

Cryptic species in the mountaintops: species delimitation and taxonomy of the *Bembidion breve* species group (Coleoptera: Carabidae) aided by genomic architecture of a century-old type specimen

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The *breve* species group includes closely related *Bembidion* Latreille ground beetles commonly found at high elevation in the mountains of western North America. For several decades, the group has been considered to consist of two species. Here, we present evidence from morphological, molecular and geographic data that the group contains nine species: *Bembidion ampliatum*, *B. breve*, *B. geoppearlis*, *B. laxatum*, *B. lividulum*, *B. oromaia*, *B. saturatum*, *B. testatum* and *B. vulcanix*. We describe three species (*B. geoppearlis*, *B. oromaia* and *B. vulcanix*) as new and resurrect four previously synonymized names (*B. ampliatum*, *B. lividulum*, *B. saturatum* and *B. testatum*). Species diversity is highest throughout the Cascades in Oregon and Washington, and Sierra Nevada of California, where up to seven species can occur in sympatry. We resolved challenging nomenclatural issues through analysis of sequences obtained from century-old type specimens by using a novel application of rDNA copy number analysis – an approach that may prove useful for other historical specimens.

ADDITIONAL KEYWORDS: Bembidiini – high-throughput sequencing – historical specimens – morphology – phylogeny – rDNA signature – species delimitation – systematics – type specimens.

INTRODUCTION

The *breve* species group is a complex of closely related *Bembidion* Latreille ground beetles (Figs 1–3) of the subgenus *Plataphus* Motschulsky, which are common in the mountains of western North America. Members of the species group are dark-bodied, medium-sized *Bembidion* and live along margins of receding snow patches and shorelines of streams and lakes.

For several decades, the *breve* group has been considered to consist of two species, called *B. breve* (Motschulsky) and *B. laxatum* Casey. However, the last worker to treat the group formally noted high levels of intraspecific variation, distinctive geographic forms and the need for a thorough revision (Lindroth, 1963). As we gathered and sequenced *breve* group material,

we noted distinctive molecular forms from the Sierra Nevada in California that appeared to have subtle correlations with external morphological structures. We then examined male genitalia, found morphological variation that corroborated molecular patterns and strongly suggested the existence of additional species. Through these early findings of distinct forms in California, and because Lindroth's work targeted only the northern geographic range of the group (primarily Canada and Alaska), we began a concentrated sampling effort throughout the western USA, and set out to delimit species and revise the taxonomy of group.

We faced a challenge in assigning names to some of the species, as two critical, century-old type specimens (those of *Bembidion saturatum* Casey and *B. lividulum* Casey) are female and lack male genitalic characters required for confident morphological identification. As part of another project, these types were sequenced with next-generation sequencing and assigned to species concepts (Sproul & Maddison, 2017). Although the assignment of the *B. saturatum* lectotype was unambiguous

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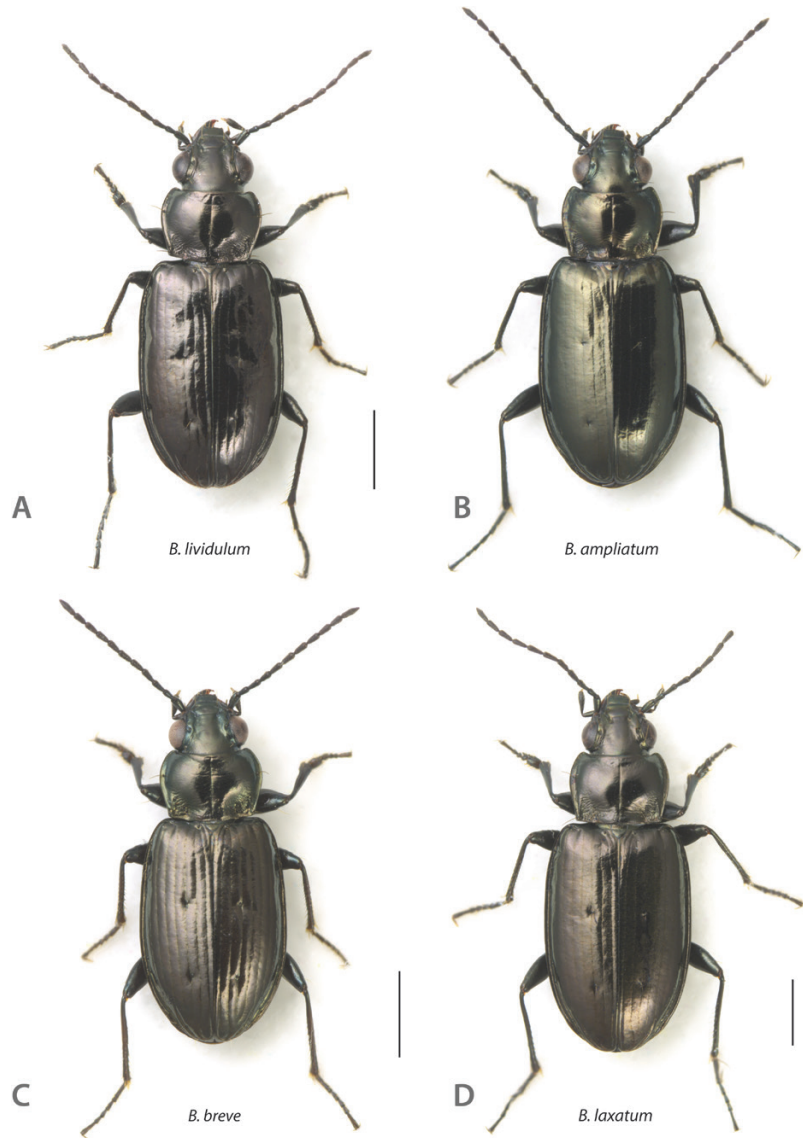


Figure 1. Habitus of *breve* group adults. A, *Bembidion lividulum*, USA: California: Tuolumne Co., Deadman Creek, DRM voucher V100961. B, *Bembidion ampliatum*, USA: Oregon: Harney Co., Steens Mts., snowfield at Kiger Gorge, DRM voucher V100963. C, *Bembidion breve*, USA: Washington: King Co., Snoqualmie River at Alpentel, DRM voucher V100955. D, *Bembidion laxatum*, USA: California: Mono Co., Mammoth Mountain Ski Area, DRM voucher V101128. Scale bar is 1 mm.

across the trees of several genes, the sequences obtained from the *B. lividulum* lectotype were of poor quality (likely due to degradation), such that placement of that specimen was less certain. We provide an additional line of evidence that corroborates the previous assignment (Sproul & Maddison, 2017) of the *B. lividulum* type. We use evidence of genomic architecture in the form of ribosomal DNA (rDNA) copy number variation within the rDNA cistron (the region of rDNA containing 18S, 5.8S and 28S rRNA genes and their spacer regions). Given that our approach relies on sequences of multi-copy genes, which show promising rates of recovery in projects

attempting to sequence old specimens (Wandeler, Hoeck & Keller, 2007; Guschanski *et al.*, 2013; Staats *et al.*, 2013; Burrell, Disotell & Bergey, 2015; Kanda *et al.*, 2015; Sproul & Maddison, 2017), it has potential broader application, which we are presently investigating.

Here, we present species delimitation and taxonomic revision of the *breve* group using morphological, molecular and geographic data. We present multiple lines of evidence that indicate the group contains at least nine species: six were previously described and three we describe as new. We then synthesize our results and observations in species descriptions, identification tools and distribution maps.

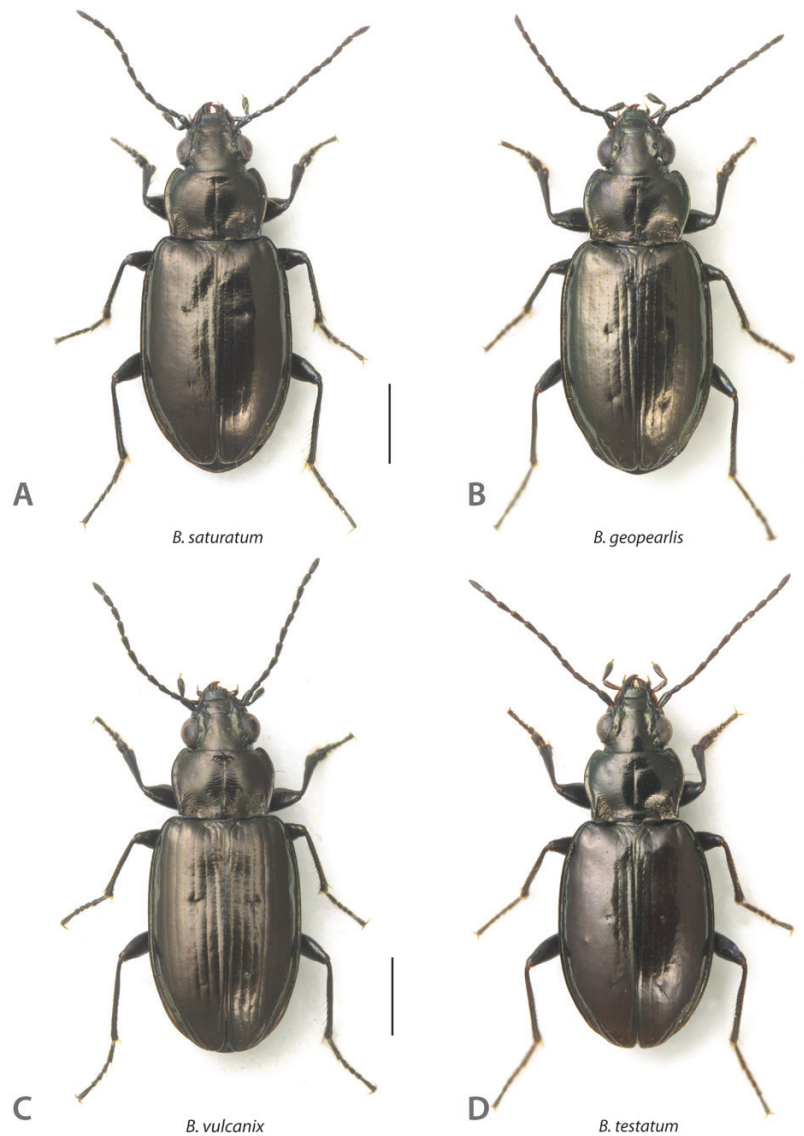


Figure 2. Habitus of *breve* group adults. A, *Bembidion saturatum*, USA: California: Fresno Co., Kaiser Pass Meadow, DRM voucher V100942. B, *Bembidion geoppearlis*, USA: Montana: Glacier Co., Glacier N.P., east slope Clements Mtn., DRM voucher V100957. C, *Bembidion vulcanix*, USA: Oregon: Deschutes Co., Stream east of Todd Lake, DRM voucher V101129. D, *Bembidion testatum*, USA: California: El Dorado Co., Lily Lake, DRM voucher DNA3062. Scale bar is 1 mm.

METHODS

We examined over 2000 specimens of the *breve* group that are from or will be deposited in the collections listed below. Each collection's listing begins with the code used in the text.

BMNH The Natural History Museum, London
 CAS California Academy of Sciences, San Francisco
 CSCA California State Collection of Arthropods, Sacramento

EMEC Essig Museum Entomology Collection, University of California, Berkeley

MNHN Muséum National d'Histoire Naturelle, Paris

OSAC Oregon State Arthropod Collection, Oregon State University, Corvallis

UAM University of Alaska Museum, University of Alaska, Fairbanks

UASM E.H. Strickland Entomological Museum, University of Alberta, Edmonton



Bembidion oromaia

Figure 3. Habitus of *Bembidion oromaia*. USA: California: Tulare Co., snowfield below White Chief Lake, DRM voucher V100959. Scale bar is 1 mm.

USNM National Museum of Natural History, Smithsonian Institution, Washington, DC

ZMH Zoological Museum, University of Helsinki, Helsinki

ZMMU Zoological Museum, Moscow State University, Moscow

TAXON SAMPLING AND DNA EXTRACTION FOR MOLECULAR ANALYSIS

We sampled for molecular study 141 specimens from 94 localities across the known range of the species group. We included five specimens of *B. (Plataphus) aeruginosum* (Gebler) sampled from five localities in Russia as outgroups, as this species is the sister group of a monophyletic *breve* group in a broader analysis of bembidiines (Maddison *et al.*, unpublished). Sample localities are summarized in Supporting Information, Table S1. Specimens were collected into 95–100% ethanol and then stored at -20°C until DNA was extracted. We extracted DNA from most specimens (those preserved in ethanol) using the Qiagen DNeasy (Qiagen) extraction kits and the manufacturer's recommended protocol. We extracted DNA from a few additional pinned specimens in a clean room with

minor protocol modifications as described in Kanda *et al.* (2015).

POLYMERASE CHAIN REACTION AMPLIFICATION AND SANGER DNA SEQUENCING

We amplified portions of five genes via polymerase chain reaction (PCR): 28S: ~1039 bases of nuclear ribosomal subunit 28S; *COI*: 658 bases of mitochondrial protein-coding cytochrome *c* oxidase subunit I; *CAD*: 811 bases of nuclear protein-coding carbamoyl phosphate synthetase domain of the rudimentary gene; *Topo*: 742 bases nuclear protein-coding gene topoisomerase I; and *MSP*: 923 bases of nuclear protein-coding gene muscle-specific protein 300. We conducted PCR on an Eppendorf Mastercycler ProS using TaKaRa Ex Taq with thermal profiles and PCR primers as explained in Maddison (2012) (for 28S, *COI*, *CAD* and *Topo*) and Maddison & Cooper (2014) (for *MSP*). PCR products were purified and sequenced at the University of Arizona's Genomic and Technology Core Facility using either a 3730 or 3730 XL Applied Biosystems automatic sequencer.

ASSEMBLY, ALIGNMENT, AND MOLECULAR ANALYSIS

Following sequencing, we assembled chromatograms using Phred (Green & Ewing, 2002) and Phrap (Green, 1999) via the Chromaseq package in Mesquite v3.2 (Maddison & Maddison, 2016, 2017). Final sequence editing was conducted manually in Chromaseq. We aligned sequences from protein-coding genes in Mesquite; no insertion or deletion events need be presumed in the history of the sequences examined. The ribosomal gene (28S) was aligned in MAFFT 7.130b (Katoh & Toh, 2008) with the G-INS-I algorithm as implemented in Mesquite v3.2 (Maddison & Maddison, 2017). Following alignment, data matrices for each gene were prepared for downstream analysis using Mesquite. We performed model selection for all genes using jModelTest v2.1.4 (Darrriba *et al.*, 2012) and identified optimal models using the Bayesian Information Criterion. We inferred gene trees for each locus through maximum likelihood (ML) analysis across 100 search replicates in GARLI v2.0 (Zwickl, 2006).

We inferred the species tree using STACEY v1.2.2 (Jones, 2017), as implemented in BEAST v2.4.5 (Bouckaert *et al.*, 2014). STACEY uses a multi-species coalescent approach (Yang, 2002; Rannala & Yang, 2003; Degnan & Rosenberg, 2009; Edwards, 2009) similar to *BEAST (Heled & Drummond, 2010) to simultaneously infer gene trees and the species tree, except that it does not require *a priori* assignment of individuals to species, or guide trees. We set all individuals as separate species in order to view the clustering of individuals in the species trees and set the epsilon value to $1 \times$

10^{-4} , following guidelines in the software documentation. We set the CollapseWeight parameters to 0.5 and 10 with a Beta prior and ran the first replicate run for 1 billion iterations, followed by two additional replicate runs for 500 million iterations, logging every 10 000th iteration. We evaluated sampling sufficiency using effective sample size values in Tracer v1.6, combined independent runs using LogCombiner after excluding 10% of trees as burn in and summarized output trees using TreeAnnotator (Bouckaert *et al.*, 2014).

rDNA COPY NUMBER VARIATION ANALYSIS OF THE *BEMBIDION LIVIDULUM* CASEY LECTOTYPE

We compared the signature of rDNA copy number variation observed in the lectotype of *B. lividulum* Casey to those of newly sequenced specimens. There are three species to which the lectotype could belong based on morphological evidence and preliminary analysis of sequences presented in Sproul & Maddison (2017). We sequenced two ethanol-preserved specimens from each of the three candidate species (specimens DNA3593, DNA4149, DNA4165, DNA4245, DNA4918, DNA5032). The extraction and sequencing methods used for the *B. lividulum* lectotype are described in Sproul & Maddison (2017).

We prepared the newly sampled specimens for sequencing with NEBNext DNA Ultra II Library Prep Kits (New England Biolabs) using the manufacturer's recommended protocol. The dual-indexed samples were then pooled and sequenced on an Illumina HiSeq 3000 maintained by the Oregon State University Center for Genome Research and Biocomputing, and for each sample, we allocated approximately 1/30 of a 150 paired-end lane. Demultiplexing was performed using CASAVA v1.8 (Illumina). Paired-end reads were imported into CLC Genomic Workbench v8.5.1 (CLC Bio, referred to below as CLC GW), with failed reads removed during import. We trimmed and excluded adapter sequences from reads in CLC GW. We then mapped reads to a reference sequence of *B. aeruginosum* using the 'Map Reads to Reference' tool and visualized pileups of mapped reads in CLC GW. To reduce the chance of mapping spurious reads, the mismatch penalty was increased to four and the length and similarity fraction cutoffs were both increased to 0.85. The resulting rDNA signatures were then compared to that of the *B. lividulum* lectotype, which we obtained through the same methods. We estimated the maximum number of rDNA copies for any point across the rDNA cistron by dividing the maximum read coverage depth of the rDNA cistron by the average coverage depth of 67 putatively single-copy nuclear protein-coding genes (Regier *et al.*, 2008) which we mapped from the same set of reads, using the same parameters.

MORPHOLOGICAL METHODS

Basic methods for studying adult structures, and terms used, are given in Maddison (1993). 'Dorsal setae' of the elytra refers to setae ed3 and ed5, with 'dorsal punctures' referring to the regions of the elytra at which those setae are attached.

We performed genitalic dissections on all male specimens whose DNA was extracted. The genitalia were cleared in KOH and mounted in Euparal on small cards, which were then pinned under the specimen. We studied external and genitalic structures of all specimens using a Leica M165C stereo microscope with a ring light, and a Leica DM5500B compound microscope. With the exception of six specimens whose genitalia were damaged or lost, we examined genitalia of all male DNA voucher specimens and recorded to which of several genitalic forms each belonged. This was done without active consideration of other data, thus allowing us to test how well genitalic forms corroborated molecular patterns. In addition to studying male genitalia from DNA vouchers, we examined genitalia from 63 additional specimens from key geographic localities.

We photographed habitus, pronotum and elytral microsculpture of DNA vouchers using a Leica Z6 Apo lens and DMC4500 camera. We photographed male genitalia with a Leica Z6 Apo lens and DMC4500 camera, or a Leica DM5500 compound microscope and DFC 425 C camera. We generated stacks of images at various focal depths using Leica Application Suite v4.8 (Leica Microsystems) and merged the stacks using Zerene Stacker (Zerene Systems) with either the PMax or DMap algorithms.

RESULTS

MOLECULAR RESULTS

GenBank accession numbers for newly acquired DNA sequences are KY950685–KY951331. GenBank accession numbers for previously published sequences are given in Supporting Information, Table S3. Models of evolution used for gene tree and STACEY analyses are listed in Supporting Information, Table S2.

Most inferred species were monophyletic in the majority of ML gene trees (Figs 4, 5). Six species were monophyletic in at least three of five gene trees, and three species were monophyletic in two or fewer gene trees (Table 1). Sequences from species pairs *B. ampliatum* + *B. laxatum* and *B. saturatum* + *B. vulcanix* showed the lowest differentiation among inferred species and were interdigitated in multiple gene trees for both species pairs (Figs 4, 5).

Specimens of *B. saturatum* showed genetic structure in *COI* consistent with geographic patterns (Fig. 4C), with specimens from the Ruby Mountains in Nevada

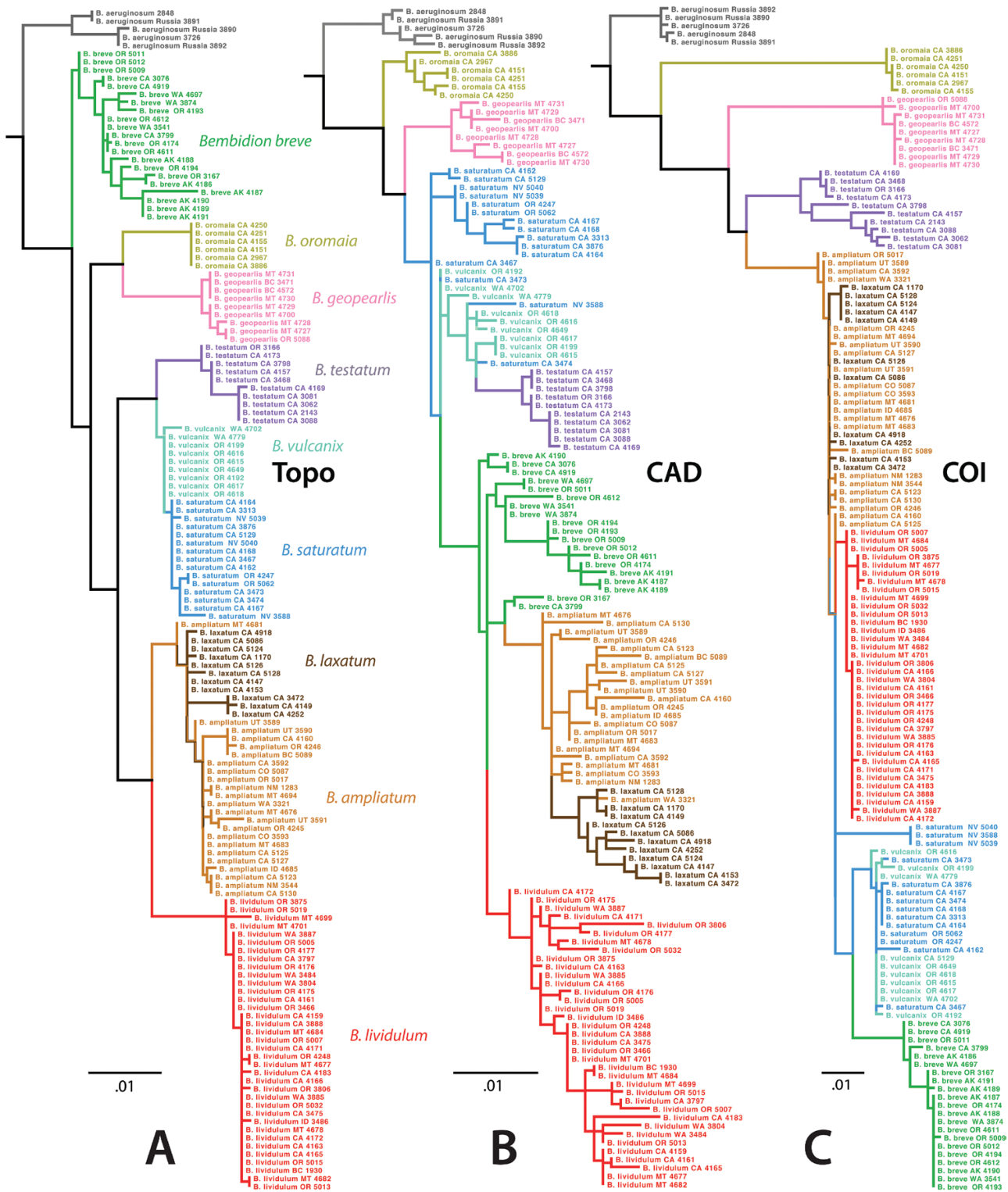


Figure 4. Maximum likelihood trees for three genes: A, *Topo*; B, *CAD*; C, *COI*. Branch length shown is proportional to relative divergence with scale bars indicating 0.01 units. Outgroup taxa are shown in grey.

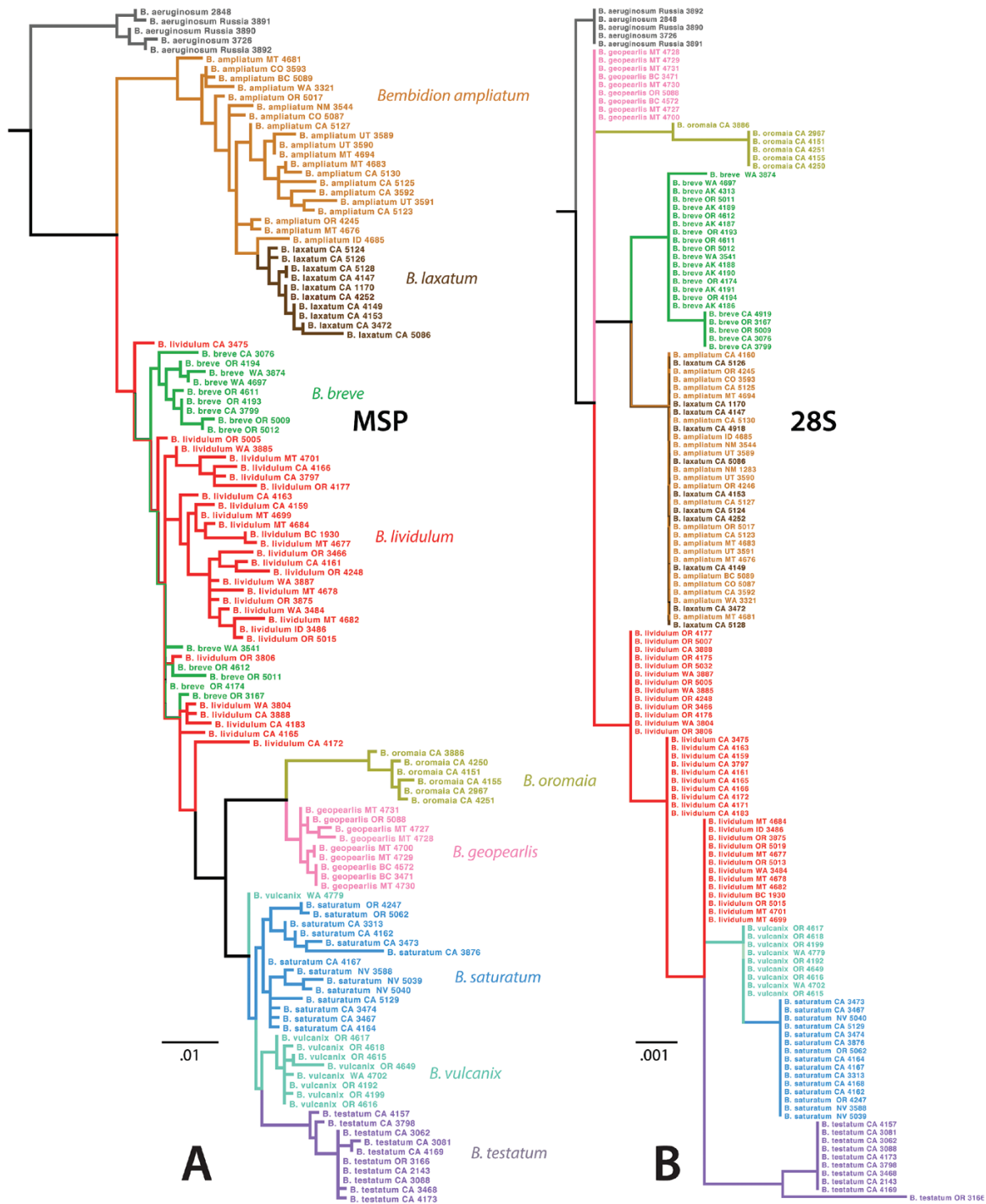


Figure 5. Maximum likelihood trees for two genes: A, *MSP*; B, *28S*. Branch length shown is proportional to relative divergence with scale bars indicating 0.01 units for *MSP* and 0.001 units in *28S*. Outgroup taxa are shown in grey.

(specimens 5040, 3588 and 5039) showing a notably divergent haplotype (with a unique base at ten sites) that renders the inferred species paraphyletic in *COI*. We also note genetic variants for *B. oromaia* (specimen 3886) and *B. testatum* (specimen 3166) in *28S*

(Fig. 5B). For both species, we sampled a single specimen from outside the Sierra Nevada (Trinity Alps in northwest California for *B. oromaia* and Mount Ashland in southern Oregon for *B. testatum*) that differed by at least two bases in *28S* (all other sequences

Table 1. Monophyly of inferred *breve* group species

Species	# gene trees	STACEY tree
<i>Bembidion geoparlis</i>	5	yes
<i>B. oromaia</i>	5	yes
<i>B. testatum</i>	5	yes
<i>B. breve</i>	3	yes
<i>B. lividulum</i>	3	yes
<i>B. saturatum</i>	3	yes
<i>B. laxatum</i>	2	yes
<i>B. ampliatum</i>	0	yes
<i>B. vulcanix</i>	0	yes

#Gene trees: indicates the number gene trees in which the species in monophyletic; STACEY tree: indicates if the species was monophyletic in the STACEY topology.

were identical in 28S for both species), but lacked notable differences in the remaining genes.

The STACEY analysis revealed nine major clades with high Bayesian posterior probability (BPP) support (>94%) that corresponded to the nine distinct genitalic forms (Fig. 6). There are some clades found within these nine clades, but none of those corresponded to distinct morphological forms. Two of these clades had BPP of at least 85%: the two *B. saturatum* from the Steens Mountains, Oregon, grouped together with BPP of 85%, and two of the northern Sierra Nevada *B. breve* grouped with BPP of 90%. No other within-species clades in the STACEY analysis had BPP greater than 70%, and none of the within-species clades show evident morphological distinctiveness.

Our analysis of rDNA copy number variation within the rDNA cistron corroborated the previous assignment of the *B. lividulum* Casey (1918) lectotype made by Sproul & Maddison (2017). The type specimen showed dramatic inflation of copy number in the second internal transcribed spacer (ITS2) and 28S gene regions (Fig. 7G). We observed this same signature of copy number inflation in both fresh specimens sequenced (from distinct geographic localities) of the species herein referred to as *B. lividulum* (Fig. 7E, F). We observed less region-specific copy number variation overall in specimens of the other two candidate species (*B. laxatum* and *B. ampliatum*), and different signatures in visualized read pileups (Fig. 7A–D).

MORPHOLOGICAL RESULTS

Genitalic dissections of DNA vouchers revealed nine distinct genitalic forms (Figs 8–10). Overlaying these forms on the STACEY tree showed that each form corresponds to one of the major clades recovered in the STACEY analysis (Fig. 6). We found shape and location of sclerite ‘St’ (Lindroth, 1963) and length of the flagellum to be diagnostic characters that corroborated

molecular evidence (Fig. 10), with a single exception. Specimen DNA3321 showed a unique genitalic form that made it difficult to place as either *B. ampliatum* or *B. laxatum*. The weight of molecular evidence, including the STACEY results, places this specimen with *B. ampliatum* (but note this specimen’s placement within *B. laxatum* in the *CAD* gene tree of Fig. 4B). As this is our only specimen assigned to that species from the Cascades, additional sampling is needed to confirm the distinctiveness and meaning of morphological and molecular patterns.

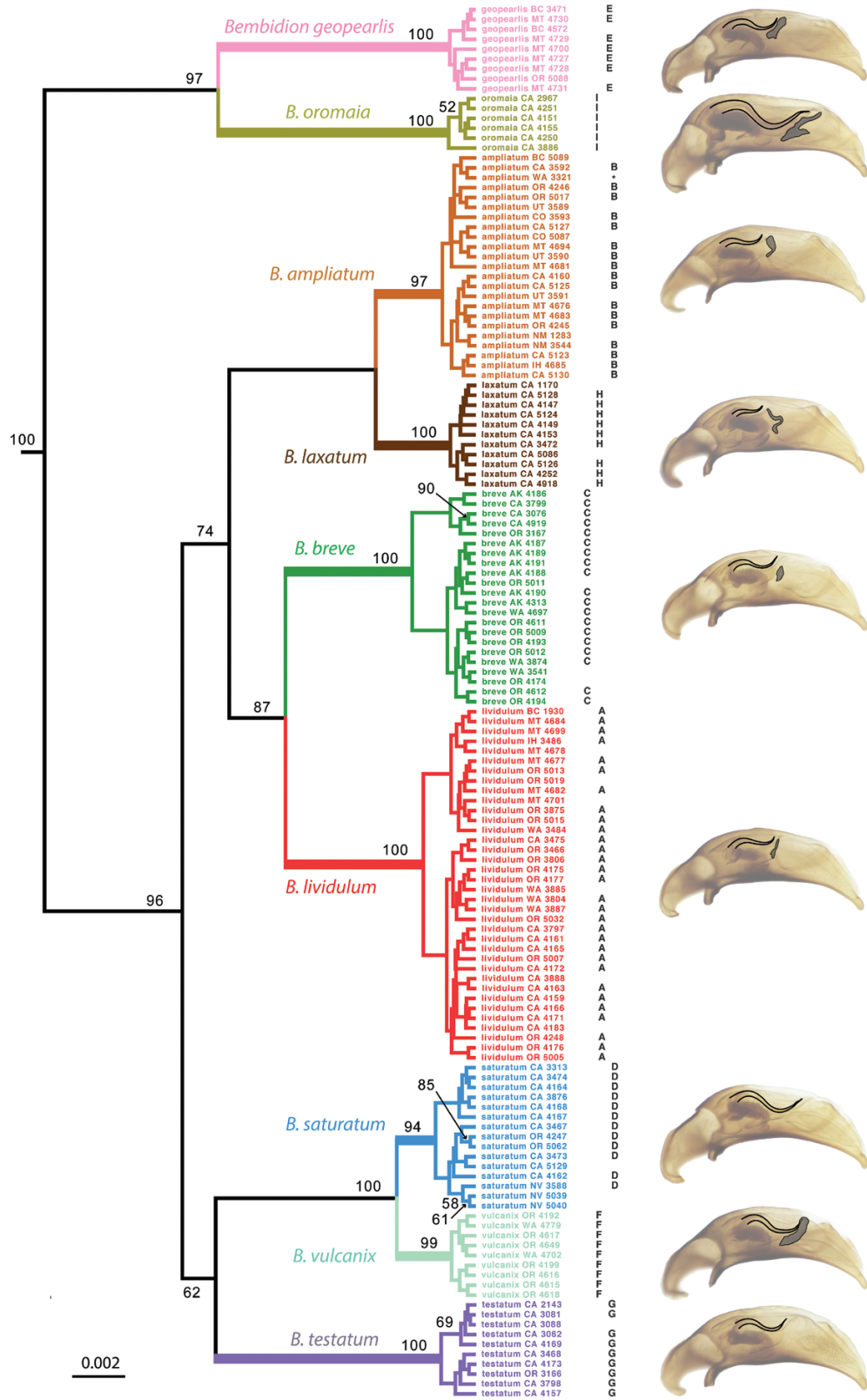
In addition to genitalic characters, characters in the pronotum (Figs 11, 12), elytra (Figs 1–3) and the extent of microsculpture (Fig. 13) corroborated patterns in gene trees and male genitalia, but are subject to more intraspecific variation than genitalic characters. The remaining results of the morphological investigation are presented in ‘Species Description and Identification’ below.

DISCUSSION

SPECIES DELIMITATION

We consider as species separately evolving metapopulation lineages (de Queiroz, 2007). We delimit these lineages using evidence provided by patterns of gene trees, morphological data and geographic data that suggest a lack of gene flow between entities so delimited, but presence of gene flow within such entities. Given patterns in individual gene trees (Figs 4, 5), results of the STACEY analysis (Fig. 6) and corroborating morphological characters, with many apparent species pairs so delimited found in sympatry (Table 2), we find multiple independent lines of evidence supporting the nine species recognized herein.

The weakest support for species we consider distinct is for the separation of *B. ampliatum* from *B. laxatum* and for *B. vulcanix* from *B. saturatum*. In both cases, the specimens within each pair are interdigitated in multiple gene trees (e.g. Figs 4B, C, 5B). Despite the lack of reciprocal monophyly for these species in individual gene trees, the multi-gene tree inference in STACEY recovered each species as monophyletic with high support (Fig. 6). Morphological evidence further corroborates these groupings; in particular, evidence from the male genitalia supports the STACEY analysis (Figs 8–10). Finally, geographic data provide additional support for the separation of *B. ampliatum* and *B. laxatum*, as the two forms are microsympatric in the Sierra Nevada (Table 2). We sequenced both species from each of two localities (see localities for DNA vouchers 4160, 5123, 5125, 5127 and 5130 in *B. ampliatum* and 4918, 4153, 5124, 5126 and 5128 in *B. laxatum* in Supporting Information, Table S1). The fact that morphological and genetic distinctiveness are



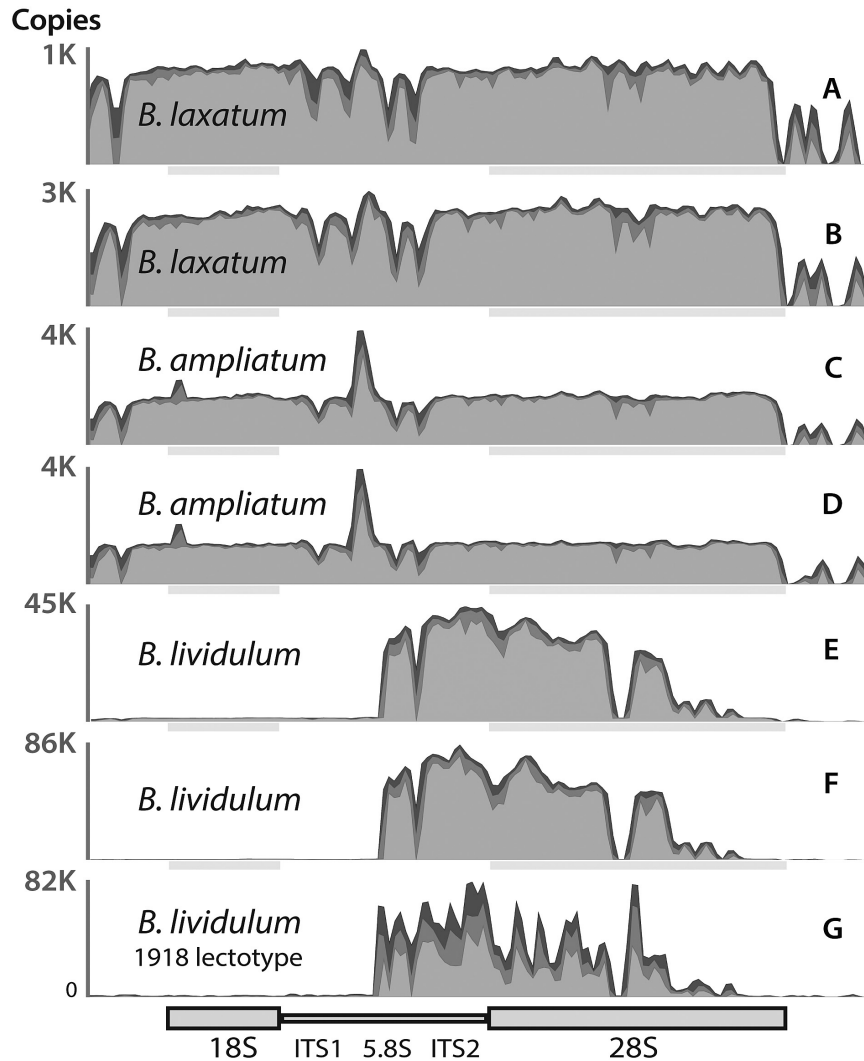


Figure 7. Plots of copy number variation across the rDNA cistron of: A, *Bembidion laxatum*, California: Alpine Co., Sonora Pass, 2900 m 38.3323°N 119.6401°W, DRM voucher DNA4918. B, *Bembidion laxatum*, California: Tulare Co., snowfield below White Chief Lake, 2912 m, 36.417°N 118.5941°W, DRM voucher DNA4149. C, *Bembidion ampliutum*, Oregon: Harney Co., Steens Mts., snowfield at Kiger Gorge, 2618 m, 42.7152°N 118.5786°W, DRM voucher DNA4245. D, *Bembidion ampliutum*, Colorado: Mesa Co., Grand Mesa, route 65 at FS100, 3243 m, 39.0316°N 108.0561°W, DRM voucher DNA3593. E, *Bembidion lividulum*, California: Fresno Co., Kaiser Pass Meadow, 2783 m, 37.2948°N 119.1006°W, DRM voucher DNA4165. F, *Bembidion lividulum*, Oregon: Klamath Co., Crater Lake NP, Sun Notch, 2163 m, 42.9009°N 122.0988°W, DRM voucher DNA5032. G, *Bembidion lividulum* Casey 1918, lectotype, California: Placer Co. ‘Copies’ indicates the maximum copy number observed for any portion of the rDNA cistron. For example, there are approximately 1000 copies of most of the cistron present in the specimen shown in (A); for the specimen shown in (F), there are almost 86 000 copies for a region from 5.8S through part of 28S. The position of mapped reads relative to the boundaries of rRNA genes within the rDNA cistron is provided along the bottom of the figure.

Figure 6. Species tree inferred by STACEY using five loci. Terminal taxa (and subtending branches) are coloured according to species as inferred in this study based on all evidence; branches giving rise to each inferred species’ clade are thickened. Posterior probabilities are shown above all branches with at least 50% support. Letters in columns to the right of most terminals indicate the coding of male genital characters assigned independent from the molecular data, and correspond to the letters in Fig. 10 (e.g. a specimen with an ‘E’ corresponds to the genital form illustrated by Fig. 10E). Most terminals lacking a letter code are female; the remaining six were uncoded males due to the genitalia being damaged or lost. Branch length shown is proportional to relative divergence with scale bars indicating 0.01 units.

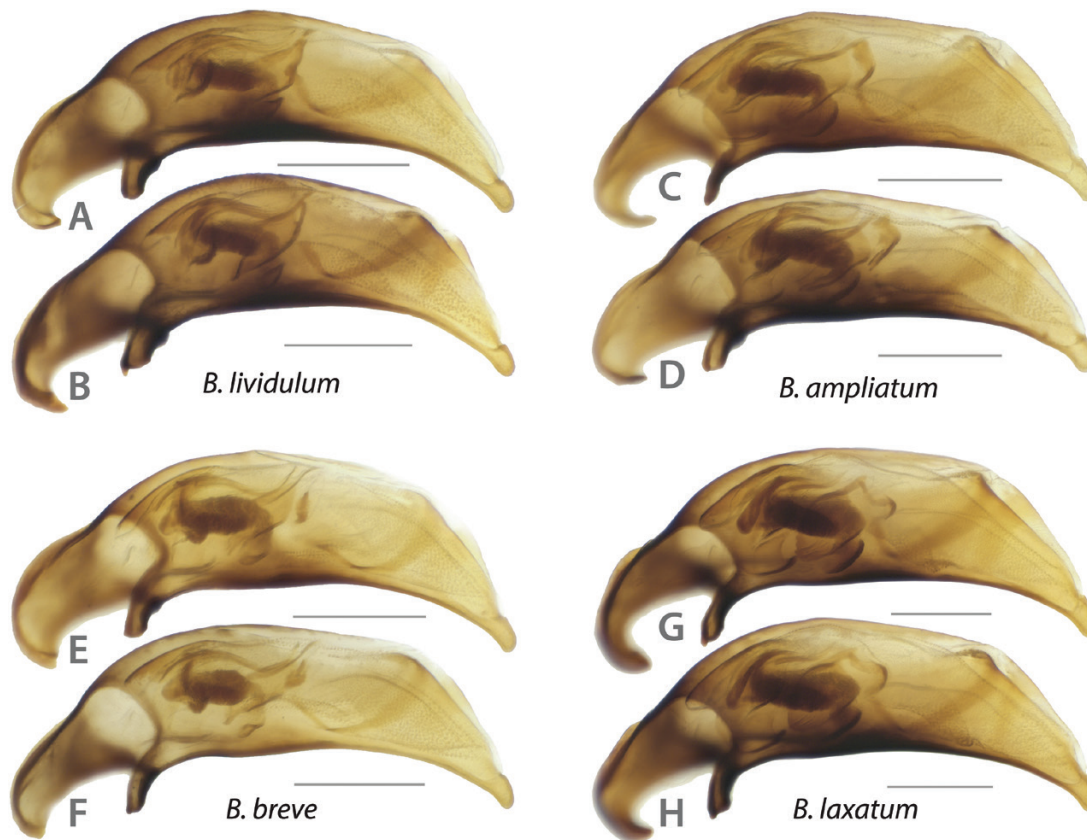


Figure 8. Male genitalia, left side. A, *Bembidion lividulum*, USA: California: Alpine Co., pond below Ebbetts Pass, DRM voucher DNA4161. B, *Bembidion lividulum*, USA: Montana: Ravalli Co., snow above Bailey Lake, DRM voucher DNA4684. C, *Bembidion ampliatum*, USA: Utah: San Juan Co., Geyser Pass Rd nr Horse Ck, La Sal Mtns, DRM voucher DNA3590. D, *Bembidion ampliatum*, USA: California: Mono Co., snow field above Ellery Lake, DRM voucher DNA4160. E, *Bembidion breve*, USA: Alaska: Juneau, Heintzleman Ridge, DRM voucher DNA4187. F, *Bembidion breve*, USA: Washington: Whatcom Co., Bagley Lakes, Mt Baker, Snoqualmie NF, DRM voucher DNA4697. G, *Bembidion laxatum*, USA: California: Fresno Co., South Fork Kings River, DRM voucher DNA4147. H, *Bembidion laxatum*, USA: California: Tulare Co., snowfield below White Chief Lake, DRM voucher DNA4149. Scale bar is 0.25 mm.

maintained by individuals in microsympatry is additional evidence that validates their separation.

Finally, the morphological evidence, in particular the male genitalic characters, and extensive sympatry provide evidence that differences inferred with genetic data are not simply due to population structure within the same species, which is a core concern of any study delimiting species using standard coalescent analyses (Knowles & Carstens, 2007; Carstens *et al.*, 2013; Sukumaran & Knowles, 2017).

ASSIGNING PRIMARY TYPE SPECIMENS TO SPECIES CONCEPTS

We were able to confidently assign most primary type specimens to species concept using morphological analysis. We assigned primary types of *Bembidion adumbratum* Casey, *B. ampliatum* Casey, *B. improvisum*

Casey, *B. laxatum* Casey, *B. lividulum* Casey, *B. testatum* Casey, *Notaphus incertus* Motschulsky and *Peryphus brevis* Motschulsky using male genitalic characters and external structure. Female types of *Bembidion blanditum* Casey and *B. raineri* Hatch could be assigned confidently based upon external structure and geography. The primary type of *Peryphus tetraglyptus* Mannerheim was not examined; however, only *B. breve* is known from the latitudes at which it was collected, and it likely belongs as a synonym under *B. breve*.

Our efforts to assign morphologically ambiguous type specimens to species concept benefitted from the analysis of DNA sequences from the century-old primary type specimens of *B. saturatum* Casey and *B. lividulum* Casey. Although Sproul & Maddison (2017) unambiguously placed the former specimen through gene tree analysis, the latter showed evidence of sequence degradation that prevented its fine-scale

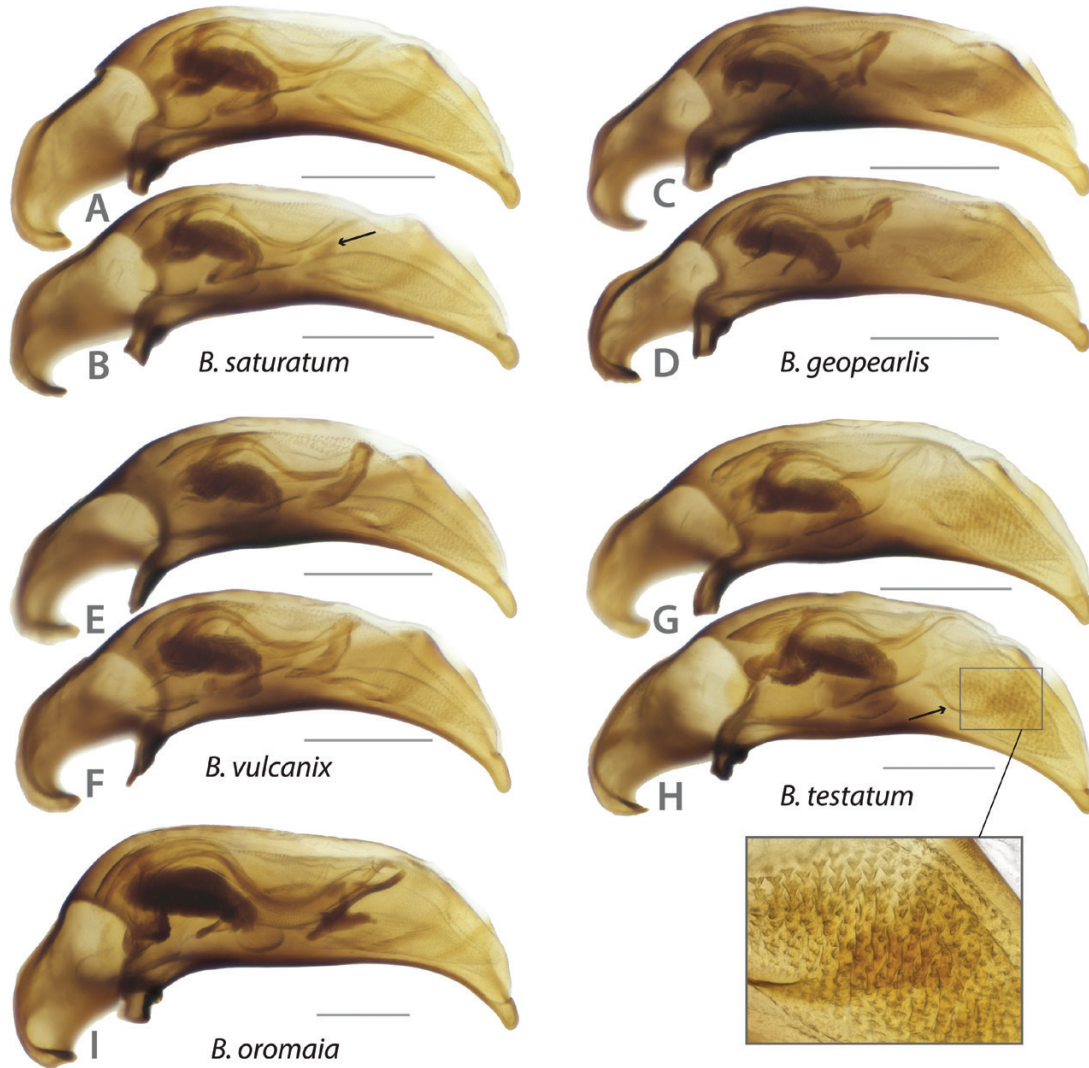


Figure 9. Male genitalia, left side. A, *Bembidion saturatum*, USA: California: Fresno Co., Kaiser Pass Meadow, DRM voucher DNA4168. B, *Bembidion saturatum*, arrow indicates the region that is occasionally lightly sclerotized in some specimens, USA: California: Tulare Co., snow field above Emerald Lake, DRM voucher DNA4162. C, *Bembidion geoppearlis*, USA: Montana: Glacier Co., Glacier N.P., east slope Clements Mtn., DRM voucher DNA4728. D, *Bembidion geoppearlis*, USA: Montana: Glacier Co., Glacier N.P., east slope Clements Mtn., DRM voucher DNA4730. E, *Bembidion vulcanix*, USA: Oregon: Deschutes Co., Stream east of Todd Lake, DRM voucher DNA4617. F, *Bembidion vulcanix*, USA: Oregon: Deschutes Co., Stream east of Todd Lake, DRM voucher DNA4615. G, *Bembidion testatum*, arrow indicates abbreviated ostial flag, USA: California: Tulare Co., 2.5 km N Sherman Pass, Sequoia NF, DRM voucher DNA3798. H, *Bembidion testatum* with closeup of distinctive scales in apical third that cause a darkened patch unique to this species, USA: California: Trinity Co., Canyon Creek, DRM voucher DNA4173. I, *Bembidion oromaia*, USA: California: Tulare Co., Upper East Fk. Kaweah River, DRM voucher DNA4251. Scale bar is 0.25 mm.

placement. [Sproul & Maddison \(2017\)](#) analyzed large fractions of the mitochondrial genome and rDNA and used sequence similarity at distinguishing sites as evidence to assign the *B. lividulum* lectotype to species.

In further exploring sequences obtained from the *B. lividulum* lectotype, we noted a striking pattern of read depth variation across the rDNA cistron. Although the 18S rRNA gene showed poor coverage (~0–10× coverage depth) across its length, the 28S rRNA gene,

just a few thousand bases downstream, showed greater than 400× coverage, which is equivalent to a copy number of over 80 000 based on the average coverage of putatively single-copy nuclear protein-coding genes obtained from the same specimen ([Fig. 7G](#)).

In sequencing fresh specimens belonging to the candidate species we call herein *B. lividulum*, we observed the same dramatic signature of rDNA copy number seen in the *B. lividulum* Casey lectotype ([Fig. 7E–G](#)); in contrast, specimens

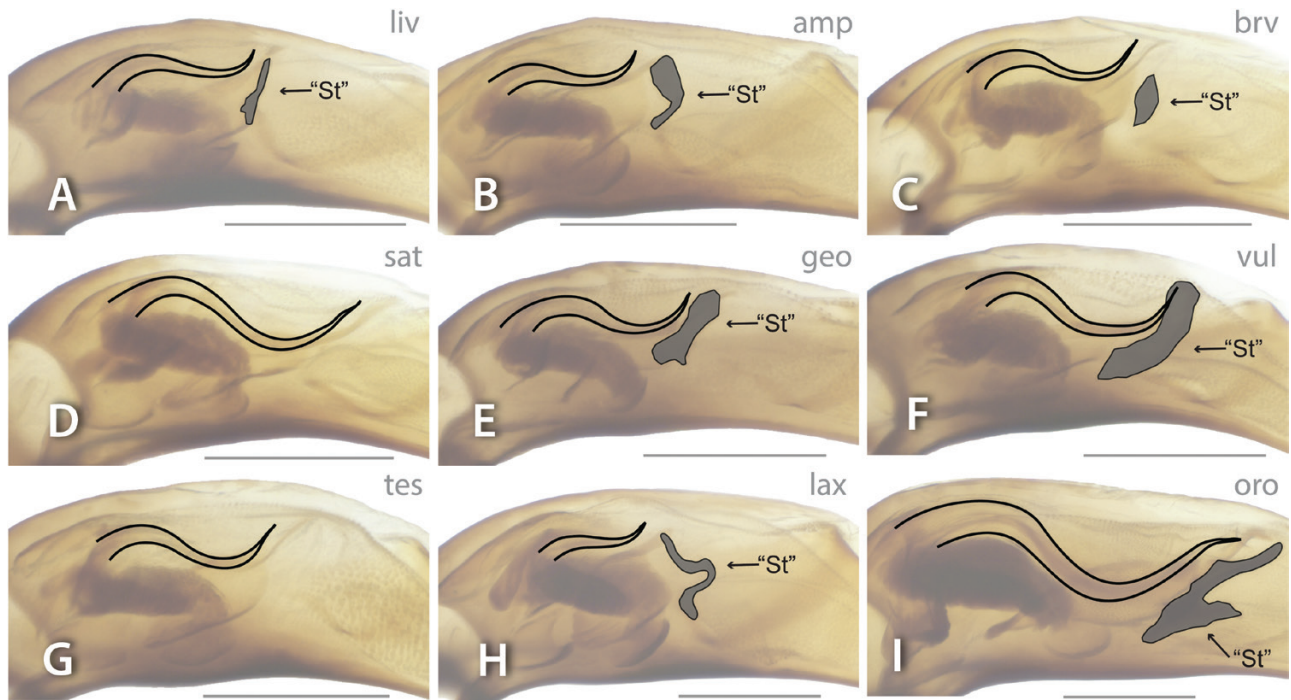


Figure 10. Male genitalia (left side) with emphasis added to sclerite 'St' and flagellum shape. A, *Bembidion lividulum*, see panel (A) in Fig. 8 caption. B, *Bembidion ampliatum*, see panel (C) in Fig. 8 caption. C, *Bembidion breve*, see (E) in Fig. 8 caption. D, *Bembidion saturatum*, see (A) in Fig. 9 caption. E, *Bembidion geoparlis*, see (D) in Fig. 9 caption. F, *Bembidion vulcanix*, see (E) in Fig. 9 caption. G, *Bembidion testatum*, see (G) in Fig. 9 caption. H, *Bembidion laxatum*, see (G) in Fig. 8 caption. I, *Bembidion oromaia*, see (I) in Fig. 9 caption. Scale bar is 0.25 mm.

of the other candidate species each showed a different, species-specific signal (Fig. 7A–D). This result confirms the previous assignment of Sproul & Maddison (2017) and strengthens our taxonomic conclusions. Although several cytogenetic studies document region-specific copy number variation the rDNA cistron between closely related species (Raskina *et al.*, 2008), we are unaware of any studies that have measured this signal via high-throughput sequencing as a line of evidence for assigning specimens to species concept. In the present application, this signature was a valuable source of data because it allowed us to detect a clear signal in the sequence data of a specimen that was too degraded to allow for placement through analysis of individual sequences. We have additional studies underway to explore the broader potential of rDNA copy number variation as a tool for species delimitation and taxonomy.

SPECIES DESCRIPTION AND IDENTIFICATION

CHARACTERISTICS OF THE *BREVE* GROUP

The *breve* group belongs to *Bembidion* subgenus *Plataphus* (Lindroth, 1963; Maddison, 2012). Historically the group was placed within subgenus *Plataphodes* Ganglbauer, 1891 (e.g. Lindroth, 1963), a name now

considered a junior synonym of *Plataphus* (Maddison, 2012). Distributed across high-elevation western North America (Figs 14–17), most species have notably broad prothoraces basally compared to other *Plataphus*, with dark forebodies (dark brown to black, in some specimens with a metallic aeneous or blue hue) and elytra either the same colour as the forebody, or dark brown to dark reddish brown. Legs and antennae are dark, similar in colour to forebody and elytra. All species in the group show recurvature of the lateral bead at the base of the elytron (Fig. 12B), a character used to define the former subgenus *Plataphodes*. They are distinguishable from other *Plataphus* by the broad width of the base of the pronotum relative to elytral width, combined with the re-curved elytral bead (Fig. 12B).

In subalpine habitats in western North America, particularly along creeks, many dark-bodied bembidiines may be present, including those of the genus *Lionepha* Casey, as well as other members of *Bembidion* subgenus *Plataphus*, and members of the *Ocydromus* complex of *Bembidion*. Two *Bembidion* commonly found alongside species of the *breve* group in alpine habitats are *B. (Plataphus) complanulum* (Mannerheim) and *B. (Ocydromus complex) commotum* Casey. *Bembidion complanulum* is smaller, flatter and narrower than any *breve* group, and *B. commotum* lacks microsculpture on

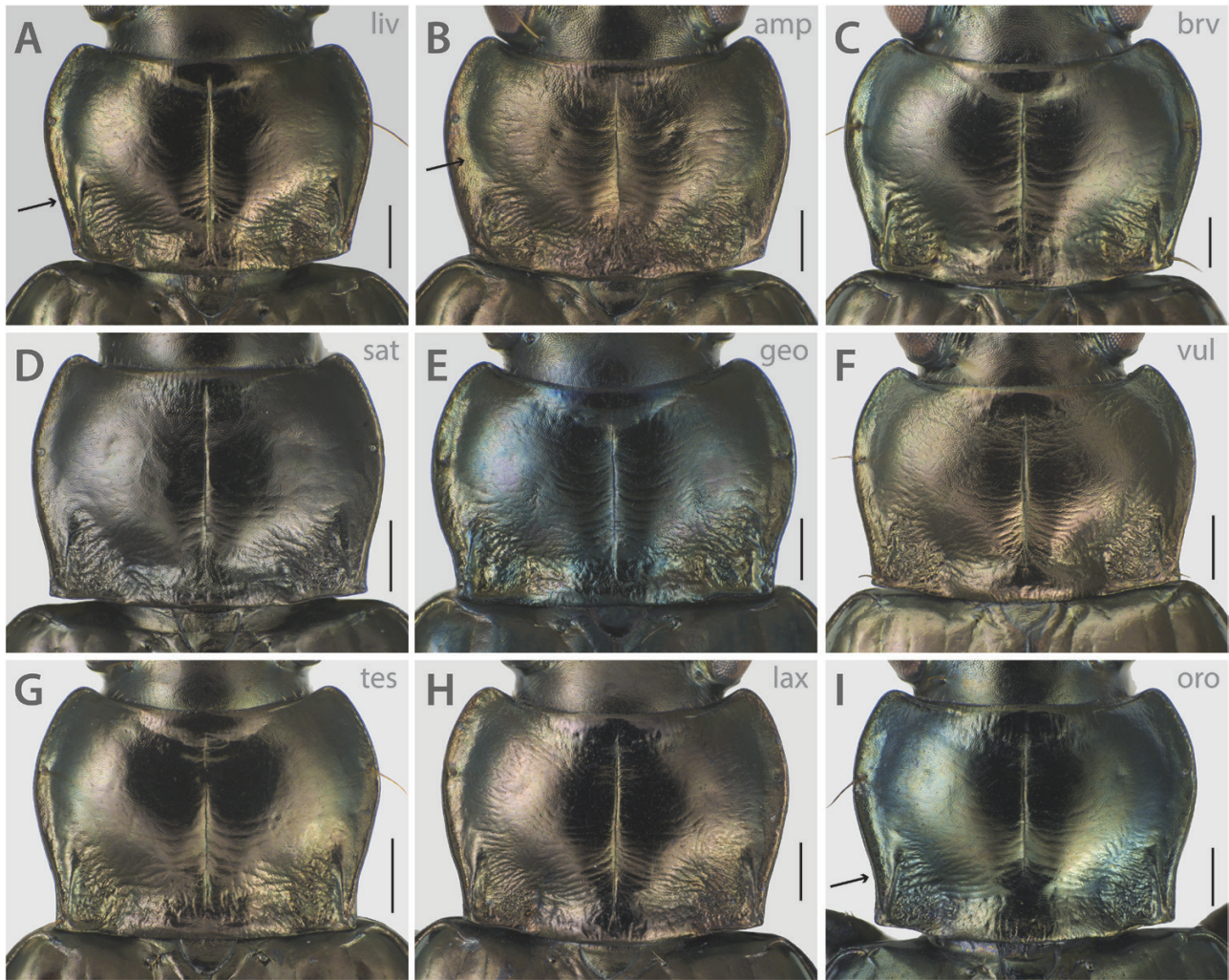


Figure 11. Pronota of the *breve* group species. A, *Bembidion lividulum*, arrow indicates the non-sinuate lateral border, compare with (H) and (I) below, USA: California: Fresno Co., Kaiser Pass Meadow, DRM voucher DNA4165. B, *Bembidion ampliatum* indicating the broad lateral explanation, USA: New Mexico: Santa Fe Co., Santa Fe Ski Basin, DRM voucher DNA3544. C, *Bembidion breve*, USA: Washington: King Co., Snoqualmie River at Alpentel, DRM voucher V100955. D, *Bembidion saturatum*, USA: California: Tulare Co., outlet of Emerald Lake, DRM voucher DNA4164. E, *Bembidion geoppearlis*, USA: Montana: Ravalli Co., Lost Horse Creek, DRM voucher DNA4700. F, *Bembidion vulcanix*, USA: Oregon: Deschutes Co., Stream east of Todd Lake, DRM voucher V101130. G, *Bembidion testatum*, USA: California: Sierra Co., creek above Tamarack Lake, DRM voucher DNA4169. H, *Bembidion laxatum*, USA: California: Tulare Co., snow field above Emerald Lake, DRM voucher DNA4252. I, *Bembidion oromaia*, arrow indicates the strongly sinuate lateral border, compare with (A) and (B) above, USA: California: Tulare Co., snowfield below White Chief Lake, DRM voucher V100959. Scale bar is 0.25 mm. Note: we removed forelegs and painted the background in Photoshop in order to reduce visual complexity around the margins of the pronotum.

the elytra. Members of the *breve* group are easily confused with *B. manningense* Lindroth (1969) and other similar *Plataphus*; *breve* group members can be distinguished by having a broader pronotum basally and being more convex with less pronounced elytral striae.

IDENTIFICATION OF SPECIES

Identifying specimens to species within the *breve* group is challenging, especially if only external structure is

used, and several species pairs or trios can be difficult to separate. Although differences in external structure discussed herein generally hold within a species, notable intraspecific variation (e.g. in pronotal shape) is common. The external characters presented in the following key can aid in identification; however, examination of internal sac details of cleared male genitalia, or DNA sequences, may be needed to place some specimens with certainty.

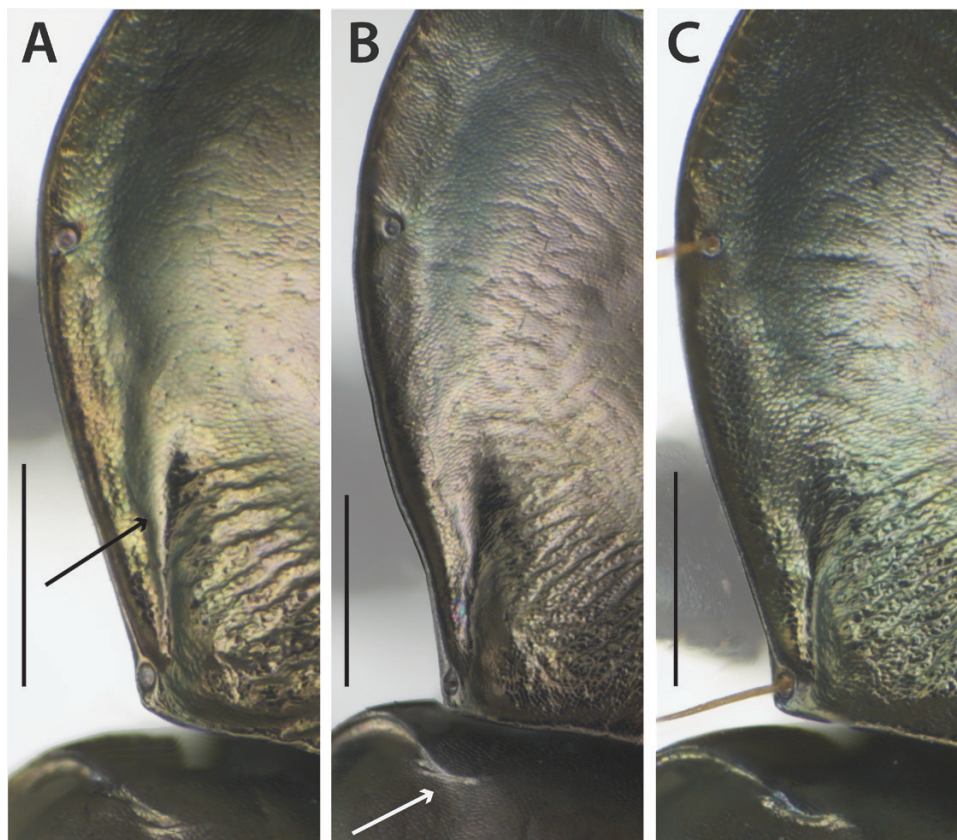


Figure 12. Laterobasal carina of pronota. Black arrow indicates the location of the carina, white arrow indicates the recurved elytral bead typical of *breve* group members and some other *Plataphus*. A, *Bembidion lividulum*, USA: California: Fresno Co., Kaiser Pass Meadow, DRM voucher DNA4165. B, *Bembidion laxatum*, USA: California: Tulare Co., snow field above Emerald Lake, DRM voucher DNA4252. C, *Bembidion ampliatum*, the small shadow along the right margin of the carina is due to the shallower basal fovea relative to (A) and (B), USA: California: Mono Co., snow field above Ellery Lake, DRM voucher DNA4160. Scale bar is 0.25 mm.

KEY TO SPECIES OF THE *BREVE* GROUP

- 1 Elytra notably convex, with lateral margins strongly rounded, resulting in inflated appearance (Fig. 2D); striae 3 and 4 partially disappeared or very weak (Fig. 2D). Pronotum sinuate laterally (Fig. 11G, see also Fig. 11I). Hindbody dark brown or reddish brown and generally slightly paler than the forebody. Male genitalia with darkened patch of scales apically (Fig. 9H, see inset) and lacking sclerite ‘St’ (Figs 9G, H, 10G); 4.6–5.1 mm *B. testatum*
- Elytra flat or convex, or with lateral margins somewhat rounded, but not strongly rounded laterally and therefore lacking inflated appearance, striae weak, or not. Pronotum sinuate laterally, or not. Male genitalia lacking darkened patch of scales apically.....2
- 2 (1) Pronotum with hind angles near 90° (Fig. 11D–F, I), or slightly obtuse (Fig. 11H)3
- Pronotum with hind angles strongly obtuse (Fig. 11A, B).....8
- 3 (2) Pronotum broad (Fig. 2A–C), with broad lateral explanation and broad base (Fig. 11D–F). Body convex, with a stout appearance. Smaller, most specimens less than 4.8 mm (although can be up to 5 mm)....4
- Pronotum broad or not (Fig. 11H, I). Body convex or somewhat flattened, but greater than 4.8 mm (females > 5.1 mm).....7

4 (3)	Male genitalia with expanded sclerite ‘St’ as in Figure 10E . Montana, southeastern British Columbia, southwestern Alberta, northeastern Oregon (and likely northern Idaho) (Fig. 16B); 4–4.5 mm <i>B. geoparlis</i>	
–	Male genitalia with sclerite ‘St’ as in Figure 10C, D, F . California, Nevada, western and southeastern Oregon, western Washington, western and southeastern British Columbia, north to Alaska.....5	
5 (4)	Elytra with lateral margins somewhat rounded and narrowed at shoulder, dorsal punctures strongly foveate and striae often pronounced (Fig. 1C). Pronotum fairly large relative to elytral length, broad lateral explanation generally lacking (specimens from the Sierra Nevada may have a broad lateral explanation). Male genitalia with medium-length flagellum and diamond-shaped sclerite ‘St’ (Figs 8E, F, 10C). Smaller, 3.7–4.5 mm <i>B. breve</i>	
–	Elytra fairly parallel-sided, broad at shoulder, often tapering towards apex, dorsal punctures weakly foveate. Male genitalia with apical half narrower and more tapered, third and long, sinuate flagellum (Fig. 9A, B, E, F).....6	
6 (5)	Male genitalia lacking large arcuate sclerite ‘St’ (Figs 9A, B, 10D). Sierra Nevada in California, Nevada, southeastern Oregon (Fig. 16A); 4–4.6 mm..... <i>B. saturatum</i>	
–	Male genitalia with a large, arcuate sclerite ‘St’ (Figs 9E, F, 10F). Northwestern California, and the Cascades from Oregon to southern British Columbia (Fig. 16A); 4.1–5.0 mm, most ≤ 4.8 mm <i>B. vulcanix</i>	
7 (3)	Pronotum narrow and strongly sinuate laterally (Figs 3, 11I). Legs and antennae elongate and slender. Elytra widest behind middle. Forebody and hind body generally unicolourous black (in rare specimens, elytral disc brown or reddish brown), in some specimens with a bluish hue; 5.2–6 mm <i>B. oromaia</i>	
–	Pronotum broad (at least as broad as one elytron); in some specimens, slightly sinuate laterally (Figs 1D, 11H). Legs and antennae less elongate and slender. Elytra more or less parallel-sided, widest near middle and generally tapering towards apex. Elytral microsculpture often granulate in females (Fig. 13F). Forebody and hindbody unicolourous dark brown (occasionally black) with aeneous hue. Male genitalia not bent basally, flagellum short and weakly sinuate; 4.9–5.6 mm <i>B. laxatum</i>	
8 (2)	Male genitalia as in Figures 8C, D and 10B . Elytral dorsal punctures weakly foveate (especially in specimens from California and Oregon) (Fig. 1B). Pronotum relatively flat with a broad lateral explanation (Fig. 11B), weak laterobasal carina and shallow basal fovea (Fig. 12C); 4.0–5.3 mm, most ≥4.5 mm <i>B. ampliatum</i>	
–	Elytral dorsal punctures strongly foveate (Fig. 1A, C). Pronotum with strong laterobasal carina, basal fovea somewhat deeper with less broad lateral explanation (Figs 11A, G, 12A).9	
9 (8)	Body parallel-sided and relatively flat (Fig. 1A). Male genitalia as in Figures 8A, B and 10A ; 3.7–4.9 mm, most ≥4.1 mm..... <i>B. lividulum</i>	
–	Pronotum and elytra quite convex. Elytra with somewhat rounded lateral margin (Fig. 1C). Male genitalia as in Figures 8E, F and 10C . Smaller, 3.7–4.5 mm..... <i>B. breve</i>	

SPECIES ACCOUNTS

BEMBIDION LIVIDULUM CASEY

(Figs 1A, 8A, B, 10A, 11A, 12A, 13B, 14A)

Bembidion lividulum Casey, 1918: 25. Lectotype female, designated by Lindroth (1975: 117), in USNM, labelled ‘Placer Co. CAL.’ [white paper], ‘CASEY bequest 1925’ [white paper], ‘TYPE USNM

36830’ [red paper], ‘lividula Csy.’ [white paper, hand written], ‘LECTOTYPE saturatum Csy. By C.H. Lindroth’ [white paper, partly handwritten]. Type locality: Placer Co., California. Examined, including DNA sequences. Extracted DNA is deposited at the USNM and associated with the GUID of the type specimen: ark:/65665/3fa4e0e6d-4705-4d96-b32b-83af093df729.

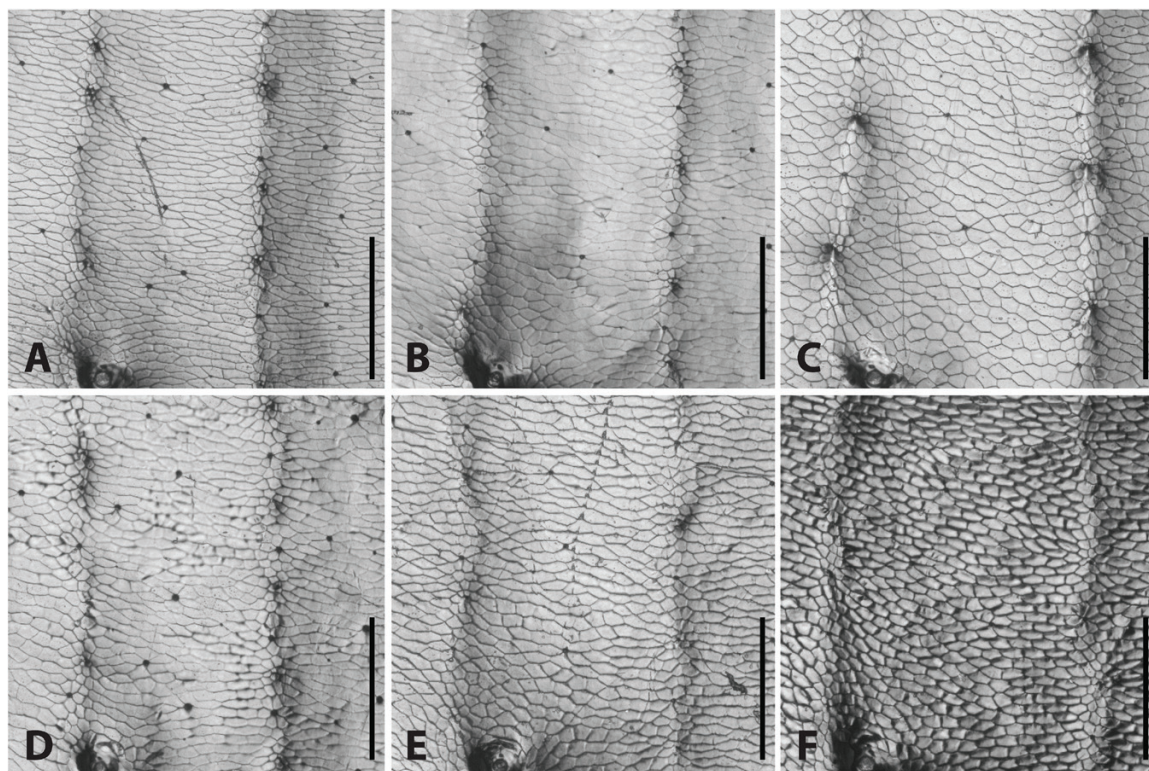


Figure 13. Elytral microsculpture. A, *Bembidion breve*, USA: Alaska: Dall Isl., karst shallow pond, male, DRM voucher DNA4188. B, *Bembidion lividulum*, male, USA: California: Tuolumne Co., Deadman Creek, DRM voucher V100961. C, *Bembidion oromaia*, male, USA: California: Tulare Co., Upper East Fk. Kaweah River, DRM voucher V101127. D, *Bembidion ampliutum*, male, USA: Colorado: Mesa Co., Grand Mesa, route 65 at FS100, DRM voucher DNA3593. E, *Bembidion laxatum*, male, USA: California: Tulare Co., snowfield below White Chief Lake, DRM voucher DNA4149. F, *Bembidion laxatum*, female, USA: California: Mono Co., pond above Tioga Lake, DRM voucher V101126. Scale bar is 0.1 mm.

Table 2. Spatial relationships among *breve* group species

	<i>B. ampliutum</i>	<i>B. breve</i>	<i>B. geoppearlis</i>	<i>B. lividulum</i>	<i>B. laxatum</i>	<i>B. oromaia</i>	<i>B. saturatum</i>	<i>B. testatum</i>
<i>B. breve</i>	S							
<i>B. geoppearlis</i>	M	273 km						
<i>B. lividulum</i>	M	M	M					
<i>B. laxatum</i>	M	S	289 km	M				
<i>B. oromaia</i>	M	S	587 km	M	M			
<i>B. saturatum</i>	M	M	258 km	M	M	S		
<i>B. testatum</i>	S	M	484 km	M	S	S	M	
<i>B. vulcanix</i>	M	M	273 km	M	M	S	141 km	S

M, specimens are microsypatric (i.e. collected at the same locality); S, specimens that are broadly sympatric, but not known to occur at the same locality. Cells with numbers indicate the distance in kilometres between the nearest confirmed localities for non-sympatric species

Nomenclatural notes: This is the species referred to as ‘*Bembidion* “Ebbets Pass”’ in Sproul & Maddison (2017) and *B. breve* (specimen 1930) in Maddison (2012).

Diagnosis: A shiny, medium-sized, parallel-sided, relatively flat species with strongly foveate dorsal punctures (Fig. 1A). Forebody and elytra black or

dark brown often with aeneous hue. Pronotum widest anterior to middle with obtuse hind angles; not sinuate laterally; laterobasal carina strong (Figs 11A, 12A). Elytra parallel-sided; dorsal punctures strongly foveate. Elytral disc commonly with uneven surface (caused by a depression in elytral surface) in basal third anterior to dorsal punctures (see pattern of

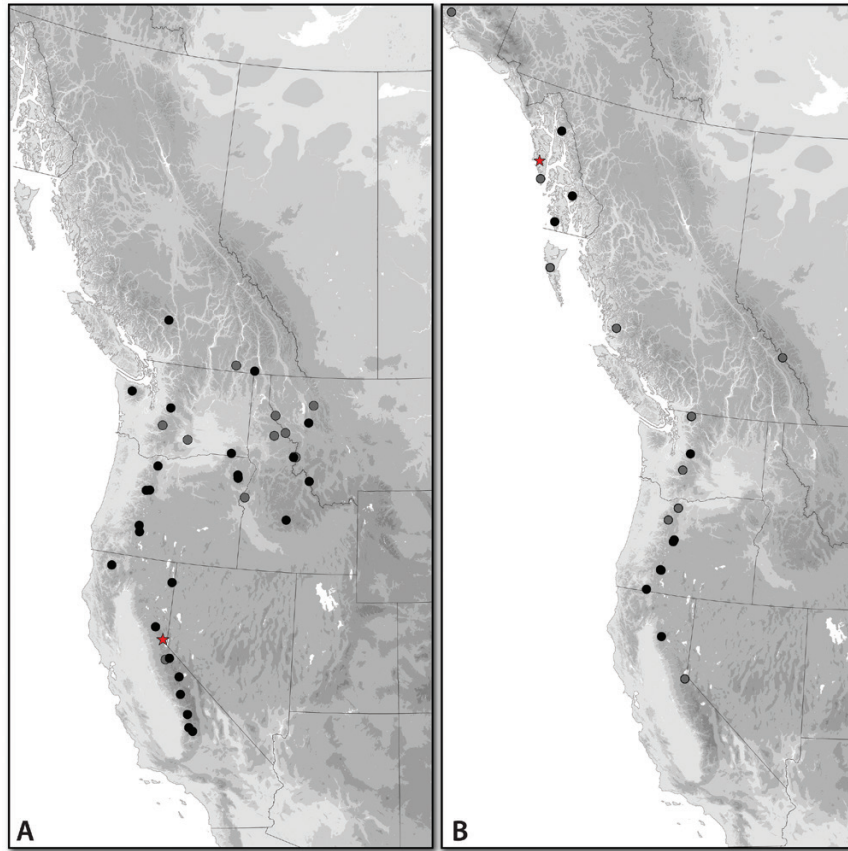


Figure 14. Distributions of *Bembidion lividulum* (A) and *B. breve* (B). Black circles indicate specimens identified through analysis of DNA (male and female) and genitalic characters (males only). Grey circles indicate specimens identified using male genitalic characters. Red stars indicate the type localities estimated using label data. The locality for a specimen of *B. breve* specimen (DNA4313) from the Aleutian Islands is not shown.

shadows in the basal third of elytral disc in Fig. 1A). Microsculpture weakened in males (Fig. 13B) causing shiny appearance and making foveate dorsal punctures easily visible without magnification. Male genitalia with flagellum sinuate and moderately long; sclerite ‘St’ slender, commonly hourglass-shaped (Figs 8A, B, 10A).

Comparison with similar species: Most easily confused with *B. ampliatus* and *B. breve*. Can be distinguished from the former by having a slightly more convex pronotum and stronger laterobasal carina, more strongly foveate dorsal punctures (particularly where they co-occur with *B. ampliatus* in California) and unevenness in the basal third of the elytral disc anterior to the dorsal punctures, and a longer, more sinuate flagellum and more slender sclerite ‘St’. Distinguished from the latter by having more parallel-sided elytra, the pronotum widest anterior to middle and a slender sclerite ‘St’. May also be confused with *B. laxatum* from which it is distinguished by having a smaller body size, weaker microsculpture, a narrower

pronotum relative to elytral width and sclerite ‘St’ lacking U-shaped recurvature.

Geographic distribution: From southern British Columbia along the Cascade Range to the southern Sierra Nevada. East through Idaho to the Rocky Mountains in Montana and southeastern British Columbia (Fig. 14A).

Habitat: Known from a wider range of environments than most other species in the group. It is often abundant in the damp soil below receding snow patches on alpine slopes. Also present along the shoreline of streams and lakes at high elevation. Common along the shorelines of moderate-sized rivers at somewhat lower elevation with increasing latitude (e.g. the Pacific Northwest and Alaska).

Geographic variation: This species is fairly variable across its range and within populations. Notable size variation is common in multiple locations of the Oregon Cascades and in Montana, with very small

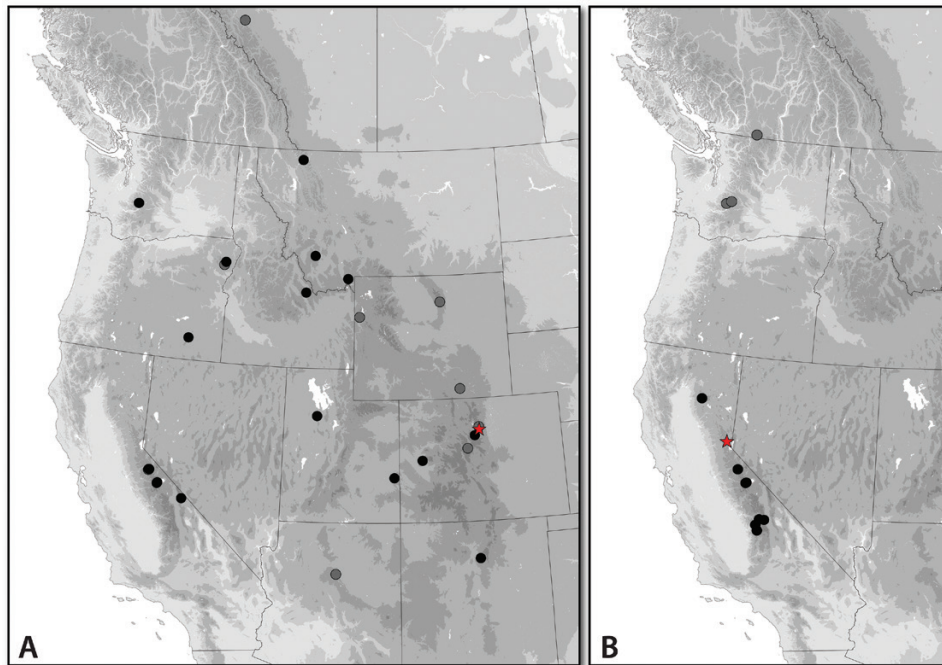


Figure 15. Distributions of *Bembidion ampliatum* (A) and *B. laxatum* (B). Black circles indicate specimens identified through analysis DNA (male and female) and genitalic characters (males only). Grey circles indicate specimens identified using male genitalic characters. Red stars indicate type localities estimated using label data.

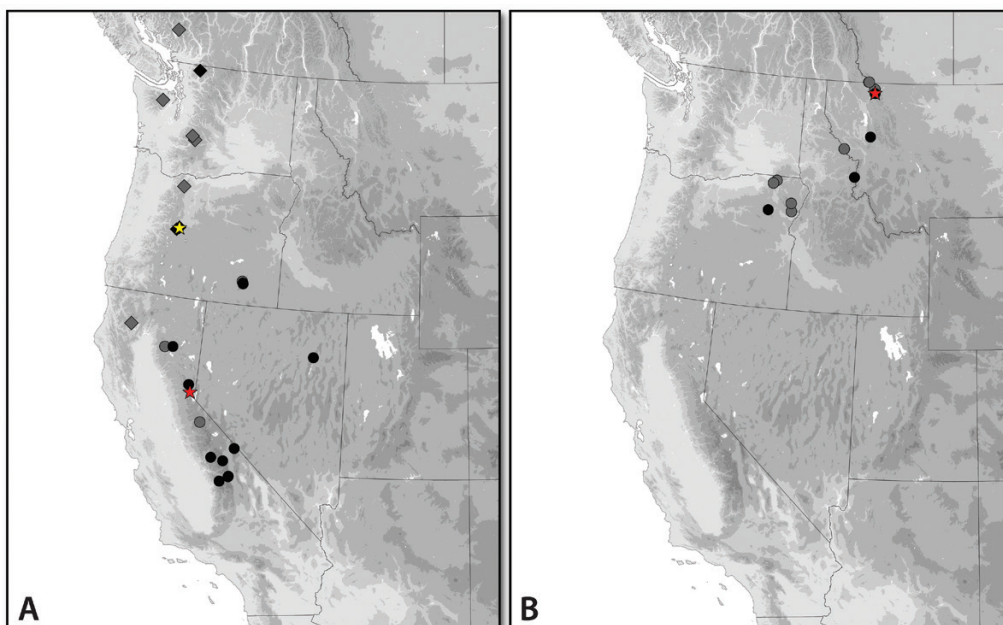


Figure 16. Distributions of *Bembidion saturatum* (indicated by circles) and *B. vulcanix* (indicated by diamonds) (A) and *B. geoparlis* (B). Black shapes indicate specimens identified through analysis DNA (male and female) and genitalic characters (males only). Grey shapes indicate specimens identified using male genitalic characters. Red stars indicate type localities of *B. saturatum* (A) and *B. geoparlis* (B), the former being estimated using label data. The yellow star indicates the type locality of *B. vulcanix* (A).

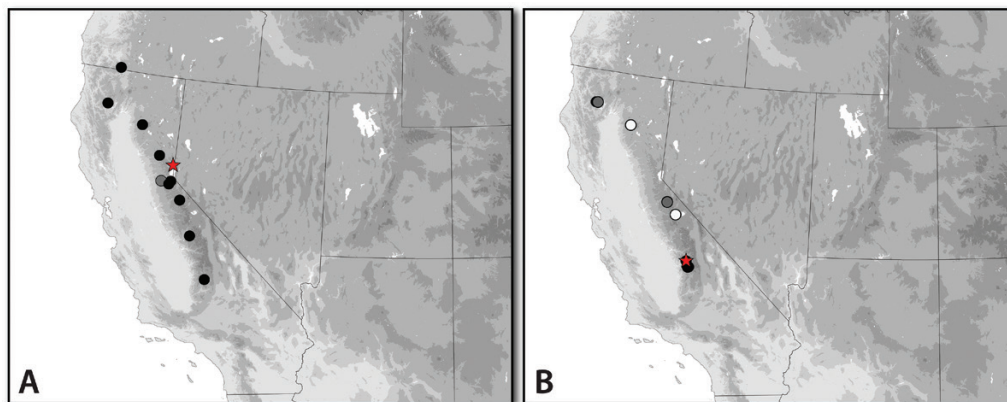


Figure 17. Distributions of *Bembidion testatum* (A) and *B. oromaia* (B). Black circles indicate specimens identified through analysis DNA (male and female) and genitalic characters (males only). Grey circles indicate specimens identified using male genitalic characters. White circles indicate specimens identified with external morphological characters. Red stars indicate type localities (estimated using label data in the case of *B. testatum*).

females in some populations (those sequenced do not show obvious differences in the genes examined).

BEMBIDION AMPLIATUM CASEY

(Figs 1B, 8C, D, 10B, 11B, 12D, 15A)

Bembidion ampliatum Casey, 1918: 24. Lectotype male, designated by Lindroth (1975: 117), in USNM, labelled 'Col' [white paper], [male symbol, hand drawn on white paper], 'CASEY bequest 1925' [white paper], 'TYPE USNM 36828' [red paper], 'ampli-atum Csy.' [white paper, handwritten], 'LECTOTYPE ampli-atum Csy. By C.H. Lindroth' [white paper, partly handwritten]. Type locality: Colorado. Examined, including genitalia.

Bembidion improvisum Casey, 1918: 24. Lectotype male, designated by Lindroth (1975: 117), in USNM, labelled 'Col' [white paper], [male symbol, hand drawn on white paper], 'CASEY bequest 1925' [white paper], 'TYPE USNM 36832' [red paper], 'improvisum Csy.' [white paper, handwritten], 'LECTOTYPE improvisum Csy. By C.H. Lindroth' [white paper, partly handwritten]. Type locality: Colorado. Examined, including genitalia.

Diagnosis: This medium-sized species is parallel-sided with a distinctly flat pronotum. Forebody and hindbody black or very dark brown, often with aeneous or metallic hue. Pronotum relatively flat; hind angles obtuse; widest anterior to middle with broad lateral explanation and weak laterobasal carina (as the basal fovea beside it is not as deep, and thus the carina does not stand out so prominently); not sinuate laterally (Figs 11B, 12C). Elytra are more or less parallel-sided and broadly rounded at apex; elytral disc with smooth appearance (particularly in California and Oregon), in part due

to weak striae and weakly foveate dorsal punctures (Fig. 1B) which are not easily observed without magnification. Microsculpture meshes moderately etched in females, but weakly or at least unevenly etched in most males, often partially disappeared (13D). Male genitalia with the ventral portion of sclerite 'St' angled anteriorly such that the ventral extremity is even with, or anterior to, the dorsal extremity; flagellum short and somewhat sinuate (Figs 8C, 13D, 10B).

Comparison with similar species: Most easily confused with *B. lividulum* from which it can be distinguished by having a flatter pronotum with weak laterobasal carina (especially reliable in California), less pronounced elytral striae with weakly foveate dorsal punctures and a smooth elytral disc in the basal third anterior to the dorsal punctures (though often less so in specimens east of California and Oregon), and the male genitalia having a more laterally expanded sclerite 'St' with the ventral portion angled anteriorly. May also be confused with *B. laxatum* from which it can be separated through the weakened elytral microsculpture in both sexes, by having a flatter pronotum with obtuse hind angles and weakened laterobasal carina, and lacking the U-shaped recurvature in sclerite 'St' of the male genitalia.

Geographic distribution: Throughout the Sierra Nevada and White Mountains in California and north throughout the eastern mountains of Oregon (Steens and Wallowas) to Alberta. Easterly through the Great Basin and Rocky Mountains to Montana, Wyoming, and Colorado, south to New Mexico and Arizona. The only *breve* group species known from Utah, Wyoming, Colorado, New Mexico and Arizona (Fig. 15A). A single questionable specimen is reported from Washington (see note below about 'Geographic variation').

Habitat: Open alpine slopes, commonly below patches of melting snow.

Geographic variation: Specimens east of California and Oregon (e.g. Great Basin and Rocky Mountains) are generally smaller-bodied, especially males, and show more intraspecific variation in external structures. Notable within-population size variation has been observed in the Manti La Sal Mountains of eastern Utah, and the Sangre de Cristo Mountains of New Mexico. The single specimen from Washington reported herein (DNA3321) is a morphological and molecular outlier that we doubtfully include within this species.

BEMBIDION BREVE (MOTSCHULSKY)
(FIGS 1C, 8E, F, 10C, 11C, 13A, 14B)

Peryphus brevis Motschulsky, 1845: 28. Lectotype male, designated by Bousquet & Laroche (1993: 16), in ZMMU, labelled 'Plataphus brevis Motsch [illegible]' [green paper, handwritten], 'B. brevis Mtsch spec. interto proxima cfr. et. tetraglyptum dt. Netolitzky' [white paper, handwritten], 'interto' is likely a misspelling of 'incerto', [red rectangle], 'LECTOTYPE *Peryphus brevis* Motschulsky Des. by Y. Bousquet' [red paper, partly handwritten]. Type locality: Sitka, Alaska. Examined, including genitalia.

Notaphus incertus Motschulsky, 1845: 350. Lectotype male, designated herein, in ZMMU, labelled 'Sitka' [green paper, handwritten], 'Plataphus incertus Motsc Am.b. [illegible] Sitka' [green paper, handwritten], 'B. incertum Mts spec. [illegible] det Netolitzky' [white paper, handwritten], 'LECTOTYPE *Notaphus incertus* Mtsch. designated Sproul & Maddison 2014' [red and white paper, partly handwritten]. Type locality: Sitka, Alaska. Examined, including genitalia.

Peryphus tetraglyptus Mannerheim, 1853: 151. Lectotype male, designated by Lindroth (1963: 273), in ZMH.

Bembidion blanditum Casey, 1918: 23. Lectotype female, designated by Lindroth (1975: 116), in USNM, labelled 'Metlakatla B. Col. Keen' [white paper], 'CASEY bequest 1925' [white paper], 'blandita Csy.' [white paper, handwritten], 'TYPE USNM 36829' [red paper], 'LECTOTYPE *blanditum* Csy. By C.H. Lindroth' [white paper, partly handwritten]. Type locality: Metlakatla, British Columbia. Examined.

Nomenclatural notes: Lindroth's concept of '*Bembidion incertum*', as a widespread species distributed from the Pacific states to Colorado, included this species, *B. ampliatum*, *B. lividulum* and *B. saturatum*.

Diagnosis: A small-bodied, convex species with strongly foveate dorsal punctures. Forebody dark brown, hindbody dark brown or reddish brown, often lighter than forebody; forebody and hindbody commonly with a

metallic hue. Pronotum with fairly rounded lateral margin, widest at middle or just anterior to middle; laterobasal carina strong but often short (not proceeding far anteriorly) due to convexity of pronotum; basal fovea deep; hind angles slightly obtuse (Fig. 11C). Elytral striae generally pronounced and dorsal punctures strongly foveate; elytra fairly short relative to length of pronotum (Fig. 1C). Microsculpture with meshes moderately etched in both sexes (Fig. 13A) (but note geographic variation below). Male genitalia with medium-length flagellum; sclerite 'St' more or less diamond-shaped (Figs 8E, F, 10C).

Comparison with similar species: Most easily confused with *B. lividulum*, *B. saturatum* and *B. vulcanix*. Distinguished from *B. lividulum* by having more convex pronotum and elytra with somewhat rounded lateral margin of elytra, pronotum widest closer to middle, and male genitalia with a longer, more sinuate flagellum and broader sclerite 'St'. Distinguished from *B. saturatum* and *B. vulcanix* by having a slightly smaller body size, more rounded lateral margin of elytra that narrows at the shoulder and more strongly foveate dorsal punctures, with pronotum larger relative to elytra and with a short laterobasal carina, and male genitalia with a shorter flagellum.

Geographic distribution: The northernmost species, ranging from the Aleutian Islands south along the coastal mountains of British Columbia, and in the Cascades of Oregon and Washington, south throughout California in the Sierra Nevada (Fig. 14B). Also known from one locality in Yoho National Park in eastern British Columbia.

Habitat: Most common on small, subalpine creeks in the southern part of its range. In the north, it occurs along creeks, rivers or open slopes at high elevation.

Geographic variation: Some specimens are larger-bodied in the Sierra Nevada with broader pronota relative to northern localities. Notable intraspecific variation (in body size, pronotum shape, forebody and elytral coloration) is present in northern populations (e.g. southeast Alaska). Microsculpture shape and intensity is variable; in particular, some specimens may have notably transverse meshes (e.g. Queen Charlotte Islands, British Columbia), or less deeply etched meshes such that portions of cells are partially disappeared in males (e.g. Snoqualmie Pass, Washington). Some individuals from Yoho National Park are notably small in size.

BEMBIDION LAXATUM CASEY

(FIGS 1D, 8G, H, 10H, 11H, 12B, 13E, F, 15B)

Bembidion laxatum Casey, 1918: 24. Lectotype male, designated by Lindroth (1975: 117), in USNM,

labelled 'Placer Co. CAL.' [white paper], 'CASEY bequest 1925', 'TYPE USNM 36833' [red paper], 'laxata Csy.' [white paper, handwritten], 'LECTOTYPE laxatum Csy. By C.H. Lindroth' [white paper, partly handwritten]. Type locality: Placer Co., California. Examined, including genitalia.

Bembidion adumbratum Casey, 1918: 26. Lectotype male, designated by Lindroth (1975: 117), in USNM, labelled 'Placer Co. CAL.' [white paper], 'Oct.' [white paper], 'CASEY bequest 1925' [white paper], 'TYPE USNM 36827' [red paper], 'adumbrata Csy.' [white paper, handwritten], 'LECTOTYPE adumbratum Csy. By C.H. Lindroth' [white paper, partly handwritten]. Type locality: Placer Co., California. Examined, including genitalia.

Bembidion rainieri Hatch, 1950: 97. Holotype female in USNM, labelled 'Mt. Rainier, WASH. Sunrise Park Sept. 6, 1934 M. H. Hatch' [white paper], 'ADP 115747' [white paper], 'Bembidion (Plataphodes) laxatum Csy. M. Hatch-1969' [white paper, handwritten], 'TYPE Bembidion (Trechonepha) rainieri 1948.-M. H. Hatch' [red paper, hand written]. Examined. Type locality: Sunrise Park, Mount Rainier Pierce Co., Washington.

Diagnosis: Large and heavy-bodied, this parallel-sided species is distinct in its dull appearance, particularly females. Forebody and hindbody dark brown, rarely black, typically with aeneous, or bluish, metallic hue. Pronotum broad and somewhat convex; widest near middle with hind angles near 90° or slightly obtuse (Fig. 11H); laterobasal carina strong (Fig. 12B). Elytra widest near middle, often narrowing towards apex; elytral striae somewhat pronounced, and dorsal punctures moderately foveate (Fig. 1D). Microsculpture strongly etched in males; very strongly etched, often granulate, in females (Fig. 13E, F). Male genitalia with short, weakly sinuate flagellum; sclerite 'St' with U-shaped recurvature ventrally (Figs 8G, H, 10H).

Comparison with similar species: Most easily confused with *B. oromaia* from which it is distinguished by having a wider pronotum relative to width of the elytra, the elytra being widest near middle, having larger protarsomeres in males, less slender, elongate legs and antennae, and by various characters in the male genitalia. May also be confused with *B. lividulum* and *B. ampliutum*. It can be distinguished from both by its larger, more convex body, duller appearance due to strong microsculpture in both sexes, broadened pronotum relative to the elytra with hind angles near 90°, and the U-shaped recurvature of sclerite 'St' in the male genitalia.

Geographic distribution: Throughout the Sierra Nevada in California, north along the Cascade Range to Washington and southern British Columbia (Fig. 15B).

Habitat: Open alpine slopes commonly below patches of melting snow or along small alpine creeks.

Geographic variation: Sclerite 'St' in males from California localities is fairly elongate (Fig. 9G, H). Sclerite 'St' in the few Washington and British Columbia specimens available for examination appears slightly less elongate and more broadened than in California specimens.

BEMBIDION SATURATUM CASEY

(Figs 2A, 9A, B, 10D, 11D, 16A)

Bembidion saturatum Casey, 1918: 24. Lectotype female, designated by Lindroth (1975: 117), in USNM, labelled 'Placer co. Cal.' [white paper], 'CASEY bequest 1925' [white paper], 'TYPE USNM 36831' [red paper], 'saturata Csy.' [white paper, handwritten], 'LECTOTYPE saturatum Csy. By C.H. Lindroth' [white paper, partly handwritten]. Type locality: Placer Co., California. Examined, including DNA sequences.

Nomenclatural notes: This is the species referred to as '*Bembidion* "University Peak"' in Sproul & Maddison (2017). Extracted DNA is deposited at the USNM and associated with the GUID of the type specimen: ark:/65665/380c4cce2-3007-4a2d-9d40-5865c9760b4f.

Diagnosis: This small-bodied convex species has a broad pronotum and stout appearance (Fig. 2A). Forebody and hindbody dark brown, some specimens with an aeneous reflection. Pronotum very broad basally with hind angles near 90°; lateral explanation broad (Fig. 11D), laterobasal carina somewhat weak as the basal fovea beside it is quite shallow, and thus the carina does not stand out so prominently, although carina may still extend far anteriorly. Elytra long relative to length of pronotum; tapered apically in some specimens; dorsal punctures weakly foveate (Fig. 2A). Microsculpture meshes strongly etched in females and moderately etched in males (except for populations in eastern Oregon and Nevada where microsculpture is notably weakened). Male genitalia with long sinuate flagellum (Figs 9A, B, 10D); sclerite 'St' either lacking (Fig. 9A), or with a hint of sclerotization in the position of sclerite 'St' (Fig. 9B).

Comparison with similar species: Most easily confused with *B. vulcanix* and *B. geoparlis*, from which it is extremely difficult to separate using only external structures. It is most easily distinguished using characters in the male genitalia and geography. It is separated from both by lacking sclerite 'St' (but see note on 'Geographic variation' below), and by having a southern and southeastern distribution (Fig. 16). May also be confused with *B. breve*, from which it is distinguished by a slightly larger body with more parallel-sided

elytra, weakly foveate dorsal punctures, and a longer, more sinuate flagellum of the male genitalia.

Geographic distribution: From Washington south to the southern Sierra Nevada, east to the Ruby Mountains in Nevada and Steens Mountains in Oregon (Fig. 16A).

Habitat: A variety of damp environments at or below the tree line including damp meadows, along lake-shores or small creeks, or on open alpine slopes below patches of melting snow.

Geographic variation: Populations from the Steens Mountains in eastern Oregon and the Ruby Mountains in Nevada (the ‘eastern form’) are smaller-bodied, shinier and more black than dark brown, with slight sclerotization in the position of sclerite ‘St’ evident (e.g. Fig. 9B), but not nearly so expanded as in *B. geoparlis* (Figs 9C, D, 10E) or *B. vulcanix* (Figs 9E, F, 10F). A slight patch of sclerotization has also been observed occasionally in individuals from California (Fig. 9B).

BEMBIDION GEOPEARLIS SP. NOV.

(Figs 2B, 9C, D, 10E, 11E, 16B)

Holotype male (in OSAC) here designated, labelled: ‘USA: Montana: Glacier N.P., east slope Clements Mtn., 2129 m, 48.692°N 113.7292°W, 12 August 2015. JSS 2015.107-1 [-1’ handwritten]. J.S. Sproul & family’ [white paper], ‘David R. Maddison DNA4727 DNA Voucher’ [pale green paper], ‘HOLOTYPE *Bembidion geoparlis* Sproul + Maddison 2017’ [partly handwritten, red paper], ‘Oregon State Arthropod Collection OSAC_0002000000 [matrix code]’ [printed on both sides of white paper]. Genitalia mounted in Euparal on small card labelled ‘DNA4727’ beneath the specimen; extracted DNA stored separately. GenBank accession numbers for DNA sequences of the holotype are: KY950786 (28S); KY950914 (*CAD*); KY951044 (*COI*), KY951174 (*MSP*), KY951301 (*Topo*).

Type locality: USA: Montana: Glacier National Park, east slope Clements Mountain near Logan Pass, 2129 m, 48.69204°N 113.72920°W.

Paratypes: Thirty-eight paratypes from the following localities, with specimens deposited in OSAC and USNM: USA: Montana: Glacier National Park, east slope Clements Mountain near Logan Pass, 2129 m, 48.69204°N 113.72920°W (25); USA: Montana: Glacier Co., Glacier National Park, Iceberg Lake (2); USA: Montana: Glacier Co., Glacier National Park, Logan Pass (1); USA: Montana: Glacier National Park (1); USA: Montana: Mineral Co., Hoodoo Creek, 1780 m (1); USA: Montana: Missoula Co., inlet to Heart Lake, 1891 m, 47.3801°N 113.8501°W (1); USA: Montana: Ravalli Co., Lost Horse Creek, 1760 m, 46.1417°N 114.4863°W

(1); USA: Montana: Ravalli Co., Lost Horse Creek, 1660 m, 46.1402°N 114.4371°W (1); USA: Montana: Flathead Co., Glacier National Park, Sperry Chalets (1); Canada: British Columbia: Akamina Pass, 1740 m, 49.0261°N 114.0611°W (3); Canada: Alberta: Waterton Lakes National Park, Cameron Lake, 5440 m (1).

Derivation of specific epithet: The name ‘geoparlis’ is derived from an informal combination of letters taken from the names of JSS’s children ‘George’ (*geo*) and ‘Pearl’ (*pearl*) with an ending (*is*) to make the name euphonic. The name recognizes the contribution of George (age 10) and Pearl (age 7) to the present work. They have accompanied JSS on over 6500 miles of high-intensity collecting road trips, hiked over 40 miles to high-elevation habitats in California, Oregon and Montana, and helped collect hundreds of specimens including members of the type series of this species. JSS is indebted to them for their companionship and support. The components of the name also reference the organism: ‘geo’ evokes something of the earth; thereby, ‘earth pearl’ or ‘a precious thing from the earth’ is descriptive of these seldom-collected ground beetles.

Diagnosis: A small, convex species with a notably broad prothorax (Fig. 2B). Forebody dark brown or black; hind body dark brown or reddish brown. Pronotum variable but generally very broad basally with a broad lateral explanation; hind angles near 90°; sinuate laterally in some specimens (Fig. 11E). Elytra widest behind middle; often but not always broadly rounded at apex; dorsal punctures weakly foveate. Microsculpture meshes strongly etched in females and moderately etched in males. Genitalia somewhat parallel-sided in outer shape having a short taper towards the apex; flagellum sinuate and moderately long; sclerite ‘St’ expanded (Figs 9C, D, 10E).

Comparison with similar species: Most similar in appearance to *B. saturatum*, *B. vulcanix* and *B. breve*. Most reliably distinguished from all three with male genitalic characters and by its eastern geographic distribution. In particular, distinguished from *B. saturatum* and *B. vulcanix* by having elytra widest behind middle resulting in a more rounded apex of the elytra, and by the apical half of the aedeagus being less curved and elongate. Distinguished in male genitalia from *B. saturatum* by the presence of an expanded sclerite ‘St’. Distinguished from *B. vulcanix* by having a less expanded, non-arcuate sclerite ‘St’. Distinguished from *B. breve* by having a broader pronotum, more weakly foveate elytral dorsal punctures, the shape and position of sclerite ‘St’.

Geographic distribution: Known only from the Rocky Mountains of Montana, the Waterton National Parks area of southern Alberta and British Columbia, and the Wallowa and Blue Mountains in northeastern Oregon (Fig. 16B).

Habitat: Collected in abundance on open slopes above the tree line at the type locality. Small series or singletons have also been collected on small creeks or depressions with damp soil below the timberline.

Geographic variation: none noted.

***BEMBIDION VULCANIX* SP. NOV.**

(Figs 2C, 9E, F, 10F, 11F, 16A)

Holotype male (in OSAC) here designated, labelled: 'USA: Oregon: Deschutes Co., Stream east of Todd Lake, 1952 m, 44.0282°N 121.6709°W, 24.v.2015. JSS.2015.030-1 [-1' handwritten]. J.S., E.C., G.S., & P.E. Sproul' [white paper], 'David R. Maddison DNA4615 DNA Voucher' [pale green paper], 'HOLOTYPE *Bembidion vulcanix* Sproul + Maddison 2017' [partly handwritten, red paper], 'Oregon State Arthropod Collection OSAC_0002000001 [matrix code]' [printed on both sides of white paper]. Genitalia mounted in Euparal on small card labelled 'DNA4615' beneath the specimen; extracted DNA stored separately. GenBank accession numbers for DNA sequences of the holotype are: KY950767 (28S), KY950895 (CAD), KY951025 (COI), KY951155 (MSP), KY951282 (Topo).

Type locality: USA: Oregon: Deschutes Co., Deschutes National Forest, stream east of Todd Lake, 1952 m, 44.0282°N 121.6709°W.

Paratypes: Seventy-two paratypes from the following localities, with specimens deposited in BMNH, CAS, CSCA, EMEC, MNHN and USNM: USA: Oregon: Deschutes Co., stream east of Todd Lake, 1952 m, 44.0282°N 121.6709°W (57); USA: Oregon: Deschutes Co., Creek below Little Three Creek Lake, 2018 m, 44.1057°N 121.6347°W (6); USA: Oregon: Deschutes Co., NE Todd Lake, Deschutes NF road 370, 2067 m, 44.038°N 121.6718°W (1); USA: Oregon: Deschutes Co., E Todd Lake, Deschutes NF road 370, 1976 m, 44.0306°N 121.6683°W (1); USA: Oregon: Hood River Co., Mt. Hood, Hood River Meadow Ski Area, 5300 ft. (2); USA: Washington: Whatcom Co., Bagley Lakes, Mt Baker, Snoqualmie NF, 1326 m, 48.8528°N 121.6886°W (1); USA: Washington: Whatcom Co., Bagley Lakes, Mt Baker, Snoqualmie NF, 1290 m, 48.8534°N 121.6948°W (1); USA: Washington: Pierce Co., Mt. Rainier, Tipsoo Lake (1); USA: Washington: Pierce Co., Mt. Rainier, Yakima Park (1); Canada: British Columbia: Garibaldi Provincial Park, S. slope Black Tusk (1).

Derivation of specific epithet: Informally derived by combining the two Latin words *Vulcanis*, the blacksmith god of fire and volcanoes from Roman mythology, and *nix*, meaning snow. The name references

snow-covered volcanoes of the Cascade Range where this species can be commonly found at high elevation.

Diagnosis: This convex species is recognized by a broad pronotum basally, elongate elytra and distinctive male genitalia. Forebody and hindbody dark brown in some specimens aeneous. Pronotum very broad basally; hind angles near 90°; lateral explanation broad; laterobasal carina fairly weak due to shallow adjacent basal fovea, but may extend far anteriorly nearly parallel to lateral margin of pronotum (Fig. 11F). Elytra long relative to length of pronotum and somewhat bullet-shaped in that they are parallel-sided, and tapering toward apex (although not readily obvious in Fig. 2C); dorsal punctures weakly foveate; striae often pronounced (Fig. 2C). Microsculpture strongly etched in females and moderately etched in males. Aedeagus is strongly curved, with an elongate taper towards the narrow apex; sclerite 'St' in apical half, large and arcuate; flagellum long and sinuate (Figs 9E, F, 10F).

Comparison with similar species: Most easily confused with *B. saturatum* and *B. geoppearlis*. It is separated from both by having an expanded, arcuate sclerite 'St' in the apical half (which is absent in the former, and not as expanded, arcuate, or apically positioned in the latter), and by its northwestern distribution (Fig. 16). May also be confused with *B. breve* from which it is distinguished by its generally larger body size, more parallel-sided elytra, weakly foveate dorsal punctures, and longer, more sinuate shape of the flagellum and large of sclerite 'St' in the male genitalia.

Habitat: Along the shorelines of subalpine small creeks or lakes.

Geographic distribution: From southwestern British Columbia and the Olympic Peninsula in Washington, south along the Cascades of Washington and Oregon to the Trinity Alps in northwestern California (Fig. 16A).

Geographic variation: None noted.

***BEMBIDION TESTATUM* CASEY**

(Figs 2D, 9G, H, 10G, 11G, 17A)

Bembidion testatum Casey, 1918: 30. Lectotype male, designated by Erwin (1984: 174), in USNM, labelled 'Ca[a vertical line crossed by two shorter horizontal lines]' [white paper], (male symbol) [hand drawn on white paper], 'CASEY bequest 1925' [white paper], 'TYPE USNM 36842' [red paper], 'LECTOTYPE [male symbol] *Bembidion testatum* Csy. By Erwin '77' [white paper, partly handwritten]. Examined. Type locality: Lake Tahoe, California.

Nomenclatural notes: This is the species referred to as '*Bembidion* "Lily Lake Creek"' in Sproul & Maddison (2017).

Diagnosis: This fairly large-bodied species is most easily recognized by the inflated appearance of the elytra and narrow pronotum relative to width of the elytra (Fig. 2D). Forebody dark brown or black; hind body from dark brown to reddish brown, often paler than forebody. Pronotum narrow relative to the width of the elytra; strongly sinuate laterally (Figs 2D, 11G); hind angles near 90° with basal fovea deeply excavated (Fig. 11G). Elytra notably convex with strongly rounded lateral margin resulting in an inflated appearance; dorsal punctures weakly foveate, elytral striae weak with striae three and four partially disappeared in some specimens. Microsculpture strongly etched in females and moderately etched in males. Male genitalia with darkened patch of membranes apically; sclerite 'St' lacking; flagellum sinuate and moderately long; ostial flag with an abbreviated sinuation not nearing the ventral surface of the aedeagus, and not extending far anteriorly (Figs 9G, H, 10G).

Comparison with similar species: Can be confused with *B. saturatum* and *B. breve* in the northern Sierra Nevada. Distinguished from both by a larger body size, more convex elytra, and a darkened patch of scale-like structures apically in the male genitalia (see inset in Fig. 9H). Further distinguished from the former by having a narrower pronotum at the base. Distinguished from the latter by having very weakly foveate dorsal punctures and weak striae of the elytra.

Geographic distribution: Known from the Sierra Nevada and Trinity Alps in California, as well as a single locality in southern Oregon (Fig. 17A).

Habitat: Appears to be restricted to small, subalpine creeks.

Geographic variation: Some specimens from the Trinity Alps in northwestern California have a slightly longer flagellum in male genitalia. The single individual we sampled from Mount Ashland, Oregon has four distinctive bases in 28S (three of which are ambiguities in DNA4173, but non-ambiguous and different in all other specimens), but is not notably distinct in the other genes. The Oregon specimen also has less sinuate later margins of the pronotum than typical California specimens.

BEMBIDION OROMAIA SP. NOV.

(Figs 3, 9I, 10I, 11I, 13C, 17B)

Holotype male (in OSAC) herein designated, labelled 'USA: California: Tulare Co., snow field above Emerald Lake, 2851 m, 36.5959°N 118.6756°W, 21.vi.2014. JSS 2014.064-12 [-12' handwritten]. J.S. Sproul & Family' [white paper], 'David R. Maddison DNA4250 DNA Voucher' [pale green paper], 'HOLOTYPE *Bembidion oromaia* Sproul + Maddison 2017' [partly handwritten, red paper], 'Sequoia and Kings Canyon National Parks

SEKI 23092' [green paper], 'Oregon State Arthropod Collection OSAC_0002000002 [matrix code]' [printed on both sides of white paper]. Genitalia mounted in Euparal on small card labelled 'DNA4250' beneath the specimen; extracted DNA stored separately. GenBank accession numbers for DNA sequences of the holotype are KY950760 (28S), KY950889 (CAD), KY951019 (COI), KY951149 (MSP), KY951276 (Topo).

Type locality: USA: California: Tulare Co., snowfield above Emerald Lake, 2851 m, 36.5959°N 118.6756°W.

Paratypes: Thirty-two specimens from the following localities, with specimens deposited in CAS, OSAC, and USNM: USA: California: Tulare Co., Upper East Fk. Kaweah River, 2812 m, 36.4189°N 118.5927°W (12); USA: California: Tulare Co., snow field above Emerald Lake, 2851 m, 36.5959°N 118.6756°W (6); USA: California: Tulare Co., snowfield below White Chief Lake, 2912 m, 36.417°N 118.5941°W (3); USA: California: Tulare Co., Lower Franklin Lake, 36.4203°N 118.5614°W (1); USA: California: Tuolumne Co., Blue Canyon Creek, 2750 m, 38.3151°N 119.6613°W (2); USA: California: Tuolumne Co., stream draining N. face Leavitt Peak, 2930 m, 38.3098°N 119.6619°W (2); USA: California: Tuolumne Co., Deadman Creek, 2700 m, 38.3188°N 119.6634°W (1); USA: California: Tuolumne Co., Hwy. 108, stream SE of Chipmunk Flat, 2440 m (1); USA: California: Tuolumne Co., Deadman Creek at junction with Blue Canyon Creek, 2665 m, 38.3174°N 119.6652°W (1); USA: California: Mono Co., snow field above Ellery Lake, 37.9345°N 119.2318°W (2); USA: California: Mono Co., H. M. Hall Natural Area, Lee Vining Creek, 3020 m, 37.9591°N 119.2838°W (1).

Derivation of specific epithet: Derived from Greek roots, with 'oro' meaning 'mountain', and 'maia' meaning 'good mother', or 'caregiver'. Thus, the name connotes 'good mother of the mountain' or 'caregiver of the mountains'. The name recognizes the contribution of Elizabeth C. Sproul to the present work. Mother of George and Pearl mentioned above, Elizabeth has spent hundreds of hours traveling to *breve* group localities, guiding young legs up steep trails, waiting at trailheads, recording locality data, and collecting and processing specimens in support of JSS's dissertation research, and to enable positive associations for George and Pearl. Her steady support has added tremendous physical and emotional energy to the sampling efforts and overall scope of this work. The specific epithet also references the beetles. Distributed at higher elevation than any other members of the group, their elegant appearance inspires imagery of a maternal caregiver high in mountains.

Diagnosis: This large-bodied black species with long, slender appendages is the most distinctive member

of the group (Fig. 3). Forebody and hindbody black, commonly with greenish or bluish metallic lustre. Pronotum narrow relative to elytral width and sinuate laterally; hind angles near 90° (Fig. 11I). Elytra widest behind middle with microsculpture meshes strongly etched in females and moderately etched in males (Fig. 13C). Legs and antennae black, long and slender; first protarsomeres in males quite small (not illustrated as Fig. 3 is a female). Male genitalia distinctive with outer shape having the appearance of being bent basally; flagellum long and sinuate; sclerite 'St' elongate, bilobed and heavily sclerotized (Figs 9I, 10I).

Comparison with similar species: Most easily confused with *B. laxatum* from which it is distinguished by having a pronotum which is narrower relative to the elytra, and more sinuate laterally, longer and more slender legs and antennae, smaller first protarsomeres in males, opaque black coloration, and by various male genitalic characters.

Geographic distribution: Throughout the Sierra Nevada, north to Mount Lassen and the Trinity Alps in California (Fig. 17A).

Habitat: Open alpine slopes under fairly large rocks on soil, commonly below patches of melting snow. Also present along small alpine creeks.

Geographic variation: The single specimen we sampled from the Trinity Alps in California has two distinctive bases in 28S (a gene for which all other specimens have identical sequences), but is not notably distinctive in other genes.

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REFERENCES

- Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ. 2014.** BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* **10**: e1003537.
- Bousquet Y, Laroche A. (1993).** Catalogue of the Geadephaga (Coleoptera: Trachypachidae, Rhysodidae, Carabidae including Cicindelini) of America north of Mexico. *Memoirs of the Entomological Society of Canada No. 167*. 397 pp.
- Burrell AS, Disotell TR, Bergue CM. 2015.** The use of museum specimens with high-throughput DNA sequencers. *Journal of Human Evolution* **79**: 35–44.
- Carstens BC, Pelletier TA, Reid NM, Satler JD. 2013.** How to fail at species delimitation. *Molecular Ecology* **22**: 4369–4383.
- Casey TL. 1918.** *A review of the North American Bembidiinae. Memoirs on the Coleoptera. VIII.* Lancaster: The New Era Printing Company, 1–223.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012.** jModel-Test 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772.
- Degnan JH, Rosenberg NA. 2009.** Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology & Evolution* **24**: 332–340.
- De Queiroz K. 2007.** Species concepts and species delimitation. *Systematic Biology* **56**: 879–886.
- Edwards SV. 2009.** Is a new and general theory of molecular systematics emerging? *Evolution* **63**: 1–19.
- Erwin TL. 1984.** Studies of the tribe Bembidiini (Coleoptera: Carabidae): lectotype designations and species group assignments for *Bembidion* species described by Thomas L. Casey and others. *The Pan-Pacific Entomologist* **60**: 165–197.
- Ganglbauer L. 1891.** Die Käfer von Mitteleuropa. Die Käfer der österreichisch-ungarischen Monarchie, Deutschlands, der Schweiz, sowie des französischen und italienischen Alpengebietes. Ester Band. Familienreihe Caraboidea. *Carl Gerold's Sohn*: iii + 557.
- Green P. 1999.** Phrap. Version 0.990329. Available at: <http://phrap.org> (accessed 15 June 2016).

- Green P, Ewing B. 2002.** Phred. Version 0.020425c. Available at: <http://phrap.org> (accessed 15 June 2016).
- Guschanski K, Krause J, Sawyer S, Valente LM, Bailey S, Finstermeier K, Sabin R, Gilissen E, Sonet G, Nagy ZT, Lenglet G, Mayer F, Savolainen V. 2013.** Next-generation museomics disentangles one of the largest primate radiations. *Systematic Biology* **62**: 539–554.
- Hatch MH. 1950.** Studies on the Coleoptera of the Pacific Northwest. II: Carabidae: Bembidiini. *The Pan-Pacific Entomologist* **26**: 97–106.
- Heled J, Drummond AJ. 2010.** Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution* **27**: 570–580.
- Jones G. 2017.** Algorithmic improvements to species delimitation and phylogeny estimation under the multispecies coalescent. *Journal of Mathematical Biology* **74**: 447–467.
- Kanda K, Pflug JM, Sproul JS, Dasenko MA, Maddison DR. 2015.** Successful recovery of nuclear protein-coding genes from small insects in museums using illumina sequencing. *PLoS ONE* **10**: e0143929.
- Katoh K, Toh H. 2008.** Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* **9**: 286–298.
- Knowles LL, Carstens BC. 2007.** Delimiting species without monophyletic gene trees. *Systematic Biology* **56**: 887–895.
- Lindroth CH. 1963.** The ground beetles (Carabidae, excl. Cicindelinae) of Canada and Alaska, Part 3. *Opuscula Entomologica Supplementum XXIV*: 201–408.
- Lindroth CH. 1969.** The ground beetles (Carabidae, excl. Cicindelinae) of Canada and Alaska, Part 6. *Opuscula Entomologica Supplementum* **34**: 945–1192.
- Lindroth CH. 1975.** Designation of holotypes and lectotypes among ground beetles (Coleoptera, Carabidae) described by Tomas L. Casey. *The Coleopterists Bulletin* **29**: 109–147.
- Maddison DR. 1993.** Systematics of the holarctic beetle subgenus *Bracteon* and related *Bembidion* (Coleoptera: Carabidae). *Bulletin of the Museum of Comparative Zoology* **153**: 143–299.
- Maddison DR. 2012.** Phylogeny of *Bembidion* and related ground beetles (Coleoptera: Carabidae: Trechinae: Bembidiini: Bembidiina). *Molecular Phylogenetics and Evolution* **63**: 533–576.
- Maddison DR, Cooper KW. 2014.** Species delimitation in the ground beetle subgenus *Liocosmius* (Coleoptera: Carabidae: *Bembidion*), including standard and next-generation sequencing of museum specimens. *Zoological Journal of the Linnean Society* **172**: 741–770.
- Maddison DR, Maddison WP. 2016.** Chromaseq: a Mesquite module for analyzing sequence chromatograms. Version 1.2. Available at: <http://mesquiteproject.org/packages/chromaseq> (accessed 15 June 2016).
- Maddison WP, Maddison DR. 2017.** Mesquite: a modular system for evolutionary analysis. Version 3.2. Available at: <http://mesquiteproject.org> (accessed 7 October 2017).
- Mannerheim CG. 1853.** Dritter Nachtrag zur Kaefer-Fauna der nord-amerikanischen Laender des russischen Reiches. *Bulletin de la Société Impériale des Naturalistes de Moscou* **26**: 95–273.
- Motschulsky V. 1845.** Remarques sur la collection de coléoptères Russes de Victor de Motschulsky. *Bulletin de la Société Impériale des Naturalistes de Moscou* **18**: 3–127.
- Motschulsky V. 1864.** Enumération des nouvelle espèces de coléoptères rapportés de ses voyages. 4-ème article. *Bulletin de la Société Impériale des Naturalistes de Moscou* **37**: 171–240.
- Rannala B, Yang Z. 2003.** Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics* **164**: 1645–1656.
- Raskina O, Barber JC, Nevo E, Belyayev A. 2008.** Repetitive DNA and chromosomal rearrangements: speciation-related events in plant genomes. *Cytogenetic and Genome Research* **120**: 351–357.
- Regier JC, Shultz JW, Ganley AR, Hussey A, Shi D, Ball B, Zwick A, Stajich JE, Cummings MP, Martin JW, Cunningham CW. 2008.** Resolving arthropod phylogeny: exploring phylogenetic signal within 41 kb of protein-coding nuclear gene sequence. *Systematic Biology* **57**: 920–938.
- Sproul JS, Maddison DR. 2017.** Sequencing historical specimens: successful preparation of small specimens with low amounts of degraded DNA. *Molecular Ecology Resources*. doi:10.1111/1755-0998.12660
- Staats M, Erkens RH, van de Vossenberg B, Wieringa JJ, Kraaijeveld K, Stielow B, Geml J, Richardson JE, Bakker FT. 2013.** Genomic treasure troves: complete genome sequencing of herbarium and insect museum specimens. *PLoS One* **8**: e69189.
- Sukumaran J, Knowles LL. 2017.** Multispecies coalescent delimits structure, not species. *Proceedings of the National Academy of Sciences* **114**: 1607–1612.
- Wandeler P, Hoeck PE, Keller LF. 2007.** Back to the future: museum specimens in population genetics. *Trends in Ecology & Evolution* **22**: 634–642.
- Yang Z. 2002.** Likelihood and Bayes estimation of ancestral population sizes in hominoids using data from multiple loci. *Genetics* **162**: 1811–1823.
- Zwickl DJ. 2006.** *Garli*. Version 2.0. Available at: <https://code.google.com/archive/p/garli/> (accessed 10 June 2016).

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Localities of *breve* group specimens examined in molecular studies. Four-digit numbers in the first column are DRM voucher numbers for DNA extracted specimens.

Table S2. Optimal substitution models for phylogenetic analysis estimated in jModelTest. Differences between the model used in Garli and STACEY are because not all models are available in STACEY, and thus the next best fitting model that is available was chosen.

Table S3. GenBank accession numbers for previously published sequences.

SHARED DATA

Sequence assembly files are deposited in Dryad.

doi:10.5061/dryad.10qj1.

DNA sequences included in phylogenetic analyses are deposited in Genbank [KY950685–KY951331]. Sequence read files are deposited in NCBI Sequence Read Archive [SRR5514451–SSR5514456]. Matrices and results from phylogenetic analysis are deposited in Dryad. doi:10.5061/dryad.10qj1.