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A New Subgenus of *Bembidion* Latreille from México and Guatemala, with Descriptions of Two New Species (Coleoptera: Carabidae)

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A new subgenus of *Bembidion, Geocosmius* subg. nov., is described from forests of México and Guatemala. *Geocosmius* includes *Bembidion nahuala* Erwin as its type species, as well as *B. franiae* Erwin, *B. elescarabajo* sp. nov., and additional forms similar to *B. nahuala.* In México this subgenus is widespread, ranging from Chiapas and Oaxaca north and west to Jalisco, Querétaro, and Hidalgo; most specimens are from cloud forests between 1700–2700m in elevation. A molecular phylogenetic analysis based upon eight genes indicates that *Geocosmius* is the sister group of subgenus *Liocosmius*, from western North America. In addition, a new species of subgenus *Liocosmius*, *B. cachagua* sp. nov., is described from three localities in California. Illumina sequencing was used to acquire DNA sequences of six pinned, dried specimens, which ranged in age from 24 to 57 years, allowing the phylogenetic placement of four of the studied species.

KEYWORDS: Ground beetle, Trechinae, Bembidiini, historical DNA, sequencing museum specimens, new species

George E. Ball would begin each of his many expeditions to México in Edmonton, Canada, by starting the engine of the truck that had been transformed into his custom-built field vehicle and camper. Beginning in August 1965 and over the course of the next several decades, three generations of campers carried George, his students, and colleagues south from Edmonton to the mountains of México in search of carabid beetles. George called the first of his campers *El Escarabajo*, and its successors *El Escarabajo Grande* and *El Escarabajo Oro*. The thousands of specimens George and his fellow explorers collected during his travels in the campers across many miles of roads throughout México are mostly now housed at the University of Alberta Strickland Museum (UASM), where they serve as a primary repository of information about the Mexican ground beetle fauna.

Within that wealth of material are about 300 specimens of small, brown *Bembidion* (Fig. 1A–C) somewhat similar in appearance to members of the North American subgenus *Hydriomicrus* Casey; in the UASM, George labelled these specimens "*Hydriomicrus*" or "*Hydriomicroides*". George and colleagues collected most of these specimens in leaf litter at a total of nearly 50 localities in the mountains of Chiapas, Oaxaca, Veracruz, Puebla, Guerrero, Michoacan, Jalisco, Querétaro, and Hidalgo. Although widespread (Fig. 2) and apparently common, this group of beetles is unreported from México.

As part of an effort to infer the relationships of the world *Bembidion* fauna using DNA

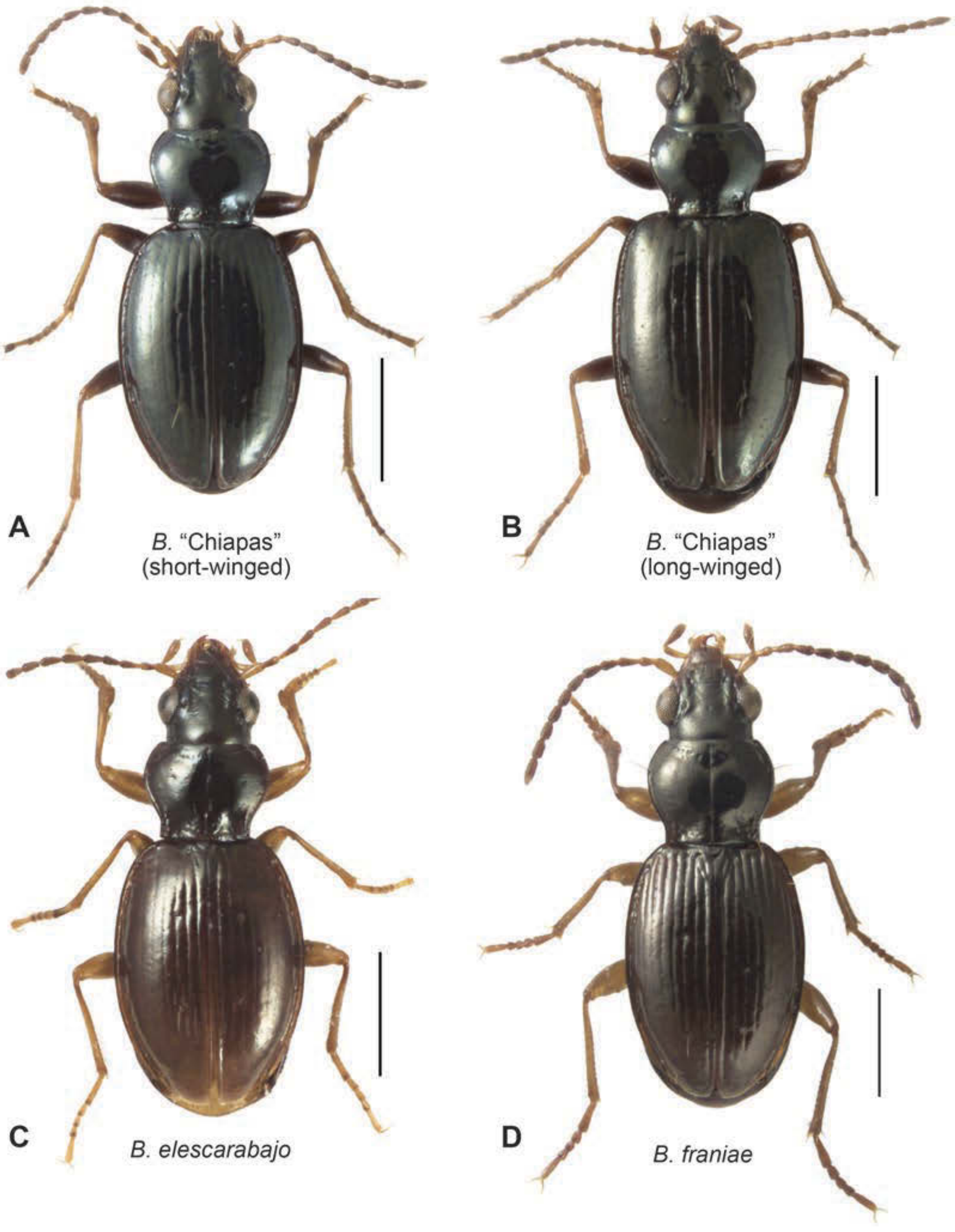


FIGURE 1. Habitus of *Geocosmius* species. (A) *Bembidion* “Chiapas”, brachypterous form, voucher V101274. (B) *B.* “Chiapas”, fully winged form, voucher V101273. (C) *B. elescarabajo*, paratype, voucher V101491. (D) *B. franiae*, specimen UASM371160. Note: *B. nahuala* (not shown) is extremely similar externally to *B.* “Chiapas”. Scale bar 1 mm.

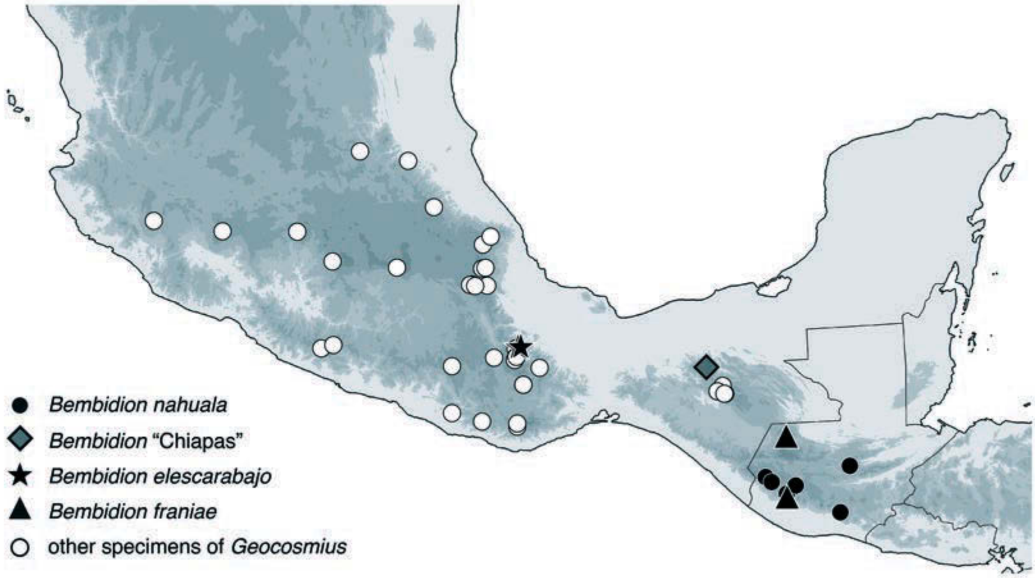


FIGURE 2. Geographic distribution of *Geocosmius* in México and Guatemala.

sequence data, I sought to sequence members of this group, as well as other enigmatic members of the Central American fauna of *Bembidion* including *Bembidion nahuala* Erwin and *Bembidion franiae* Erwin, two species from Guatemala placed in their own species groups by Erwin when he described them in 1982. Luca Toledano had noted (pers. comm. 2009) the similarity of George's Mexican "*Hydriomicroides*" to *Bembidion nahuala*, and Erwin (1982) noted the genitalic similarity of *B. nahuala* and *B. franiae*. In addition, Erwin (1982) noted that "both are similar to diverse members of the subgenus *Peryphus* [*Ocydromus* complex *sensu* Maddison (2012)] or *vernale* group".

In this paper I report that Mexican "*Hydriomicroides*", *Bembidion nahuala*, and *Bembidion franiae* are closely related, and form a distinct lineage within *Bembidion*, well separated from others, and unrelated to subgenus *Peryphus*. The sister group of this distinct lineage is the subgenus *Liocosmius*, a group of delicate, spotted, riparian beetles found in western North America (Maddison and Cooper 2014). I describe the Mexican — Guatemalan lineage as a new subgenus, *Geocosmius*, and describe one new species within the group. In addition, a curious and rare species of *Liocosmius* was discovered after our revision of that subgenus (Maddison and Cooper, 2014) was published. I take this opportunity to describe that species, and include it in the phylogenetic analysis of the *Geocosmius* + *Liocosmius* clade.

MATERIALS AND METHODS

Members of *Bembidion* were examined from the collections listed below. Each of the collections listed begins with the code used in the text.

CAS	California Academy of Sciences, San Francisco, USA
CMNH	Carnegie Museum of Natural History, Pittsburgh, USA
CTVR	Luca Toledano Collection, Verona, Italy
CUIC	Cornell University Insect Collection, Ithaca, USA
EMEC	Essig Museum Entomology Collection, University of California, Berkeley, USA

MCZ	Museum of Comparative Zoology, Harvard University, Cambridge, USA
NHMUK	The Natural History Museum, London, UK
NMNH	National Museum of Natural History, Smithsonian Institution, Washington, USA
OSAC	Oregon State Arthropod Collection, Oregon State University, Corvallis, USA
UASM	University of Alberta Strickland Museum, Edmonton, Alberta, Canada
UIUC	University of Illinois Urbana-Champaign, Urbana and Champaign, Illinois, USA
UNAM	Universidad Nacional Autónoma de México, Mexico City, México

Morphological methods. General methods of specimen preparation for morphological work, and terms used, follow Maddison (1993; 2008). Genitalia were prepared, after dissection from the body, by treatment in 10% KOH at 65°C for 10 minutes followed by multi-hour baths of distilled water, 5% glacial acetic acid, distilled water, and finally 100% ethanol. Male genitalia were then mounted in Euparal between two small coverslips attached to archival-quality heavyweight watercolor paper, and, once dried, pinned beneath the specimen.

Photographs of entire beetles were taken with a Leica Z6Apo lens and DMC4500 camera, and of male genitalia with a Leica DM5500B compound microscope and DMC425C camera, with Leica Application Suite v4.9 software capturing each image. Microsculpture photographs were taken with a DMC425C camera attached to a DM5500B compound scope equipped with an X-Cite 110LED light source, which provides co-axial illumination, and a 20X epi-illumination objective lens. For all photographs, a stack of images from different focal positions was merged using the PMax procedure in Zerene Systems's Zerene Stacker; the final images thus potentially have some artifacts caused by the merging algorithm. Measurements were made using Leica Application Suite v4.9 from images acquired using these imaging systems.

Taxon sampling for DNA studies. We obtained DNA sequence data for seven specimens of subgenus *Geocosmius,* as well as one specimen of the new species of subgenus *Liocosmius* (Table 1). We combined these with previously published data from all described taxa of *Liocosmius* (*Bembidion cooperi* Maddison, *B. darlingtonielum* Cooper & Maddison, *B. festivum festivum* Casey, *B. festivum hilare* Casey, *B. horni* Hayward, *B. mundum* (LeConte), *B. orion* Cooper & Maddison), two species of subgenus *Trechonepha* (*B. iridescens* (LeConte) and *B. trechiforme* (LeConte)), and 191 additional *Bembidion* species as well as 20 Bembidiini in other genera. These data came from

TABLE 1. Specimens of *Bembidion* subgenera *Geocosmius* and *Liocosmius* whose DNA was sequenced for this study. Four-digit numbers in the "#" column are D.R. Maddison DNA voucher numbers. "Wing state" indicates whether the specimen sequenced is fully winged (w+) or brachypterous (w-).

	#	Wing state	Locality
Bembidion franiae	4623	w-	Guatemala: Quetzaltenango: Cerro Zuni, Fuentes Georginas, cloud forest 2400m
Bembidion nahuala	5033	w+	Guatemala: Quetzaltenango: Fuentes Georginas, 2400m, 14.7506°N 91.4804°W
Bembidion nahuala	5034	w-	Guatemala: Quetzaltenango: Fuentes Georginas, 2400m, 14.7506°N 91.4804°W
Bembidion nahuala	5946	w+	Guatemala: San Marcos, 27.0 km NW San Marcos, 15°04'N 091°52'W, 2800-2900mts [type locality; this is a paratype in NMNH]
Bembidion "Chiapas"	4882	w+	México: Chiapas, Yerba Buena Hosp. 1.5 mi N Pueblo Nuevo, 7200', cloud forest
Bembidion "Chiapas"	5317	w-	México: Chiapas, Yerba Buena Hosp. 1.5 mi N Pueblo Nuevo, 7200', cloud forest
Bembidion elescarabajo	5945	w-	México: Oaxaca: 27.5 mi.s. Valle Nacional, 5600'
Bembidion cachagua	4758	w+	USA: California: Pinnacles National Park

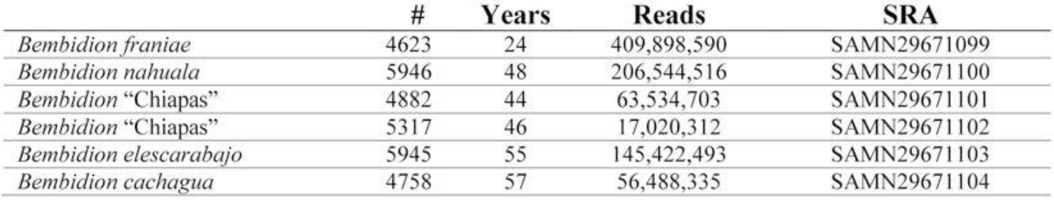
Maddison (2012), Maddison and Cooper (2014), Maddison and Maruyama (2019), Maddison and Sproul (2020), Maddison et al. (2019a), Sproul et al. (2020), and references cited therein. In addition, we sequenced a fragment of the MSP gene for 94 species outside of *Geocosmius* and *Liocosmius* that had not been previously sequenced for that gene. The full list of taxa sampled is present in the files deposited on Data Dryad (see "Data availability", below). The newly sequenced specimens are deposited in OSAC, except for *Bembidion nahuala* DNA5946 (NMNH specimen number USNM:Ent01474002) and *Bembidion cachagua* DNA4758 (EMEC specimen number 348099).

DNA sequencing. Genes studied, and abbreviations used in this paper, are: **28S:** 28S ribosomal DNA (D1-D3 domains); **18S**: 18S ribosomal DNA (near full-length); **COI**: cytochrome c oxidase subunit I; **CAD**: part 4 of carbamoyl phosphate synthetase domain of the *rudimentary* gene; **MSP**: Muscle Specific Protein 300; **Topo**: topoisomerase I; **wg**: wingless; **ArgK**: arginine kinase.

For specimens collected into 95-100% ethanol (*Bembidion nahuala* specimens 5033 and 5034), DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit. Fragments for the seven genes were amplified using the Polymerase Chain Reaction on an Eppendorf Mastercycler Pro Thermal Cycler, using TaKaRa Ex Taq and the basic protocols recommended by the manufacturers. Primers and details of the cycling reactions used are given in Maddison (2012) and Maddison and Cooper (2014). 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 and Ewing, 2002) and Phrap (Green, 1999) as orchestrated by Mesquite's Chromaseq package (Maddison and Maddison, 2021a; Maddison and Maddison, 2021c), with subsequent modifications by Chromaseq and manual inspection. Multiple peaks at a single position in multiple reads were coded using IUPAC ambiguity codes.

DNA extraction and sequencing of the six dried, point-mounted specimens (Table 2) follow the protocols of Kanda et al. (2015; specimen 4623) and Sproul and Maddison (2017; remaining five specimens). In brief, DNA in specimen 4623 was extracted using the Qiagen DNeasy Blood & Tissue Kit, with a single-index library prepared using an NEBNext Ultra™ DNA Library Prep Kit for Illumina (New England BioLabs), which was then sequenced by itself on an Illumina HiSeq 2500 100-base paired-end lane at Oregon Health Sciences University. The remaining five specimens were extracted using the Qiagen QIAmp Micro Kit (using the standard protocol with carrier RNA added), with dual-index libraries prepared using the NEBNext DNA Ultra II kit (New England BioLabs), which were then sequenced on an Illumina HiSeq 3000, multiplexed on either a 100-base (specimens 4758 and 4882) or 150-base (the other specimens) paired-end run at the

TABLE 2. Dried, pinned specimens sequenced using Illumina sequencing. Four-digit numbers under "#" are D.R. Maddison DNA voucher numbers. "Years" indicates the number of years between specimen collection and DNA extraction, that is, the approximate number of years the specimens sat pinned in a drawer in a museum. The last two columns indicate the number of Illumina reads acquired for samples after trimming, and the accession number of reads in NCBI's Sequence Read Archive.



	#	Years	Reads	SRA
Bembidion franiae	4623	24	409,898,590	SAMN29671099
Bembidion nahuala	5946	48	206,544,516	SAMN29671100
Bembidion "Chiapas"	4882	44	63,534,703	SAMN29671101
Bembidion "Chiapas"	5317	46	17,020,312	SAMN29671102
Bembidion elescarabajo	5945	55	145,422,493	SAMN29671103
Bembidion cachagua	4758	57	56,488,335	SAMN29671104

Oregon State University Center for Quantitative Life Sciences. Between 17.0 million and 409.9 million reads were obtained for the samples (Table 2).

For the six specimens on which Illumina sequencing was performed, sequences of the studied genes were obtained as follows. Reads were processed in CLC Genomics Workbench (CLCGW) versions 8.5.1–20.0.4. Reads were trimmed to eliminate low-quality ends (limit=0.05 for 4623, 4882, and 5317; limit=0.000316 for 5945 and 5946), and to remove adapter sequences. The number of reads left after trimming for each sample is shown in Table 3. *De novo* assemblies were generated using Genomics Workbench from paired, trimmed reads using an automatic word and bubble size, with the minimum contig length set to 200. The *de novo* assemblies were converted to BLASTable databases using NCBI's makeblastdb tool, and BLASTed using Mesquite's (Maddison and Maddison, 2021c) local BLAST tool (1E-80 as the e-value cutoff for nuclear protein-coding genes except for specimens 4758, 5945, and 5946, for which the cutoff used was 1E-40; the cutoff for COI and ribosomal genes was 1E-100) using the sequences of *Asaphidion yukonense* as query sequences. All contigs that were returned as hits were BLASTed to NCBI's GenBank, and rejected if the top hits were not beetles. Contigs that matched beetle sequences on GenBank were accepted according to the following rules, with one exception: if there was only one contig returned from the BLASTable local database using *Asaphidion* as the query sequence, that contig was accepted as valid and included in the analysis; if two hits, and they overlapped with no differences in the overlap region, the contigs were merged and accepted; if two or more hits, but one was at least five times longer than the others, and fully contained the others, then only the long contig was accepted. One exception to this were the two contigs returned for ArgK for *Bembidion* "Chiapas" 5317, which BLASTed to carabid sequences, but which preliminary analyses suggested were not orthologous to *Bembidion* ArgK. They are presumed to be paralogs and were rejected. The other exception was for CAD for *Bembidion nahuala* 5946, which overlapped with the analyzed region by only 126 bases, and so was excluded from consideration. For the *Geocosmius* specimens, this protocol yielded accepted sequences for 28S, 18S, and COI for all three dried specimens, but nuclear protein-coding genes for only *B. franiae* (Table 3). For *B. cachagua* 4758, reference-based assembly was also conducted in CLCGW for wingless and MSP, using *Asaphidion yukonense* sequences from Maddison (2012) as references. For each of wingless and MSP, the reference-based sequence was merged with the *de novo* fragments after preliminary phylogenetic analyses showed that the referenced-based and *de novo* fragments form a clade in the phylogenetic tree.

TABLE 3. Genes studied for specimens of subgenera *Geocosmius* and *Liocosmius*. Four-digit numbers under "#" are D.R. Maddison DNA voucher numbers. Voucher numbers and other information for previously published sequences are available in their respective publications. Newly obtained sequences are those with GenBank accession numbers in the range ON525450 through ON525588.

	#	28S	18S	COI	CAD	MSP	Topo	wg	ArgK
Subgenus *Geocosmius*									
Bembidion franiae	4623	ON525474	ON525482	ON525466	ON525453	ON525583	ON525458	ON525450	ON525463
B. nahuala	5033	ON525475		ON525467	ON525454	ON525584	ON525459		
B. nahuala	5034	ON525476	ON525483	ON525468	ON525455	ON525585	ON525460	ON525451	
B. nahuala	5946	ON525477	ON525484	ON525469		ON525586			
B. "Chiapas"	4882	ON525478	ON525485	ON525470	ON525456	ON525587	ON525461		
B. "Chiapas"	5317	ON525479	ON525486	ON525471					
B. elescarabajo	5945	ON525473	ON525481	ON525465		ON525582			
Subgenus *Liocosmius*									
B. cachagua	4758	ON525480	ON525487	ON525472	ON525457	ON525588	ON525462	ON525452	ON525464
B. cooperi		KJ624158	KJ624356	KJ624318	KJ624207	KJ624364	KJ624273	KJ624243	KJ624254
B. darlingtonielum		KJ624161	KJ624357	KJ624321	KJ624210		KJ624276	KJ624245	KJ624255
B. festivum festivum		KJ624173		KJ624332	KJ624221	KJ624373	KJ624287	KJ624250	KJ624259
B. festivum hilare		JN170338		JN171034	KJ624217		KJ624283	KJ624249	JN170559
B. horni		JN170356	JN170182	KJ624338	JN170824	KJ624379	JN171228	JN171426	JN170578
B. mundum		JN170386	JN170205	JN171072	JN170858	KJ624382	JN171253	JN171458	JN170614
B. orion		KU233798	KU233698	KU233848	KU233986	KJ624385	KU234083	KU233873	KU234036

All told, sequences of between three and eight genes were obtained for each specimen of *Geocosmius* and *Liocosmius* (Table 3).

Alignment and data exclusion. Alignment was not difficult for any of the protein-coding genes. There were no insertion or deletions (indels) evident in the sampled CAD, ArgK, Topo, or COI sequences. In wingless there was a 6-base insertion in three species within subgenus *Odontium*, and a separate three-base insertion within the subgenus *Omotaphus*. Thus, the protein-coding genes could be aligned manually. Alignments of 28S and 18S was conducted in MAFFT version 7.130b (Katoh and Standley, 2013), using the L-INS-i search option and otherwise default parameter values.

Sites in 28S and 18S were chosen to be excluded from consideration using the modified GBLOCKS analysis present in Mesquite with the following options: minimum fraction of identical residues for a conserved position = 0.2, minimum fraction of identical residues for a highly-conserved position = 0.4, counting fraction within only those taxa that have non-gaps at that position, maximum number of contiguous non-conserved positions = 4, minimum length of a block = 4, and allowed fraction of gaps within a position = 0.5.

Phylogenetic analyses. Maximum likelihood analysis was conducted for each gene individually using IQ-TREE version 2.1.3 (Nguyen et al., 2015), as orchestrated by Mesquite's Zephyr package (Maddison and Maddison, 2021b; Maddison and Maddison, 2021c). The ModelFinder feature within IQ-TREE (Kalyaanamoorthy et al., 2017) was used to find the optimal character evolution models. The MFP model option was used for 28S and 18S, and the TESTMERGE option for protein-coding genes. The TESTMERGE option sought the optimal partition of sites, beginning with the codon positions in different parts. In addition, analyses with the concatenated data were conducted, with the TESTMERGE option also being used, beginning with each codon position for each gene as a separate part (thus, the analysis began allowing for up to 17 parts, three for each of the five protein-coding genes, and one for each of 28S and 18S). Fifty searches were conducted for the maximum-likelihood tree for each of the single-gene matrices, and 100 searches for the concatenated matrix; for standard, non-parametric bootstrap analyses, 500 replicates were used.

Data availability. Sequences of the studied genes have been deposited in GenBank with accession numbers ON525450 through ON525588. Illumina reads are archived on NCBI's Sequence Read Archive under the accession numbers SAMN29671099 through SAMN29671104 (Table 2). Files containing the entire (untrimmed) gene sequences for each specimen as well as the inferred trees for each gene have been deposited in Dryad (data available from the Dryad Digital Repository at https://doi.org/10.5061/dryad.b5mkkwhgc).

PHYLOGENETIC RESULTS

Both *Geocosmius* and *Liocosmius* (with the later including *B. cachagua*, sp. nov.) are monophyletic (Fig. 3, 4A), with strong or very strong support from seven of the eight genes studied (Fig. 4B). Within *Bembidion*, *Geocosmius* is sister to subgenus *Liocosmius* (Figs 3, 4A), with moderate to very strong support from 28S, CAD, MSP, and Topo, and some support from COI (Fig. 4B). There is moderate support for the sister of *Geocosmius* + *Liocosmius* being the western North American subgenus *Trechonepha*, with two recognized species, *Bembidion iridescens* LeConte and *B. trechiforme* LeConte. Evidence for this relationship comes primarily from 28S and CAD, with some evidence provided by ArgK. Within *Geocosmius*, there is strong support for *Bembidion nahuala* being sister to *B.* "Chiapas", and weaker support for their sister being *B. elescarabajo*. Within *Liocosmius*, there is weak support for *B. cachagua* being sister to the remaining *Liocosmius*.

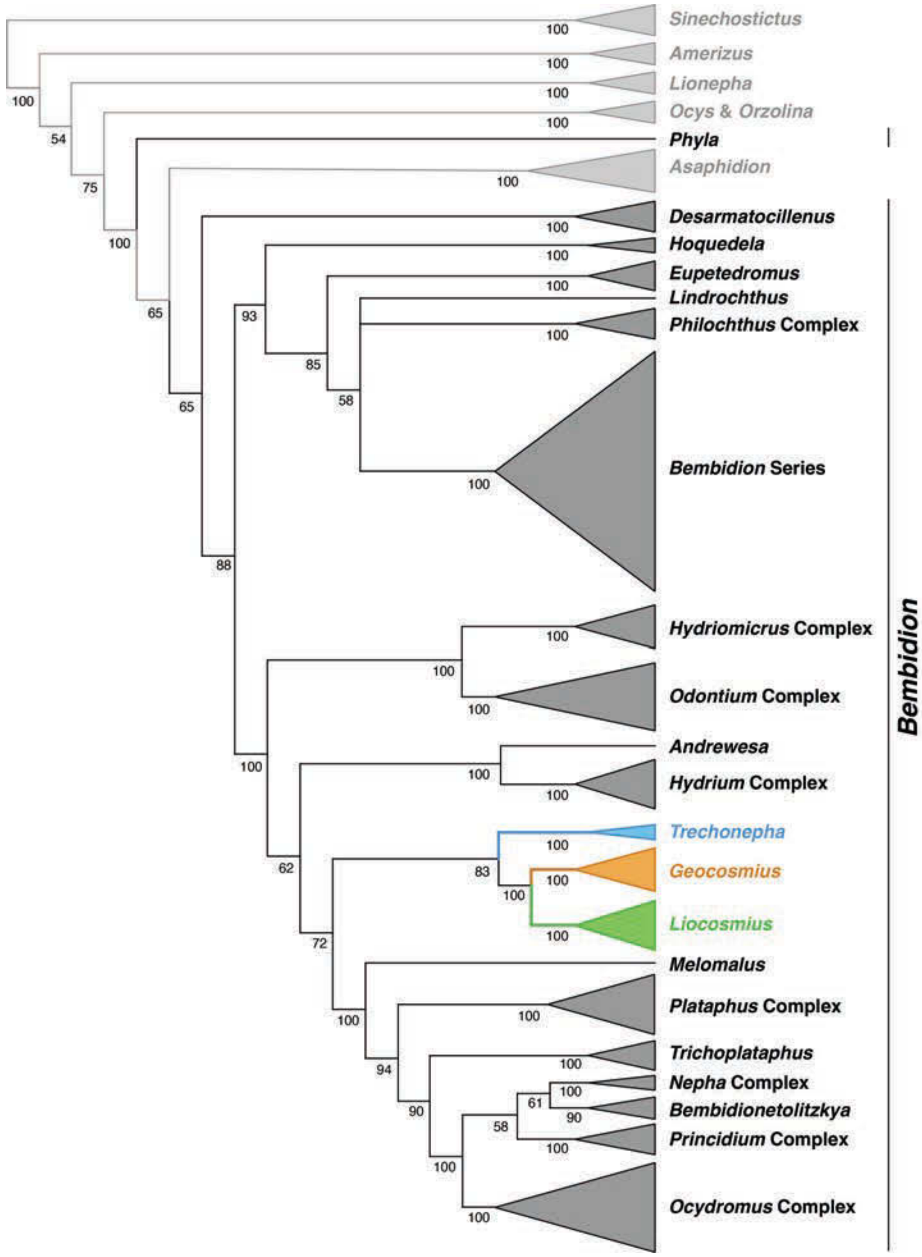
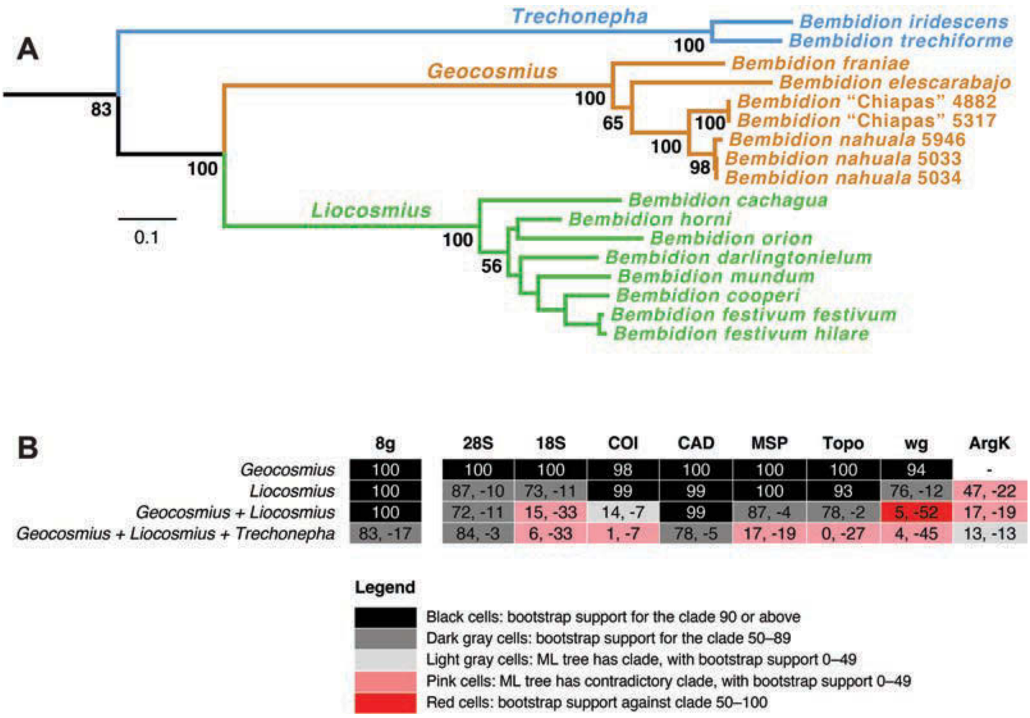


FIGURE 3. Overview of maximum likelihood bootstrap tree of Bembidiini based upon the concatenated, eight-gene matrix, showing position of the subgenera *Geocosmius*, *Liocosmius*, and *Trechonepha*. Taxon names in paler gray are genera of Bembidiini other than *Bembidion*; all other taxa are subgenera, subgeneric complexes, or subgeneric series of *Bembidion*. Triangles (clades) have their area approximately proportional to the number of species included in the analysis. Details within each clade are presented in the files submitted to Data Dryad (see Data availability section). Values below branches are the bootstrap support for that clade, expressed as a percentage.



	8g	28S	18S	COI	CAD	MSP	Topo	wg	ArgK
Geocosmius	100	100	100	98	100	100	100	94	-
Liocosmius	100	87, -10	73, -11	99	99	100	93	76, -12	47, -22
Geocosmius + Liocosmius	100	72, -11	15, -33	14, -7	99	87, -4	78, -2	5, -52	17, -19
Geocosmius + Liocosmius + Trechonepha	83, -17	84, -3	6, -33	1, -7	78, -5	17, -19	0, -27	4, -45	13, -13

Legend

- ⬛ Black cells: bootstrap support for the clade 90 or above
- ▩ Dark gray cells: bootstrap support for the clade 50–89
- ░ Light gray cells: ML tree has clade, with bootstrap support 0–49
- ▨ Pink cells: ML tree has contradictory clade, with bootstrap support 0–49
- 🟥 Red cells: bootstrap support against clade 50–100

FIGURE 4. Phylogenetic results, focusing on subgenera *Geocosmius*, *Liocosmius*, and *Trechonepha*. (A) A portion of the maximum likelihood tree inferred from the concatenated, eight-gene matrix. Numbers of the branches are maximum likelihood bootstrap percentages; bootstrap values within most of *Liocosmius* not shown. Other bembidiines omitted from the image. Scale bar 0.1 units, as calculated by IQ-TREE. (B) Support for various clades, measured with bootstrap support percentages from maximum likelihood phylogenetic analyses. One or two values are given in each cell. If the bootstrap support percentage is 90 or more, only that value is listed. If bootstrap support is less than 90, two values are listed: the bootstrap support for the clade, followed by a negative value which is the bootstrap support against the clade, as measured by the bootstrap value for the contradictory clade with the highest support value. The "8g" column shows the support values for the concatenated, eight-gene matrix. "-" indicates only one species is included, and thus support for that clade cannot be measured.

TAXONOMY

Geocosmius Maddison, new subgenus

Type species *Bembidion nahuala* Erwin, 1982

Derivation of name. The first portion of the name, "Geo", consists of the first three letters of George E. Ball's given name, and is included to honor George's long-term commitment to discovering and documenting the biodiversity of México, and his role in collecting most known specimens of this subgenus. In addition, "Geo" is a Greek prefix referring to the Earth, and evokes the interesting relative geographic distributions of *Geocosmius* and its sister group, *Liocosmius*. The last portion of the name, "cosmius", was chosen to provide a linguistic connection to its sister group.

Habitus. Beetles of the subgenus *Geocosmius* have the general appearance of typical members of non-riparian cloud forest *Bembidion*; that is, they are small, dark, unspotted, and convex; most specimens lack hind wings and have rounded shoulders. A similar form has evolved multiple times within *Bembidion* in similar habitats, as in the subgenus *Ecuadion* Moret & Toledano from

Central and South America (Moret and Toledano, 2002), *Nesocidium* Sharp from Hawaii (Liebherr, 2008), subgenus *Hypsipezum* Alluaud from Africa, and some members of the *Ocydromus* complex in Central America (e.g., *B. chiriqui* Erwin) (Maddison et al., 2019b).

Morphological Diagnosis. Small to medium (2.6–3.5 mm), shiny *Bembidion,* without colored markings on the elytra. Frontal furrows single, broad, shallow, well-marked (*Bembidion franiae*), or broad, shallow, and disrupted by surface rugosity (other species), not convergent or extended onto clypeus. Eyes of normal size for *Bembidion* (*B. franiae*) or slightly reduced (other species). Mentum with anterior lateral region complete, triangular; mentum tooth triangular.

Posterior angle of pronotum with a posterolateral carina, although it is interrupted posteriorly in *B. franiae* and some specimens of other species. Posterior margin of pronotum not strongly sinuate laterally; basal transverse furrow weak, disrupted by shallow punctures, and in many specimens interrupted by the medial furrow extending posteriorly to the hind margin of the pronotum; hind angles right or slightly acute.

Elytron with lateral bead ending at humerus, not prolonged onto base, although in *B. franiae* a short carina extends from the end of the bead toward the center of the elytral disc at an angle, giving the impression of an angulate shoulder bead. Striae vary in depth, from anteriorly deep in *B. franiae* to much shallower in most other species, with lateral striae increasingly evanescent; all striae absent or nearly so in the posterior quarter of the elytra, except for the first stria; two discal setae in third stria. Elytra slightly iridescent in *B. franiae*, because of the transverse microsculpture, and slightly dull in *B. elescarabajo*, because of the nearly isodiametric sculpticells; elytral microsculpture absent from specimens of the *nahuala* group. Mesoventral process without subapical setae. Metaventral process unmargined.

Apex of last visible abdominal sternite with two setae in males, four setae in females. Apices of each paramere normally with three setae, but some individuals have two or four setae on the left paramere, and one has four setae on the right paramere.

Within the geographic range of *Geocosmius* live several other species of *Bembidion* with small, convex adults without spots: *B. purulha* Erwin (a member of the *Ocydromus* complex), as well as *B.* (*Cyclolopha*) *cyclodes* Bates and *B.* (*Cyclolopha*) *championi* Bates. *Bembidion purulha* can be distinguished by the elytral striae, which abruptly transition at about the halfway point from large, distinct punctures anteriorly to fully effaced striae posteriorly, as well as a fully margined metaventral process. The two *Cyclolopha* species can be distinguished by the much broader pronotum with very rounded hind angles, with the posterior lateral seta notably anterior to the posterior margin of the pronotum at the midline.

All known specimens of *B. franiae* and *B. elescarabajo* lack hind wings. *Bembidion nahuala* and *B.* "Chiapas" are wing dimorphic, with most specimens lacking hind wings, and with more rounded elytra and notably rounded shoulders (Fig. 1A). A minority of specimens of both species are fully winged, with more parallel-sided elytra and less rounded shoulders, giving these specimens a rather different appearance (Fig. 1B).

Habitat. Based upon the available label data, species in this group live in cloud forests (Guatemala, Chiapas, Oaxaca, Guerrero, and Puebla), oak-pine forests (Guatemala, México, Oaxaca), pine forests (Veracruz), pine-alder forests (Veracruz), wet oak forests (Puebla), and oak-alder forests (Guerrero).

Geographic distribution. This subgenus is known from southern México and Guatemala (Fig. 2).

Composition. The known species of subgenus *Geocosmius* are *Bembidion franiae* Erwin, *Bembidion elescarabajo* Maddison, sp. nov., and then a group of similar forms (the *nahuala* group) including *Bembidion nahuala* Erwin and *B.* "Chiapas". I have examined 5 specimens of *B. frani-*

ae, 13 of *B. elescarabajo*, and 311 specimens of the *nahuala* group (UASM, CUIC, CMNH, NMNH, UNAM, CTVR, and OSAC).

Members of the *nahuala* group are very similar to one another. With the exception of a form with deeper elytral stria from Volcán de Colima in Jalisco, and the differences associated with wing dimorphism and gender, this widespread complex shows no obvious patterns of external morphological variation. *Bembidion nahuala* Erwin and *B.* "Chiapas" appear to be different species based upon DNA sequences (Fig. 4) and male genitalia (Fig. 5). The two forms differ by 4 bases in 28S, and by 27 bases or 4.1% in COI; these nucleotide differences in COI imply two amino acid differences between the species.

Key to adults of species of *Bembidion* subgenus *Geocosmius*

1. Elytra with evident microsculpture 2
— Elytra without evident microsculpture . . . . *nahuala* group

2. Entire dorsal surface with evident microsculpture, including on the top of the head and disc of pronotum; throughout the disc of the pronotum there are clearly and completely edged sculpticells. Elytral microsculpture more transverse (Fig. 6A). All striae deep in anterior quarter of the elytra, with intervals notably convex in this region. Eyes of normal size for *Bembidion* (Fig. 1D) . . . . *Bembidion franiae* Erwin
— Dorsal surface of head shiny, without microsculpture, at least at center; pronotal disc shiny, without clear microsculpture. Elytral microsculpture more or less isodiametric (Fig. 6B). Lateral striae absent or nearly so, even in anterior region of elytra. Eyes slightly less protruding than typical for *Bembidion* (Fig. 1C) . . . . ***Bembidion elescarabajo*** sp. nov.

***Bembidion elescarabajo*, sp. nov.**

(Figures 1C, 5C, 5D, 6B)

Holotype male, in UASM, labeled "MEX. Oaxaca Rte. 175. 28 mi. s. Valle Nacional. 2040 m. Aug. 25, 1974" [handwritten], "MIDDLE AMER. EXP. 1974, H. Frania & G.E. Ball collectors", "David R. Maddison V101490 Voucher Specimen", "HOLOTYPE Bembidion elescarabajo David R. Maddison [partly handwritten, on red paper]", "UASM# 410000" [printed on white paper]. Genitalia mounted in Euparal in between coverslips pinned with specimen.

Paratypes (6 males, 6 females). "Mexico: Oaxaca. 27.5 mi s. Valle Nacional. 5600' VIII.15-16.65", "George E. Ball and D.R. Whitehead collectors" (2: UASM, CTVR). "MEXICO: Oaxaca. 27.5 mi s. Valle Nacional. 5600' V.4-5.1966", "George E. Ball and D.R. Whitehead collectors" (5: UASM, UNAM, OSAC, NHMUK, NMNH). "MEXICO Oaxaca 36.2 km s. Valle Nacional, Rte. 175 cloud forest ca. 2010 m., April 27, 1977", "MEXICAN EXP. 1977 J.S. Ashe, H.E. Frania, D. Shpeley coll." (1: UASM). "MEX. Oaxaca Rte. 175. 28 mi. s. Valle Nacional. 2040 m. Aug. 28, 1974", "MIDDLE AMER. EXP. 1974, H. Frania & G.E. Ball collectors" (1: UASM). "MEX. Oaxaca 35.1 mi.n. Istlan de Juarez, Rte. 175. 2050m, 22.07.1975", "MEX. EXP. 1975, H. Frania & G.E. Ball collectors" (3: UASM, UNAM, OSAC).

Type locality. México: Oaxaca: 28 miles (45 km) south of Valle Nacional (San Juan Bautista Valle Nacional) along route 175; this is approximately 17.594°N 96.427°W.

Derivation of specific epithet. This species is named in honor of George Ball's three *El Escarabajo* campers. George and his colleagues used those trusty vehicles to explore the regions of México inhabited by *Geocosmius*, and on these journeys most known specimens of the subgenus were collected, including the type series of this species. The name is to be treated as a noun in apposition.

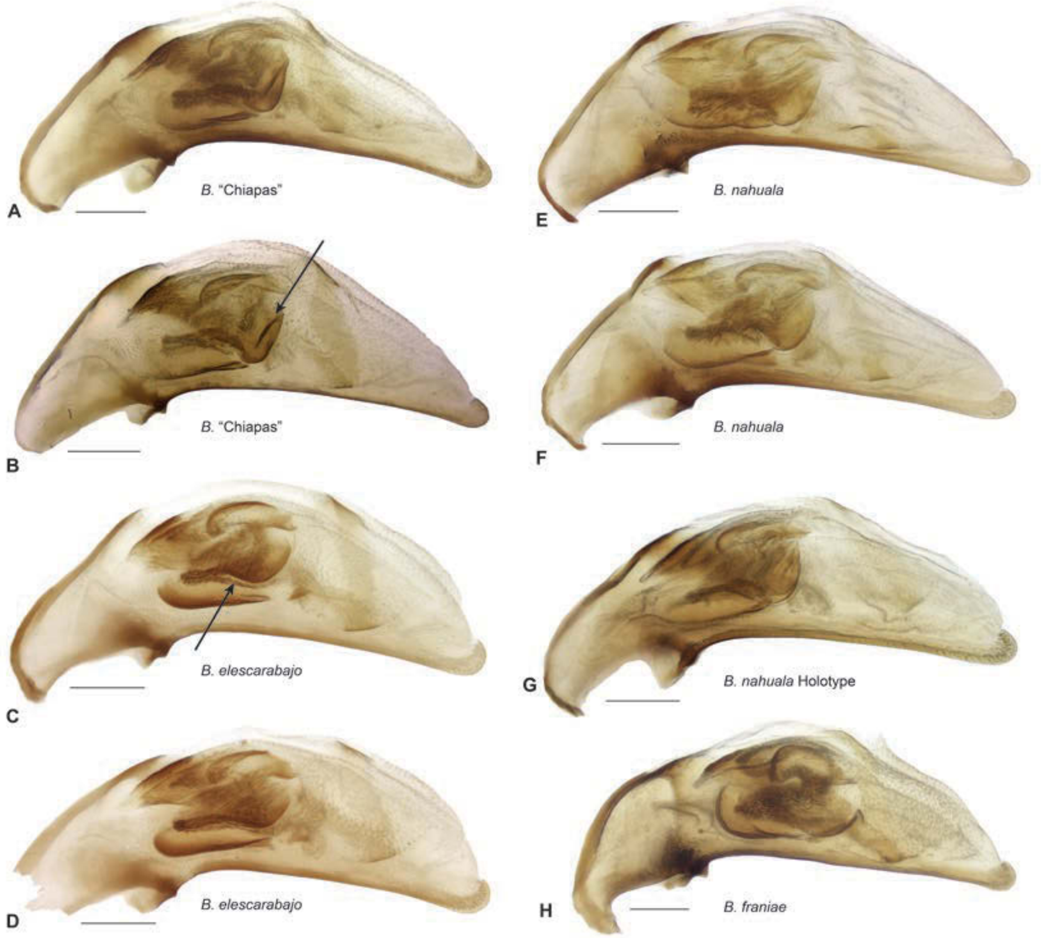


FIGURE 5. Aedeagi of *Geocosmius*. (A) *Bembidion* "Chiapas", voucher DNA5317. (B) *B*. "Chiapas", voucher DNA4882. Arrow indicates the prominent ridge characteristic of this species. (C) *B. elescarabajo*, voucher V101490. Arrow indicates the less curved sclerite margin characteristic of this species. (D) *B. elescarabajo*, voucher V101489. (E) *B. nahuala* voucher DNA5033. (F) *B. nahuala* voucher DNA5034. (G) *B. nahuala*, holotype. (H) *B. franiae*, voucher DNA4623. Scale bar 100 µm.

Description. Body length 3.25–3.90 mm. Body brown, with head and pronotum slightly darker than elytra. Legs uniform in color, testaceous or slightly infuscated; antennomere 1 testaceous, antennomeres 2 and 3 with distal half slightly infuscated, remaining antennomeres slightly infuscated; penultimate maxillary palpomere infuscated, antepenultimate testaceous. Mentum with anterior lateral regions large, triangular; medial tooth triangular with rounded tip; frontal furrows weakly defined, somewhat rugose; eyes not prominent; prothorax with greatest width in front of middle; sides sinuate, with sides in front of hind angle straight; hind angle approximately right; posterolateral carina evident, moderately long, and straight; posterior region of pronotum slightly rugose with poorly defined punctures. Elytra with lateral bead not prolonged medially at shoulder; inner three striae well-defined around ed3; striae 4 and 5 faint, even anteriorly, with striae 6 and 7 extremely faint; only first stria evident to apex, with other striae more or less absent from apical fourth. Microsculpture absent from dorsal surface of head at center and from pronotal disc; evident in both sexes over entire surface of elytra, nearly isodiametric (Fig. 6B). Pronotum with two lateral setae on each side; elytron with two setae in third stria. Aedeagus (Fig. 5C, D) similar to members of the *nahuala* group (Fig. 5A, B, E, F, G), but with ventral margin of the large central sclerite less abruptly bent.

Additional characteristics. All thirteen specimens examined lack hind wings.

Geographic distribution. Known only from four closely proximate localities in Oaxaca along route 175 between San Juan Bautista Valle Nacional and Ixtlán de Juárez, between about 17.604°N 96.381°W and 17.594°N 96.460°W (Fig. 2, star). These localities are at most 9 km apart.

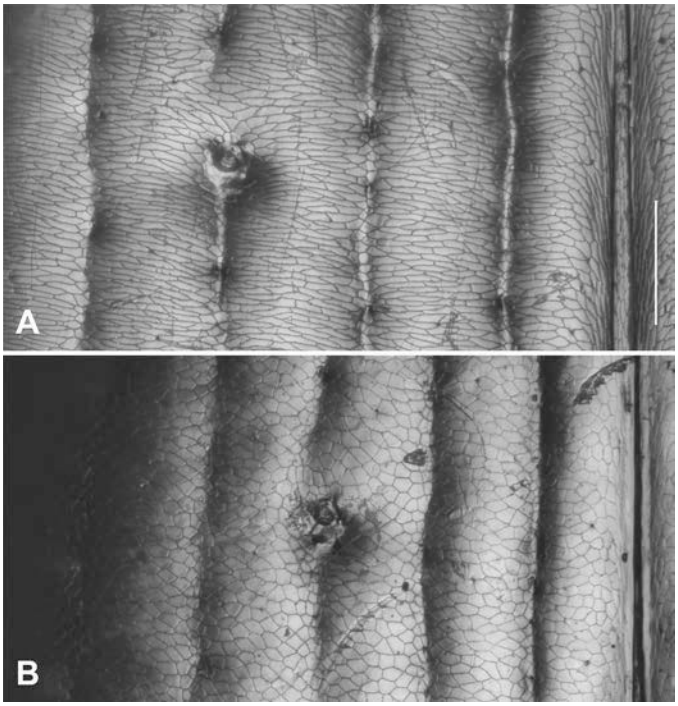


FIGURE 6. Microsculpture of *Geocosmius*. (A) *Bembidion franiae,* voucher DNA4623, (B) *B. elescarabajo*, voucher V101491. Scale bar 100 µm.

Geographic relationships with other species. One specimen of a *nahuala*-group species (perhaps *Bembidion* "Chiapas") was collected at the same place and time as five of the paratypes of *B. elescarabajo*: "Mexico: Oaxaca. 27.5 mi s. Valle Nacional. 5600' VIII.15-16.65".

Habitat. Based upon imagery in Google Earth, and the annotation "cloud forest" on the label of the 1977 specimen, the known localities are all in cloud forest.

Subgenus *Liocosmius* Casey

Type species *Ochthedromus mundus* LeConte (1852), designated by Lindroth (1963).

A revision of *Liocosmius* is provided in Maddison and Cooper (2014). The species described here as *Bembidion cachagua* became known shortly before the publication of that work, but it was not recognized as a *Liocosmius* until after a genitalic dissection and DNA sequencing revealed that it belonged to the subgenus. Within *Liocosmius*, *B. cachagua* is unusual for its microsculptured pronotum and head, rugose pronotum (at least in the posterior portion), low-contrast color pattern on the elytra, and slight metallic sheen on elytra. The species can be identified in the key provided by Maddison and Cooper (2014) if an additional couplet is added at the beginning:

0. Evident microsculpture throughout dorsal surface of head (except, in some specimens, between eyes near midline), and over most of the pronotum; microsculpture near apex of elytra evident as distinct sculpticells that are only slightly transverse. Ground color of elytra brown, slightly depigmented, contrasting only weakly with pale spots (Fig. 7A). Entire dorsal surface with slight yellow-green or brown-green metallic sheen, including on the elytra.

Appendages infuscated. Posterior region of pronotum evidently wrinkled, at least medially, and with many punctures (Fig. 7B) . . . . ***B. cachagua*** sp. nov.

— Most of the dorsal surface of head without microsculpture; pronotum with at most weak, very transverse microsculpture restricted to lateral margins; microsculpture absent from apex of elytra or consisting of very transverse sculpticells. If ground color of elytra brown and contrasting only weakly with pale spots, then appendages testaceous, not infuscated (Maddison and Cooper 2014, Fig. 2). Elytra without metallic sheen. Posterior region of pronotum smoother (e.g., Maddison and Cooper 2014, Fig. 7) 1

Bembidion cachagua, sp. nov.
(Figures 7, 8)

Holotype male, in CAS, labeled: “U.S.A.: CALIFORNIA: Monterey Co., Hastings Nat. History Reserve. J.M. Linsdale Colln.”, “C.A.S. Accession 29-V-48 Cachagua Creek”, “David R. Maddison DNA3842 DNA Voucher” [pale green paper], “HOLOTYPE Bembidion cachagua David R. Maddison” [partly handwritten, on red paper]. Genitalia mounted in Euparal in between coverslips pinned with specimen; extracted DNA stored separately.

Paratypes (2 males, 4 females). Same data as holotype (3: CAS, OSAC). “Pinnacles Nat’l Mon. 4.19.58 Toschi” (1: EMEC; this is D.R. Maddison DNA voucher number 4758, and EMEC specimen number 348099). “Santa Barbara Cal. May ’91 L.E.R.” (1: MCZ, Hayward Collection). “S. Cal.” (1: UIUC).

Type locality. Cachagua Creek does not flow through Hastings Natural History Reserve, although a tributary, Finch Creek, does. At its nearest Cachagua Creek is approximately 2.7 km from the boundary of the Reserve. It is reasonable to presume that the upper label on the holotype, “U.S.A.: CALIFORNIA: Monterey Co., Hastings Nat. History Reserve. J.M. Linsdale Colln.”, was a generic label used for all specimens from the Linsdale collection, as J.M. Linsdale was the Director of the Hastings Natural History Reserve at the time (Alagona, 2012). The lower label presumably allowed customization for each specimen, and the locality information on the second label is thus likely more accurate. Thus, the type locality would more accurately be stated to be “Cachagua Creek near Hastings Natural History Reserve”.

Derivation of specific epithet. Named after Cachagua Creek, the locality at which four of the seven known specimens were found. Treated as a noun in apposition.

Description. Body length 3.45–3.90 mm. Forebody dark brown or piceous, with metallic greenish-yellow or brassy sheen; elytra pale chestnut brown, with the four testaceous elytral spots contrasting only slightly against the ground color (Fig. 7). In four specimens, the antennae are infuscated throughout; in the other three specimens, two of which appear to be teneral, the basal 2.5 antennomeres are testaceous; legs pale reddish brown, slightly infuscated. Frontal furrows shallow, not prolonged onto clypeus. Mentum with anterior lateral regions triangular, large; central tooth of mentum triangular, slightly rounded. Prothorax narrow, only slightly wider than head; hind angles slightly obtuse; posterolateral carina of pronotum long and more or less straight; posterior region of pronotum rugose, wrinkled and with evident punctures (Fig. 7). Elytra with lateral bead not prolonged medially at shoulder; striae shallow, poorly defined, with extremely small punctures. Microsculpture evident throughout the dorsal surface of the forebody in most specimens, more or less isodiametric or slightly transversely stretched, in some specimens effaced in the center of the head between the eyes, and in the center of the pronotal disc; microsculpture effaced from most of elytra, but evident behind the second dorsal discal seta (ed5) as defined, slightly transverse sculpticells. Pronotum with two lateral setae on each side; elytron with two setae in third stria. Aedea-

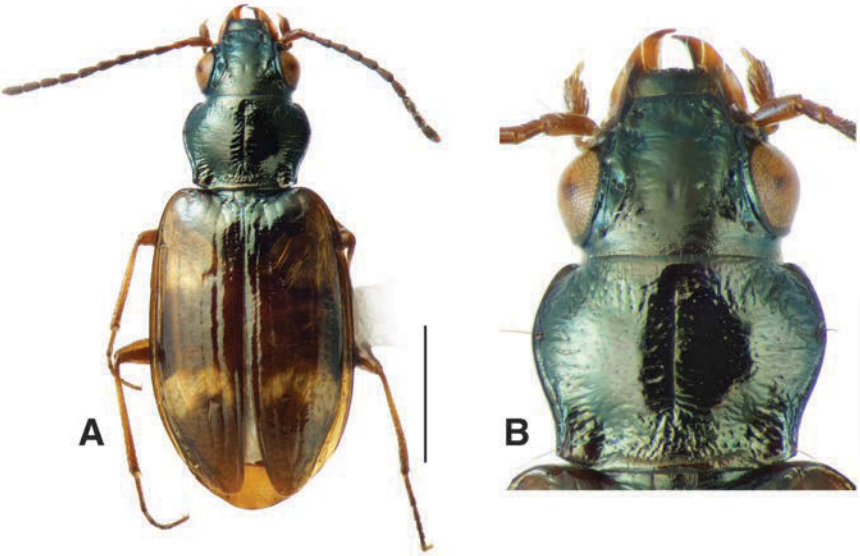


FIGURE 7. Holotype of *Bembidion cachagua*. (A) Habitus, scale bar 1 mm. (B) Forebody, scale bar 0.5 mm.

gus (Fig. 8) with a small brush sclerite similar to that of *B. horni*, but with a long, slightly curved flagellum, most similar to that of *B. festivum*.

Most easily distinguished from other *Liocosmius* by the stronger and more widespread microsculpture, the greenish-yellow metallic sheen on the dorsal surface, and the relatively low-contrast pattern on the elytra. In comparison to *Bembidion horni* in particular, the pronotum in *B. cachagua* is much narrower, with straighter sides.

Variation. Some specimens have numerous punctures on the dorsal surface of the head and in anterior regions of the pronotum.

Geographic Distribution. Known only from California from Monterey and San Benito Counties south to Santa Barbara County (Fig. 9).

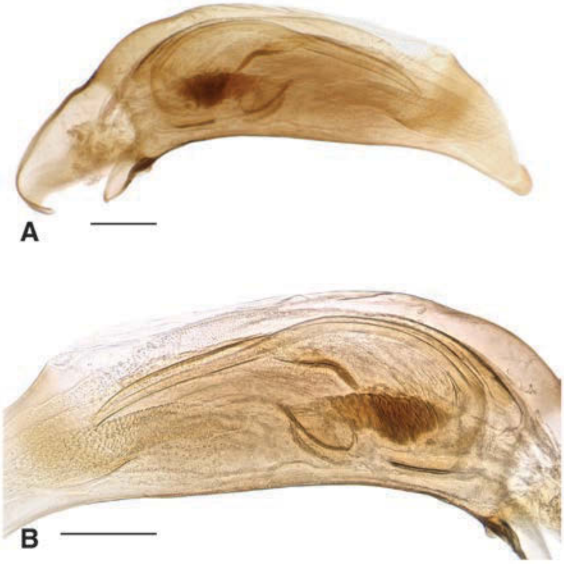


FIGURE 8. Aedeagus of holotype of *Bembidion cachagua*. (A) Left lateral view. (B) Right lateral view. Scale bar 100 µm.

Geographic relationships with other species. Found sympatrically with four other species of subgenus *Liocosmius*: *Bembidion horni*, *B. mundum*, *B. darlingtonielum*, and *B. festivum*.

Habitat. Unknown. It may occur in similar habitats as other *Liocosmius*, that is, on sandy shores of creeks, most often in shaded or partly shaded areas, and in general where the sand banks are relatively steep.

Notes. I have seen no specimens of this species collected since 1958. In late April and early

May 2014, John Sproul and I searched at multiple known localities for this species, including at Hastings Natural History Reserve, Pinnacles National Park, around Santa Barbara, and at many other localities in between. We searched in typical *Liocosmius* microhabitats (including steep sand banks in partial shade), with no success. Along Finch Creek in Hastings Natural History Reserve, the only *Bembidion* in those microhabitats was *B. iridescens* LeConte; in nearby microhabitats along the creek shore were three species of *Bembidion*: *B. californicum* Hayward, *B. perspicuum* LeConte, and *B. lugubre* LeConte.

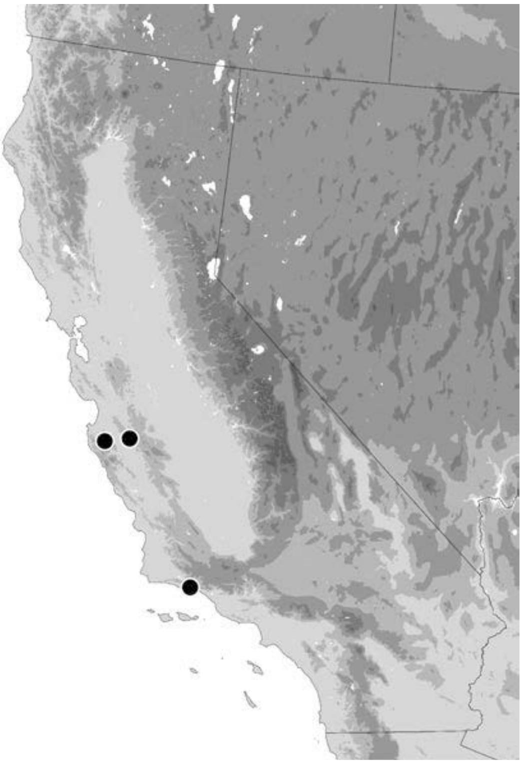


FIGURE 9. Map of California showing the geographic distribution of *Bembidion cachagua*.

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Numerous other people helped this project in important ways, and I am very thankful to all of them. John S. Sproul, Olivia F. Boyd, and R. Antonio Gomez prepared the libraries for Illumina sequencing. Kojun Kanda collected the first available specimens in ethanol of *Geocosmius*. John Sproul and Paul G. Johnson helped look for *Bembidion cachagua*. Vincent Voegeli of the Hastings Natural History Reserve gave permission to search for *B. cachagua* there, and gave advice about habitats. Both David H. Kavanaugh and Wayne Maddison helped in numerous ways over the years, including providing valuable advice.

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