

A preliminary characterization of *Bembidion perspicuum* LeConte, with a reclassification of related species (Coleoptera, Carabidae) north of México

David R. Maddison¹, Alexander P. Swanson²

1 Department of Entomology, University of Arizona, Tucson, AZ 85721, USA. Current address: Department of Zoology, 3029 Cordley Hall, Oregon State University, Corvallis, OR 97331, USA **2** Department of Entomology, University of Arizona, Tucson, AZ 85721, USA. Current address: 4415 Olive Ave., Long Beach, CA 90807, USA

Corresponding author: David R. Maddison (david.maddison@science.oregonstate.edu)

Academic editor: Terry Erwin | Received 20 January 2010 | Accepted 24 March 2010 | Published 13 April 2010

Citation: Maddison DR, Swanson AP (2010) A preliminary characterization of *Bembidion perspicuum* LeConte, with a reclassification of related species (Coleoptera, Carabidae) north of México. ZooKeys 43: 15–31. doi: 10.3897/zookeys.43.390

Abstract

Bembidion perspicuum LeConte is a species distinct from *B. transversale* Dejean, the names of which have been synonymized in the past. In the United States, *B. perspicuum* occurs from Oregon through Texas along the shores of rivers and creeks. We show that it is consistently different at 40 bases in DNA sequences of cytochrome oxidase I and 4 bases in 28S ribosomal DNA, as well as in subtle morphological characters. In addition, we review the classification of related species in the *B. transversale* and *B. mexicanum* species groups in America north of México. In the United States and Canada, the *B. transversale* group includes *B. transversale*, *B. perspicuum*, and *B. sarpedon* Casey; the *B. mexicanum* group includes *B. mexicanum* Dejean, *B. lugubre* LeConte, and *B. pernotum* Casey.

Keywords

Bembidiini, *Ocydromus*, COI

Introduction

Members of the *Bembidion transversale* group are large (6–8mm) *Bembidion* occurring along creek and river shores throughout much of western North America, east across Canada and adjacent United States to Nova Scotia and Newfoundland. A recent catalogue (1993) lists two species within the group, *B. pernotum* Casey (known from Colorado and New Mexico) and the widespread *B. transversale* Dejean.

The related *Bembidion mexicanum* group contains very similar members whose combined range extends from South Dakota to Central America; in the United States this group is considered to consist of only the nominotypical species (Bousquet and Laroche 1993).

In sequencing 28S rDNA (28S) and cytochrome oxidase I (COI) from specimens of the *B. transversale* group from several North American localities, and examination of morphological characters, it became evident that “*B. transversale*” is in reality several species.

This paper is the first of several that will attempt to resolve the complexity of both the *B. transversale* and *B. mexicanum* groups. We herein report the discovery that the common form of the *B. transversale* group throughout much of the southwest United States is a separate species, *B. perspicuum* LeConte, distinct both morphologically and molecularly from the widespread and more northern *B. transversale*. We also briefly document the other species in the two species groups, so that a revised classification is available for future publications. A later paper (Maddison, in prep.) will revise the group in America north of México, including forms along the west coast of North America, and give a fuller treatment of all species. In advance of this more complete revision, however, we wish to document the distinctiveness of *B. perspicuum*, as this is one of the primary species studied in a forthcoming paper (Wild, Kanda, McKenna, Farrell, and Maddison, in prep.).

Methods

Members of the *B. transversale* and *B. mexicanum* groups were examined from or are deposited in the collections listed below. Each collection’s listing begins with the coden used in the text.

- DRM** David R. Maddison, Department of Zoology, Oregon State University, Corvallis, OR, 97331 U.S.A.
MCZ Museum of Comparative Zoology, Harvard University
MNHN Muséum National d’Histoire Naturelle, Paris
OSAC Oregon State Arthropod Collection, Oregon State University
USNM National Museum of Natural History, Smithsonian Institution
ZMUM Zoological Museum, Moscow State University

Methods for studying adult structures, and terms used, are given in Maddison (1993). Photographs of body parts were taken with a Leica Z6 and JVC KY-F75U camera using Microvision's Cartograph software for extended depth of field (EDF) processing; the images thus potentially have some artifacts caused by the EDF algorithm.

In the section on classification, we do not list which junior synonyms are so considered for the first time. All names listed under *B. transversale* have previously been considered synonyms of *B. transversale*; the same applies to *B. mexicanum*. However, we have not conducted the historical analysis required to determine if any of the names herein considered junior synonyms of *B. lugubre* have ever been considered as such previously, although they and *B. lugubre* have been considered synonyms of *B. mexicanum*; the same applies to *B. perspicuum* and *B. sarpedon* (which have been considered synonyms of *B. transversale*, as have all of their synonyms).

Taxon sampling for DNA studies. We sequenced DNA from 28 specimens of the *B. transversale* group from Nova Scotia, Alberta, British Columbia, as well as 10 states in western U.S.A. (Table 1). We have included one specimen each of *B. mexicanum*, *B. commotum* Casey, and *B. sejunctum semiaureum* Fall as outgroups to this study. Preliminary analyses of multiple genes across *Bembidion* (Maddison, unpublished) indicate that the *B. mexicanum* group and *B. transversale* group are closely related, forming a clade along with a few other members of *Ocydromus* (*sens. lat.*), including *B. commotum* and *B. sejunctum*. Vouchers are housed in the David Maddison voucher collection at Oregon State University.

DNA sequencing. Methods for obtaining DNA sequences are described in Maddison (2008). In brief, we obtained ca. 1000 bases of sequence data in the D1 through D3 domains of 28S and between 600 and 1400 bases of COI. Fragments for these genes were amplified using the Polymerase Chain Reaction on an Eppendorf Mastercycler Thermal Cycler, using either Eppendorf Hotmaster Taq or TaKaRa Ex Taq and the basic protocols recommended by the manufacturers. Primers and details of the cycling reactions used are given in Maddison (2008). In particular, we used the primer pair LS58F and LS998R and the pair NLF184/21 and LS1041R to amplify and sequence 28S rDNA. For COI, two amplification strategies were used: amplification with B1490 and Pat, and sequencing with those primers plus Bcoi2R and Jerry (see Maddison, 2008), or amplification and sequencing with the LCO1490 and HCO2198 primers (Hebert et al. 2003). Amplified products were cleaned, quantified, 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 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 2009a; Maddison and Maddison 2009b), with subsequent modifications by Chromaseq and manual inspection. Multiple peaks at a single position in both reads were coded using IUPAC ambiguity codes.

Table 1. Specimens examined for 28S rDNA and COI. #: Maddison lab DNA voucher number. Sex: male (“m”) or female (“f”). St: State or Province.

Species	#	Sex	St	Locality	Latitude	Longitude
<i>B. transversale</i>	2161	m	AB	Lethbridge	49.7043°N	112.866°W
<i>B. transversale</i>	2186	m	AB	Lethbridge	49.7043°N	112.866°W
<i>B. transversale</i>	2191	f	BC	Creston	49.1395°N	116.6489°W
<i>B. transversale</i>	2164	f	ID	Baker Creek	43.7511°N	114.5627°W
<i>B. transversale</i>	2184	f	ID	Baker Creek	43.7511°N	114.5627°W
<i>B. transversale</i>	2163	m	MT	Divide Creek	48.7314°N	113.4216°W
<i>B. transversale</i>	2185	m	MT	Galena Gulch	46.2562°N	112.1843°W
<i>B. transversale</i>	2359	m	MT	Bozeman	45.7077°N	110.9743°W
<i>B. transversale</i>	2158	f	NS	Bass River	45.4125°N	64.0560°W
<i>B. transversale</i>	2160	m	NS	Hantsport	45.0487°N	64.1835°W
<i>B. transversale</i>	2346	m	NV	Dunphy	40.7052°N	116.5312°W
<i>B. transversale</i>	2183	f	UT	Diamond Fork	40.0737°N	111.426°W
<i>B. transversale</i>	2251	f	UT	Diamond Fork	40.0737°N	111.426°W
<i>B. transversale</i>	2097	m	WY	Laramie	41.2897°N	105.6224°W
<i>B. transversale</i>	2157	m	WY	Laramie	41.2897°N	105.6224°W
<i>B. transversale</i>	2481	m	CO	Texas Creek	38.4106°N	105.5844°W
<i>B. transversale</i>	2486	m	CO	Texas Creek	38.4106°N	105.5844°W
<i>B. perspicuum</i>	2482	m	CO	Texas Creek	38.4106°N	105.5844°W
<i>B. perspicuum</i>	2485	m	CO	Texas Creek	38.4106°N	105.5844°W
<i>B. perspicuum</i>	1120	f	AZ	Charleston	31.6300°N	110.1774°W
<i>B. perspicuum</i>	2156	m	AZ	McGuireville	34.6376°N	111.813°W
<i>B. perspicuum</i>	2159	f	AZ	Mammoth	32.7413°N	110.6458°W
<i>B. perspicuum</i>	2182	m	NM	Gila	32.9691°N	108.5872°W
<i>B. perspicuum</i>	2173	m	NV	Weeks	39.2866°N	119.2778°W
<i>B. perspicuum</i>	2318	m	CA	Miller Canyon	34.2717°N	117.2892°W
<i>B. perspicuum</i>	2319	m	CA	Pine Creek	32.8548°N	116.5228°W
<i>B. perspicuum</i>	2320	m	CA	Pine Creek	32.8548°N	116.5228°W
<i>B. perspicuum</i>	2321	m	CA	Pine Creek	32.8548°N	116.5228°W
<i>B. mexicanum</i>	2192	m	NM	Bonito Creek	33.4557°N	105.7470°W
<i>B. commotum</i>	2136	m	CA	Sonora Pass	38.3323°N	119.6401°W
<i>B. sejunctum</i>	1817	f	WA	Ilwaco	46.29°N	124.08°W

Sequences have been deposited in GenBank with accession numbers GU454737 through GU454797. In addition, the 28S sequence for *B. transversale* voucher number DNA2157 is from GenBank, number EU677688.

Alignment and phylogenetic analysis

Alignment was not difficult for either gene, as there was no evidence of insertion or deletions in the history of these sequences. Aligned matrices are available at <http://bembidion.org/transversaleGroup>. Three matrices were examined: (1) 28S rDNA; (2)

COI with all data; (3) COI with only the first ca. 600 bases that were sequenced for all specimens, the so-called “barcode” piece (Hebert, Cywinska, Ball and DeWaard 2003). The second matrix (full-length COI) has the last half of COI missing for 10 of the 28 *B. transversale* group specimens as well as *B. sejunctum* and *B. commotum*; only 77.7% of the cells have data in them, as opposed to 98.6% for the shorter COI matrix and 99.6% for the 28S matrix. Phylogenetic analysis was conducted using parsimony, likelihood, and Bayesian methods.

Most-parsimonious trees were sought using PAUP* (Swofford 2002). For each search, 4000 replicates were conducted, each beginning with a starting tree formed with the random addition sequence option; each replicate was allowed to save no more than 25 trees. The number of most parsimonious trees found for each matrix ranged from 2 to 63,341; shortest trees were found in at least 2000 of the replicates. Bootstrap parsimony analyses were conducted using 1000 bootstrap replicates, with each replicate consisting of five search replicates beginning with starting trees formed with the random addition sequence option, and with each search replicate allowed to save no more than 25 trees.

Models of nucleotide evolution were chosen with the aid of ModelTest (Posada 2005). The model chosen by the Akaike Information Criterion (AIC) for 28S rDNA was an HKY 2-parameter rate matrix with a proportion of sites being invariant (HKY+I). For COI, models were inferred treating each codon position separately as well as pooling all positions; phylogenetic analyses were conducted under both model structures. First positions are best fit by a General Time Reversible (GTR) rate matrix with a proportion of sites being invariant (GTR+I); second positions a 1-parameter F81 rate matrix with a proportion of invariant sites (F81+I), and third positions with an HKY rate matrix and site-to-site rate variation following a gamma distribution (HKY+ Γ). If instead all positions were pooled for COI, the model chosen was GTR+I.

Maximum Likelihood analyses were conducted with GARLI version 0.96 (Zwickl 2006). A GTR+I model was used for all COI sites. The maximum likelihood tree was sought using 500 search replicates. Maximum likelihood bootstrap analyses with 500 replicates were also conducted for all three matrices.

Bayesian analyses were conducted using MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2005). For each of the two COI matrices, the analyses were done both with separate models for each codon position, as well as GTR+I for all sites; thus, four Bayesian analyses were conducted for COI and one for 28S. A single MrBayes MCMCMC search was conducted for each analysis, each with two runs of four chains each, for 10 million generations, with trees sampled every 1000 generations. All searches converged, as judged by an average standard deviation of split frequencies of less than 0.006 (Huelsenbeck and Ronquist 2005) and by stabilization of the likelihood scores and all parameter values as judged by visualization tools in Tracer (Rambaut and Drummond 2004), except for the full-length COI matrix with codon positions treated separately. That analysis did not converge after 100 million generations, and was discarded. A burn-in sample of the first 7.5 million generations was excluded from the other analyses; the number of trees sampled for each analysis was thus 5,000.

Results of molecular analyses

The two forms, *B. transversale* and *B. perspicuum*, consistently differ by 4 bases in 28S, and 40 bases in COI (of which 14 fall within the first 600 bases of the gene; Table 2). The base differences in COI yield predictions of two sites at which amino acids differ in the protein. These fixed differences in both genes are also evident between the two specimens of *B. transversale* and two of *B. perspicuum* sampled microsympatrically from the same shore of the Arkansas River at Texas Creek, Colorado (38.4106°N 105.5844°W).

As a cautionary note, there is evidence (in the form of double peaks at sites in the chromatograms of both reads) for multiple copies of COI in one *Bembidion perspicuum* from Arizona (specimen 1120), one *B. transversale* from Montana (specimen 2163) and another from Utah (specimen 2183). This is likely to indicate nuclear copies of COI, and casts some doubt on the location of any particular sequenced copy. Nonetheless, the consistency within each species in the sequenced COI provides evidence of lack of gene flow even if some copies are nuclear.

The phylogenetic analyses (Fig. 1; Table 3) indicate clearly that the two forms are reciprocally monophyletic. This is supported in all analyses for both genes. The geographic distributions of sequenced individuals of these two forms are shown in Fig. 2.

We should note that while it appears from Fig. 1B that the California specimens of *B. perspicuum* are distinctive, there are no nucleotide sites in either gene at which there are consistent differences between California specimens and the remainder.

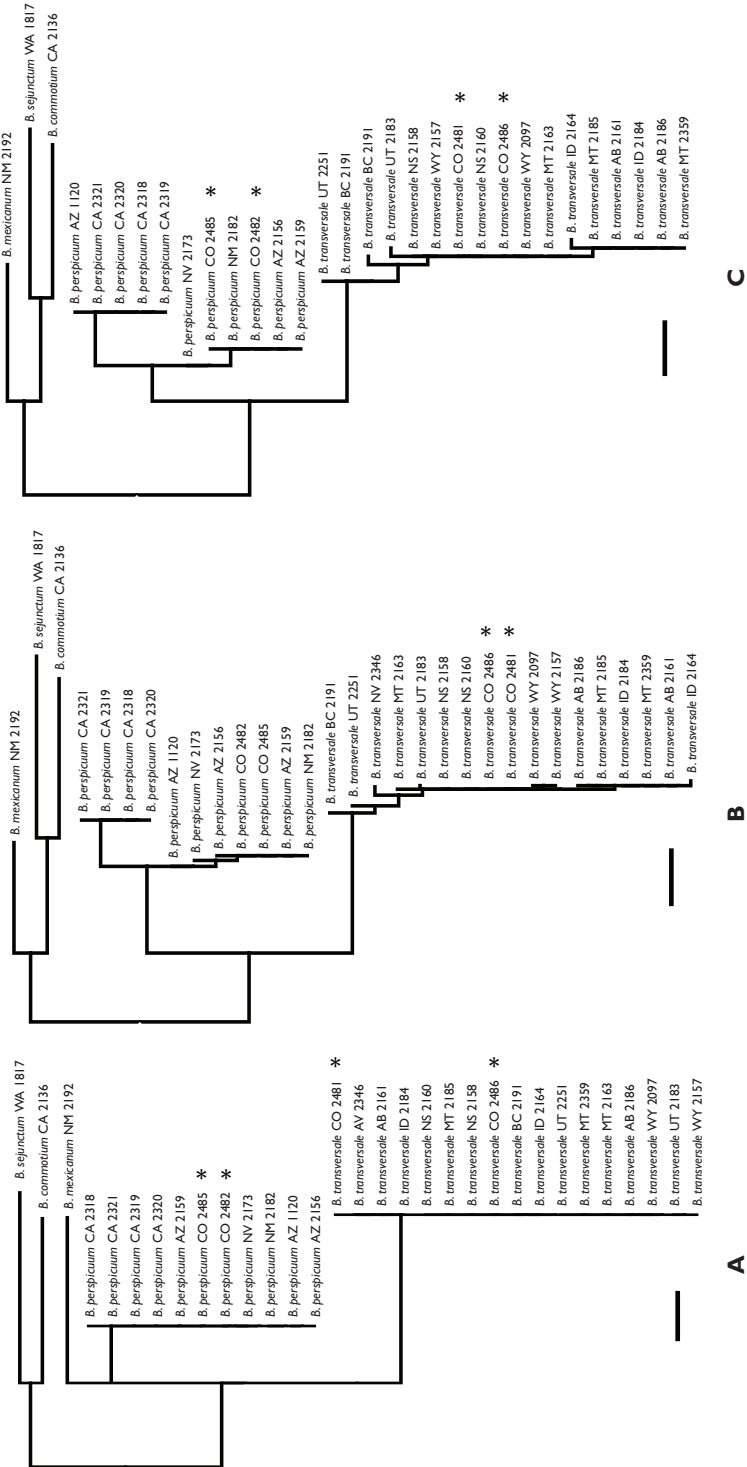
Morphological differences

Once the two forms became evident from the molecular analyses, specimens were separated and examined for morphological differences. *B. transversale* shows more contrast in the dorsal color pattern, and *B. perspicuum* tends to have a more orange hue (Fig. 3).

Table 2. Number of fixed differences between *B. transversale* and *B. perspicuum* for each gene. Only those specimens whose sequences were complete for the length indicated where used in the comparison. For example, there are 40 sites in the entire COI gene at which all 13 examined *B. transversale* have a different nucleotide than all 5 examined *B. perspicuum*, and these nucleotide differences imply two consistent differences in amino acids.

	28S	COI (entire)	COI (first portion)
<i>B. transversale</i> , n=	17	13	17
Fixed nucleotide differences	4	40	14
Fixed amino acid differences	–	2	0
Total nucleotides	914	1417	614
<i>B. perspicuum</i> , n=	11	5	11

Figure 1. Maximum likelihood trees inferred from gene sequences. Branch lengths as reconstructed by Garli. **A** 28s rDNA; scale bar 0.001 substitutions per site **B** COI, all sites; scale bar 0.01 substitutions per site **C** COI, first ca. 600 sites; scale bar 0.01 substitutions per site. Asterisks indicate specimens collected microsym-
 patrically on a gravel bank along the shores of the Arkansas River at Texas Creek, Colorado (38.4106°N 105.5844°W).



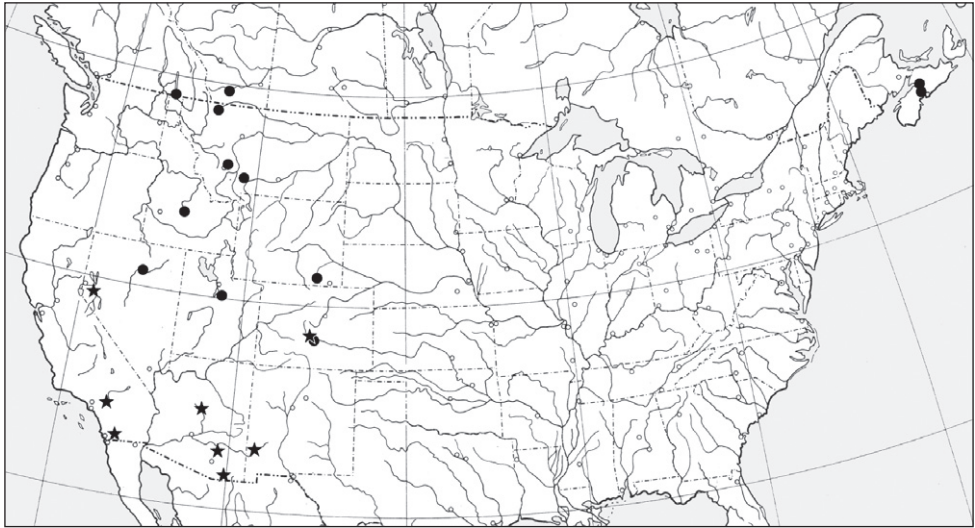


Figure 2. Geographic distribution of specimens sampled for DNA sequences. Circles: *Bembidion transversale*; stars: *Bembidion perspicuum*. More complete data for these localities are available at <http://bembidion.org/transversaleGroup/>, as a KML file.

Table 3. Support values for monophyly of each of *B. transversale* and *B. perspicuum*. BPP: Bayesian Posterior Probability; ML Boot: likelihood bootstrap percentage; Pars Boot: parsimony bootstrap percentage. “partitioned” indicates those analyses for which the gene was partitioned into codon positions with different models used for first, second, and third positions, as opposed to analyses in which all positions were pooled, and subject to a single model.

		<i>B. transversale</i>	<i>B. perspicuum</i>
28S	BPP	0.78	0.76
	ML Boot	96	65
	Pars Boot	98	65
COI (entire)	BPP (partitioned)	–	–
	BPP (pooled)	1.00	0.99
	ML Boot	99	95
	Pars Boot	100	100
COI (partial)	BPP (partitioned)	1.00	0.96
	BPP (pooled)	1.00	0.72
	ML Boot	97	68
	Pars Boot	100	94

The most consistent external differences are in the pronotum: *B. transversale* has a slightly more convex and cordate pronotum, with a smoother basal region, in contrast to the flatter pronotum of *B. perspicuum*, which has less rounded sides, and with the basal region more evidently punctate (Fig. 4). In the localities from which DNA sequences were obtained (Fig. 2), specimens of *B. transversale* have in addition a very

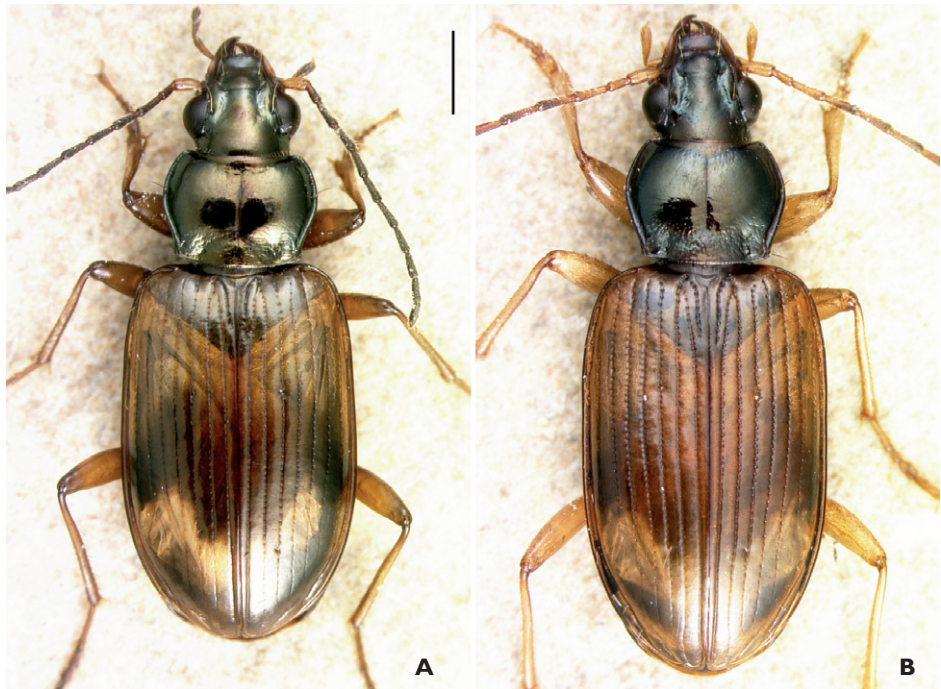


Figure 3. Adult habitus. **A** *B. transversale*, DNA2481, USA: Colorado: Fremont Co., Arkansas River at Texas Creek, 38.4106°N 105.5844°W **B** *B. perspicuum*, DRM Voucher V100552, USA: Colorado: Huerfano Co., Huerfano River at Badito, 37.7285°N 105.0167°W. Scale bar is 1 mm.

distinctive mentum, with much reduced lateral lobes (Fig. 5A), in contrast to the more normal mentum and typical lateral lobes of *B. perspicuum* (Fig. 5B). However, there are specimens in the west (western British Columbia south to California) that match *B. transversale* in all regards, including in DNA sequences and other morphological characters, and yet have a more typical mentum with large lateral lobes (Maddison, unpublished). In addition to these differences in external traits between *B. transversale* and *B. perspicuum*, the ostial flag of *B. transversale* extends further ventrally and has a more gentle curvature (arrow in Fig. 6A) than that of *B. perspicuum* (arrow in Fig. 6C).

The differences observed in morphological characters, congruent with the consistent molecular differences between two unlinked genes, even where the two forms are microsympatric in Colorado, provides strong evidence that the two forms are not exchanging genes, and are two different species.

As an aid for identification, *Bembidion perspicuum* can be incorporated into Lindroth's (1963) key by replacing couplet 145 (on page 223) with the following two couplets:

- 145. Prothorax (Figs. 168a–b) without or with very faint, oblique latero-basal carina ... 145A
- Prothorax with latero-basal carina well developed, less oblique..... 146

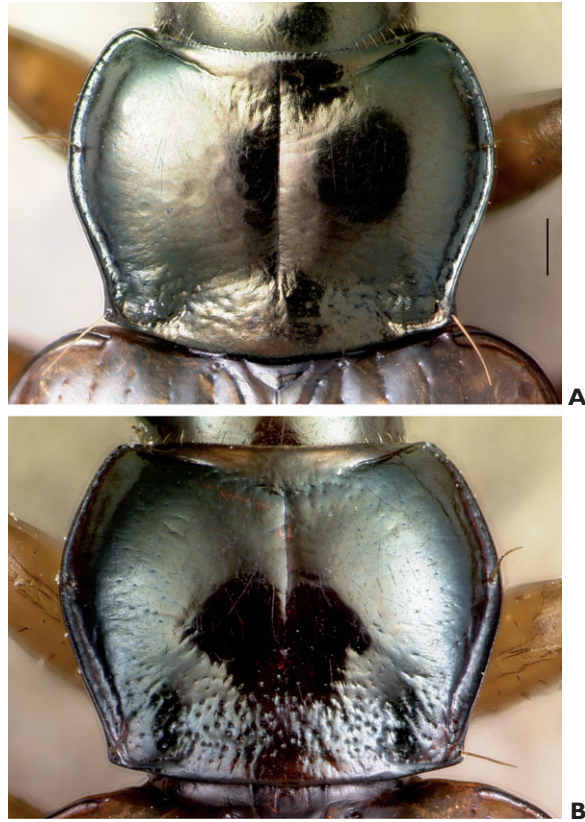


Figure 4. Dorsal view of pronotum. **A** *B. transversale*, DNA2481, USA: Colorado: Fremont Co., Arkansas River at Texas Creek, 38.4106°N 105.5844°W **B** *B. perspicuum*, DRM Voucher V100552, USA: Colorado: Huerfano Co., Huerfano River at Badito, 37.7285°N 105.0167°W. Scale bar is 0.25 mm.

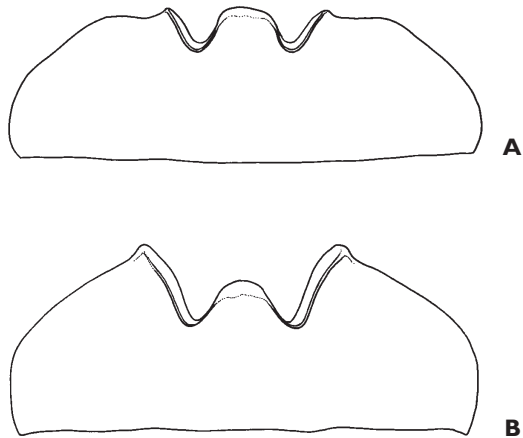


Figure 5. Mentum, ventral view. Both specimens from USA: Colorado: Fremont Co., Arkansas River at Texas Creek, 38.4106°N 105.5844°W. **A** *B. transversale*, DRM Voucher V100555 **B** *B. perspicuum*, DRM Voucher V100556.

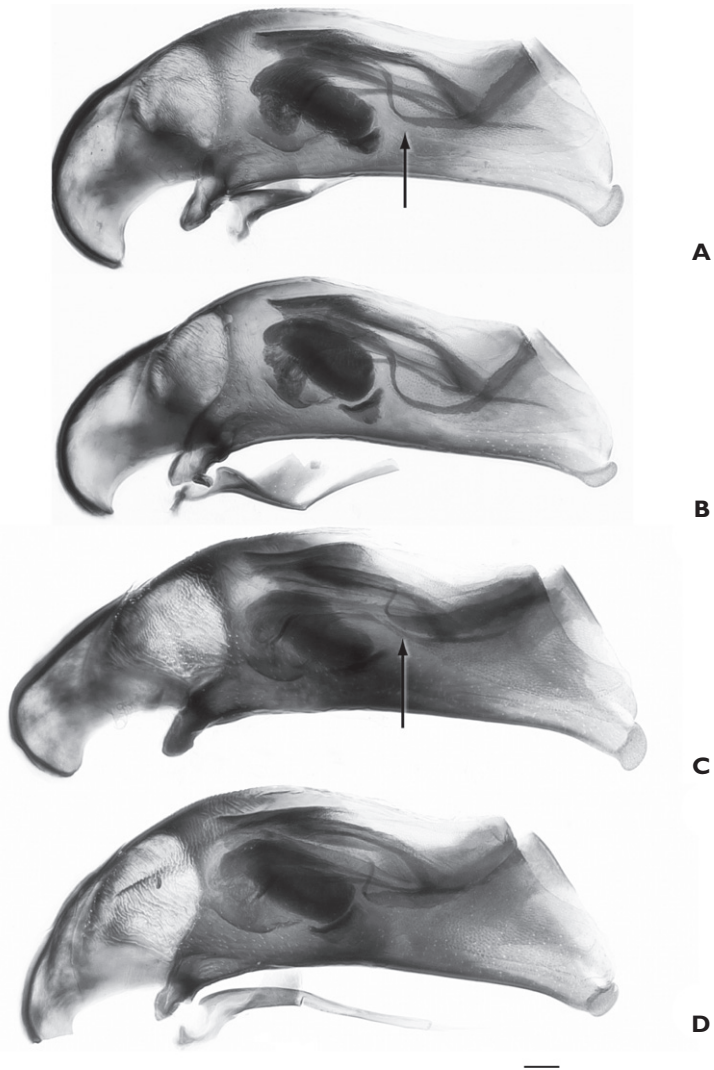


Figure 6. Male aedeagus, left lateral view. All specimens from USA: Colorado: Fremont Co., Arkansas River at Texas Creek, 38.4106°N 105.5844°W. **A** *Bembidion transversale*, DRM Voucher DNA2481 **B** *B. transversale*, DRM Voucher DNA2486 **C** *B. perspicuum* DRM Voucher DNA2482 **D** *B. perspicuum* DRM Voucher DNA2485. All to same scale; scale bar is 0.1 mm. Arrows indicate the ostial flag, which differs in form between the species.

- 145A. Prothorax slightly more cordate, flatter, with a smoother basal region (this paper, Fig. 4A); aedeagus with ostial flag extending further ventrally, and with gentler curvature (this paper, Fig. 6A)*B. transversale*
 – Prothorax slightly less cordate, with more notable punctures in the basal region (this paper, Fig. 4B); aedeagus with ostial flag more dorsal, and with a more abrupt curve at its anterior end (this paper, Fig. 6B) *B. perspicuum*

Classification and species notes

Our studies indicate that the *Bembidion transversale* and *B. mexicanum* groups contain a combined six species in America north of México:

B. transversale group

B. transversale Dejean

B. perspicuum LeConte

B. sarpedon Casey

B. mexicanum group

B. mexicanum Dejean

B. lugubre LeConte

B. pernotum Casey

Bembidion transversale Dejean, 1831

Bembidium transversale Dejean, 1831:110. Type locality restricted to Nipigon, Ontario, by Lindroth (Lindroth 1963). Holotype female, in MNHN, examined by Kipling Will, who provided to us photographs, including of the mentum, allowing us to confirm the identification.

Peryphus eros Motschulsky, 1850:10. Type locality California. Lectotype, designated by Bousquet and Larochelle (1993), in ZMUM. Synonymy tentative.

Ochthedromus mannerheimii LeConte, 1852:190. Type locality San Diego, California. Three specimens in the MCZ in the *Bembidion transversale* unit tray in the LeConte collection have gold discs (signifying California), and include syntypes for this name. The first specimen is a female labeled as follows: [gold dot] / “O. mannerheimii Lec. S. D. transversale ‡ Man” [in LeConte’s handwriting] / “transversale 27” [handwritten], and is certainly a syntype. The two following specimens, a male and female, are labeled with a gold dot in addition to a label of “transversale 28” and “transversale 29” respectively, and are quite possibly also syntypes. As the first specimen is the only one that is surely a syntype, it is herein designated as the lectotype, MCZ type number 35571. All specimens externally match the western form (see below) of *B. transversale*. We have examined the aedeagus of the male, and it matches that of *B. transversale*.

Bembidium haplogonum Chaudoir, 1868: 241. Type locality California. Location of types unknown. Synonymy tentative.

Bembidion marinicum Casey, 1918:57. Type locality Marin County, California. Holotype female in USNM (type number 36919), examined. Synonymy tentative.

Bembidion tuolumne Casey, 1924:30. Type locality Tuolumne, California. Lectotype male, designated by Lindroth (1975), in USNM (type number 36917), examined. Synonymy tentative.

As currently conceived, this species is a mix of different forms. The typical form (including the holotype of *B. transversale* Dejean, and all specimens sequenced for this

study) has an unusual mentum with much-reduced lateral lobes, which do not project anteriorly much beyond the mental tooth (Fig. 5A). This form occurs from Nova Scotia west to Alaska, south through eastern British Columbia, eastern Oregon, Idaho, Nevada, Utah, Colorado, and Wyoming. Western British Columbia, western Oregon, and California contain another form, with no consistent differences in COI or 28S from the typical form (Maddison, unpublished), with a normal *B. transversale* pronotum and aedeagus, but with full lateral lobes on the mentum. The mentum of this western form is thus similar to that of *B. perspicuum*, Fig. 5B, and in contrast to eastern *B. transversale*. The western form itself is rather variable morphologically, and is being examined in more detail (Maddison, in prep.). It may be a complex of multiple species, and thus the five younger names mentioned above are only tentatively synonymous with *B. transversale*. In addition, while it is clear that the LeConte name and the two Casey names are not synonyms of *B. perspicuum*, the Motschulsky and Chaudoir names might be junior synonyms of *B. perspicuum*; examination of the types will be required to confirm the synonymies.

Morphological characters distinguishing these species from *B. perspicuum* are described above, under “Morphological Differences”.

***Bembidion perspicuum* LeConte, 1848**

Ochthedromus perspicuus LeConte, 1848: 466. Type locality “Rocky Mountains”. Holotype male, in MCZ (type number 5510), external structure and aedeagus examined.

Bembidion acomanum Casey, 1918: 59. Type locality Jemez Springs, New Mexico, so restricted by Lindroth (1975). Lectotype female, designated by Lindroth (1975), in USNM (type number 36916), examined.

Bembidion excursum Casey, 1918: 59. Type locality Tucson, Arizona. Holotype female, in USNM (type 36915), examined.

This species is known from Texas, Kansas, Colorado, New Mexico, Arizona, Utah, Nevada, California, and Oregon. Its range in México has not been examined. Specimens from northern California and Oregon are dark, with only elytral apices being pale. They are thus very similar in appearance to paler specimens of *Bembidion lugubre*, from which they are most readily distinguished by genitalic characters.

***Bembidion sarpedon* Casey 1918**

Bembidion sarpedon Casey, 1918: 58. Type locality Cañon City, Colorado. Lectotype male, designated by Lindroth (1975), in USNM (type number 36914); external structure and aedeagus examined.

Bembidion animatum Casey, 1918: 62. Type locality Jemez Springs, New Mexico, so restricted by Lindroth (1975). Lectotype female, designated by Lindroth (1975), in USNM (type number 36918), examined.

This species is known from northeastern Arizona, Utah, northern New Mexico, Colorado, and Wyoming. It is characterized by a shiny, slightly more cordate pronotum than other members of the group, without notable basal punctuation, relatively flat elytral intervals, with small punctures in the elytral striae, and distinctive male genitalia. As first revisers, we choose *B. sarpedon* Casey as the valid name of this species.

***B. mexicanum* Dejean, 1831**

Bembidium mexicanum Dejean, 1831:126. Type locality México. Lectotype male, designated by Erwin (1982), in MNHN; external structure and aedeagus examined.

Bembidium stabile LeConte, 1879: 508. Type locality La Veta, Colorado. Lectotype male, designated by Erwin (1984), in MCZ; external structure and aedeagus examined.

Bembidion lugubre vafrum Casey, 1918:60. Type locality Arizona. Lectotype male, designated by Erwin (1984), in USNM; external structure and aedeagus examined.

Bembidion badiipenne Casey, 1918: 60. Type locality between Fort Wingate and Jemez Springs, New Mexico. Lectotype female, designated by Erwin (1984), in USNM (type number 36913); external structure examined (spermatheca absent, as most of the abdomen is missing).

This species is known from South Dakota, Colorado, New Mexico, and Arizona, south at least to Guatemala. In the United States, specimens have unicolorous, dark elytra, a smoother pronotum, and tend to be larger than those of *B. lugubre*; some specimens from México and Central America have pale elytral apices. The two species can only be reliably distinguished by characters of the male genitalia, female spermatheca, or DNA sequences. The two species are microsympatric, occurring together on the same creek or river shore at numerous localities in Arizona and New Mexico.

***B. lugubre* LeConte, 1857**

Bembidium lugubre LeConte, 1857:6, described as a “variety” of *B. mexicanum* Dejean. Type locality “Valley of the Rio Grande”. Holotype female in MCZ (type number 5511); external structure and spermatheca examined.

Bembidium sallaei Bates, 1882:148. Type locality not specified, with localities specified in several states of México as well as Belize and Guatemala. Lectotype male, designated by Erwin (1982), in MNHN; external structure and aedeagus examined.

Bembidion canonicum Casey, 1918:61. Type locality Arizona. Holotype male in USNM (type number 36920); external structure and aedeagus examined.

Bembidion retectum Casey, 1918:61. Type locality St George, Utah. Lectotype male, designated by Erwin (1984), in USNM (type number 36923); external structure and aedeagus examined.

This species is known from Texas, New Mexico, Arizona, Utah, and California south at least to Oaxaca, México. In the United States specimens of this species tend to be paler than those of *B. mexicanum*, with some specimens having a pale elytral apex, and with more notable punctures at the base of the pronotum. Male genitalia, female spermathecae, and DNA sequences are distinctive.

***B. pernotum* Casey, 1918**

Bembidion pernotum Casey, 1918: 62. Type locality Jemez Springs, New Mexico. Lectotype female, designated by Erwin (1984), in USNM (type number 36922), examined.

This species is known from northern New Mexico and southern Colorado. Individuals are colored similarly to *B. transversale*, but are brighter and more reddish, and are more convex and shinier than other members of the group, with larger punctures in the elytral striae, and more convex intervals. While their color pattern is similar to members of the *B. transversale* group, male genitalia and DNA sequences indicate a much closer relationship to *B. mexicanum* and *B. lugubre*.

Acknowledgements

For aid in collecting specimens, we thank A. Elizabeth Arnold (University of Arizona, Tucson), Wayne P. Maddison and Julia H. Amerongen Maddison (University of British Columbia, Vancouver), Janine N. Caira (University of Connecticut, Storrs), Kathleen L. Prudic and Jeffrey C. Oliver (Yale University, New Haven), Kipling W. Will (University of California at Berkeley), Ainsley Seago (CSIRO, Australia), Matthew C. Brandley (Yale University, New Haven), and Michael A. Ivie (Montana State University, Bozeman).

For making the specimens in their care available, we heartily thank Thierry Deuve (MNHN), Terry L. Erwin (USNM), and Philip D. Perkins (MCZ).

We also thank two anonymous reviewers for their helpful comments.

Kip Will's examination and photography of the type of *Bembidum transversale* Dejean was necessary to confirm the valid name of the northern species, and we are most grateful to him for his efforts. In addition, we are very thankful to Yves Bousquet (Agriculture and Agri-Food Canada) for his help in clarifying the status of the syntypes of *Ochthedromus mannerheimii* LeConte.

References

Bates HW (1882) Insecta. Coleoptera. Vol. I. Part 1. In Godman FD, Salvin O (Eds) *Biologia Centrali-Americana*. Taylor and Francis, London, 41–152.

- Bousquet Y, Laroche A (1993) Catalogue of the Geodephaga (Coleoptera: Trachypachidae, Rhysodidae, Carabidae including Cicindelini) of America north of Mexico. *Memoirs of the Entomological Society of Canada* 167: 1–397.
- Casey TL (1918) A review of the North American Bembidiinae. *Memoirs on the Coleoptera* 8: 1–223.
- Casey TL (1924) Additions to the known Coleoptera of North America. *Memoirs on the Coleoptera* 11: 1–347.
- Chaudoir M, de (1868) Observations synonymiques sur les Carabiques de l'Amérique septentrionale et descriptions d'espèces nouvelles de ce pays. *Revue et Magazin de Zoologie, Series 2* 20: 239–245.
- Dejean PFMA (1831) *Spécies général des Coléoptères de la collection de M. le Comte Dejean*. Paris, I–VIII, 1–384 pp.
- Erwin TL (1982) Small terrestrial ground-beetles of Central America (Carabidae: Bembidiina and Anillina). *Proceedings of the California Academy of Sciences* 42: 455–496.
- 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. *Pan-Pacific Entomologist* 60: 165–187.
- Green P (1999) Phrap. Version 0.990329. <http://phrap.org>
- Green P, Ewing B (2002) Phred. Version 0.020425c. <http://phrap.org>
- Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B-Biological Sciences* 270: 313–321.
- Huelsenbeck J, Ronquist F (2005) MrBayes. Version 3.1.2. <http://www.mrbayes.net>
- LeConte JL (1848) A descriptive catalogue of the geodephagous Coleoptera inhabiting the United States east of the Rocky Mountains. *Annals of the Lyceum of Natural History of New York* 4: 173–233, 334–474.
- LeConte JL (1852) Descriptions of new species of Coleoptera, from California. *Annals of the Lyceum of Natural History of New York* 5: 185–216.
- LeConte JL (1857) Catalogue of the species of *Bembidium* found in the United States and contiguous northern regions. *Proceedings of the Academy of Natural Sciences of Philadelphia* 9: 2–6.
- LeConte JL (1879) The Coleoptera of the alpine Rocky Mountain regions. – Part II. *Bulletin of the United States Geological and Geographical Survey of the Territories* 5: 499–520.
- Lindroth CH (1963) The ground-beetles (Carabidae, excl. Cicindelinae) of Canada and Alaska. Part 3. *Opuscula Entomologica Supplementum XXIV*: 201–408.
- Lindroth CH (1975) Designation of holotypes and lectotypes among ground beetles (Coleoptera, Carabidae) described by Thomas 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 (2008) Systematics of the North American beetle subgenus *Pseudoperiphys* (Coleoptera: Carabidae: *Bembidion*) based upon morphological, chromosomal, and molecular data. *Annals of Carnegie Museum* 77: 147–193.

- Maddison DR, Maddison WP (2009a) Chromoseq: a Mesquite module for analyzing sequence chromatograms. Version 0.97. <http://mesquiteproject.org/packages/chromaseq>
- Maddison WP, Maddison DR (2009b) Mesquite: a modular system for evolutionary analysis. Version 2.71. <http://mesquiteproject.org>
- Motschulsky TV, de (1850) Die Kaefer Russlands. Gautier, Moscow, iv + xi + 91 pp.
- Posada D (2005) Modeltest: A tool to select the best-fit model of nucleotide substitution. Version 3.7. <http://darwin.uvigo.es>
- Rambaut A, Drummond A (2004) Tracer. Version 1.3. <http://evolve.zoo.ox.ac.uk/software.html?id=tracer>
- Swofford DL (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10. Sinauer Associates.
- Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD Dissertation, Austin, Texas: The University of Texas at Austin.