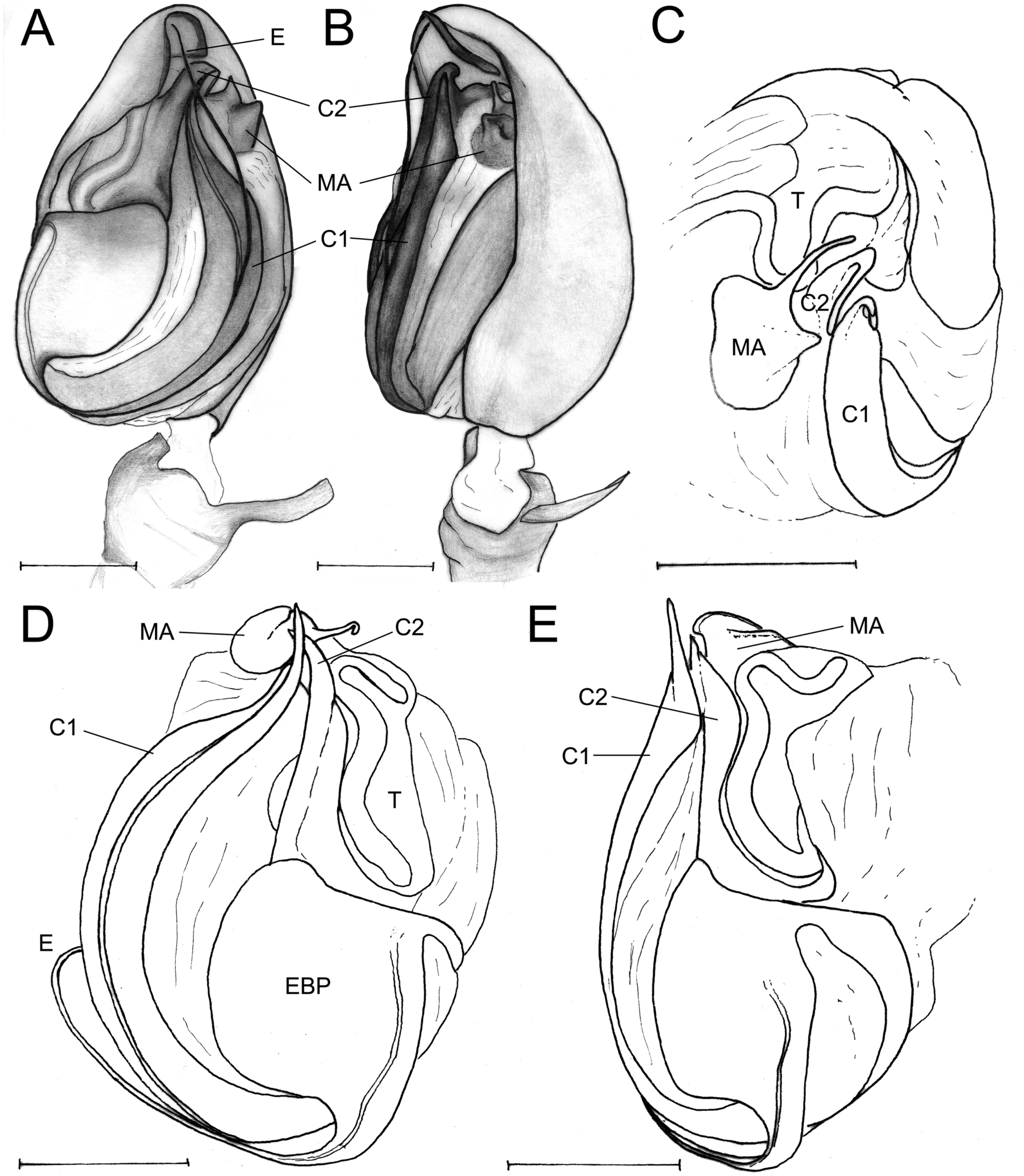

FIGURE 2. *Coptoprepes laudani* new species (MHNS, male holotype): **A**–**B** left palp; **A** ventral view; **B** retrolateral view. **C**–**E** right palp, expanded; **C** apical view; **D** ventro-lateral view; **E** prolateral view. Scale bars = 200 µm. Abbreviations: C1, primary conductor; C2, secondary conductor; E, embolus; EBP, embolus basal process; MA, median apophysis; T, tegulum.

Coptoprepes ecotono, Werenkraut & Ramírez
Figs 3–4

Coptoprepes ecotono Werenkraut & Ramírez 2009: 9 (male holotype MACN-Ar 2218, female paratype excluded).

Note. Our analysis of the COI barcoding sequence indicates that a male with the characteristics of *Coptoprepes ecotono* (CASENT 9029781) share identical sequences with a still undescribed female. Both specimens were collected in the same locality in Chiloé, southern Chile, and have similar somatic morphology. Thus we consider them as conspecific. In comparison, the minimum distance we found between *Coptoprepes* species is 5.3%, between *C. flavopilosus* and *C. bellavista*. The female originally described as *C. ecotono* is here identified as *C. laudani* n. sp.

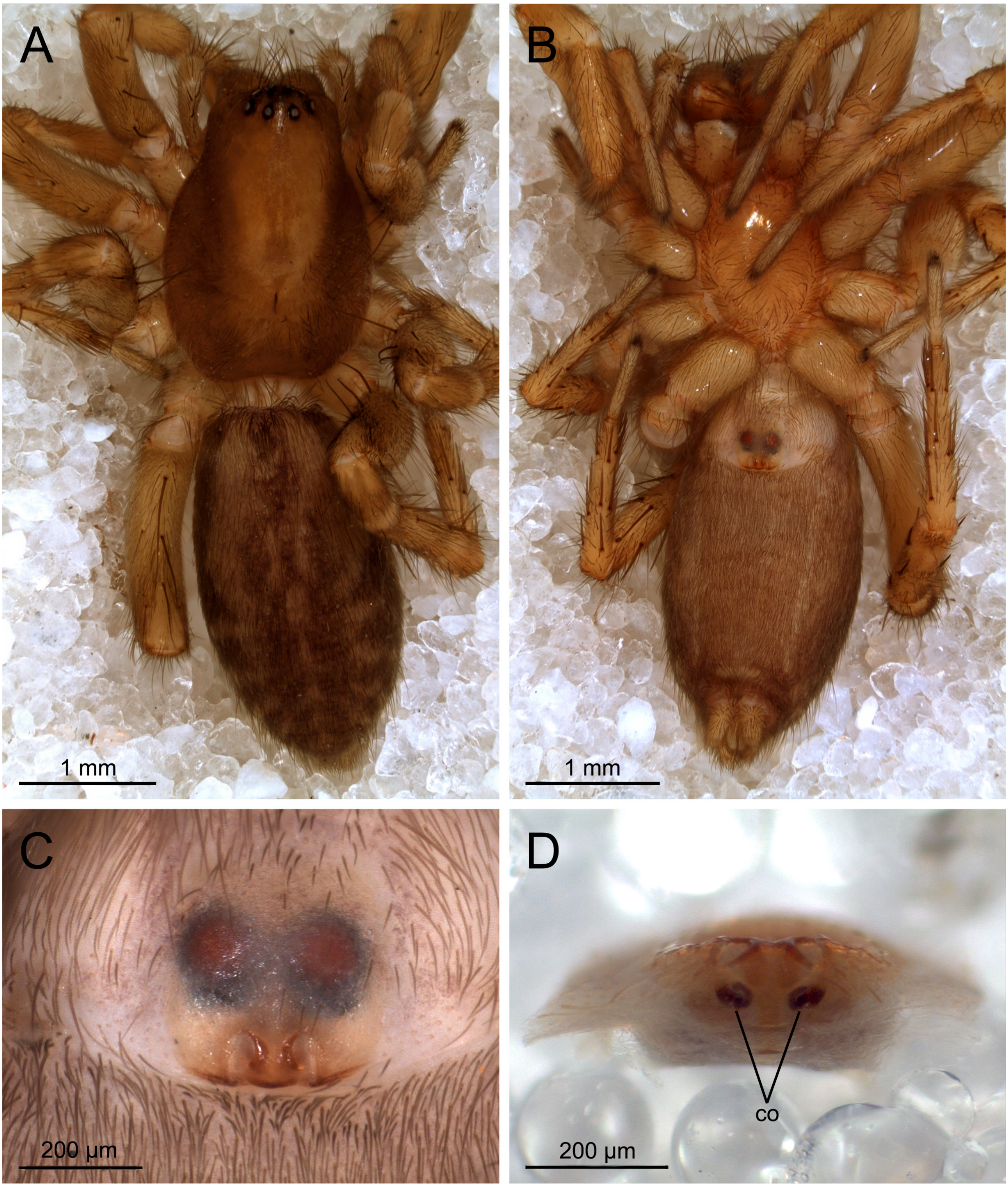

FIGURE 3. *Coptoprepes ecotono* Werenkraut & Ramírez (MACN-Ar 19397, temporary preparation VIW-00007), female: **A**–**B** habitus; **A** dorsal; **B** ventral; **C**–**D** epigyne; **C** ventral view; **D** posterior view. Abbreviation: CO, copulatory opening.

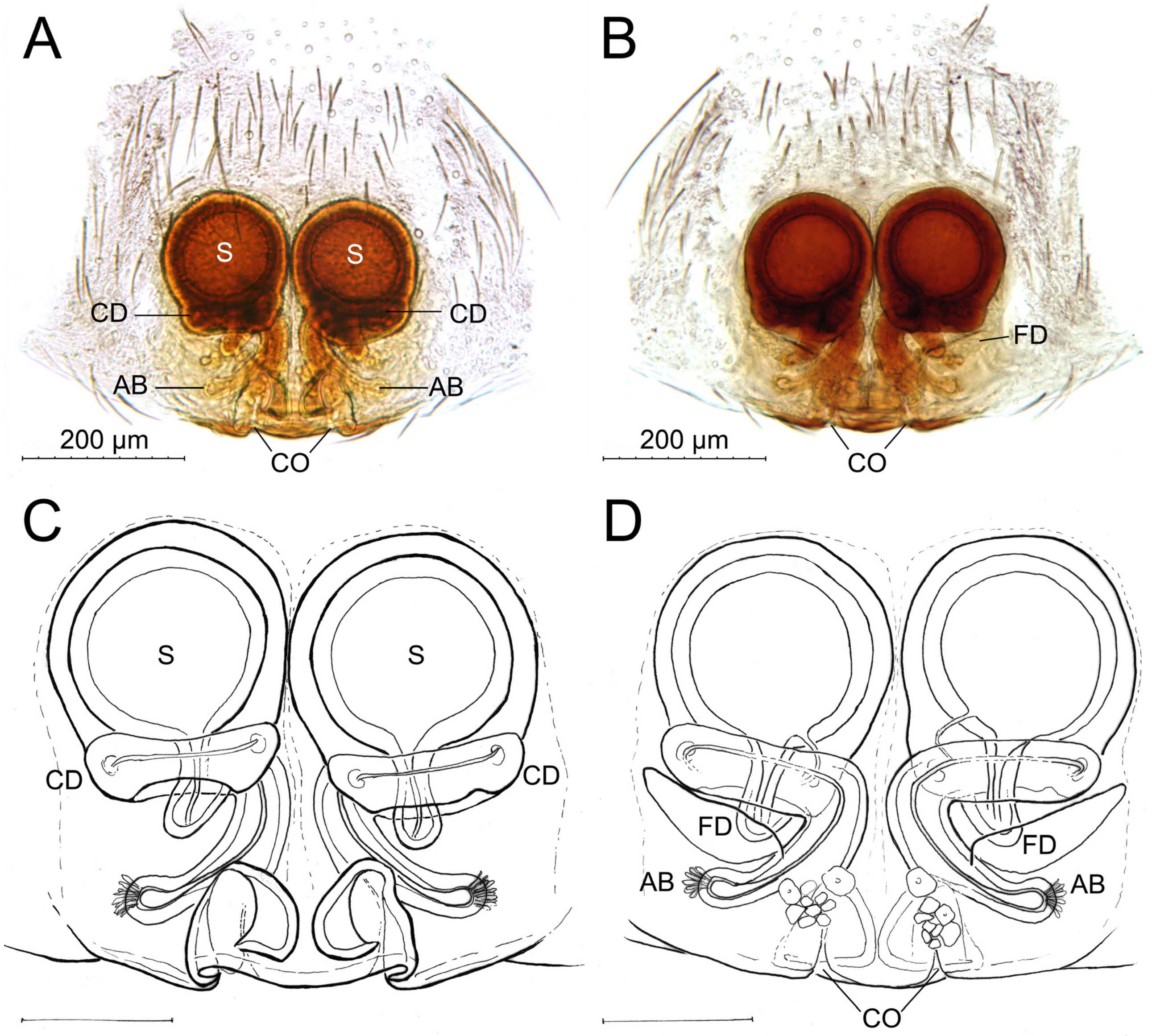

FIGURE 4. *Coptoprepes ecotono* Werenkraut & Ramírez (MACN-Ar 19397, temporary preparation MLB 00902), cleared epigyne: **A, C** ventral view; **B, D** dorsal view. Scale bars: **A– B** 200 µm; **C–D** 100 µm; Abbreviations: AB, accessory bulb; CD, copulatory duct; CO, copulatory opening; FD, fertilization duct; S, spermatheca.

Emended diagnosis. Males are similar to those of *C. campanensis* by virtually lacking RTA, but can be distinguished by having the median apophysis smaller, not bifid (Werenkraut & Ramírez 2009: figs 7A–F, 23A–C). Females are easily distinguished from those of all other *Coptoprepes* by having two small superficial pouches close to the epigastric furrow and spherical, contiguous spermathecae, with the copulatory ducts posterior to them (Fig. 4). *Coptoprepes casablanca* has roughly spherical spermathecae, but with the copulatory ducts lateral to them (see Werenkraut and Ramírez 2009: figs 24G–H).

Description. *Male* (holotype). Described in Werenkraut & Ramírez (2009: 9, figs 5, 7A–F, 23A–C).

Female (MACN-Ar 19397). Total length 5.19. Carapace length 2.39, width 1.60. Length of tibia/metatarsus I—1.00/0.87; II—0.93/0.87; III—0.80/0.93; IV—1.30/1.40. Palpal tarsus length 0.63. Chelicerae with three promarginal and four retromarginal teeth. Sternum length 1.30, width 0.93. Abdomen length 2.87, width 1.47. Tracheal spiracle 0.23 from spinnerets, 1.50 from epigastric furrow. *Spines*: leg I—femur d 1-1-1, p d2ap; metatarsus v 2bas. II—femur d 1-1-1, p d1ap; metatarsus v 2bas. III—femur d 1-1-1, p 0-d1-d1, r d1ap; patella r d1; tibia p 1-d1-1-0, r 0-d1-1-0, v p1-2-2; metatarsus d 0-p1-2, p and r d1-1-1, v 2-0-(2+thick setae). IV—femur d 1-1-1, p 0-d1-d1, r d1ap; patella r d1; tibia p and r 1-d1-1-0, v 2-2-2; metatarsus d 0-2-2, p and r d1-1-1, v 2-2-

(2+thick setae). Color in ethanol: Carapace and sternum reddish brown, legs lighter than prosoma. Dorsal abdomen light brown with symmetrical pattern of dark brown patches, ventral abdomen light brown. Sternum reddish brown, darker than legs (Figs 3A–B). Epigyne with two superficial pouches close to epigastrium, spermathecae spherical, contiguous to each other (Figs 3C, 4). Copulatory openings close to epigastrium (Figs 3D, 4), accessory bulbs oriented posteriorly, connection with copulatory ducts apparently close to the beginning of the latter. Copulatory ducts describing a loop from dorsal to ventral at base of spermathecae. First portion of fertilization duct emerging from the base of spermathecae, running posteriorly (Fig. 4).

Variability. Rarely five teeth on promargin of chelicerae. Spines slightly variable between right-left sides. Ventral spines of metatarsus in females absent or r 1bas. Leg III—femur r 0-d1-d1; tibia r 1-d1-1-0; metatarsus d 0-2-2, v x-2-x or x-p1-x. IV—femur p d1ap or none; tibia v p1-2-2. Frequently dorsal side of patella and tibia with long apical and basal bristles.

Distribution. Known from Nahuelbuta Natl. Park in Chile, Región IX (Araucanía) and Chiloé Island in Region X (Los Lagos), also in Río Negro province in Argentina.

Other material examined. ARGENTINA: Río Negro: Bariloche: El Bolsón, Reserva Forestal Loma del Medio-Río Azul, S41.97726° W71.55416°, Malaise trap, April 2002, P. Sackmann, 1♀ (MACN-Ar 19397, temporary preparations MLB 04167, VIW-00007, MLB 00902). **CHILE: Región IX (Araucanía): Malleco:** Nahuelbuta Natl. Park, S37.78464° W72.95329°, elev. 1200 m, fogging *Nothofagus dombeyi*, 12.II.2005, J.E. Barriga, 3♀ (MACN-Ar 31360, MACN-Ar 31361, MACN-Ar 31359). **Región X (Los Lagos): Chiloé:** Isla Chiloé, Chinquén, S42.61830° W74.10970°, elev. 60 m, fogging *Nothofagus nitida* and *Amomyrtus luma*, 04.III.2008, Arias et. al., 1♀ 1♂ (CASENT 9029781, temporary preparations MLB 04087, MLB 04088); fogging *Nothofagus nitida* and *Lonicera sempervirens* 1♀ (CASENT 9034099); elev. 31 m, fogging *Nothofagus nitida*, 01.III.2008, 1♀ (CASENT 9034041).

Genetic barcoding. The COI sequence of a male (CASENT 9029781) is identical as one of the females (CASENT 9034099), and diverge 0.8% with the other female sequenced (CASENT 9034041). This group of sequences diverge 6.1% with the nearest neighbor, the only record of *C. campanensis* (MACN-Ar 28847).

Coptoprepes bellavista Werenkraut & Ramírez, 2009

Diagnosis and description. Werenkraut & Ramírez (2009: 14)

New records. CHILE: Región IX (Araucanía): Cautín: Villarrica Natl. Park, 8.45 km S Pucón, 780 m, S39.35000° W71.97900°, 18.III.2008, H. Wood, 1♀ (CASENT 9028167).

Genetic barcoding. The only specimen sequenced for COI (CASENT 9028167) (Table 1) was established by BOLD as the founder of an operational taxonomic unit (BIN) (Fig. 5), and diverge 5.78% from the sequence of the nearest neighbor, a *C. flavopilosus* female (MACN-Ar 29569).

Coptoprepes campanensis Ramírez, 2003

Figs 7C–D

Diagnosis. Werenkraut & Ramírez (2009: 14)

Description. Ramírez (2003: 87)

Natural History. Most specimens were collected manually or with pitfall traps, on relict forests (Figs 7C–D).

New records. CHILE: Región VII (Maule): Curicó: Ruta J-55, 30 km W de Los Queñes, 1406 m (Figs 7C–D), S35.06316° W70.51248°, 08.II.2012, M.J. Ramírez, M.A. Izquierdo, P. Michalik, C. Wirknler & K. Huckstorf, 1♀ (MACN-Ar 29165). **Región V (Valparaíso): Quillota:** La Campana Natl. Park, Palma de Ocoa, 500 m, S32.96404° W71.08176°, 17.II.2005, M.J. Ramírez & F.M. Labarque, 1♂ (MACN-Ar 28847).

Genetic barcoding. The only specimen sequenced for COI (MACN-Ar 28847) (Table 1) was established by BOLD as the founder of an operational taxonomic unit (BIN) (Fig. 5), and diverge 6.34% from the sequence of the nearest neighbor, the only male of *C. ecotono* sequenced (CASENT 9029781).

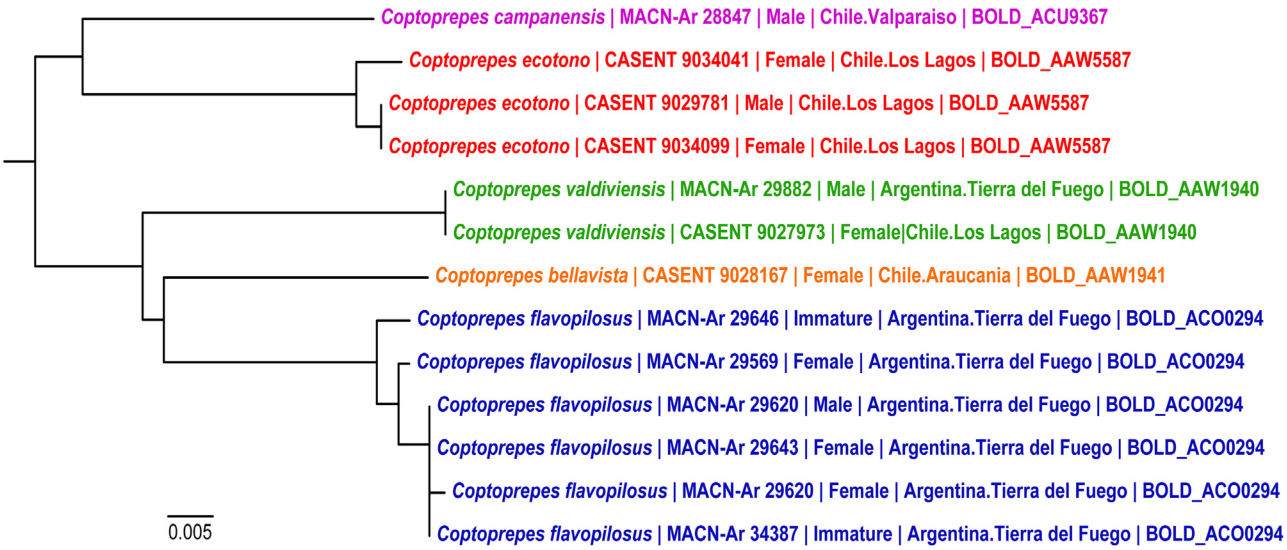

FIGURE 5. Neighbour-joining tree obtained using K2P distances, downloaded from BOLD, showing the relationships for *Coptoprepes* Simon. Data presented as: Species name | Collection number | Sex | Country. Region or province | BIN number. Each color represents a different Barcode Index Number (BIN).

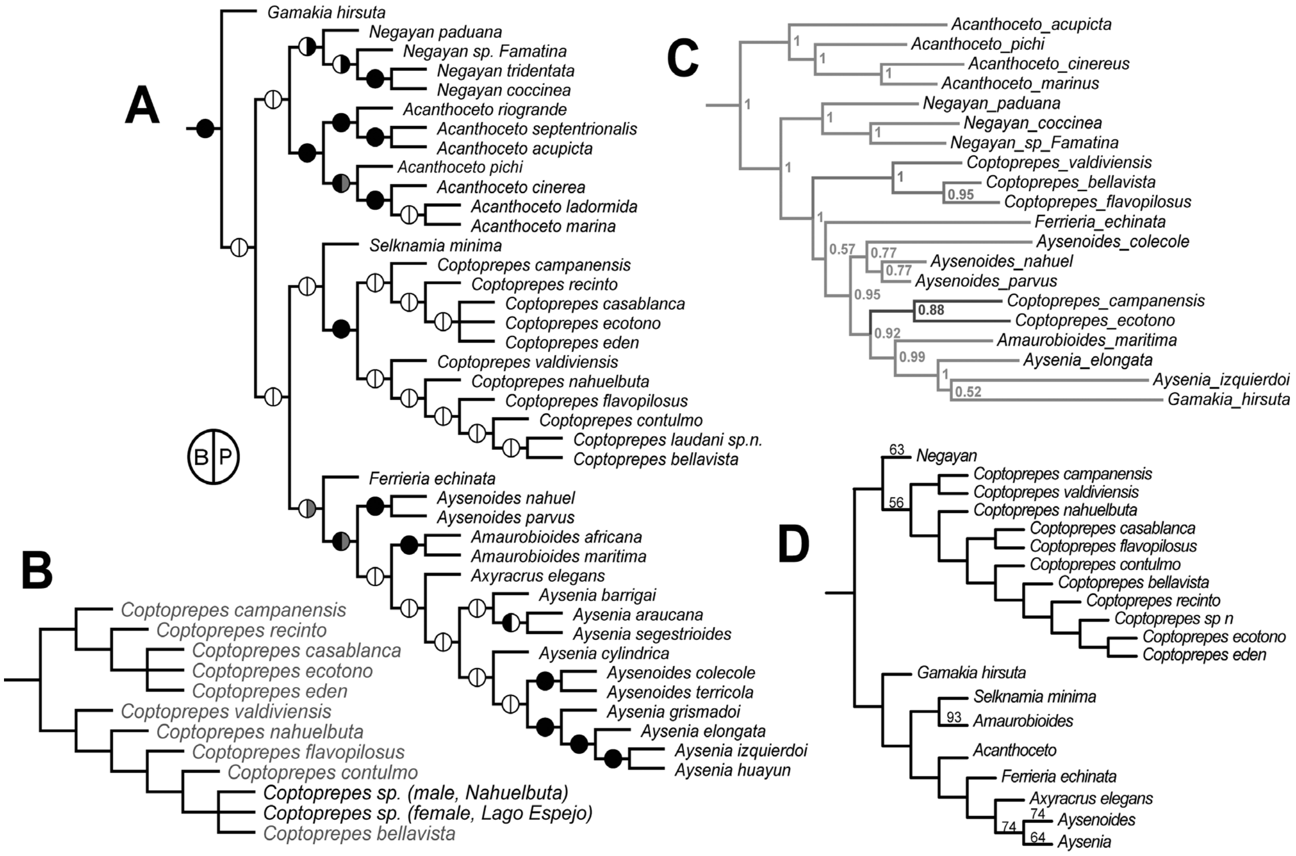

FIGURE 6. A Phylogenetic tree obtained in the parsimony analysis under implied weights of the combined molecular alignments and morphology (only Amaurobioidini shown). Circles at nodes indicate support levels by analysis (B, Bayesian; P, Parsimony). Black indicates posterior probabilities >0.95 and jackknifing proportions >0.75; grey indicates that the clade was recovered but with lower support than the previous values; white indicates that the clade was not recovered. **B** Dual cladistic analysis of the split matrix showing the proximity of the undescribed male from Nahuelbuta and the female from Lago Espejo (only *Coptoprepes* shown). **C** Bayesian tree from sequence data only (posterior probabilities on nodes); note the non-monophyly of *Coptoprepes* (colored clades) (only Amaurobioidini shown). **D** Summary of parsimony tree from the morphology data only (implied weights, jackknifing values on nodes) (only Amaurobioidini shown).

Coptoprepes flavopilosus **Simon, 1884**
Figs 7A–B, E–F

Diagnosis. Werenkraut & Ramírez (2009: 4)

Description. Ramírez (2003: 81)

Natural History. This species constructs retreats under logs in very humid localities (Figs 7 A, E, F).

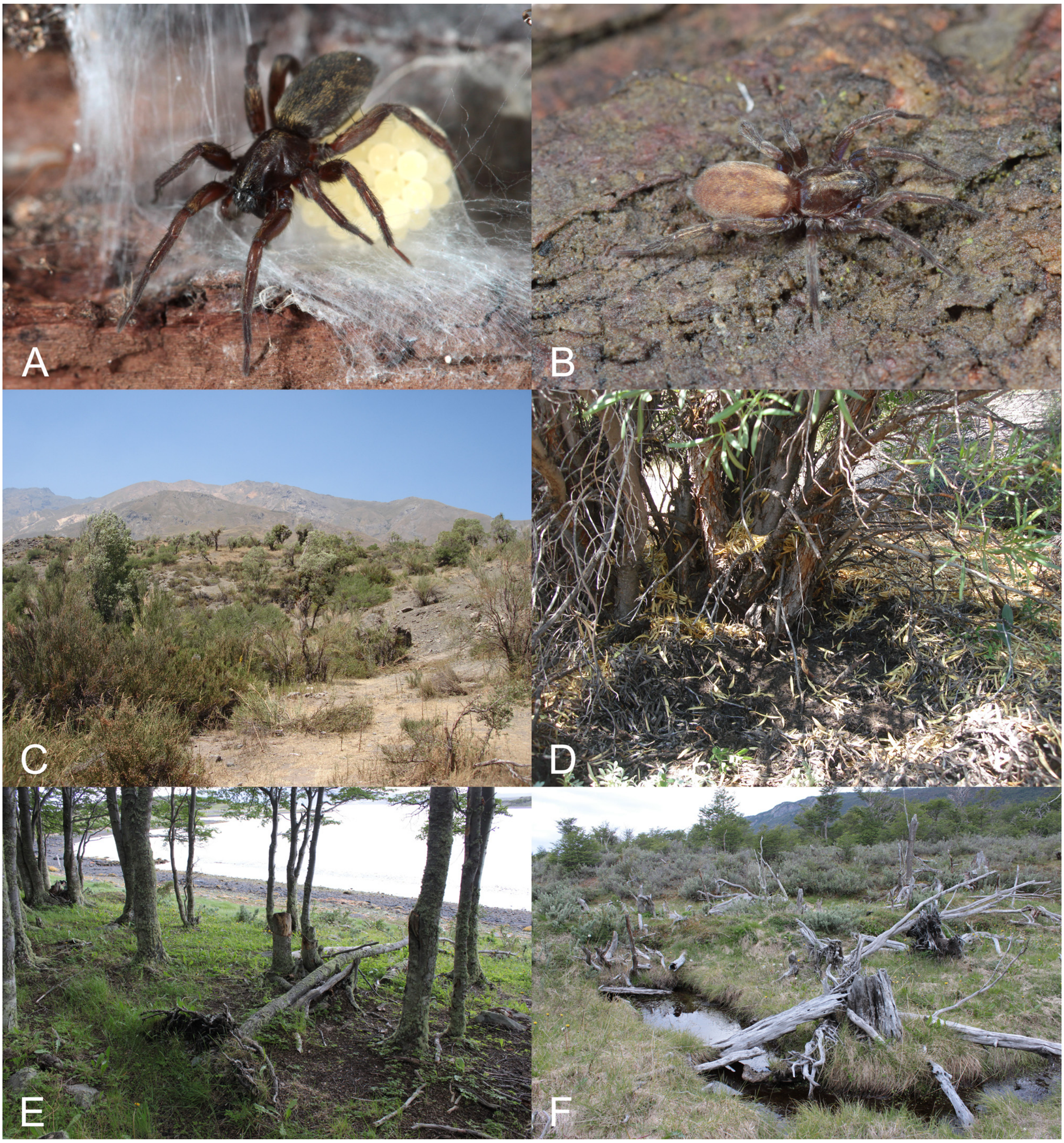

FIGURE 7. A–B Living specimens of *Coptoprepes flavopilosus* from Tierra del Fuego; **A** Female with eggsac (MACN-Ar 29808); **B** Immature (MACN-Ar 29646). **C** Shrubland 30 km W Los Queñes, central Chile, habitat of *Coptoprepes campanensis*. **D** Same, detail of leaf litter where the specimen was captured. **E** Forest in Moat, Tierra del Fuego, habitat of *Coptoprepes flavopilosus*. **F** Old beaver dam SW of Laguna Negra, Tierra del Fuego, habitat of *Coptoprepes flavopilosus*.

New records. ARGENTINA: Tierra del Fuego: Ushuaia: Laguna sobre Ruta Complementaria J, camino a Moat, 71 km E de Ushuaia, 35 m, S54.88383° W67.21405° (Fig. 7E), 05.XII.2012, M.J. Ramírez, C.J. Grismado, A. Ojanguren & E.M. Soto, 5♀ (MACN-Ar 29569, temporary preparation MLB 04096; MACN-Ar 29545, MACN-Ar 31145); Ruta Complementaria J camino a Moat, paraje Poste Fierro al pie del Cerro No Top, 75 km E de Ushuaia, 1 m, S54.89321° W67.15962°, 05.XII.2012, M.J. Ramírez, C.J. Grismado, A. Ojanguren & E.M. Soto, 1♀ (MACN-Ar 29603); Tierra del Fuego Natl. Park, castorera SW de Laguna Negra, 17 km WSW de Ushuaia, 9 m (Fig. 7F), S54.85033° W68.59602°, 03–11.XII.2012, M.J. Ramírez, C.J. Grismado, A. Ojanguren & E.M. Soto, 4♀ [plus 1 imm] (MACN-Ar 29643, temporary preparation MLB 04097; MACN-Ar 29647, MACN-Ar 31146; MACN-Ar 29646, temporary preparation MLB 04094); turbal sobre Ruta Nacional N° 3, 13 km WSW de Ushuaia, 89 m, S54.83991° W68.51438°, 09.XII.2012, M.J. Ramírez, C.J. Grismado, A. Ojanguren & E.M. Soto, 1♀ (MACN-Ar 29808); turbal SW de Laguna Negra, 17 km WSW de Ushuaia, 8 m, S54.84814° W68.59558°, pitfall trap, 03–11.XII.2012, M.J. Ramírez, C.J. Grismado, A. Ojanguren & E.M. Soto, 1♀ 2♂ [plus 1 imm] (MACN-Ar 29620, temporary preparation MLB 04098 and MLB 04099; MACN-Ar 31147; MACN-Ar 34387 temporary preparation MLB 04090).

Genetic barcoding. All the *C. flavopilosus* sequences are included in a single BIN (Fig. 5). The average distance between the sequences is 0.49%, and the distance from the nearest neighbor, the only record of *C. bellavista* (CASENT 9028167), is 5.3%.

Coptoprepes valdiviensis Ramírez, 2003

Diagnosis. Werenkraut & Ramírez (2009: 9)

Description. Ramírez (2003: 90)

New records. ARGENTINA: Tierra del Fuego: Ushuaia: Tierra del Fuego Natl. Park, turbal SW de Laguna Negra, 17 km WSW de Ushuaia, 8 m, S54.84814° W68.59558°, pitfall trap, 03–11.XII.2012, M.J. Ramírez, C.J. Grismado, A. Ojanguren & E.M. Soto, 1♂ (MACN-Ar 29882 temporary preparation MLB 04089). **CHILE: Región X (Los Lagos): Llanquihue:** Alerce Andino Natl. Park, 25.31 km E Puerto Montt, 120 m, S41.46700° W72.65000°, 15.III.2008, H. Wood & C. Griswold, 1♀ (CASENT 9027973).

Genetic barcoding. We were able to sequence specimens of both sexes (male MACN-Ar 29882, female CASENT 9027973); they have identical sequences, even when they come from distant localities (Fig. 5, Table 1). The divergence from the nearest neighbor, a female of *C. flavopilosus* (MACN-Ar 29569), is 5.94%. This result supports the matching of the sexes assigned in Ramírez (2003) for this species.

Phylogenetic analysis

The simultaneous analysis of sequences and morphology retrieves the monophyly of *Coptoprepes* with good support (Fig. 6A). The intraspecific relationships are however poorly supported, and there is little in common with the previous results from morphological data alone (Werenkraut & Ramírez 2009), which also generally depicted low support values. As expected, the morphological data in isolation produces results similar as in that study (Fig. 6D), but the sequence data did not retrieve *Coptoprepes* as a monophyletic group (Fig. 6C), although only the clade of *C. valdiviensis*, *C. bellavista* and *C. flavopilosus* has good support (Fig. 6C). It should be noted however that two of the species (*C. ecotono* and *C. flavopilosus*) are sampled only by a single marker added here (COI) (see Table 1), while the other *Coptoprepes* species have data for the four markers (see Labarque *et al.*, 2015, table S1).

Discussion

Our findings agree with those of Rubio & Ramírez (2015) about that standard barcoding marker provides useful evidence for matching sexes in anyphaenid spiders, combined with morphology and locality data. The same marker is informative also for phylogenetic inference, although with the logical limitations of a single piece of evidence. Upon comparison of the phylogenetic trees from the combined, sequences-only, and morphology-only datasets

(Figs 6A, C, D, respectively), it is clear that there is conflict in the two data sources, and that none of them provides decisive support for the relationships of *Coptoprepes*. The resolution provided by the sequence data, although poorly supported, is however interesting; the two species that are set apart from the genus (*C. campanensis* and *C. ecotono*) are the only ones with a vestigial or altogether absent retrolateral tibial apophysis (RTA) in the male palp. Further analyses with a better sampling of molecular markers and additional species, in combination with new morphological characters, may bring light to the relationships of *Coptoprepes*.

TABLE 1. Data for COI genetic barcoding sequences of *Coptoprepes* species.

Species	BOLD Sample ID	BOLD Process ID	Museum ID	Country / Province	GenBank Acc. Number
C. campanensis	MACN-Ar 28847_copca002	SPDCH437-15	MACN-Ar 28847	Chile, Región V	KX536683
C. ecotono	CASENT 9034041	SPDCH123-11	CASENT 9034041	Chile, Región X	KX536677
C. ecotono	MLB 04088	SPDCH435-14	CASENT 9029781	Chile, Región X	KX536675
C. ecotono	CASENT 9034099	SPDCH156-11	CASENT 9034099	Chile, Región X	KX536674
C. valdiviensis	MLB 04089	SPDAR494-14	MACN-Ar 29882	Argentina, Tierra del Fuego	KX536684
C. valdiviensis	CASENT 9027973	SPDCH022-11	CASENT 9027973	Chile, Región X	KX536679
C. bellavista	CASENT 9028167	SPDCH025-11	CASENT 9028167	Chile, Región IX	KX536680
C. flavopilosus	MLB 04094	SPDAR499-14	MACN-Ar 29646	Argentina, Tierra del Fuego	KX536673
C. flavopilosus	MLB 04096	SPDAR501-14	MACN-Ar 29569	Argentina, Tierra del Fuego	KX536682
C. flavopilosus	MLB 04090	SPDAR495-14	MACN-Ar 34387	Argentina, Tierra del Fuego	KX536685
C. flavopilosus	MLB 04098	SPDAR503-14	MACN-Ar 29620	Argentina, Tierra del Fuego	KX536676
C. flavopilosus	MLB 04099	SPDAR504-14	MACN-Ar 29620	Argentina, Tierra del Fuego	KX536681
C. flavopilosus	MLB 04097	SPDAR502-14	MACN-Ar 29643	Argentina, Tierra del Fuego	KX536678

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