*Zootaxa* 3636 (4): 547–560
www.mapress.com/zootaxa/

**Article**

ISSN 1175-5326 (print edition)
**ZOOTAXA**
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3636.4.3
http://zoobank.org/urn:lsid:zoobank.org:pub:CE50C436-232D-4539-A887-4F6723938B7B

# Phylogenetic relationships of the South American ground beetle subgenus *Chilioperyphus* Jeannel (Coleoptera: Carabidae: Trechinae: Bembidiini: *Bembidion* Latreille)

DAVID R. MADDISON[1,5], LUCA TOLEDANO[2], SOLEDAD SALLENAVE[3] & SERGIO ROIG-JUÑENT[4]

[1] *Department of Zoology, Oregon State University, Corvallis, OR 97331, USA. E-mail: david.maddison@science.oregonstate.edu*
[2] *Museo Civico di Storia Naturale, Lungadige Porta Vittoria, 9 I - 37129 Verona, Italy. E-mail: lucatole2@libero.it*
[3] *Secretaría de Ambiente y Desarrollo Sustentable, 7mo piso de Casa de Gobierno, Peltier 351 - Ciudad de Mendoza, Mendoza, Argentina. E-mail: soledad.sallenave@gmail.com*
[4] *IADIZA, CCT-CONICET, Mendoza. Laboratorio de Entomología. CC 507, 5500 Mendoza, Argentina*
*E-mail: saroig@mendoza-conicet.gov.ar*
[5] *Corresponding author*

## Abstract

The boundaries and relationships of the ground beetle group *Chilioperyphus* Jeannel (a subgenus of the cosmopolitan genus *Bembidion* Latreille) are examined using DNA and morphological data. DNA sequence data from seven genes (six nuclear and one mitochondrial) indicates that *Chilioperyphus* (as newly defined) is monophyletic, and is related to the subgenera *Antiperyphanes* Jeannel and *Plocamoperyphus* Jeannel, within the South American *Antiperyphanes* Complex. *Chilioperyphus* includes two described species, *B. mendocinum* Jensen-Haarup and *B. orregoi* Germain. *Bembidion cassinense* Roig-Juñent and Gianuca as well as *Bembidion cuyanum* Roig-Juñent and Scheibler, formerly placed in subgenus *Chilioperyphus*, are transferred to subgenus *Antiperyphanes*. *Bembidion cuyanum* is considered a junior synonym of *B. hirtipes* Jeannel. The male genitalia of *Chilioperyphus* is unique in having a very long flagellum that is folded twice, allowing it to fit much of its length within the walls of the median lobe. However, the brush sclerite and basal part of the flagellum are not contained within the median lobe, as they extend anterior to its base.

**Key words:** South America, DNA, morphology

## Resumen

Se analizaron los límites y las relaciones del grupo de coleópteros *Chilioperyphus* Jeannel (un subgénero del género cosmopolita *Bembidion* Latreille) usando los datos de la secuencia del ADN y las características morfológicas. Las secuencias del ADN indican que *Chilioperyphus* es monofilético y se relaciona con los subgéneros *Antiperyphanes* Jeannel y *Plocamoperyphus* Jeannel, dentro del complejo de *Antiperyphanes* que se halla en América del Sur. *Chilioperyphus* incluye dos especies descritas, *B. mendocinum* Jensen-Haarup y *B. orregoi* Germain. Por su parte, *Bembidion cassinense* Roig-Juñent y Gianuca y *Bembidion cuyanum* Roig-Juñent y Scheibler, anteriormente situados en el subgénero *Chilioperyphus*, se transfieren al subgénero *Antiperyphanes*. *Bembidion cuyanum* se considera un sinónimo de *B. hirtipes* Jeannel. El endofalo de *Chilioperyphus* es único al tener un flagelo muy largo que se dobla dos veces, lo que le permite alojarse enteramente dentro del edeago. Sin embargo, el esclerito en cepillo y la parte basal del flagelo no se sitúan en el lóbulo medio, sino que están por delante de su base.

**Palabras clave:** América del Sur, DNA, morfología

*Accepted by J. Serrano: 18 Feb. 2013; published: 8 Apr. 2013*

## Introduction

In 1962, René Jeannel revised the carabid beetle subfamily Trechinae (as Trechidae) from southern South America, and in the process described a new subgenus, *Chilioperyphus*, which he placed in *Peryphus* Dejean. The latter is now considered to be a subgeneric complex within the large genus *Bembidion*. The two species he placed in *Chilioperyphus* have medium-sized members, and are brown and spotted, with distinctive, convergent grooves on the dorsal surface of the head (Figs. 1–2).

Jeannel's concept of *Peryphus* included a large number of northern Hemisphere forms, and a small radiation of beetles of similar appearance in South America, among others. Maddison (2012) showed that the sampled members of the South American radiation (including the subgenus *Antiperyphanes*) were not closely related to true *Peryphus* (confirming Toledano's (2002; 2008) hypothesis), but were instead part of the *Bembidion* Series, and were related more closely to *Bembidion (s. str.),* subgenus *Notaphus*, and other South American *Bembidion*. Although members of subgenus *Chilioperyphus* were not sampled in Maddison (2012), the expectation, based in part upon the figures of the male genitalia Jeannel provided, was that these beetles would be closely related to subgenus *Antiperyphanes* and other members of the South American *Bembidion* Series, and not related to northern Hemisphere subgenus *Peryphus* or its relatives.

However, a few years ago, as we examined the types and other material of *B. (Chilioperyphus) mendocinum* Jensen-Haarup and *B. (Chilioperyphus) orregoi* Germain, we discovered that Jeannel's drawings of the male genitalia were in error, and that the true genitalic form did not match that of other *Antiperyphanes* complex members. Most curiously, the aedeagus of *Chilioperyphus* shares derived characteristics with the subgenus *Peryphanes* Jeannel, a northern Hemisphere group related to true *Peryphus* (Maddison, 2012). This raised the possibility that *Chilioperyphus* is related to *Peryphanes* and thus *Peryphus* as Jeannel had proposed, rather than being a member of the *Bembidion* Series like all of the other South American *Bembidion*.

The errors in Jeannel's drawings not only hid the true nature of the genitalia of *Chilioperyphus* for decades, but they also misled Roig-Juñent and Gianuca (2001) and Roig-Juñent and Scheibler (2004) to attribute two species to the subgenus *Chilioperyphus: B. cassinense* Roig-Juñent and Gianuca and *B. cuyanum* Roig-Juñent and Scheibler. Both of these species have male aedeagi very similar to those shown in Jeannel's (incorrect) figures of *Chilioperyphus*. In this paper, we remove these species from *Chilioperyphus,* and place them in subgenus *Antiperyphanes*; we also establish *B. cuyanum* as a junior synonym of *B. hirtipes* Jeannel.

To resolve the relationships of *Chilioperyphus*, we here examine DNA sequences of the two original members of subgenus *Chilioperyphus* (*B. mendocinum* and *B. orregoi*), as well as an undescribed species from northern Argentina, in the context of data from other species of *Bembidion*. We conclude that *Chilioperyphus* is related to other South American *Antiperyphanes* Complex members, and that the genitalic similarities to subgenus *Peryphanes* are convergences.

## Methods

Approximately 130 specimens of *Bembidion* (*Chilioperyphus*) were examined from the collections listed below; each collection listing begins with the codon used in the text. Included in the examined material were the syntypes of *B. mendocinum* (ZMUC, MACN) and *B. orregoi* (MNNC). We have also examined the type material of *B. cassinense* (FURG) and *B. cuyanum* (IADIZA).

| | |
|---|---|
| FURG | Departamento de Oceanografía of the Fundação Universidade Federal do Rio Grande |
| IADIZA | Instituto Argentino de Investigaciones de las Zonas Aridas, Mendoza, Argentina |
| MACN | Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina |
| MLP | Museo de La Plata, La Plata, Buenos Aires, Argentina |
| NHMW | Naturhistorisches Museum, Vienna |
| MNHN | Muséum National d'Histoire Naturelle, Paris |
| MNNC | Museo Nacional de Historia Natural, Santiago, Chile |
| OSAC | Oregon State Arthropod Collection, Oregon State University |
| ZMUC | Natural History Museum of Denmark, University of Copenhagen |

**Collecting methods.** Specimens were collected by hand or using an aspirator; specimens were found during the day in their habitat after splashing the soil with water, or with the aid of a headlamp at night, when the beetles are more actively moving on the surface.

Specimens for morphological studies were killed and preserved in *Acer* sawdust to which ethyl acetate was added. Specimens for DNA sequencing were collected into 95% or 100% ethanol, with best results obtained when the abdomen was slightly separated from the rest of the body to allow better penetration.

**TABLE 1.** Sampling of the *Antiperyphanes* Complex. In the columns for each gene, four-digit numbers are D.R. Maddison DNA voucher numbers for the newly sequenced specimens. Other entries are GenBank numbers of previously published sequences from Maddison (2008;2012), Hildebrandt & Maddison (2011), Maddison & Ober (2011), and Wild & Maddison (2008). Thus, all cells with entries have DNA sequences of that gene for that species. Further information about the newly sequenced specimens is given in Table 3. The species number is listed in the "#" column; these numbers are used in Table 4.

| # | | CAD | wg | ArgK | Topo | 28S | 18S | COI |
|---|---|---|---|---|---|---|---|---|
| | **Subgenus *Chilioperyphus* Jeannel** | | | | | | | |
| 1 | *B. mendocinum* Jensen-Haarup | | 2332 | | 2332 | 2332 | | 2332 |
| | *B. mendocinum* Jensen-Haarup | 2333 | 2333 | 2333 | 2333 | 2333 | 2333 | 2333 |
| 2 | *B. orregoi* Germain | 2337 | 2337 | 2337 | 2337 | 2337 | 2337 | 2337 |
| | *B. orregoi* Germain | | 2338 | | 2338 | 2338 | | 2338 |
| 3 | *B. (Chilioperyphus)* n. sp. "Cal" | 2700 | 2700 | 2700 | 2700 | 2700 | 2700 | 2700 |
| | *B. (Chilioperyphus)* n. sp. "Cal" | 2704 | 2704 | 2704 | 2704 | 2704 | | 2704 |
| | *B. (Chilioperyphus)* n. sp. "Cal" | 2706 | 2706 | 2706 | 2706 | 2706 | | 2706 |
| | **Subgenus *Antiperyphanes* Jeannel** | | | | | | | |
| 4 | *B. caoduroi* Toledano | JN170771 | JN171375 | JN170525 | JN171188 | JN170305 | JN170158 | JN171006 |
| 5 | *B. zanettii* Toledano | 2679 | 2679 | 2679 | 2679 | 2679 | 2679 | 2679 |
| 6 | *B. chilense* Solier | JN170779 | JN171382 | JN170533 | JN171196 | JN170313 | | JN171014 |
| 7 | *B.* sp. nr. *chilense* Solier | JN170920 | GU556037 | JN170677 | JN171297 | GU556088 | JN170236 | JN171117 |
| 8 | *B. spinolai* Solier | JN170925 | JN171523 | JN170682 | JN171302 | JN170448 | | JN171122 |
| 9 | *B. hirtipes* (Jeannel) | JN170822 | JN171424 | JN170576 | JN171227 | JN170354 | | JN171045 |
| 10 | *B. rufoplagiatum* Germain | JN170902 | JN171501 | JN170659 | JN171282 | JN170426 | JN170227 | JN171102 |
| | **Subgenus *Plocamoperyphus* Jeannel** | | | | | | | |
| 11 | *B. mandibulare* Solier | EU677545 | EU677669 | JN170603 | EU677643 | EU677689 | JN170200 | JN171065 |
| | **Subgenus *Ecuadion* Moret and Toledano** | | | | | | | |
| 12 | *B. rawlinsi* Moret and Toledano | JN170893 | JN171492 | JN170650 | JN171275 | JN170418 | | JN171096 |
| 13 | *B. rogersi* Bates | JN170897 | JN171496 | JN170654 | JN171279 | JN170422 | JN170225 | JN171100 |
| | **Subgenus *Pacmophena* Jeannel** | | | | | | | |
| 14 | *B. melanopodum* Solier | JN170853 | JN171453 | JN170609 | JN171249 | JN170383 | JN170202 | JN171069 |
| 15 | *B. scitulum* Erichson | JN170911 | JN171510 | JN170668 | JN171288 | JN170435 | | JN171109 |
| | **Subgenus *Notholopha* Jeannel** | | | | | | | |
| 16 | *B. rugosellum* (Jeannel) | JN170903 | JN171502 | JN170660 | JN171283 | JN170427 | JN170228 | JN171103 |
| 17 | *B. sexfoveatum* Germain | JN170916 | JN171515 | JN170673 | JN171293 | JN170439 | JN170233 | JN171113 |
| 18 | *B. (Notholopha)* sp. 1 | JN170747 | JN171352 | JN170500 | 2046 | JN170281 | | 2046 |
| | **Subgenus *Nothonepha* Jeannel** | | | | | | | |
| 19 | *B. lonae* Jensen-Haarup | JN170844 | JN171444 | JN170599 | JN171242 | JN170374 | JN170196 | JN171061 |
| 20 | *B. sp. nr. lonae* Jensen-Haarup | JN170921 | JN171519 | JN170678 | JN171298 | JN170444 | | JN171118 |

**TABLE 2.** Sampling of other species of Bembidiina. See legend of Table 1 for more details.

| | | CAD | wg | ArgK | Topo | 28S | 18S | COI |
|---|---|---|---|---|---|---|---|---|
| OTHER *BEMBIDION* SERIES | | | | | | | | |
| | **Subgenus *Bembidion* Latreille** | | | | | | | |
| 21 | *B. quadrimaculatum dubitans* (LeConte) | JN170890 | JN171489 | JN170647 | JN171274 | JN170415 | JN170223 | JN171093 |
| | **Subgenus *Zemetallina* Lindroth** | | | | | | | |
| 22 | *B. parviceps* Bates | JN170872 | JN171472 | JN170629 | JN171261 | JN170400 | JN170211 | JN171079 |
| | **Subgenus *Nothocys* Jeannel** | | | | | | | |
| 23 | *B. anthracinum* Germain | JN170756 | JN171360 | JN170510 | JN171177 | JN170290 | JN170149 | JN170994 |
| | **Subgenus *Notaphus* Dejean** | | | | | | | |
| 24 | *B. solieri* Gemminger and Harold | JN170919 | JN171518 | JN170676 | JN171296 | JN170442 | JN170235 | JN171116 |
| 25 | *B. varium* Olivier | JN170938 | JN171536 | JN170696 | JN171311 | JN170459 | JN170246 | JN171132 |
| | **Subgenus *Trepanedoris* Netolitzky** | | | | | | | |
| 26 | *B. fortestriatum* (Motschulsky) | JN170808 | JN171410 | JN170562 | JN171217 | JN170341 | JN170174 | JN171036 |
| | **Subgenus *Notaphemphanes* Netolitzky** | | | | | | | |
| 27 | *B. ephippium* (Marsham) | JN170801 | JN171403 | JN170555 | JN171211 | JN170334 | JN170169 | JN171030 |
| | **Subgenus *Emphanes* Motschulsky** | | | | | | | |
| 28 | *B. vile* (LeConte) | JN170942 | JN171540 | JN170700 | JN171314 | JN170463 | JN170248 | JN171135 |
| *OCYDROMUS* SERIES | | | | | | | | |
| | **Subgenus *Peryphanes* Jeannel** | | | | | | | |
| 29 | *B. maroccanum* Antoine | JN170851 | JN171451 | JN170607 | JN171247 | JN170381 | | JN171067 |
| 30 | *B. platynoides* Hayward | JN170880 | JN171479 | JN170637 | JN171268 | JN170405 | JN170217 | JN171086 |
| 31 | *B. stephensi* Crotch | JN170926 | JN171524 | JN170683 | 1758 | JN170449 | | 1758 |
| 32 | *B. texanum* Chaudoir | JN170931 | JN171529 | JN170688 | JN171306 | JN170453 | | JN171126 |
| | **Subgenus *Ocydromus* Clairville** | | | | | | | |
| 33 | *B. modestum* (Fabricius) | JN170857 | JN171457 | JN170613 | JN171252 | JN170385 | | JN171071 |
| | **Subgenus *Peryphus* Dejean** | | | | | | | |
| 34 | *B. tetracolum* Say | JN170930 | JN171528 | JN170687 | JN171305 | JN170452 | JN170240 | JN171125 |
| | **Subgenus *Princidium* Motschulsky** | | | | | | | |
| 35 | *B. punctulatum* Drapiez | JN170887 | JN171486 | JN170644 | JN171273 | JN170412 | JN170222 | JN171091 |
| | **Subgenus *Bembidionetolitzkya* Strand** | | | | | | | |
| 36 | *B. geniculatum* Heer | JN170814 | JN171416 | JN170568 | JN171222 | JN170347 | JN170179 | JN171041 |
| | **Subgenus *Nepha* Motschulsky** | | | | | | | |
| 37 | *B. genei illigeri* Netolitzky | JN170813 | JN171415 | JN170567 | JN171221 | JN170346 | JN170178 | JN171040 |
| OTHER *BEMBIDION* | | | | | | | | |
| | **Subgenus *Trichoplataphus* Netolitzky** | | | | | | | |
| 38 | *B. planum* (Haldeman) | JN170879 | JN171478 | JN170636 | JN171267 | JF800048 | JN170216 | JF800067 |
| | **Subgenus *Blepharoplataphus* Netolitzky** | | | | | | | |
| 39 | *B. hastii* Sahlberg | JN170821 | JN171423 | JN170575 | JN171226 | JN170353 | JN170181 | JN171044 |
| | **Subgenus *Metallina* Motschulsky** | | | | | | | |
| 40 | *B. properans* (Stephens) | JN170883 | JN171482 | JN170640 | JN171270 | JN170408 | JN170219 | JN171088 |
| | **Subgenus *Eupetedromus* Netolitzky** | | | | | | | |
| 41 | *B. variegatum* Say | JN170937 | JN171535 | JN170695 | JN171310 | JN170458 | JN170245 | JN171131 |
| | **Subgenus *Pseudoperyphus* Hatch** | | | | | | | |
| 42 | *B. chalceum* Dejean | EF649431 | EF649548 | EF648737 | EU677650 | EF648892 | EF648647 | EF649200 |
| GENUS *ASAPHIDION* GOZIS | | | | | | | | |
| 43 | *Asaphidion yukonense* Wickham | EU677540 | EU677666 | EU677515 | EU677638 | JN170273 | JN170139 | JN170979 |

**Morphological methods**. Photographs of body parts were taken with a Leica Z6 and JVC KY-F75U camera. For pronotal, elytral, and genitalic images, a stack of photographs at different focal planes was taken using Microvision's Cartograph software. These photographs were then merged using the PMax procedure in Zerene Systems's Zerene Stacker; the images thus potentially have some artifacts caused by the merging algorithm.

**Taxon sampling for DNA studies.** We sequenced DNA from two specimens each of *B. (Chilioperyphus) orregoi* (the type species of subgenus *Chilioperyphus*) and *B. (Chilioperyphus) mendocinum*, as well as three specimens from an undescribed species of *Chilioperyphus* from northern Argentina, similar in appearance to *B. mendocinum*, which we will call *B.* n.sp. "Cal". In addition, we sequenced specimens of *B. (Antiperyphanes) zanettii* Toledano, *B. (Notholopha)* sp. 1, and *B. (Peryphanes) stephensi* Crotch (Tables 1–3). DNA vouchers are housed in the David Maddison voucher collection at OSAC, with the exception of specimen DNA2700, which is housed at IADIZA. These new data were added to sequences previously collected from 15 other species of the *Antiperyphanes* Complex, including *Bembidion hirtipes* Jeannel, and three other species of subgenus *Peryphanes*, 18 additional species of *Bembidion*, and one species of the related genus *Asaphidion* (Tables 1–2).

**DNA sequencing.** The genes studied, and the abbreviations used in this paper, are: **28S** or **28S rDNA**: 28S ribosomal DNA; **18S** or **18S rDNA**: 18S ribosomal DNA; **COI**: cytochrome oxidase I; ***wg***: *wingless*; **CAD**: carbamoyl phosphate synthetase domain of the rudimentary gene; **ArgK**: arginine kinase; **Topo**: topoisomerase I.
Fragments for these genes were amplified using the Polymerase Chain Reaction on an Eppendorf Mastercycler Thermal Cycler, using TaKaRa Ex Taq and the basic protocols recommended by the manufacturer. Primers and details of the cycling reactions used are given in Maddison (2012). The amplified products were then cleaned, quantified, and sequenced at the University of Arizona's Genomic and Technology Core Facility using a 3730 XL Applied Biosystems automatic sequencer.

Assembly of multiple chromatograms for each gene fragment and initial base calls were made with Phred (Green & Ewing, 2002) and Phrap (Green, 1999) as orchestrated by Mesquite's Chromaseq package (Maddison & Maddison, 2011a; Maddison & Maddison, 2011b) with subsequent modifications by Chromaseq and manual inspection. Multiple peaks at a single position in multiple reads were coded using IUPAC ambiguity codes.

Sequences have been deposited in GenBank with accession numbers KC140234 through KC140285.

**Alignment.** Alignment of the protein-coding sequences was simple, as the only insertion or deletion present consists of three contiguous nucleotides, representing one amino acid, in *B. parviceps* in the *wingless* gene. The two ribosomal genes showed a richer history of insertions and deletions. Multiple sequence alignments of 28S and 18S were performed by Opal (Wheeler & Kececioglu, 2007), using default parameter values. The resulting alignment produced no region that appeared to have ambiguous alignment, and as a result no sites were excluded on this basis.

**Molecular phylogenetic analysis.** Models of nucleotide evolution where chosen with the aid of jModelTest version 0.1.1 (Darriba, et al., 2012; Guindon & Gascuel, 2003). Among the models supported by MrBayes and GARLI, the models chosen by the Bayesian Information Criterion were HKY+I+Γ (CAD, *wingless*, and 18S), GTR+Γ (ArgK, 28S), and GTR+I+Γ (Topo).

Likelihood analyses of nucleotide data were conducted using GARLI version 1.0.699 (Zwickl, 2006). Analyses were conducted on each gene individually, as well as a matrix of seven genes concatenated together, partitioned by gene. For bootstrap analyses, 1000 replicates were conducted for single-gene analyses, and 500 for the seven-gene analysis. In addition to these bootstrap analyses, a search for the maximum likelihood tree was conducted using 200 search replicates for single-gene matrices, and 100 search replicates for the seven-gene matrix.

Bayesian analyses were conducted using MrBayes version 3.1.2 (Huelsenbeck & Ronquist, 2005). Two runs of four chains each were run for between 20 million and 70 million generations, with trees sampled every 1,000 generations. Runs were terminated once the average standard deviation of split frequencies went below 0.01(Huelsenbeck & Ronquist, 2005), at which time the Effective Sample Size was greater than or equal to 470, and likelihood scores and all parameter values reached a stable plateau, as judged by the tools in Tracer (Rambaut & Drummond, 2004). For each analysis, the trees in a burn-in period of 50% of the generations were excluded, and the majority-rule consensus tree of remaining trees was calculated to determine Bayesian posterior probabilities of clades. The number of trees sampled for each analysis varied from 22,000 to 76,000.

Most-parsimonious trees (MPTs) were sought using PAUP* (Swofford, 2002). To search for most parsimonious trees, 2000 replicates were conducted, each beginning with a starting tree formed with the random addition sequence option, with each replicate saving no more than 25 trees. For parsimony bootstrap analyses in PAUP*, 2000 bootstrap replicates were examined, each of which used a heuristic search with four replicates, each beginning with a starting tree formed by the random addition sequence option, with TBR branch rearrangement, with each replicate saving no more than 25 trees; the estimated bootstrap values are reported as percentages.

**TABLE 3.** Locality data for specimens from which new sequences were obtained. The four-digit number in the "#" column is the D.R. Maddison DNA voucher number. Locality data for voucher 2046 (*B. (Notholopha)* sp. 1) and 1758 (*B. (Peryphanes) stephensi*) are given in Maddison (2012).

| | # | Locality data |
|---|---|---|
| *B. mendocinum* | 2332 | ARGENTINA: Mendoza: Rio Mendoza ca 10 km S Uspallata, 1740m, 32.6701°S 69.3652°W |
| *B. mendocinum* | 2333 | ARGENTINA: Neuquén: 4.7 km S Puente Picún Leufú, 820m, 39.2426°S 70.0765°W |
| *B. orregoi* | 2337 | ARGENTINA: Chubut: Rio Azul at Lago Puelo, 200m, 42.0933°S 71.6221°W |
| *B. orregoi* | 2338 | ARGENTINA: Neuquén: Rio Aluminé, 22.5 km S Aluminé, 850m, 39.3998°S 70.9315°W |
| *B.* n. sp. "Cal" | 2700 | ARGENTINA: Jujuy: PN Calilegua, Arroyo Tres Cruces, 1120m, 23.6939°S 64.8678°W |
| *B.* n. sp. "Cal" | 2704 | ARGENTINA: Jujuy: PN Calilegua, Arroyo Tres Cruces, 1120m, 23.6939°S 64.8678°W |
| *B.* n. sp. "Cal" | 2706 | ARGENTINA: Jujuy: PN Calilegua, Arroyo Tres Cruces, 1120m, 23.6939°S 64.8678°W |
| *B. zanettii* | 2679 | ECUADOR: Napo: Rio Angenaro near Rio Cosanga, 2200m, 0.6394°S 77.9089°W |

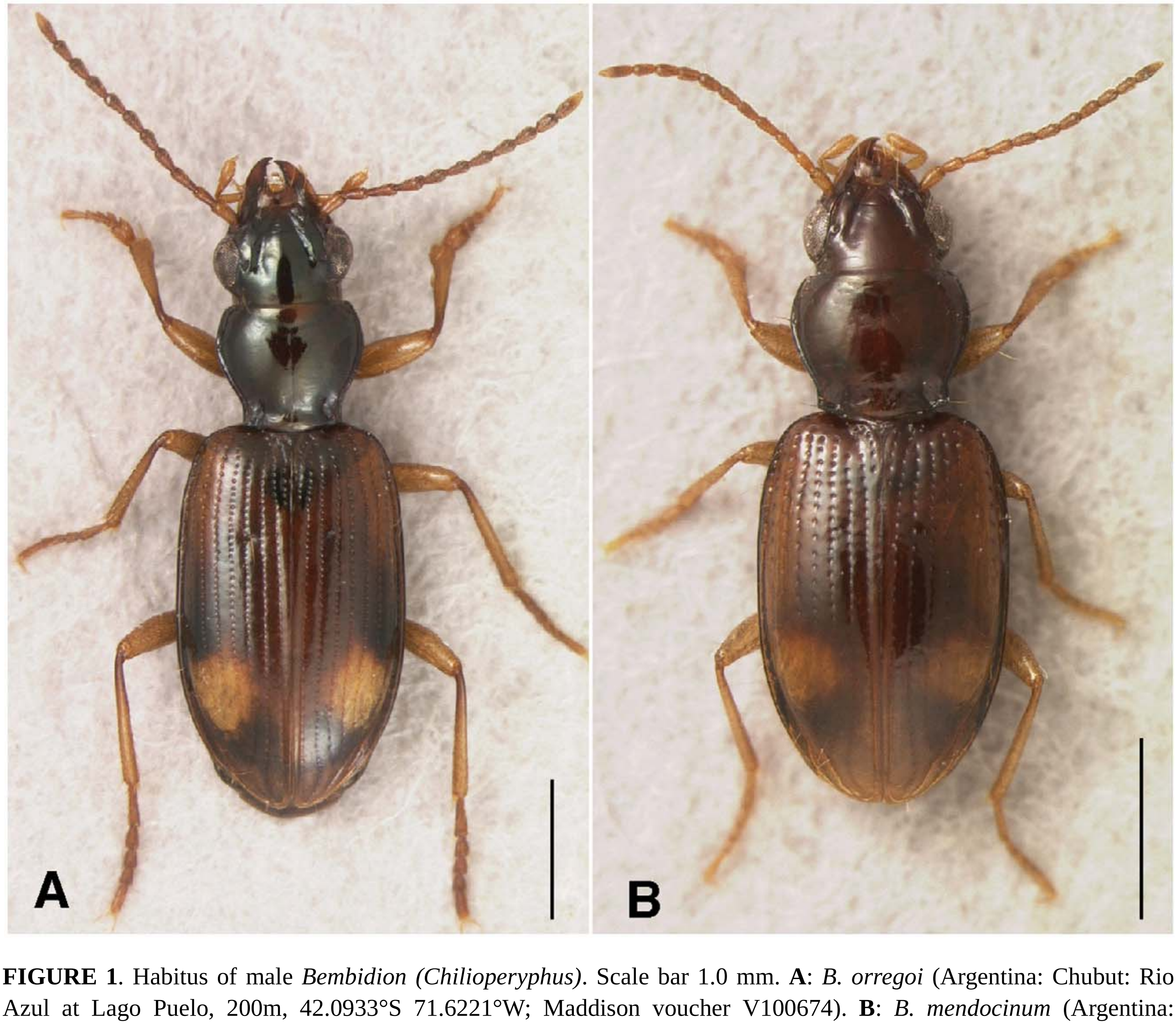

**FIGURE 1**. Habitus of male *Bembidion (Chilioperyphus)*. Scale bar 1.0 mm. **A**: *B. orregoi* (Argentina: Chubut: Rio Azul at Lago Puelo, 200m, 42.0933°S 71.6221°W; Maddison voucher V100674). **B**: *B. mendocinum* (Argentina: Neuquén: Puente Picún Leufú, 775m, 39.2112°S 70.0637°W; Maddison voucher V100673).

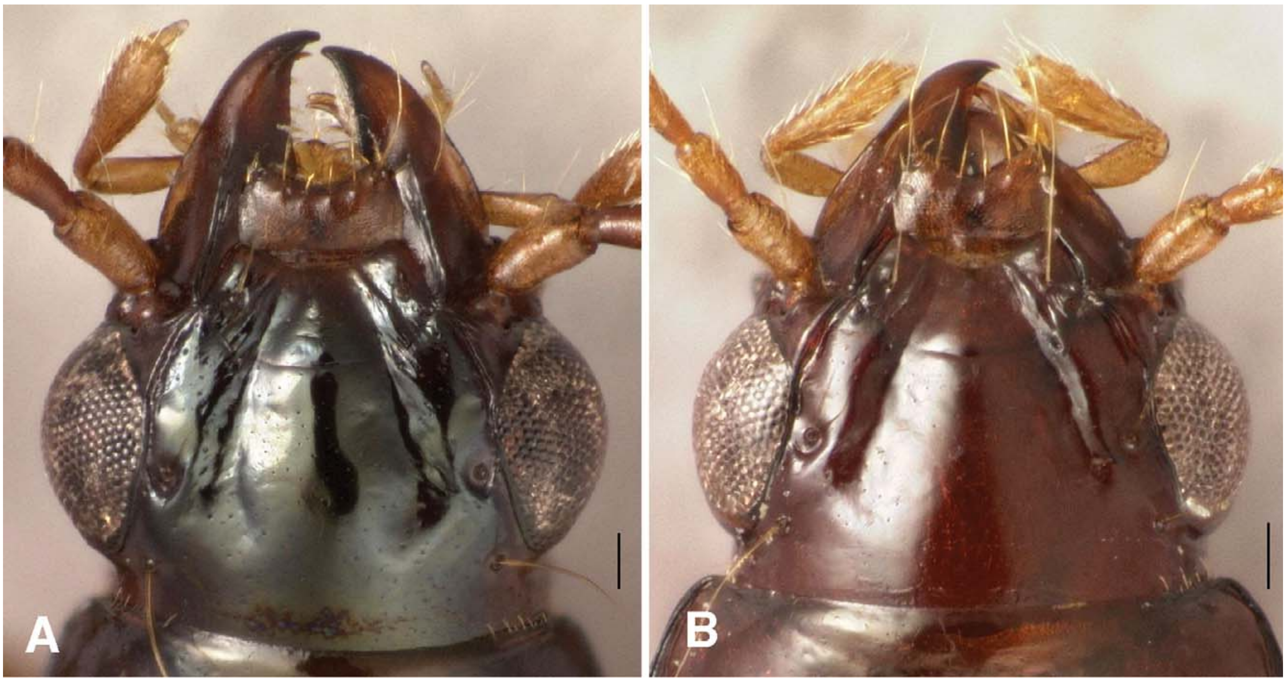

**FIGURE 2**. Dorsal view of head of males. Scale bar 0.1 mm. **A:** *B. orregoi* (Argentina: Chubut: Rio Azul at Lago Puelo, 200m, 42.0933°S 71.6221°W; Maddison voucher V100674). **B:** *B. mendocinum* (Argentina: Neuquén: Puente Picún Leufú, 775m, 39.2112°S 70.0637°W; Maddison voucher V100673).

## Results and discussion

**Species previously included in *Chilioperyphus*.** There are four species previously considered to belong to subgenus *Chilioperyphus: Bembidion orregoi*, *B. mendocinum*, *B. cassinense*, and *B. cuyanum*. Re-examination of the holotype of *Bembidion cuyanum* (IADIZA) reveals that it belongs to *Bembidion (Antiperyphanes) hirtipes* Jeannel (a genitalia of which is shown in Fig. 6H), and we therefore treat *B. cuyanum* as a junior synonym of *B. hirtipes*. Considering as well the undescribed species from Argentina, *B.* n.sp. "Cal", there are thus five known species that might be considered to belong in *Chilioperyphus*: *Bembidion orregoi*, *B. mendocinum*, *B.* n.sp. "Cal", *B. cassinense*, and *B. hirtipes*. We have sampled all of these except *B. cassinense* for DNA sequences.

**Monophyly and relationships of *Chilioperyphus*.** The inferred phylogeny, with information about the support for various clades, is shown in Figs. 3 and 4, with details of support for critical clades given in Table 4.

The monophyly of true *Chilioperyphus* (*B. orregoi* + *B.mendocinum* + *B.* n.sp. "Cal") is strongly supported by all seven genes examined, both in combined analyses and individual gene analyses, with Bayesian posterior probabilities being at least 98 for each gene (Table 4.1), and with Bayesian posterior probability, ML bootstrap, and parsimony bootstrap values of 100 for the combined matrix. However, *B. hirtipes*, considered (under the name *Bembidion cuyanus*) by Roig-Juñent and Scheibler (2004) to belong to *Chilioperyphus*, is not closely related to true *Chilioperyphus*, instead belonging to subgenus *Antiperyphanes* (Figs. 3 and 4).

It is also evident that *Chilioperyphus* belongs to the *Bembidion* Series, a result supported by six of the seven genes and the combined analysis (Table 4.2), and that it does not belong to subgenus *Peryphanes* in particular or the *Ocydromus* complex more generally (Table 4.5). Within the *Bembidion* Series, *Chilioperyphus* belongs within the *Antiperyphanes* Complex (Table 4.3), specifically in a clade with *Antiperyphanes* and *Plocamoperyphus*, a result supported by five of the seven genes individually, and strongly supported by the combined analysis (Table 4.4).

**Male genitalia.** With the boundaries of true *Chilioperyphus* evident from the phylogeny based upon DNA sequence data, we can now investigate the genitalic characteristics of the clade. Male genitalia of true *Chilioperyphus* have an extremely long flagellum, folded twice, which would be longer than the median lobe if straightened (Fig. 5). In addition, the brush sclerite ("brush" in Fig. 5A) and associated structures at the base of the flagellum are not contained within the median lobe, instead protruding from its base. The flagellum is longer, and the brush sclerite more basally protruded, in *B. mendocinum* than in *B. orregoi* (compare Fig. 5B to Fig. 5A).

**TABLE 4**. Support for and against various clades. B: Bayesian analysis; ML: Maximum likelihood analysis; P: parsimony analysis. Numbers in the body of table indicate posterior probabilities (B) or bootstrap support (ML, P) expressed as a percentage; check marks indicate that the clade is present in the optimal (maximum likelihood or most parsimonious) trees but with bootstrap value below 50; x indicates that a contradictory clade was present in the optimal (maximum likelihood or most parsimonious) trees but with bootstrap value below 50; negative values indicate bootstrap support for a contradictory clade. Boxes in gray to black indicate support for the clade; boxes in pink to red indicate support against that clade, with darker colors indicating stronger support. Blank boxes indicate no support for or against the clade because of lack of resolution in the inferred trees. Abbreviations: “inc.” = “including”, “exc.” = “excluding”. Superscript numbers indicate the species numbers from Table 1–2 contained within that group.
continued.

| | | **CAD** | | | ***wg*** | | | **ArgK** | | | **Topo** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | B | ML | P | B | ML | P | B | ML | P | B | ML | P |
| 4.1 | *Chilioperyphus* $^{1-3}$ | 100 | 97 | 99 | 100 | 92 | 96 | 99 | 72 | 72 | 100 | 99 | 100 |
| 4.2 | *Bembidion* Series (inc. *Chilioperyphus*) $^{1-28}$ | 100 | 86 | 99 | 97 | 78 | 89 | 99 | 55 | 78 | 99 | 71 | |
| 4.3 | *Antiperyphanes* Complex (inc. *Chilioperyphus*) $^{1-20}$ | 99 | 76 | 82 | 95 | 51 | x | 71 | ✓ | | 86 | ✓ | |
| 4.4 | *Chilioperyphus* + *Antiperyphanes* + *Plocamoperyphus* $^{1-13}$ | 99 | 78 | 82 | 98 | 59 | 71 | -74 | x | | 58 | 78 | 81 |
| 4.5 | *Ocydromus* Complex (exc. *Chilioperyphus*) $^{29-34}$ | 98 | 74 | 51 | 89 | ✓ | ✓ | 94 | 54 | 69 | 99 | 53 | |

| | | **28S** | | | **18S** | | | **COI** | | | **Combined** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | B | ML | P | B | ML | P | B | ML | P | B | ML | P |
| 4.1 | *Chilioperyphus* $^{1-3}$ | 98 | 68 | 82 | 100 | 98 | 99 | 100 | 93 | 99 | 100 | 100 | 100 |
| 4.2 | *Bembidion* Series (inc. *Chilioperyphus*) $^{1-28}$ | 98 | 54 | ✓ | 100 | 99 | 100 | | x | x | 100 | 100 | 100 |
| 4.3 | *Antiperyphanes* Complex (inc. *Chilioperyphus*) $^{1-20}$ | -54 | x | | -68 | x | x | | x | x | 100 | 89 | 81 |
| 4.4 | *Chilioperyphus* + *Antiperyphanes* + *Plocamoperyphus* $^{1-13}$ | 79 | 66 | 61 | 99 | 92 | 96 | | x | x | 100 | 100 | 100 |
| 4.5 | *Ocydromus* Complex (exc. *Chilioperyphus*) $^{29-34}$ | -56 | x | | 83 | 52 | | -60 | x | x | 100 | 99 | 100 |

A long flagellum with basally protruded brush sclerite is also present in the subgenus *Peryphanes* (Fig. 6A, B), which is a member of the *Ocydromus* Series of *Bembidion* (Maddison, 2012). This is in contrast to typical members of *Bembidion*, which have relatively short flagella and have the brush sclerite contained within the median lobe (Fig. 6C, D). Given their relative placement on the phylogeny (Figs. 3 and 4), we can conclude that the long flagellum, with a basally protruding brush sclerite, is convergent between *Peryphanes* and *Chilioperyphus*. Within the *Antiperyphanes* Complex, some species have typical *Bembidion* genitalia (e.g., subgenus *Ecuadion*, Fig. 6E); others (members of *Antiperyphanes*) lack the brush sclerite and have relatively long flagella (Fig. 6F–H). In some species of *Antiperyphanes*, the flagellum is long enough that it protrudes far from the base of the median lobe (Fig. 6G), but in none of these is the flagellum folded back upon itself as in *Chilioperyphus*. The long, twice-folded flagellum is thus a synapomorphy of the three species of *Chilioperyphus* we have examined.

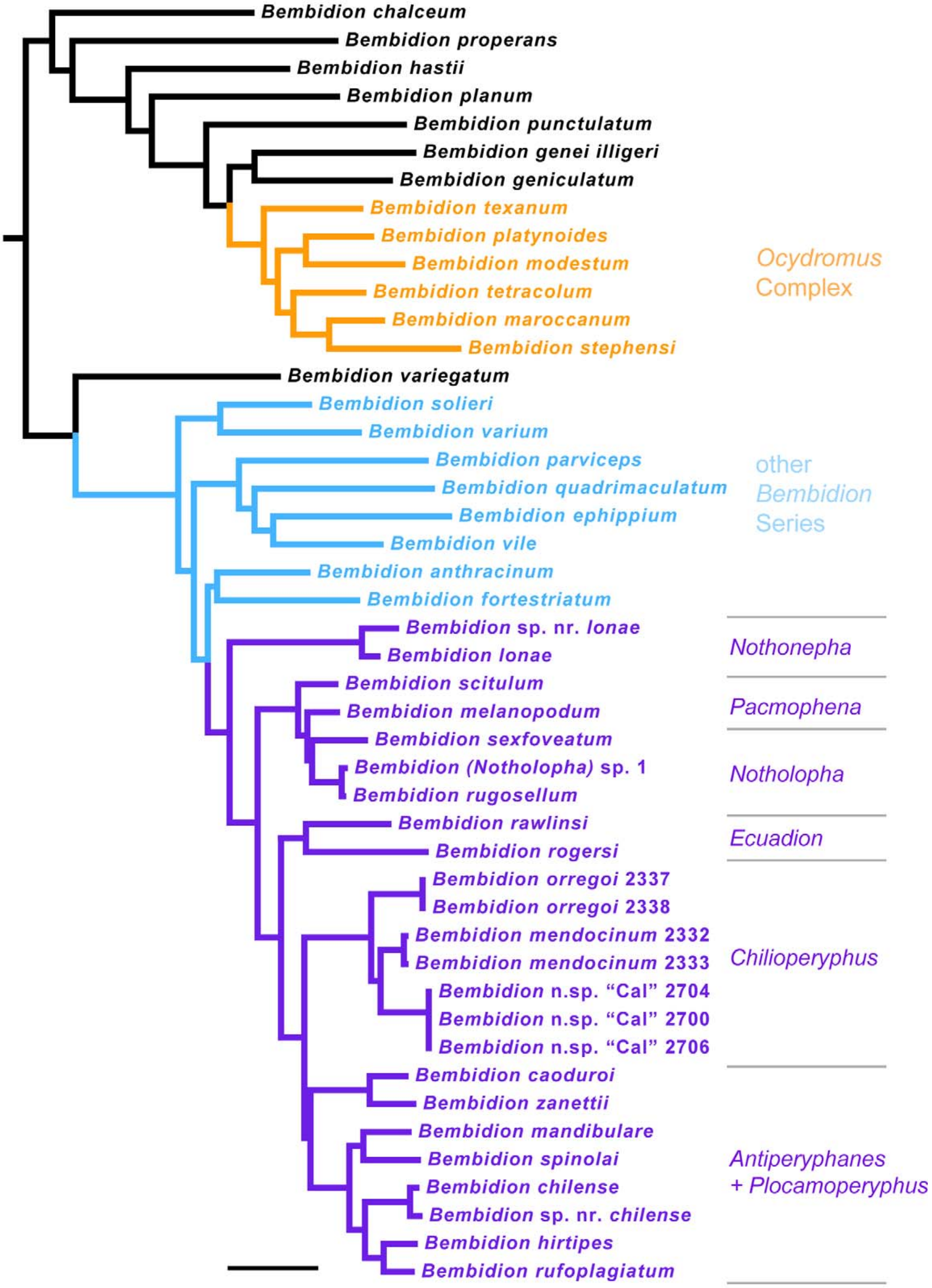

**FIGURE 3.** The tree of highest likelihood found for all seven genes combined. Labels on the right side are supra-specific taxa (subgenera and complexes) within the genus *Bembidion*. The outgroup (genus *Asaphidion*) is not shown. Scale bar: 0.1, as reconstructed by RAxML.

*Bembidion cassinense*, described as a *Chilioperyphus* because of the resemblance of its genitalia to Jeannel's figures, does not have long, twice-folded flagella (Roig-Juñent & Gianuca, 2001: 251; Roig-Juñent & Scheibler, 2004), and instead has genitalia typical of subgenus *Antiperyphanes*. Considering the lack of genitalic synapomorphies shared with *Chilioperyphus*, we therefore remove *B. cassinense* from *Chilioperyphus*, and place it into subgenus *Antiperyphanes*. We also move *B. hirtipes* to *Antiperyphanes*, because of the derived similarity of their genitalia, including the lack of a brush sclerite. This leaves *B. mendocinum* and *B. orregoi* as the only described members of *Chilioperyphus*.

Jeannel's incorrect depiction of the genitalia of the two described species of *Chilioperyphus* appears to have been caused by more than a simple confusion of drawings, as some genitalic slides are evidently mislabeled. The genitalic slide in MNHN labeled "Peryphus kuscheli n., Balmaceda", preserved separately from any specimen of *B. (Pacmophena) kuscheli* Jeannel (= *B. penai* Toledano), is almost certainly mislabeled. The label on the slide indicates the specimen is from Balmaceda. Jeannel mentions this as a locality for *B. orregoi* (1962:652), and in MNHN there is a specimen of *B. orregoi* collected by Kuschel from that locality, but Balmaceda is not a locality Jeannel mentions for *B. kuscheli*. And, indeed, the aedeagus on the slide is that of a typical *B. orregoi*, vastly different from aedeagi of members of the subgenus *Pacmophena* to which *B. kuscheli* belongs.

**FIGURE 4.** Tree showing clades in the *Antiperyphanes* Complex that have Bayesian posterior probabilities ≥ 90 for the matrix of all seven genes combined. Branches have thick horizontal bars if those clades are present in maximum likelihood and parsimony bootstrap trees with support values ≥ 95 and also have Bayesian posterior probabilities ≥ 95. The seven small vertical bars on each branch indicate support in favor (gray to black) or against (pink to red) that clade for each of the seven genes analyzed individually. The outgroup (genus *Asaphidion*) is not shown.

If the slide labeled as *B. kuscheli* contains the aedeagus of a *B. orregoi*, then where is the aedeagus of the *B. kuscheli* male? There is a slide in Jeannel's collection labeled as being that of *B. orregoi*, containing genitalia that are not those of a *B. orregoi*, but instead the genitalia is extremely similar to that of a specimen we examined belonging to *B. (Pacmophena) penai* Toledano or a very closely related species from southern Peru (NHMW). This observation suggests that the labels on the *B. orregoi* and *B. kuscheli* slides may simply have been switched. But this does not account for the images in Jeannel (1962:644) labeled as *B. mendocinum* and *B. orregoi*; these images depict genitalia of subgenus *Antiperyphanes*. There thus may be up to four mislabeled genitalic slides among Jeannel's material: the genitalia of *B. orregoi* labeled as *B. kuscheli*, the genitalia of *B. kuscheli* on the slide labeled as *B. orregoi,* and the genitalia of the two *Antiperyphanes* illustrated in Jeannel (1962, his Fig. 240 and 241) and incorrectly presented as *Chilioperyphus*. This portends future confusion about Jeannel's specimens, genitalic slides, and images, and great care should be taken when interpreting them.

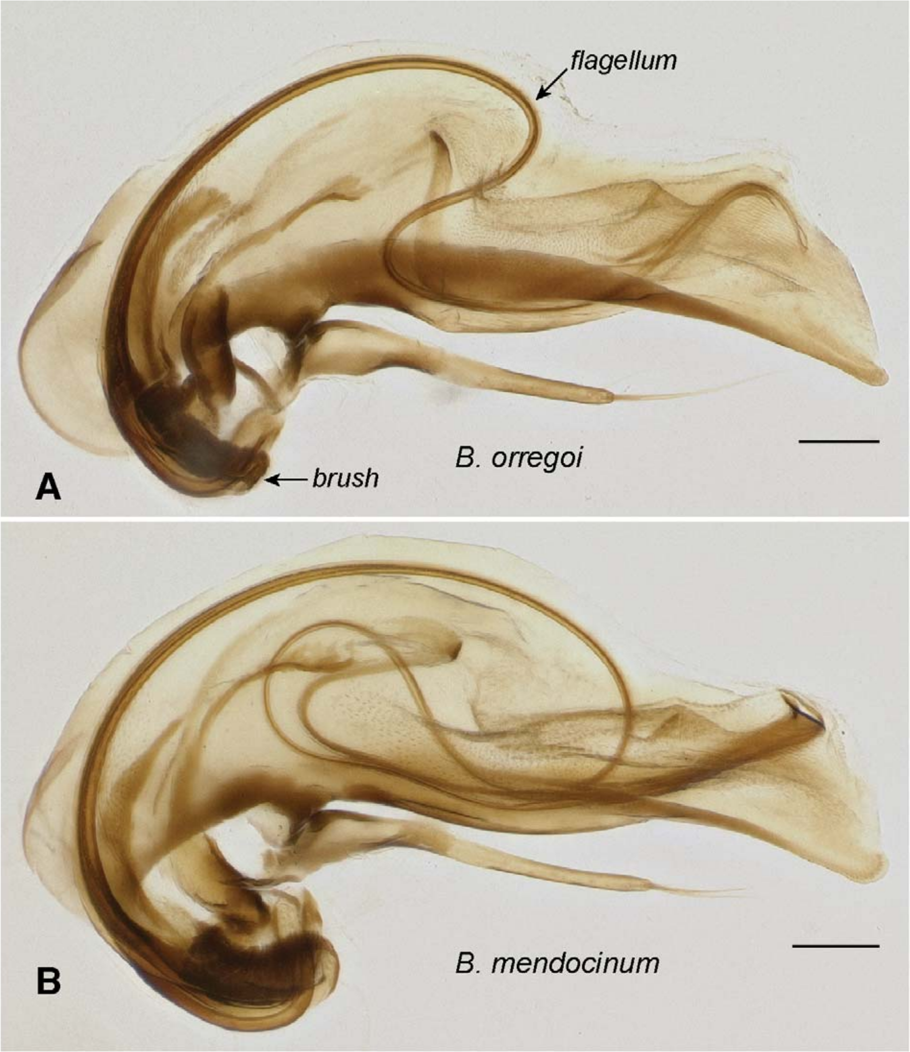

**FIGURE 5.** Male aedeagus and right paramere. Scale bar 0.1 mm. **A:** *B. orregoi* (Argentina: Chubut: Rio Azul at Lago Puelo, 200m, 42.0933°S 71.6221°W; Maddison voucher V100667. **B:** *B. mendocinum* (Argentina: Mendoza: Villa 25 de Mayo, 875m, 34.5901°S 68.5608°W; Maddison voucher V100668).

**Female genitalia.** The female genitalia of *Chilioperyphus* are also distinctive, having a very long, coiled spermathecal duct, and a large, very heavily sclerotized spermatheca (Fig. 7A, D), in contrast to the shorter spermathecal duct and less-sclerotized spermatheca of other *Bembidion* (Fig. 7B, C). The long spermathecal duct is likely a synapomorphy for the subgenus, but we have not examined enough species within the *Antiperyphanes* Complex to state this with confidence. Given the close correlation observed between the length of the male flagellum and the female spermathecal duct that has been noted across *Bembidion* species (Liebherr, 2008; Schuler, 1959), it is not surprising that *Chilioperyphus* females would have long, coiled spermathecal duct.

**External characters**. With *Chilioperyphus* restricted to *B. mendocinum*, *B. orregoi*, and *B.* n.sp. "Cal", the external form of adults of *Chilioperyphus* can be characterized. *Chilioperyphus* adults have heads with deep and convergent frontal furrows, extended upon the clypeus (Fig. 2), a trait shared in the South American fauna with members of *Bembidion (Nothonepha)* and *B. (Pseudotrepanes)*. In contrast to the former, *Chilioperyphus* have at least seven punctate striae in the basal half of the elytra; in contrast to the latter, *Chilioperyphus* have discal setae in or close to the third elytral stria. A full characterization of *Chilioperyphus* awaits the description of several unnamed species.

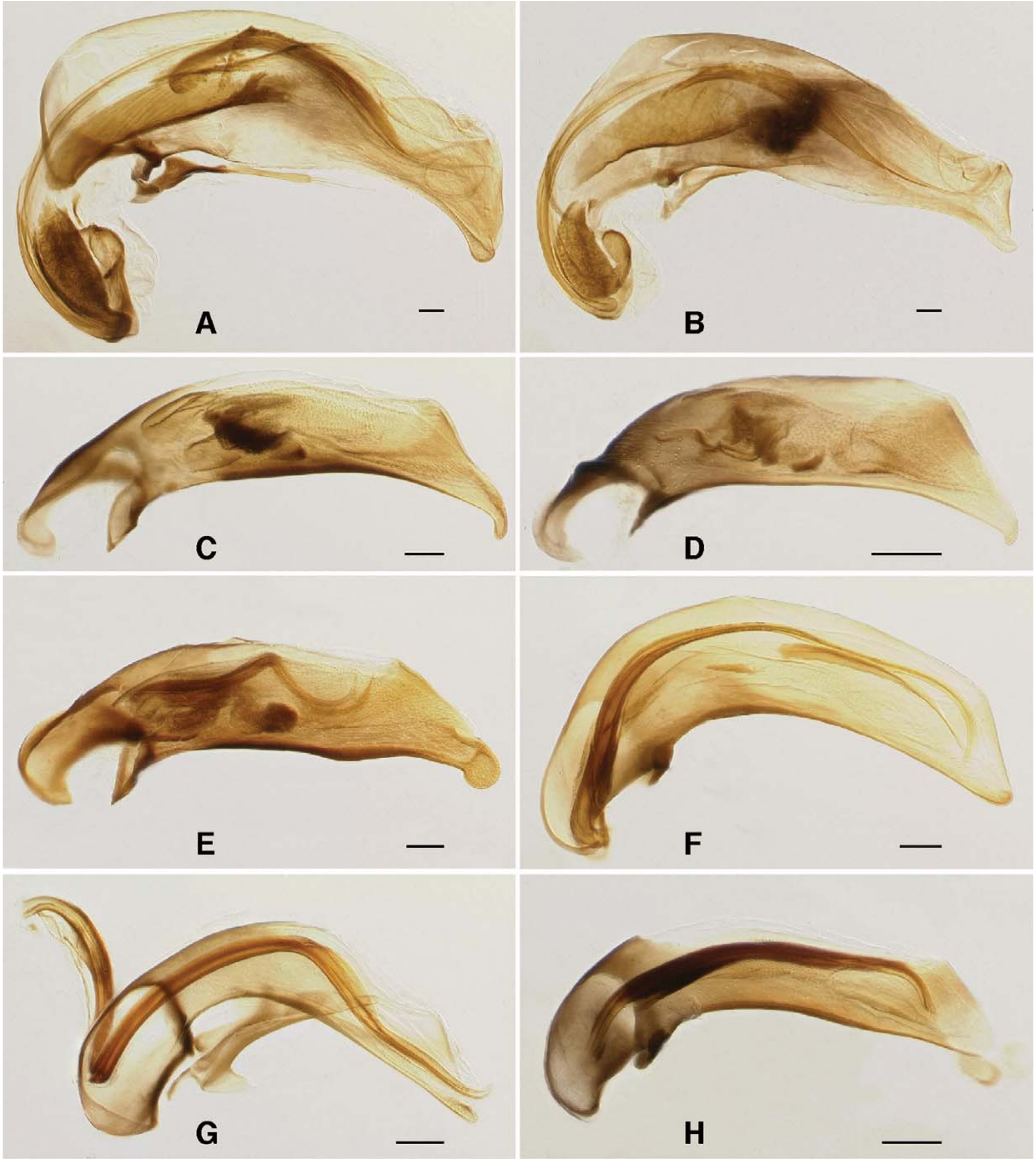

**FIGURE 6.** Male aedeagus of *Bembidion* other than *Chilioperyphus*. Scale bar 0.1 mm. **A:** *B. (Peryphanes) stephensi* (Canada: S.E. Newfoundland, Cape Broyle; OSAC specimen 0000554297). **B:** *B. (Peryphanes) maroccanum* (Morocco: Middle Atlas Mountains, highway 20, 1 km S. of Aït Kermousse (near snow barrier) 16.3 km S. of Boulemane., 33°14.38'N 4°40.92'W; Maddison voucher DNA2147). **C:** *B. (Trichoplataphus) planum* (USA: Indiana: Crawford Co., English, Camp Fork Creek, 150m 38.3334°N 86.4646°W; Maddison voucher DNA1423). **D:** *B. (Nothocys) anthracinum* (Chile: Reg. Met., La Parva, 2725m, 33.3346°S 70.2835°W; Maddison voucher DNA2228). **E:** *B. (Ecuadion) rogersi* (Costa Rica: Alajuela, Catarata del Toro, base of falls. 7 km N Bajos del Toro; Maddison voucher DNA2414). **F:** *B. (Antiperyphanes) zanettii* (Ecuador: Napo: Rio Angenaro near Rio Cosanga, 2200m, 0.6394°S 77.9089°W; Maddison voucher DNA2679). **G:** *B. (Antiperyphanes)* sp. nr. *chilense* (Peru: Pisac: Between the town of Pisac and the Pisac ruins, tributary of the Rio Urubamba, 3020 m. 13.417°S, 71.849°W; Maddison voucher DNA0714). **H:** *B. (Antiperyphanes) hirtipes* (Argentina: Mendoza: Pampa Palauco, 1975m, 35.9597°S 69.4223°W; Maddison voucher DNA2335).

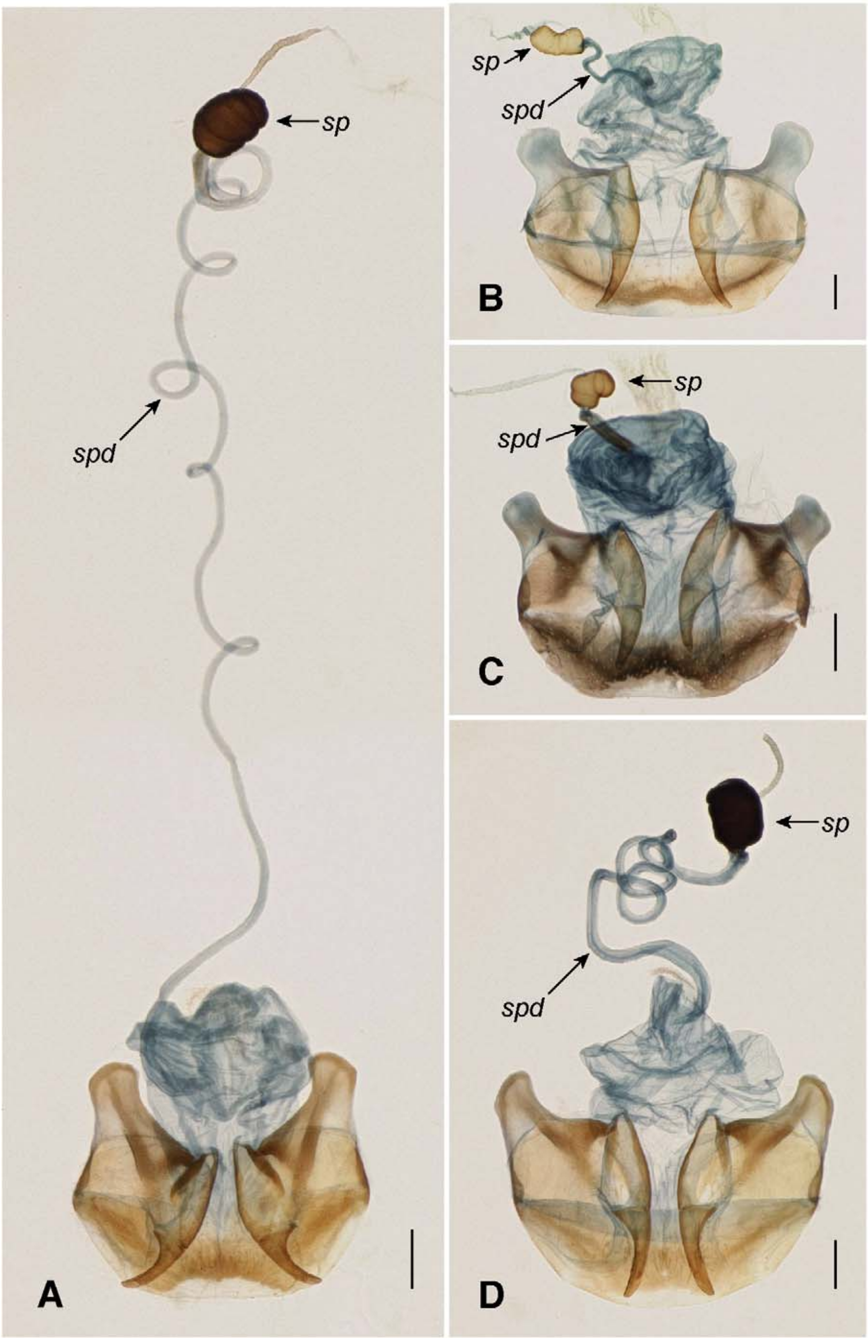

**FIGURE 7.** Female reproductive tract. ***spd***: spermathecal duct; ***sp***: spermatheca. Scale bar 0.1 mm. A: *Bembidion mendocinum* (Argentina: Neuquén: Puente Picún Leufú, 775m, 39.2112°S 70.0637°W; Maddison voucher V100678); spermathecal duct partly uncoiled. B: *B. (Trichoplataphus) planum* (Canada: Ontario: Burlington; Maddison voucher V100680). C: *B. (Antiperyphanes) hirtipes* (Argentina: Mendoza: Pampa Palauco, 1975m, 35.9597°S 69.4223°W; Maddison voucher V100681). D: *B. orregoi* (Argentina: Chubut: Rio Azul at Lago Puelo, 200m, 42.0933°S 71.6221°W; Maddison voucher V100679).

## Acknowledgements

We thank the curators of the cited collections for access to specimens in their collections.

This project was enabled by funds generously provided to DRM by the University of Arizona, and the Harold E. and Leona M. Rice Endowment Fund at Oregon State University. In addition, SRJ was supported by CONICET (PIP number 11220080101869).

## References

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9, 772.
http://dx.doi.org/10.1038/nmeth.2109

Green, P. (1999) Phrap. Version 0.990329. http://phrap.org/

Green, P. & Ewing, B. (2002) Phred. Version 0.020425c. http://phrap.org/

Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, 52, 696–704.
http://dx.doi.org/10.1080/10635150390235520

Hildebrandt, D.A. & Maddison, D.R. (2011) A new species of *Bembidion* Latreille 1802 from the Ozarks, with a review of the North American species of subgenus *Trichoplataphus* Netolitzky 1914 (Coleoptera, Carabidae, Bembidiini). *ZooKeys*, 147, 261–275.
http://dx.doi.org/10.3897/zookeys.147.1872

Huelsenbeck, J. & Ronquist, F. (2005) MrBayes. Version 3.1.2. http://www.mrbayes.net/

Jeannel, R. (1962) Les Trechides de la Paléantarctide occidentale. *In:* Deboutteville, C.D. & Rapoport, E. (Eds.) *Biologie de l'Amérique Australe*, *Études sur la Faune du Sol*, Paris, pp. 527–655.

Liebherr, J.K. (2008) Taxonomic revision of Hawaiian *Bembidion* Latreille (Coleoptera: Carabidae: Bembidiini) with a discussion of their reductive and derivative evolutionary specializations. *Annals of Carnegie Museum*, 77, 31–78.
http://dx.doi.org/10.2992/0097-4463-77.1.31

Maddison, D.R. (2008) Systematics of the North American beetle subgenus *Pseudoperyphus* (Coleoptera: Carabidae: *Bembidion*) based upon morphological, chromosomal, and molecular data. *Annals of Carnegie Museum*, 77, 147–193.
http://dx.doi.org/10.2992/0097-4463-77.1.147

Maddison, D.R. (2012) Phylogeny of *Bembidion* and related ground beetles (Coleoptera: Carabidae: Trechinae: Bembidiini: Bembidiina). *Molecular Phylogenetics and Evolution*, 63, 533–576.
http://dx.doi.org/10.1016/j.ympev.2012.01.015

Maddison, D.R. & Maddison, W.P. (2011a) Chromoseq: a Mesquite module for analyzing sequence chromatograms. Version 0.986. http://mesquiteproject.org/packages/chromaseq/

Maddison, D.R. & Ober, K.A. (2011) Phylogeny of minute carabid beetles and their relatives based upon DNA sequence data (Coleoptera, Carabidae, Trechitae). *ZooKeys*, 147, 229–260.
http://dx.doi.org/10.3897/zookeys.147.1871

Maddison, W.P. & Maddison, D.R. (2011b) Mesquite: a modular system for evolutionary analysis. Version 2.75. http://mesquiteproject.org/

Rambaut, A. & Drummond, A. (2004) Tracer. Version 1.3. http://evolve.zoo.ox.ac.uk/software.html?id=tracer

Roig-Juñent, S. & Gianuca, N.M. (2001) Species of Bembidiina (Coleoptera: Carabidae: Bembidiini) from the sandy beaches of Rio Grande do Sul, Brazil. *Revista de la Sociedad Entomologica Argentina*, 60, 249–254.

Roig-Juñent, S. & Scheibler, E. (2004) A new Argentinian species of *Bembidion (Chilioperyphus)* (Coleoptera: Carabidae: Bembidiini) with accessory setae. *Zootaxa*, 782, 1–10.

Schuler, L. (1959) Le genre *Microserrulula* Netolitzky (Bembidiini Jeannel, Col. Trechidae) les formes multispires. *Revue Française d'Entomologie*, 96–105.

Swofford, D.L. (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10. Sinauer Associates, Sunderland.

Toledano, L. (2002) Nomenclatorial revision of the supraspecific taxa of Bembidiini s.str. of South America described by Jeannel (1962) and related taxa with some descriptions of the fauna of South America (Coleoptera: Carabidae). *Koleopterologische Rundschau*, 72, 1–14.

Toledano, L. (2008) Systematic notes on the Bembidiina of the northern Andes with particular reference to the fauna of Ecuador (Coleoptera, Carabidae). *Memoirs on Biodiversity*, 1, 81–130.

Wheeler, T.J. & Kececioglu, J.D. (2007) Multiple alignments by aligning alignments. *Bioinformatics*, 23, i559–i568.
http://dx.doi.org/10.1093/bioinformatics/btm226

Wild, A.L. & Maddison, D.R. (2008) Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. *Molecular Phylogenetics and Evolution*, 48, 877–891.
http://dx.doi.org/10.1016/j.ympev.2008.05.023

Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Ph.D. Dissertation, The University of Texas at Austin, Austin, Texas