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Investigations

Species Discovery and Delimitation in Ground Beetles of the Subgenus *Trepanedoris* (Coleoptera: Carabidae: *Bembidion*)

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Abstract

We infer species boundaries in small ground beetles of the genus *Bembidion*, presenting the discoveries made by a class of students in an experiential learning course at Oregon State University. Focusing on the *Bembidion connivens* group of subgenus *Trepanedoris*, we integrate data from external structure and male genitalia, DNA sequences of one mitochondrial gene and six nuclear genes, and geographic distribution. We analyze the DNA data using Bayesian multispecies coalescent methods to help infer reproductive communities or species; SPEEDEMON tended to split species more than what our integrated approach inferred, whereas DELINEATE tended to lump more than synthetic inference. We infer 12 species in North America outside of California, including four new species: *Bembidion altipaludis* Maddison (mountain marshes of the Cascades, Sierra Nevada, and Klamath Mountains), *B. endeca* Maddison & Sproul (Oregon east of the Cascades and the eastern edge of the Sierra Nevada in California and Nevada), *B. anacalypsi* Mendez & Maddison (northeastern Oregon and western Idaho), and *B. kieranae* Maddison & Sproul (Willamette Valley of Oregon). In addition, we document morphological features of all 12 species, and present tools to enable identification.

Introduction

Smaller insects in many areas of the world are relatively poorly known. Research about their diversity often results in numerous discoveries in short order, including of new species. Thus, research projects of only a few months in length can be productive, making them suitable for an experiential course offered by an educational institution. Engaging students in all steps in this research, including collecting specimens, gathering data about their variation, and analyzing the data, can allow students to gain a better understanding of exploratory science. The students also gain a greater appreciation of science in general, in part through the feelings of excitement and accomplishment surrounding discovery.

Courses of this sort are not only valuable for the students; they can also yield valuable scientific results. This paper presents the results of research conducted during a course at Oregon State University that focused on biodiversity of beetles.

In the spring of 2015, the first two authors ran an undergraduate course called "Discovering Insect Species" (D. R. Maddison & Smith, 2026). In this course, nine undergraduate students (including the third and fourth authors) were embedded in the early stages of a research project about the diversity of subgenus *Trepanedoris*, part of the large and worldwide ground beetle genus *Bembidion*. *Bembidion* are small, predacious, terrestrial beetles, with the majority of the approximately 1,400 known species occurring along the edges of bodies of water. *Trepanedoris* are typically found in marshes, wet meadows, or the edges of slowly moving waterways (Fig. 1).

During the term of the course, the eleven of us went on field trips, including two multi-day trips to the marshes east of the Cascade Mountains in Oregon. We prepared specimens and examined them morphologically, imaged them, extracted their DNA and sequenced it, and analyzed the data phylogenetically (Figs 2, 3). On our trip to Klamath Marsh National Wildlife Refuge, we found two specimens we initially thought belonged to a known but undescribed species (here named *Bembidion altipaludis*, n. sp.). After extracting their DNA back in the lab, amplifying various genes using Polymerase Chain Reaction, and sending the products to be sequenced, we downloaded and processed the data in class. We watched together as phylogenetic analyses revealed these to be the first recognized specimens of yet another new species. The students thus fully shared in the moments of discovery, both in the field and in the lab. This new species is here named *Bembidion endeca*, n. sp. (Fig. 4), after the team of eleven people participating in the course.

Figure 1. Habitats of *Trepanedoris*.

a: Location of the class's first capture of *Bembidion endeca*. Habitat as well of *B. fortestriatum*, *B. concretum*, *B.* sp. nr. *acutifrons*, and *B. (Diplocampa) transparens* (Gebler). USA: Oregon: Klamath Co., Klamath Marsh National Wildlife Refuge, Williamson River, 42.9650°N 121.5805°W, 1382m, 10 April 2015. **b**: Type locality of *Bembidion endeca*. Habitat as well of *B. acutifrons*. USA: Oregon: Harney Co., Bridge Creek Canal pond, Malheur National Wildlife Refuge, 42.8653°N 118.8793°W, 1267m, 16 May 2015. **c**: Type locality of *Bembidion kieranae*. USA: Oregon: Lane Co., SW Eugene, 288m, 44.0031°N 123.1299°W, 5 June 2015. **d**: Type locality of *Bembidion anacalypsi*. Habitat as well of *B. endeca*, *B. siticum*, *B. cf. fortestriatum*, and *B. (Furcacampa) timidum* (LeConte). USA: Oregon: Harney Co., Dairy Creek, 43.7182°N 119.6377°W, 1375m, 14 May 2018. **e**: Lost Lake, Oregon. Habitat of *Bembidion altipaludis*, *B. connivens*, *B. fortestriatum*, *B. (Notaphus) debiliceps* Casey, *B. (Furcacampa) versicolor* (LeConte), and *B. (Plataphus) lividulum* (Casey). USA: Oregon: Linn Co., Lost Lake, 44.4345°N 121.9074°W, 1218m, 1 July 2010. **f**: Habitat of *Bembidion ampliceps* and *B. (Trechonepha) iridescens* (LeConte). USA: California: Kern Co., Stable Creek at Sawmill Rd, 35.6683°N 118.5301°W, 1350m, 30 May 2013.

Several students in the class became interested and curious enough about *Bembidion* diversity that they would collect on their own, and that led to another significant discovery. Shannon Kieran (now Blair) brought into class a specimen she had found on her parents' property in Eugene, Oregon. At first glance DRM thought it might be a *Bembidion canadianum* Casey, but subsequent morphological and molecular work revealed it was still another new

Figure 2. The class in the field.

From left to right, Danielle Mendez, Tom Clegg, Ana Vasconcelos, Elle Zeleznik, Trevin Braun, Alex Soohoo-Hui, Mamo Waianuhea, Julia Cheng (now Sliker), Shannon Kieran (now Blair), John Sproul, and David Maddison. Drawing by Arden Smith.

species, here named *B. kieranae*, n. sp. By the time the class ended in June of 2015, the eleven members of the team, including the nine undergraduate students, had thus discovered two new species that were previously unknown, and had gained a much deeper understanding of other species of *Trepanedoris*, and of the process of biodiversity discovery.

Specimens collected during the course's field trips continued to be the source of discovery after the class ended. Two of the students in the class, Danielle Mendez and Ana Caroline Vasconcelos, joined the first author's lab, and continued their work on *Trepanedoris*. While examining male genitalia of members of the *Bembidion acutifrons* LeConte species group under a compound microscope, Danielle found the first morphological evidence that some specimens the class had collected along Dairy Creek, Oregon (Fig. 1d) were a third new species. We had initially believed they were a genetically distinct population of *Bembidion acutifrons*, but Danielle's discovery made it clear that they represented yet another new species, which we name *Bembidion anacalypsi*, n. sp.

Previous research

There have been only a handful of papers examining the diversity of *Trepanedoris* in North America. The most recent catalogue (Bousquet, 2012) lists 13 species, based mainly on the work of Casey (1918, 1924) and Lindroth (1963). Casey's work is out of date, and suffers from his excessive splitting of species (Lindroth, 1963). Lindroth's revision (1963) presents a much clearer picture of *Trepanedoris* diversity, in part because of his use of male genitalic characters, but he focused on the Canadian and Alaskan fauna, and only briefly touched upon the diversity in the more species-rich areas of California and Oregon.

Trepanedoris is considered to consist of two species groups, the *fortestriatum* group and the *connivens* group (Bousquet, 2012; D. R. Maddison, 2012). These two groups are sister clades (Maddison, unpublished). The *fortestriatum* group is widely distributed in the Nearctic, and has two species in the Palearctic, *Bembidion doris* (Panzer) and *B. atripes* (Motschulsky) (Löbl & Löbl, 2017). The *connivens* group is restricted to North America, and has nine species currently recognized (Bousquet, 2012).

To date, all published work on species delimitation in *Trepanedoris* has relied upon morphological characters, with the exception of a brief mention of unpublished DNA data that led Maddison (2012) to consider *Bembidion elizabethae* Hatch to be distinct from *Bembidion connivens* (LeConte) (a decision that is reversed in this paper).

Objectives

This paper reports on research about *Trepanedoris* done within the context of the Discovering Insect Species course, focusing on species in the *connivens* group (Figs 5–7), with an emphasis on the North American fauna outside of species-rich California. The *fortestriatum* group will be examined in a later paper; delimiting species within this group has proved very difficult because of the complex pat-

Figure 3. The research team at work.

a: Sorting small beetles from soil in the cabin at Klamath Marsh National Wildlife Refuge. **b**: Preparing beetles for morphological study. **c**: Preparing PCR reactions. **d**: Examining the results of a coalescent analysis. **e**: Looking at the phylogenetic trees of individual genes. Drawings by Arden Smith.

Figure 4. Habitus photograph of *Bembidion endeca* from Dairy Creek, Harney Co., Oregon. Scale bar is 1 mm.

terns of geographic variation in DNA sequences and limited morphological variation. Several California species of the *connivens* group were discovered or became understood several years after the course was completed and will also be considered in that later paper.

Thus, the goal of this paper is to delimit and document the species in the *connivens* group in North America exclusive of California and areas of Oregon and Nevada very close to the California border. We use a combination of morphological, molecular, and geographic data. The DNA sequences we gather from 170 specimens and six genes allow us to analyze genetic patterns using coalescent-based methods, and we integrate the results of those methods into our inferences about species boundaries. In addition, we present tools to enable identification of the species.

Methods

Approximately 3,000 specimens of *Trepanedoris* were examined from the collections listed below; each collection listing begins with the code used in the text.

BYUC	Bean Life Science Museum, Brigham Young University, Provo, USA
CAS	California Academy of Sciences, San Francisco, USA
CNC	Canadian National Collections of Insects, Ottawa, Canada
CMNH	Carnegie Museum of Natural History, Pittsburgh, USA
CSCA	California State Collection of Arthropods, Sacramento, USA

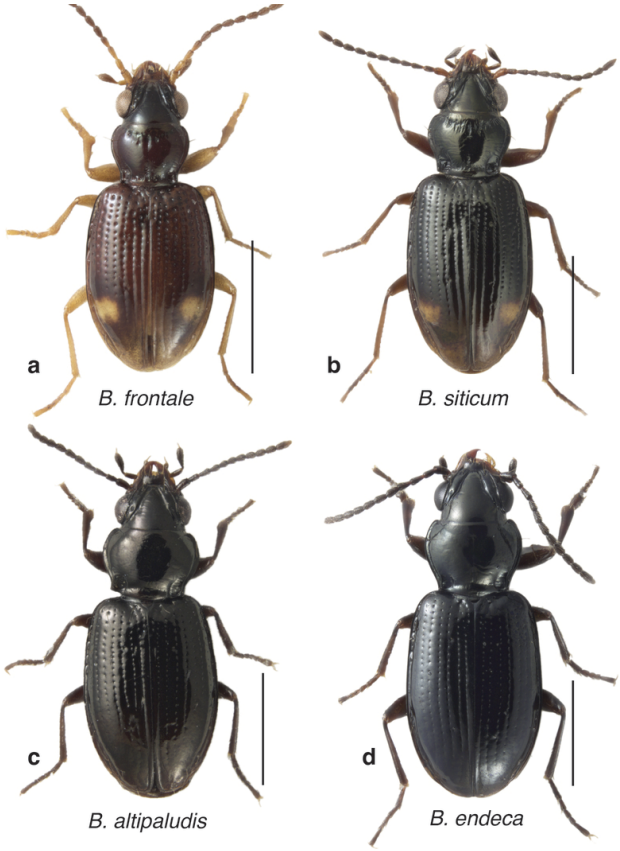

Figure 5. Habitus photographs of adults.

a: *Bembidion frontale* male (Canada: Ontario: Dwight). **b**: *B. siticum* female (USA: Oregon: Harney Co., Malheur NWR). **c**: *B. altipaludis* female (USA: Oregon: Deschutes Co., Three Creek Meadow). **d**: *B. endeca* male (USA: Oregon: Harney Co., Malheur NWR). Scale bars 1 mm.

EMEC	Essig Museum Entomology Collection, University of California, Berkeley, USA
JRB	James Bergdahl Collection, Spokane, USA
JRLC	James LaBonte Collection, Salem, USA
MCZ	Museum of Comparative Zoology, Harvard University, Cambridge, USA
MNHN	Muséum National d'Histoire Naturelle, Paris, France
MZLU	Zoological Museum, Lund, Sweden
NHMUK	The Natural History Museum, London, UK
NMNH	National Museum of Natural History, Smithsonian Institution, Washington, USA
OSAC	Oregon State Arthropod Collection, Oregon State University, Corvallis, USA
SEMUBC	Spencer Entomological Collection, University of British Columbia, Vancouver, Canada
UAIC	University of Arizona Insect Collection, Tucson, USA
UNHC	University of New Hampshire Collection, Durham, USA
WSU	MT James Entomological Collection, Washington State University, Pullman, USA

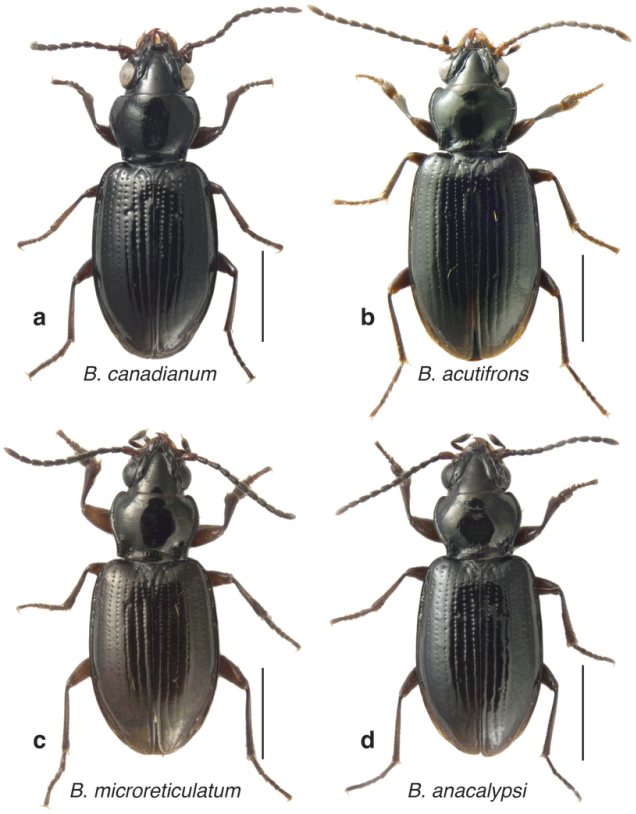

Figure 6. Habitus photographs of adults.

a: *Bembidion canadianum* female (USA: Montana: Beaverhead Co., Melrose). **b**: *B. acutifrons* male (USA: Oregon: Harney Co., Marshall Pond, Malheur NWR). **c**: *B. microreticulatum* male (USA: Washington: Lewis Co., Centralia). **d**: *B. anacalypsi* male (USA: Oregon: Harney Co., Dairy Creek). Scale bars 1 mm.

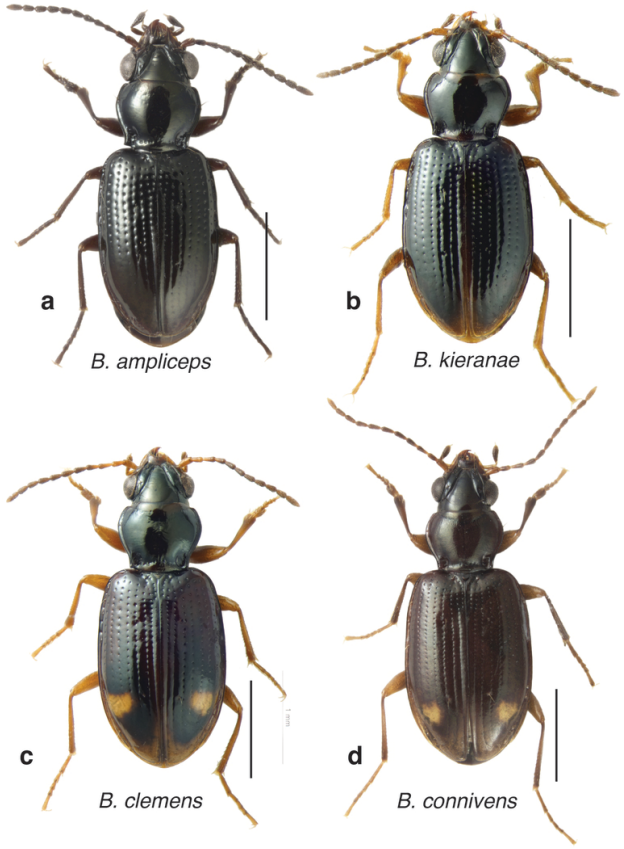

Figure 7. Habitus photographs of adults.

a: *Bembidion ampliceps* male (USA: California: Kern Co., Stable Creek). **b**: *B. kieranae* male (USA: Oregon: Lane Co., Eugene). **c**: *B. clemens clemens* male (USA: Arizona: Yavapai Co., Prescott). **d**: *B. connivens* male (USA: California: Tehama Co., Red Bluff). Scale bars 1 mm.

Collecting and storage methods

Specimens were collected by hand or using an aspirator. Many specimens were found during the day in their habitat after treading damp soil and waiting for the beetles thus disturbed to appear on the surface, after splashing the substrate with water, or by separating dead leaves and marsh vegetation near the ground to reveal the beetles hiding there. However, collecting at night was the most productive, as after dark the beetles were generally more active and walking around the surface, and were much easier to see in the light of a headlamp than among the high-contrast light and shadows of sunshine on vegetation.

Specimens for morphological studies were killed and preserved in *Acer* L. sawdust to which ethyl acetate was added. Specimens collected specifically for DNA sequencing were killed and stored in 95% or 100% ethanol, with best results obtained when the abdomen was slightly separated from the rest of the body to allow better penetration of ethanol, or when the reproductive system was dissected out through the rear of the abdomen within a few minutes of the beetle's death in ethanol. Ethanol was decanted from vials and refilled at least once within the first few weeks after death. Storage was then at -20°C.

Overview of species delimitation

We follow the retrospective reproductive community concept of species (W. P. Maddison & Whitton, 2023). We synthesize the results from DNA-based coalescent analyses that seek the boundaries of reproductive communities, with morphological data that suggests intrinsic barriers to gene flow, and with geographic evidence that suggests extrinsic barriers or helps confirm intrinsic barriers. We give such reproductive communities the rank of species if they correspond in divergence to other well-supported species in *Bembidion*.

Morphological methods

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

Morphological observations of external structures, including microsculpture, were made under a Leica M165C

dissecting scope using a ring light. Male genitalia were studied via transmitted light on a Leica DM5500B compound microscope, with most attention paid to the structures of the complex internal sac within the aedeagus.

Photographs of external features other than microsculpture were taken with a Leica M165C dissecting scope and Sony NEX-7 camera or a Leica Z6Apo lens and DMC4500 camera, and of male genitalia with a Leica DM5500B compound microscope and DMC425C camera, with the Leica Application Suite v4.9 software capturing each TIFF image. Microsculpture photographs were taken with a DMC425C camera attached to a DM5500B compound scope equipped with an X-Cite 110LED light source which provides co-axial illumination, and a 20X epi-illumination objective lens. For all photographs of specimens, a stack of images from different focal positions was merged using the PMax procedure in Zerene Systems's Zerene Stacker; the final images thus potentially have some artifacts caused by the merging algorithm.

Measurements were made using the same imaging systems with Leica Application Suite v4.9. Body length is measured from the front of the labrum to the elytral apex. Other measurements taken are the maximum width of head including eyes (HW), maximum width of pronotum (PW), width of pronotum at hind angles (PWh), and maximum width of elytra (EW).

The morphological data we gathered focused on traits that typically vary between species of *Bembidion* and related genera (e.g., *Lionepha*), and less often within species. Some of these characters are presumed to have been involved in forming intrinsic reproductive isolation barriers as they are involved in the mating process, in particular the male genitalia, protarsomeres of males, and pronotal shape. The interactions between male and female genitalia during mating make them likely candidates for morphological characteristics suggesting past and ongoing reproductive isolation, especially within the male genitalia where the structures are complex and well-sclerotized (and thus easily visible). Male genitalia in many groups of Bembidiini show patterns of variation suggesting they provide good evidence about species boundaries. Within species that have been delimited with strong support using DNA data, the internal sclerotized structures of male genitalia show little variation within species, but clear differences between species, as seen in *Lionepha* (D. R. Maddison & Sproul, 2020), *Bembidion (Liocosmius)* (D. R. Maddison & Cooper, 2014), *Bembidion (Pseudoperyphus)* (D. R. Maddison, 2008), and the *Bembidion breve* species group (Sproul & Maddison, 2017a). Protarsomeres of males and the prothoraces of both sexes are also likely body regions containing evidence of intrinsic barriers. Males use their expanded protarsomeres to hold on to the prothoraces of females during mating. There are several examples of extremely similar, closely related, sympatric pairs of distinct species that differ externally most obviously in the size of the protarsomere 1 of males, including *Lionepha sequoiae* (Lindroth) and *L. osculans* (Casey) (D. R. Maddison & Sproul, 2020), as well as *Bembidion (Bracteon) balli* Lindroth and *B. foveum* Motschulsky (D. R. Maddison, 1993). The shape of the sides of the prothorax, where males grasp females during mating, also tends to be quite constant within species but different between species (Lindroth, 1963). Although numerous, time-costly measurements and morphometric analyses of these traits might be an objective means of documenting and analyzing them, we document them instead with the traditional narrative descriptions accompanied by extensive imagery, and for analysis we rely on the 45 years of experience of the first author in visually assessing and describing the morphological data in the context of patterns throughout bembidiine carabids.

Taxon sampling

As is typical in systematic studies, the sampling of specimens that were examined in detail arose through a process of reciprocal illumination: the observed morphological and geographic variation guided the choice of specimens to be genetically sequenced, and the results of the DNA sequencing informed which specimens should be examined more thoroughly under the microscope for genitalic and other morphological characters, which suggested additional specimens to be sequenced, and so on. Eventually this cyclical process yielded sufficiently few anomalies that the patterns appeared settled, and the process was stopped.

We sampled a total of 170 specimens for DNA sequencing: 159 members of the *connivens* species group of subgenus *Trepanedoris* (Table 1, Supplementary Table S1), four other species in the *fortestriatum* species group of *Trepanedoris* (Table 2), as well as seven species of outgroups from related subgenera (Table 2). Five or six known *connivens* group species occurring in California and in Oregon and Nevada within 100 km of the California border were not sampled: *Bembidion remotum* Casey, four undescribed species, and (if it is indeed a distinct species), *B. scenicum* Casey. Our morphological and DNA data on those species indicate all but *B. scenicum* are clearly distinct from any of the species we do treat, and the inclusion of any of them, including *B. scenicum*, would not affect our conclusions about species boundaries. These six species, along with the *fortestriatum* species group, will be covered in a later paper. We sampled between four and 45 specimens per species in the *connivens* group. Outgroups were chosen as representatives of the subgenera most closely related to *Trepanedoris* (D. R. Maddison, 2012). Detailed localities of capture of the non-*connivens* group specimens are given in Maddison (2012), except for *Bembidion (Trepanedoris) doris*, which was from Russia: Leningrad reg.: 11 km NW Tolmachevo, valley of Luga R., 58.9358°N 29.8092°E.

DNA sequencing

Genes studied, and abbreviations used in this paper, are as follows: **28S** or 28S rDNA: 28S ribosomal DNA; **COI**: cytochrome oxidase I; **CAD**: carbamoyl phosphate synthetase domain of the *rudimentary* gene; **MSP**: Muscle Specific Protein 300; **Topo**: topoisomerase I; **wg**: wingless.

DNA extraction and sequencing methods varied depending upon the specimen. For specimens collected into 95–100% ethanol (all specimens except the lectotype of *Be-*

Table 1. Specimens of the *connivens* group that were sequenced, with their approximate localities. Numbers in the right column are the D.R. Maddison DNA voucher numbers of the specimens from that locality. Additional details about specimens in Supplementary Table S1.

***Bembidion frontale* (LeConte)**	
Canada: Saskatchewan: N Saskatchewan River at Borden	3388
Canada: Ontario: Arrowhead Provincial Park	1335, 1942
USA: Pennsylvania: Westmoreland Co., Powdermill Nature Reserve	2286
***Bembidion siticum* Casey**	
USA: Idaho: Lewis Co., Clearwater River near Greer	1429
USA: Oregon: Benton Co., Corvallis	3424
USA: Oregon: Harney Co., Steens Mountain Loop Road, Malheur NWR	4541
USA: California: Humboldt Co., South Fork Eel River, 2.5 km S Miranda	4331
USA: California: El Dorado Co., Lily Lake	3389
USA: California: Mono Co., SW shore Mono Lake	4370
USA: California: Fresno Co., Huckleberry Meadow	4334
USA: California: San Benito Co., Bear Gulch Reservoir	4384
***Bembidion altipaludis* Maddison**	
USA: Oregon: Deschutes Co., Three Creek Lake	2583
USA: California: Sierra Co., lower Tamarack Lake	4318
USA: California: Trinity Co., Upper Canyon Cr. Meadows	4326, 4773
USA: Oregon: Deschutes Co., N Sparks Lake, along hwy 372	4344
USA: Oregon: Linn Co., Lost Lake	4549
USA: Oregon: Klamath Co., Munson Creek, Crater Lake NP	4987
USA: California: Siskiyou Co., Scott Mountain Summit	6282
USA: California: El Dorado Co. 1 km WxNW Robbs Peak Dam	6287, 6288
USA: California: Tehama Co., Wilson Lake	6313
***Bembidion endeca* Maddison & Sproul**	
USA: Oregon: Klamath Co., Klamath Marsh NWR, Williamson R	4445
USA: Oregon: Klamath Co., Klamath Marsh NWR, Wocus Bay	4480
USA: Oregon: Harney Co., Bridge Creek Canal Pd, Malheur NWR	4528, 4531, 4543, 4557, 4559, 4563
USA: Oregon: Harney Co., Dairy Creek	4534, 4540, 4556
USA: Oregon: Harney Co., Steens Mountain Loop Road, Malheur NWR	4546
USA: Oregon: Harney Co., Marshall Pond, Malheur NWR	4552
***Bembidion canadianum* Casey**	
USA: Montana: Beaverhead Co., Melrose, Big Hole River	1445, 2255, 4458
USA: Colorado: Alamosa Co., Alamosa NWR	4317, 4325
Canada: Alberta: Gull Lake at Township Road 422	6019
***Bembidion acutifrons* (LeConte)**	
Canada: Saskatchewan: Eagle River at route 4	4371
Canada: Saskatchewan: 1 km W of Waseco	2744
USA: Colorado: Alamosa Co., Alamosa NWR	4330, 4369, 4415
USA: Utah: San Juan Co., Geyser Pass Rd nr Horse Ck	4339
USA: Utah: Kane Co., Swains Creek at highway 14	2104

USA: Oregon: Harney Co., Marshall Pond, Malheur NWR	4532, 4538, 4545, 4565
USA: Oregon: Harney Co., Buena Vista Pond, Malheur NWR	4535
USA: Oregon: Harney Co., Bridge Creek Canal Pd, Malheur NWR	4564

***Bembidion microreticulatum* Hatch**

USA: Washington: Skagit Co., Anacortes, Ship Harbor	4941
USA: Washington: Lewis Co., Fort Borst Lake, Centralia	4942, 4943, 4944
USA: Oregon: Benton Co., Corvallis	3383, 4322, 4447
USA: Oregon: Lane Co., SW Eugene	4508

***Bembidion anacalypsi* Mendez & Maddison**

USA: Oregon: Harney Co., Dairy Creek	4544, 4550, 4558, 5283, 5284, 5285, 5286, 5287, 5288
USA: Oregon: Baker Co., 4.4 km NW Haines	6087, 6088
USA: Oregon: Baker Co., Deer Creek at highway 6, Mowich Loop	6089, 6090
USA: Oregon: Grant Co., Pond near Bridge Creek, Austin Junction	6091, 6092
USA: Idaho: Boise Co., 2 mi E Idaho City	6110, 6115, 6116

***Bembidion ampliceps* Casey**

USA: California: Kern Co., Stable Creek at Sawmill Rd	4329
USA: California: Kern Co., Lake Isabella at mouth of French Gulch	4570
USA: California: Kern Co., Greenhorn Creek, Sequoia NF	4574
USA: California: Kern Co., Davis Camp, Sequoia NF	4591

***Bembidion kieranae* Maddison & Sproul**

USA: Oregon: Lane Co., SW Eugene	4507, 4576, 4577, 4586

***Bembidion clemens clemens* Casey**

USA: Colorado: Archuleta Co., E Fork San Juan River	4346
USA: New Mexico: Cibola Co., Bluewater Creek, Zuni Mtns	4355, 4412
USA: New Mexico: Grant Co., Gila River at route 211, Gila	3394
USA: Utah: Kane Co., Swains Creek at highway 14	2099
USA: Utah: Summit Co., Upper Provo River at Soapstone Basin Bridge	4351
USA: Utah: Grand Co., Moab, Matheson Wetlands Preserve	4354
USA: Utah: Washington Co., Pine Valley, Lower Fork Santa Clara R	4411
USA: Arizona: Apache Co. East Fork of Black River along FR276	4350
USA: Arizona: Yavapai Co., Prescott, Granite Creek near Watson Lake	2105, 4349
USA: Arizona: Yavapai Co., Granite Lake	4340

***Bembidion clemens disparile* Casey**

USA: California: San Diego Co., Lake Moreno	2106
USA: California: Ventura Co., Rancho Neuvo, Los Padres NF	4333
USA: California: Riverside Co., Bautista Creek E of Hemet	4569
USA: California: Kern Co., Davis Camp, Sequoia NF	4383
USA: California: Kern Co., Kern R. Cyn, Black Gulch South	3320
USA: California: Kern Co., 1 km E Davis Camp, Sequoia NF	4587
USA: California: Merced Co., San Joaquin R., Great Valley Grasslands SP	4323

USA: California: San Benito Co., Laguna Creek at Coalinga Rd	4336
USA: California: San Luis Obispo Co., San Simeon St Pk, San Simeon Creek	4321
USA: California: Monterey Co., Garrapata St Pk, Soberanes Creek	4335
USA: California: Marin Co., Nicasio Reservoir	2621

***Bembidion connivens* (LeConte)**

Northern	
USA: Washington: Whitman Co., Rock Creek just below Rock Lake	4364
USA: Oregon: Crook Co., Walton Lake	4799
USA: Oregon: Lincoln Co., Siletz River E of Kernville	1759
USA: Oregon: Benton Co., Corvallis	2490, 4467, 4451, 4454
USA: Oregon: Lane Co., SW Eugene	4584, 4485
USA: Oregon: Douglas Co., Toketee Lake	4601
USA: Oregon: Coos Co., Bullards Beach State Park, Coquille River	4362, 4395
USA: Oregon: Jackson Co., White City	6283, 6311, 6312, 6345, 6346, 6347, 6348
Central Coast	
USA: Oregon: Curry Co., Pistol River State Park	4381
USA: California: Humboldt Co., Dry Lagoon, Humboldt Lagoons St.Pk.	4366
USA: California: Humboldt Co., Little River State Beach	4414
USA: California: Humboldt Co., Mattole River near its mouth	4404
Southern	
USA: Oregon: Klamath Co., Klamath Marsh NWR, Wocus Bay	6145, 6163
USA: Oregon: Harney Co., Mud Creek brood pond, Malheur NWR	4537, 4553, 45460
USA: Oregon: Harney Co., Marshall Pond, Malheur NWR	6242
USA: Oregon: Harney Co., Malheur NWR, Buena Vista Ponds	6243
USA: California: Marin Co., Nicasio Reservoir	2107, 2598
USA: California: Tehama Co., Red Bluff, Avery Park	4343, 4495
USA: California: Sutter Co., Feather River S of Nicolaus	4324, 4496
USA: California: Butte Co., Thermalito Forebay	4338
USA: California: Merced Co., San Joaquin R., Great Valley Grasslands SP	4365
USA: California: San Luis Obispo Co., San Simeon St Pk, San Simeon Creek	4359, 4413
USA: California: San Luis Obispo Co., Pismo State Beach	4360
USA: California: Monterey Co., Arroyo Seco Cpgd, Los Padres NF	4361, 4466, 4475
USA: California: Ventura Co., Los Padres NF, Upper Rose Lake	4363

mbidion disparile Casey), DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit. Fragments for the seven genes were amplified using the Polymerase Chain Reaction on an Eppendorf Mastercycler Pro Thermal Cycler, using TaKaRa Ex Taq and the basic protocols recommended by the manufacturers. Primers and details of the cycling reactions used are given in Maddison (2012) and Maddison & Cooper (2014). The amplified products were then cleaned, quantified, and sequenced at the University of Arizona's Genomic and Technology Core Facility using a 3730 XL Applied Biosystems automatic sequencer. Assembly of multiple chromatograms for each gene fragment and initial base calls were made with Phred (Green & Ewing, 2002) and Phrap (Green, 1999) as orchestrated by Mesquite's Chromaseq package (D. R. Maddison & Maddison, 2023a; W. P. Maddison & Maddison, 2023) with subsequent modifications by Chromaseq and manual inspection. Multiple peaks at a single position in multiple reads were coded using IUPAC ambiguity codes. DNA of the historical lectotype of *Bembidion disparile* (DNA4764) was extracted using a Qiagen QIAamp Micro kit, genomic libraries prepared using New England Biolabs NEBNext DNA Ultra II and Swift Accel-NGS 1S Plus kits, sequenced on an Illumina HiSeq 3000, and genes harvested as described in Sproul & Maddison (2017b).

Of the 963 sequences examined (Supplementary Table S2), 865 were newly acquired, with 98 from previous publications (D. R. Maddison, 2012, 2023; D. R. Maddison et al.,

Table 2. Specimens sequenced other than those of the *connivens* group. The first four listed are members of the *fortestriatum* group of subgenus *Trepanedoris*, and the remainder are members of related subgenera. Numbers in the middle column are the D.R. Maddison DNA voucher numbers of the specimens.

Species		Region
Bembidion (Trepanedoris) concretum Casey	2041	USA: New Hampshire
Bembidion (Trepanedoris) doris (Panzer)	2823	Russia: Leningrad reg
Bembidion (Trepanedoris) fortestriatum (Motschulsky)	2098	Canada: British Columbia
Bembidion (Trepanedoris) pseudocautum Lindroth	1436	Canada: Nova Scotia
Bembidion (Diplocampa) assimile Gyllenhal	1421	Spain: Albacete
Bembidion (Diplocampa) transparens (Gebler)	1943	Canada: Alberta
Bembidion (Semicampa) nigrivestis Bousquet	1409	Canada: Ontario
Bembidion (Semicampa) roosevelti Pic	2050	USA: California
Bembidion (Semicampa) semicinctum Notman	2283	USA: Pennsylvania
Bembidion (Peryphodes) ephippigerum (LeConte)	1927	USA: California
Bembidion (Peryphodes) salinarium Casey	1444	Canada: Alberta

2019; D. R. Maddison & Maruyama, 2019; Sproul & Maddison, 2017b).

The matrices for 28S, COI, CAD, and Topo were 100% complete, including sequences for all 170 specimens (Supplementary Table S2). For MSP, we lacked sequences for three specimens (one *Bembidion siticum*, one *B. altipaludis*, and one *B. canadianum*). Many fewer (116) specimens were chosen to be sequenced for wg.

Sequence alignment and data exclusion

Alignment of 28S was conducted in MAFFT version 7.130b (Katoh & Standley, 2013), using the L-INS-i search option and otherwise default parameter values. Alignment was not difficult for any of the protein-coding genes, as the sampled COI, CAD, MSP, Topo, and wg sequences contained extremely few amino acid substitutions, and there were no evident insertions or deletions. Thus, the protein-coding genes could be aligned manually; to confirm, alignments of the inferred amino acids were conducted using the above-mentioned settings in MAFFT.

Sites in 28S were chosen to be excluded from consideration using the modified GBLOCKS analysis present in Mesquite with the following options: minimum fraction of identical residues for a conserved position = 0.2, minimum fraction of identical residues for a highly-conserved position = 0.4, counting fraction within only those taxa that have non-gaps at that position, maximum number of contiguous non-conserved positions = 4, minimum length of a block = 4, and allowed fraction of gaps within a position = 0.5. Other than 70 sites at the start and end of 28S where sequencing success varied, this procedure removed only 7 internal sites; the total fraction of excluded nucleotides was 0.45%.

Molecular phylogenetic analysis and species delimitation

Maximum likelihood analyses of single gene and concatenated matrices were conducted using IQ-TREE version 2.2.2.7 (Minh et al., 2020) through Mesquite's Zephyr package (D. R. Maddison & Maddison, 2023b), which performed 50 searches for maximum likelihood trees. ModelFinder (Kalyaanamoorthy et al., 2017) was used to find the optimal model of evolution. Analysis of 28S was unpartitioned and the MFP option was chosen. For single-gene analyses of protein-coding genes, each of the three codon positions was initially treated as a separate part and the TESTMERGE option was used to select the best partition scheme and model for each part. The TESTMERGE option was also used for the concatenated matrix, starting with 16 parts (one for 28S and one for each codon position of each protein coding gene). In addition, standard non-parametric bo were conducted for each gene matrix and the concatenated data, also using the TESTMERGE option, with 500 bootstrap replicates.

Three multispecies coalescent approaches were conducted to provide algorithmic analyses of boundaries of species or reproductive communities from multi-gene DNA sequence data: STACEY (Jones, 2017), SPEEDEMON (Douglas & Bouckaert, 2022), and DELINEATE (Sukumaran et al., 2021). All three of these approaches delimit the boundaries of reproductive communities; SPEEDEMON and DELINEATE also infer which of these reproductive communities are of species rank. In these analyses, COI was treated as haploid, the rest diploid; the sequences were unphased.

STACEY version 1.3.1 (Jones, 2017) was used as implemented in BEAST version 2.7.6 (Bouckaert et al., 2014, 2019), using BEAST Model Test (with the transitionTransversionSplit model set), with an optimized relaxed clock, and otherwise default parameters. Only 28S, COI, CAD, MSP, and Topo were analyzed, because those were the genes with the most extensive taxon sampling, and STACEY required all genes to be sampled for all analyzed specimens.

Taxon sampling was reduced to include only members of subgenus *Trepanedoris*. Hypothesis sampling was conducted every 50,000 generations. We evaluated sampling sufficiency using ESS values in Tracer version 1.7.2 (Rambaut et al., 2018); after the run reached 1.47 billion generations, all ESS values exceeded 240, and the run was terminated. After the first 10% of the trees were discarded as the burn-in period, this yielded a sample of 26,483 trees. The maximum clade credibility tree among these was found using TreeAnnotator (part of the BEAST package).

SPEEDEMON version 1.1.0 (Douglas & Bouckaert, 2022) using StarBeast3 version 1.1.9 (Douglas et al., 2022) was run within BEAST version 2.7.7. Data from all genes was used, including all specimens of *Trepanedoris*, but with no *Bembidion* outside of *Trepanedoris*. An HKY85 character model was used, with a three-category gamma rate distribution model, with shape and kappa estimated. The initial population structure presumed each specimen was in its own population. After the samples of trees were obtained, they were analyzed with ClusterTreeSetAnalyzer (part of SPEEDEMON). Four separate analyses were used with different epsilon values (Monjaraz-Ruedas et al., 2023), calculated using the divergence values on the maximum likelihood tree from the concatenated data. Epsilon is a threshold divergence value: populations with divergences of less than epsilon are considered part of the same species. The divergence values used, and details of the associated Bayesian analyses, are given in the following list. Runs were stopped once ESS values reached acceptable levels, as noted.

1. Minimum value between "known" species, being half of the sum of the branch lengths between the most recent common ancestor (MRCA) of all *B. canadianum* and the MRCA of all *B. acutifrons*. Epsilon value: 0.0040. Seven runs were conducted, with between 230 million (M) and 312M generations each. With 10% burn-in, all ESS values were above 200 except Tree.t:COI.height, Tree.t:COI.treeLength, and Tree.t:Topo.height; those were 172–199. After subsampling the resulting tree file (by removing all but every eighth tree) the total number of trees summarized was 21,375.
2. Maximum value within known species, being half of the maximum branch length path within *B. altipaludis*. Epsilon value: 0.0044. Four runs were conducted, with between 708M and 742M generations each. With 10% burn-in, all ESS values were above 200 except Tree.t:COI.height and Tree.t:Topo.height; those were 177–196. After subsampling one in every 12 trees, the total number of trees summarized was 21,244.
3. Maximum value within the southern form of *B. connivens* (as described below), being half of the maximum branch length path within that clade. Epsilon value: 0.0060. Four runs were conducted, with between 704M and 763M generations. With 10% burn-in, all ESS values were above 200 except Tree.t:COI.height and Tree.t:Topo.height; those were 177–196. After subsampling one in every 12 trees, the total number of trees summarized was 21,124.
4. Maximum value within the *B. siticum* (which is the species containing the most DNA sequence divergence within *Trepanedoris*), being half of the maximum branch length path within *B. siticum*. Epsilon value: 0.0102. Ten runs were conducted, with between 224M and 561M generations each. With 10% burn-in, all ESS values were above 200 except Tree.t:COI.height, Tree.t:COI.treeLength, Tree.t:Topo.treeLength, and Tree.t:Topo.height; those were 172–197. After subsampling one in every 15 trees, the total number of trees summarized was 22,636.

A DELINEATE version 1.2.3 analysis (Sukumaran et al., 2021) was conducted, beginning with inference of the guide tree using BEAST version 2.7.7 and StarBeast3 version 1.1.9, with a subsequent BPP analysis (Flouri et al., 2018), following the guidelines in A Complete Worked Example: *Lionepha* (https://jeetsukumaran.github.io/delineate/workflow1.html). The starting point for population division was to group all specimens from what was apparently one species in each locality into one population. For example, there were 21 specimens of *Trepanedoris* sequenced from Malheur National Wildlife Refuge: six *Bembidion acutifrons*, nine *B. endeca*, five *B. connivens*, and one *B. siticum*. These were considered as four populations; specimens of those same species from other localities were considered as different, additional populations. To infer the guide tree that is to be used in later steps, four MCMC runs were conducted, running between 1.20 and 1.29 billion generations each, sampling every 10,000 generations. With a 10% burn-in, ESS values of all parameters were above 200, except Tree.t:Topo.height, which was 190. After subsampling one out of every 10 of the resulting trees, the final sample of trees numbered 21,632. The maximum clade credibility tree (with mean node heights) of this sample of trees, calculated using TreeAnnotator, served as the guide tree.

BPP version 4.8.2 (Flouri et al., 2018) was then used to infer a species tree under a multispecies coalescent model, and to collapse populations into larger populations; the analysis was conducted on subsets of the full data set. In particular, the guide tree was broken up into eight pieces (Supplementary Fig. S1) and the BPP command files created for each piece using Mesquite version 4 (W. P. Maddison & Maddison, 2025). The BPP results from each component were then combined using delineate-bppsum; we chose a 95% posterior probability threshold for determining population boundaries. This resulted in the merger of several of the populations, reducing the total number of populations from 91 to 51 (Supplementary Fig. S1).

The phylogeny of these newly defined populations was then inferred using the multispecies coalescent models in StarBeast3. Four runs were conducted of between 170 million and 185 million generations, sampling every 10,000 generations. After removing the first 10% as burn-in, all ESS values were above 200. After subsampling one out of every four of the resulting trees, the resulting collection contained 15,355 trees. The maximum clade credibility tree

(with mean node ages) of this sample of trees was calculated using TreeAnnotator; this tree (Supplementary Fig. S2) was then used as input for the next step.

Finally, the "constrained" mode of the delineate-estimate program of the DELINEATE process (Sukumaran et al., 2021) was used to infer the species to which populations belonged. For every group of populations that we treat as one species, we conduct an analysis in which that particular group of populations is treated as unidentified, and all others are treated exactly as identified using the species boundaries and classification we propose. For example, to investigate whether the species boundaries for *Bembidion ampliceps* is supported by DELINEATE, we marked all specimens we call *Bembidion ampliceps* as of unknown species identity, and the remaining specimens arranged into the other 11 species in our classification. If delineate-estimate analysis inferred the "unidentified" populations to belong to a species separate from any of the other, identified species, then this means the analysis chose a model in which *B. ampliceps* is indeed a separate species; if instead the analysis inferred them to belong to another identified species, say *Bembidion endeca*, then that would provide evidence that the specimens we are calling *B. ampliceps* and *B. endeca* belong to the same species. This method uses the species distinctions present in the identified populations to estimate a parameter of the speciation model, which then allows calculations of species boundaries of highest likelihood for the unidentified populations. Such an analysis was done for each of the groups we consider species, as well as several more inclusive groups.

The ideal DELINEATE analysis would have involved many populations whose species status was well-established, and which we treated as identified, and then all remaining, more doubtful populations, treated as unidentified. Considering only well-delimited species as identified, and having many of them, would give DELINEATE enough data to infer the speciation model's parameters more accurately. The procedure we used, in which only a single species or small clade is treated as unidentified, is not ideal, as some of the other populations thereby treated in that analysis as "identified" are of uncertain status, and the specified identifications could affect the inference of the model parameters and thus the species assignments of the unidentified populations. Moreover, a species treated as unidentified in *its* analysis is necessarily treated as identified in all *other* analyses. It would be ideal to treat all populations of even the slightest level of uncertainty as unidentified in a single analysis. However, the number of populations for which we wish to test species boundaries is sufficiently high that the computational memory required for this analysis would far exceed what is available. In addition, the number of populations for which we are fully confident of the species status is limited enough that they may not provide enough structure for accurate inference of the model's parameters. For these reasons, many separate analyses were conducted, each with only one group treated as unidentified. Although the inference of species boundaries is thus biased by uncertainties in the classification we use, the method at least checks for internal consistency in the process of species delimitation; discrepancies could indicate different criteria used for different taxa, or the existence of a non-uniform model of speciation.

Integrated species delimitation

As noted above, we integrated the results of formal DNA-based species delimitation analyses with data on morphological variation (especially considering those characters involved in the mechanism of intrinsic reproductive barriers such as structures of the internal sac of male genitalia), considered in light of the geographic distributions of forms. In the Results section we outline the factors involved in each decision. Some of our more difficult decisions have been in those groups (e.g., the *acutifrons* subgroup and within *Bembidion clemens*) whose components are allopatric but with relatively small distances separating them, with uncollected land in between. The areas where these small, cryptic beetles have not yet been collected may hold telling clues about presence or absence of gene flow. In the face of uncertainties because of lack of collecting, we bias our classification to more inclusive species if there is no morphological evidence of intrinsic barriers (e.g., *B. clemens clemens* versus *B. clemens disparile*) and to separate species if there are good indications of intrinsic barriers (e.g., *B. anacalypsi* versus *B. acutifrons*).

Results in Phylogenetics and DNA-based Species Delimitation

The maximum likelihood analyses and three Bayesian methods all yielded several consistent inferences about relationships among species; these provide a context for the species delimitation analyses. The maximum likelihood tree from concatenated data (Fig. 8) and the six individual gene trees (Figs 9–11) reveal several consistent clades, including the *frontale* subgroup (*Bembidion frontale* + *B. siticum*), the *acutifrons* subgroup (*B. acutifrons* + *B. microreticulatum* + *B. anacalypsi* + *B. canadianum* + *B. endeca*, although including *B. altipaludis* in 28S), and the *connivens* subgroup (*B. connivens* + *B. clemens* + *B. kieranae*). The Bayesian analyses, which consider gene and species tree discordance through the multispecies coalescent, show similar, but not identical species relationships to the concatenated analysis (Fig. 12). The *frontale* subgroup, *acutifrons* subgroup, and *connivens* subgroup are all strongly supported by these analyses, but the details within the *acutifrons* and *connivens* subgroups differ between the analyses. However, in all analyses, the sister group of the *Bembidion canadianum* + *B. microreticulatum* + *B. acutifrons* + *B. anacalypsi* clade is *B. endeca*, with the sister of that larger clade being *B. altipaludis*, and *B. ampliceps* the next sister (Fig. 12).

The STACEY analysis infers all 12 of our species-rank taxa to be genetically separate reproductive communities with posterior probabilities of 98% or above (Fig. 13). It does find some genetic structure, possibly indicative of restricted gene flow, within several of the species, including *B. frontale* and *B. siticum* (but with posterior probabilities of

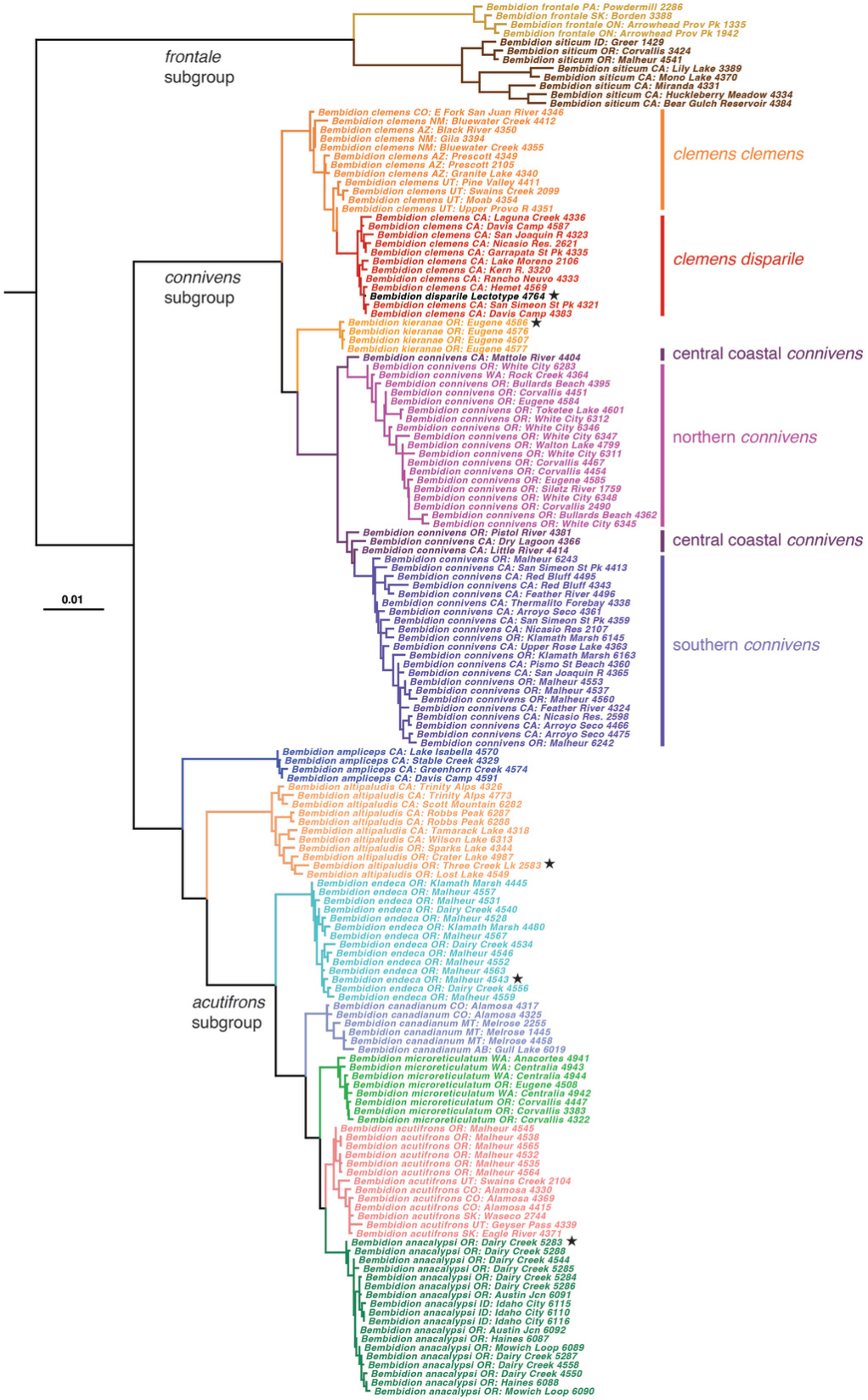

Figure 8. Maximum likelihood gene trees of six-gene concatenated matrix for the *connivens* group.

The *fortestriatum* group and outgroups were present in the analysis, but were graphically removed to simplify this figure. Primary types indicated by stars. Scale bar as reconstructed by IQ-TREE.

53% or less for groups within the taxa), as well as within *B. altipaludis* and *B. acutifrons* (but with posterior probabilities of 85–87%). The analysis shows northern *B. connivens* as a distinct population with a posterior probability of 98%, but the within-species structure is otherwise not well-supported. *B. clemens* shows the most notable struc-

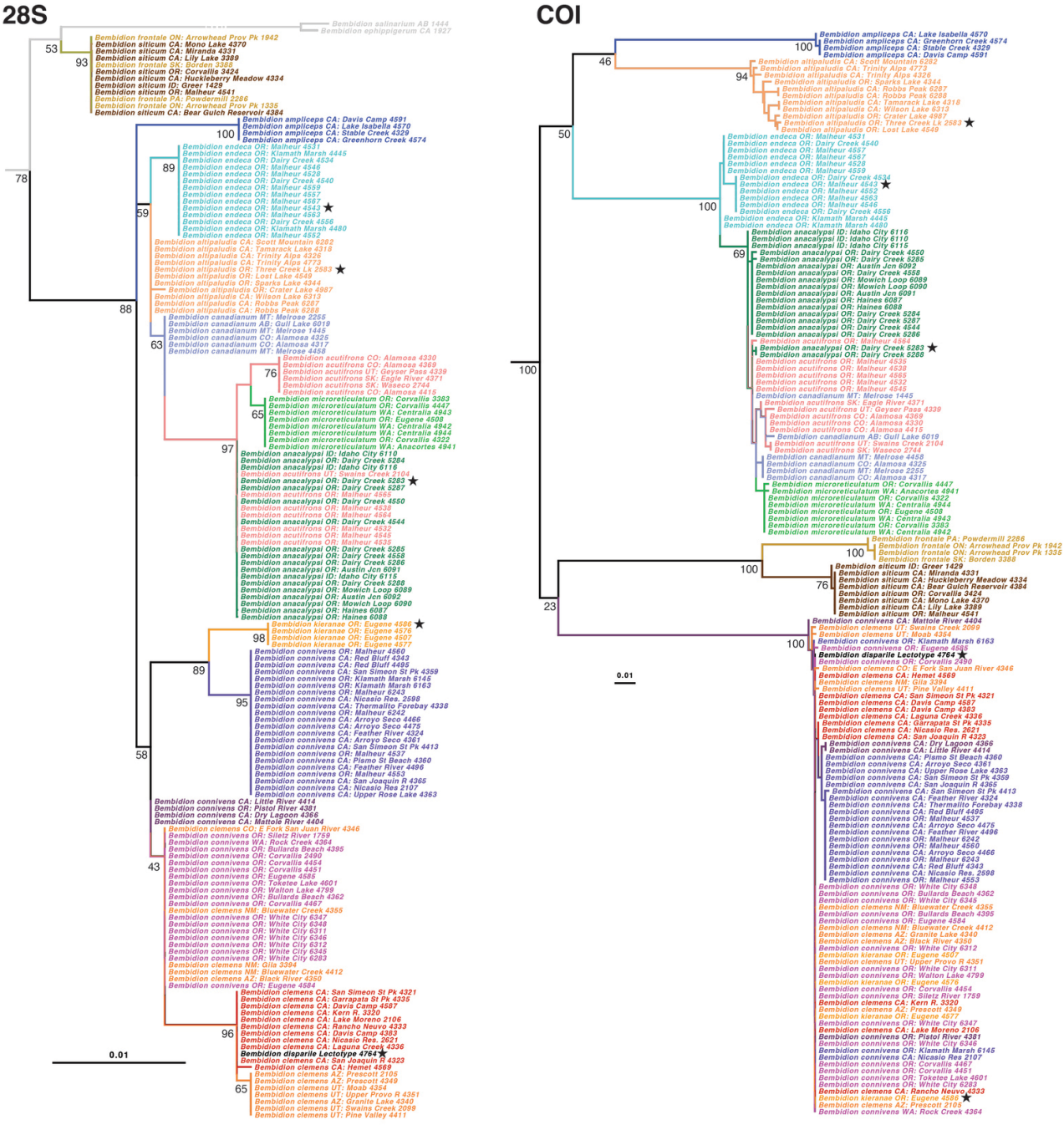

Figure 9. Maximum likelihood gene trees of 28S and COI for the *connivens* group.

The *fortestriatum* group and outgroups were present in the analysis, but, where possible, were graphically removed to simplify this figure. Numbers next to some nodes are non-parametric bootstrap support values, expressed as a percentage. Primary types indicated by stars. Scale bar as reconstructed by IQ-TREE.

ture, with *B. clemens clemens* and *B. clemens disparile* having posterior probabilities as genetically isolated populations of 99–100% (Fig. 13).

The SPEEDEMON and DELINEATE analysis assign species rank to different inferred population groups, with SPEEDEMON tending to split *Trepanedoris* into more finely divided species and DELINEATE tending to lump populations into more inclusive species.

SPEEDEMON strongly supports *Bembidion endeca*, *B. anacalypsi*, *B. microreticulatum*, *B. ampliceps*, and *B. kieranae* each as single, distinct species (Fig 14a) with posterior probabilities all above 90% for all values of epsilon, but for the other seven taxa that we consider species, the posterior probabilities are more distributed toward subgroups within our taxa, at least for lower values of epsilon. For example, in *B. frontale*, *B. canadianum*, and *B. acutifrons*, for low values of epsilon the posterior probabilities of our taxa are lower than the posterior probabilities of hypotheses in which they each consist of multiple species, but the reverse holds for higher values of epsilon. For *B. clemens*, the posterior probability that all populations belong to a single species is estimated at 0 to 0.08% (depending on epsilon), whereas the posterior probabilities that each of what we call subspecies is a separate species is 72–94% (Fig. 14a).

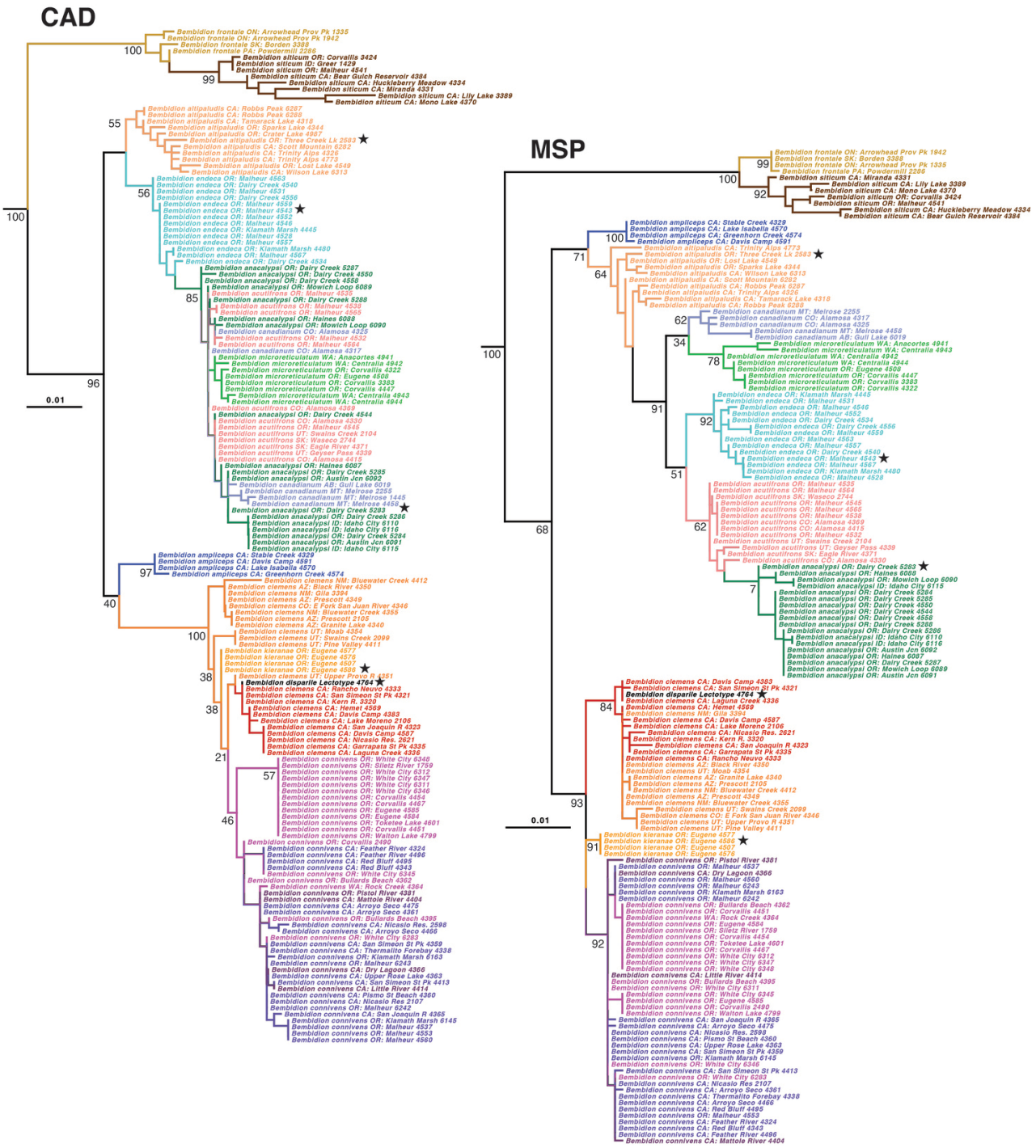

Figure 10. Maximum likelihood gene trees of CAD and MSP for the *connivens* group.

The *fortestriatum* group and outgroups were present in the analysis, but were graphically removed to simplify this figure. Numbers next to some nodes are non-parametric bootstrap support values, expressed as a percentage. Primary types indicated by stars. Scale bar as reconstructed by IQ-TREE.

At a broader scale, within the *frontale* and *acutifrons* subgroups, SPEEDEMON estimates that the probability that more inclusive groups correspond to species to be very low, less than 0.30% (Fig. 14b).

DELINEATE supports our taxonomic species as appropriate for species rank except for *B. siticum* and members of the *acutifrons* subgroup (Fig. 15a). For *B. siticum*, the maximum likelihood delimitation inferred by DELINEATE has that taxon divided into three separate species, although the arrangement with it as only a single species (as we treat it) is only slightly less probable (0.098 versus 0.107; second row in Fig. 15a). If all *B. frontale* and *B. siticum* are treated as unidentified, DELINEATE estimates that they contain four species (Fig. 15b), although the hypothesis that matches our classification is the second-best tree and is only slightly less probable (0.061 versus 0.050). For single-species analyses of the *acutifrons* subgroup exclusive of *B. endeca*, DELINEATE views that our four taxa all belong

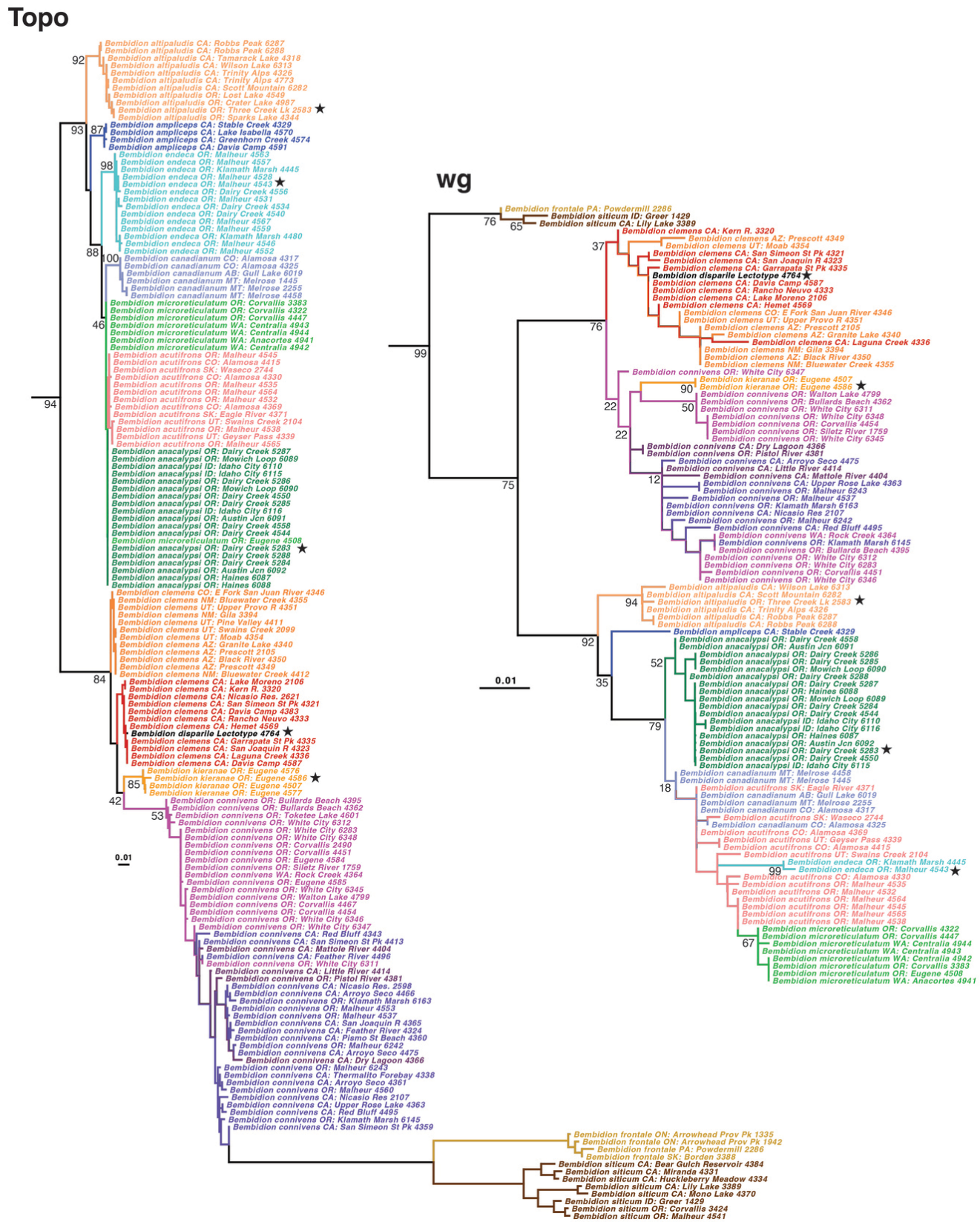

Figure 11. Maximum likelihood gene trees of Topo and wg for the *connivens* group.

The *fortestriatum* group and outgroups were present in the analysis, but were graphically removed to simplify this figure. Numbers next to some nodes are non-parametric bootstrap support values, expressed as a percentage. Bootstrap support for the monophyly of *Bembidion acutifrons* in the Topo tree is 57. Primary types indicated by stars. Scale bar as reconstructed by IQ-TREE.

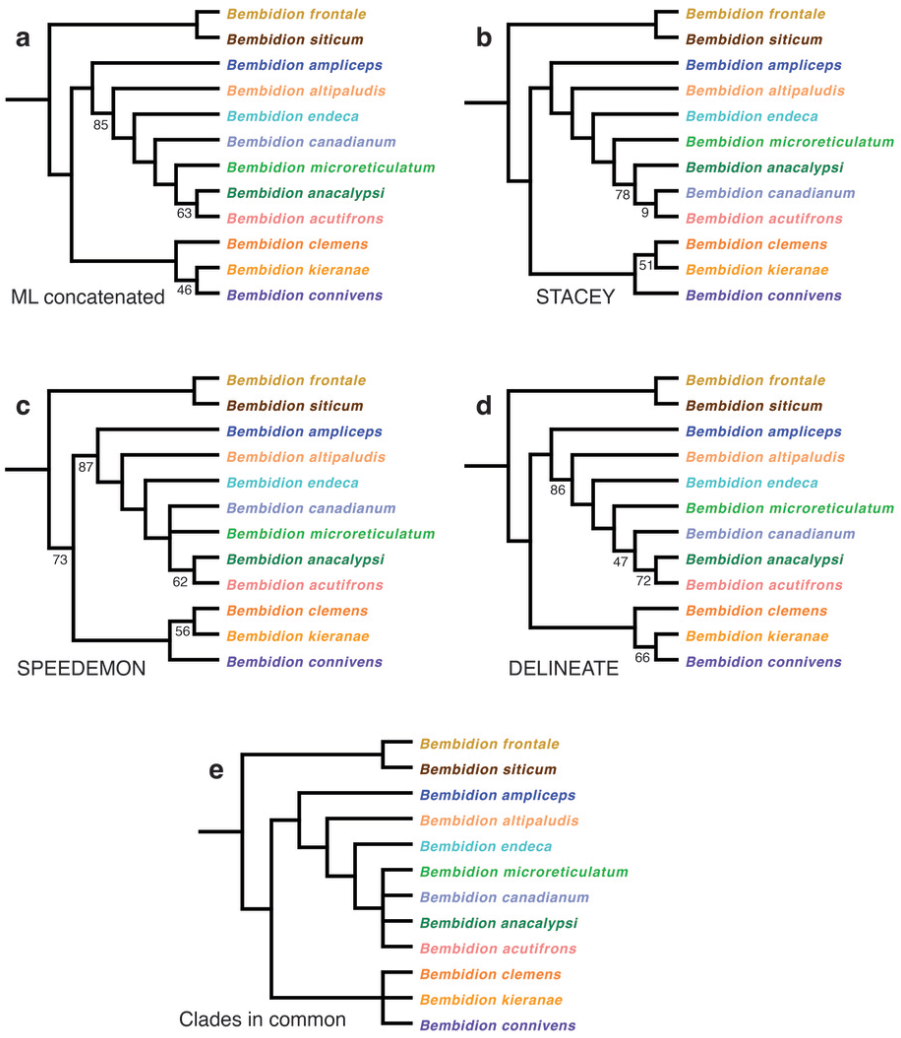

Figure 12. Species trees from various analyses.

Numbers on branches are expressed as percentages, and are either bootstrap values (**a**) or posterior probabilities (**b–d**); numbers are shown on branches only if the values are *less* than 95%. **a**: Maximum likelihood tree based upon the concatenated matrix. **b**: Maximum clade credibility tree from STACEY analysis. **c**: Maximum clade credibility tree from the SPEEDEMON analysis. Posterior probabilities shown are the lowest values across all four epsilon values. **d**: Maximum clade credibility tree from the last StarBeast3 analysis within the DELINEATE pipeline. **e**. Clades in common to species trees in **a–d**.

to two species (with *B. microreticulatum* distinct; Fig. 15a). *Bembidion canadianum*, *B. acutifrons*, and *B. anacalypsi* are considered separate species in the third-best trees. However, in treating *Bembidion canadianum*, *B. microreticulatum*, *B. acutifrons*, and *B. anacalypsi* as unidentified, DELINEATE assigns them all to a single species (Fig. 15b), with the 56th tree matching the classification we propose.

A summary of results from DNA-based species delimitation analyses, as well as morphological data (as documented in detail below), is provided in Fig. 16.

Discussion: An Integrated View of Species Boundaries

In this section we discuss the evidence for delimiting species as we have classified them. The DNA evidence and analyses are presented above. The morphological evidence is presented in detail below, in the diagnostic figures (Figs 17–19), figures showing key morphological features (Figs 20–36), and as summarized in the Taxonomic Treatment section. The geographic distributions are depicted in maps (Figs 37–43), with sympatry patterns summarized in Table 3.

The *frontale* subgroup

Our data strongly supports *B. frontale* and *B. siticum* being treated as independent species: they are differentiated by external characteristics (including in pronotal structure, proepipleural microsculpture, and color), they are sympatric in Idaho and British Columbia (Fig. 37), and there is reciprocal monophyly in COI, MSP, and Topo gene trees (Figs 9–11); STACEY supports their status as separate reproductive communities. However, there is enough genetic structure within them, in part as revealed by the SPEEDEMON and DELINEATE analyses (Figs 14, 15), that it is possible they include more than one species each. This genetic variation is primarily in nuclear protein-coding genes (Figs 10, 11) rather than 28S or COI (Fig. 9). The *frontale* sub-

Table 3. Geographic proximity of species. Approximate great-circle distances in kilometers between nearest localities of examined specimens are shown, except if the specimens are known from overlapping geographic areas, in which case the following symbols are used: S: sympatric, with overlapping ranges; M: microsympatric, collected by us within a few meters at a single locality during a single collecting event.

	frontale	*siticum*	*altipal.*	*endeca*	*canadian.*	*acutifrons*	*microret.*	*anacalyp.*	*ampliceps*	*kieranae*	*clemens*
siticum	S										
altipaludis	440	M									
endeca	400	M	30								
canadianum	S	240	690	555							
acutifrons	S	M	240	M	M						
microreticulatum	220	M	S	S	715	170					
anacalypsi	210	M	105	M	315	65	185				
ampliceps	675	S	70	65	810	235	160	285			
kieranae	610	M	100	135	840	325	M	225	350		
clemens	185	M	430	S	340	S	240	S	M	430	
connivens	S	M	M	M	370	M	M	M	S	M	M

group was not targeted for sampling in the current study; future research is warranted to sample more thoroughly and determine whether either contains sufficiently distinct populations to warrant treating them as additional species.

Bembidion altipaludis

There is also strong support for treating this lineage distinct from all others, based upon morphological (Fig. 17c) and genetic (Figs 8–11) distinctiveness. However, as with *B. frontale* and *B. siticum*, the genetic divergence within *B. altipaludis* hints that future research may support splitting this higher-elevation taxon into more than one species. The populations from western California (Trinity Alps and Scott Mountain) are especially distinctive (Fig. 14). In addition, specimens from the Trinity Alps have notably flat eyes (Fig. 28) relative to those from Scott Mountain and elsewhere. Sampling of marshes and wet meadows at higher elevation throughout northern California and adjacent Oregon is necessary to see whether there is contiguity between the Trinity Alps population and others to the north and east. There was no observed variation in male genitalia, male protarsomeres, or pronota, and thus no indication of intrinsic barriers. Based upon the data we currently have, we take the conservative approach and treat them all as belonging to one species.

The *acutifrons* subgroup

Within this subgroup of five species, two species are clearly and evidently distinct from each other and all other *Trepanedoris*: *Bembidion endeca* and *B. canadianum*. The other three species in the subgroup, *B. acutifrons*, *B. anacalypsi*, and *B. microreticulatum*, are more similar one to another.

Bembidion endeca differs in its complete lack of dorsal microsculpture, male protarsomere 1 of typical size for *Bembidion*, and details of the male genitalia. It has been found microsympatrically (within a few meters in the same microhabitat) with *B. acutifrons* and *B. anacalypsi*, and is sympatric with *B. microreticulatum* (Table 3). It is also strongly supported as a single, separate species by all DNA-based delimitation methods (Fig. 16), and we treat it as such. However, our coalescent analyses do not include any specimens from the central Sierra Nevada of California populations (Fig. 38), as we do not have DNA data from any of them. Those populations are placed in *B. endeca* based only upon morphological data. The known Oregon and California populations are about 350 km apart. This disjunction may reflect a lack of collecting, or a real gap in their distribution. If there is a large geographic gap without intervening populations, the Oregon and California populations may have a deep enough genealogical split to warrant being placed in two separate species. Future collecting could target the Warner Mountains and eastern edges of the northern and central Sierras in an attempt to find intermediate populations, and to explore DNA variation in California.

Unlike *Bembidion endeca*, specimens of *B. canadianum* have some microsculpture on the elytral apex, and larger male protarsomeres, but in contrast to *B. acutifrons*, *B. anacalypsi*, and *B. microreticulatum*, *B. canadianum* has no microsculpture on the elytral disc in females, is more convex with a narrower pronotum, has smaller male protarsomeres, and has some differences in male genitalia. Specimens of *B. canadianum* have been found around the same pond shore together with *Bembidion acutifrons* in the Alamosa National Wildlife Refuge of Colorado; the two species maintain their morphological differences there, as well as consistent sequence differences in MSP and Topo. Although there is thus no doubt that *B. canadianum* and *B. acutifrons* should be considered separate species, DELINEATE infers them as belonging to the same species (Figs 15, 16). In contrast, SPEEDEMON separates *B. canadianum* from *B. acutifrons* and near relatives and splits the two populations of *B. canadianum* (Colorado versus Montana + Alberta) into separate species. However, we could not detect differences in male genitalia between the two *B. canadianum* populations and treat all of them as a single species separate from other members of the *acutifrons* subgroup.

The other three species in this subgroup (*B. acutifrons*, *B. microreticulatum*, and *B. anacalypsi*) are quite similar to one another, with few morphological differences. All three have very large male protarsomeres, and deeply engraved microsculpture on the elytral disc in females. Although there are differences in microsculpture (especially in males), and slight differences in color and body form, the only morphological differences clearly suggesting intrinsic barriers to reproduction are in male genitalia. The three different genitalic forms are more different than the variation found within single species in other groups of *Bembidion*. Their geographic ranges do not overlap, although they are close (within 65–185 km; Table 3, Fig. 40). *Bembidion anacalypsi*'s type locality is 65 km northwest and 130 meters above the nearest known locality of *B. acutifrons*. These two localities are part of the same watershed, with the marsh in which *B. anacalypsi* is found draining downstream into Malheur Lake, where *B. acutifrons* has been found; marshes in between these two sites have not yet been sampled. In addition, the geographic range of *B. acutifrons* partly surrounds the range of *B. anacalypsi* (Fig. 40). STACEY infers these three taxa as separate reproductive communities (Fig. 13). SPEEDEMON infers *B. microreticulatum* and *B. anacalypsi* to be separate species, and divides *B. acutifrons* in two (specimens from Oregon versus east of Oregon; Fig. 14). In contrast, DELINEATE considers *B, anacalypsi*, *B. acutifrons*, and *B. canadianum* to belong to one species, potentially including *B. microreticulatum* as well (Fig. 15). Without the evidence provided by male genitalia, and the support of the SPEEDEMON and STACEY analysis, we may have treated *B. anacalypsi*, *B. acutifrons*, and *B. microreticulatum* as belonging to one species. However, considering the geographic data as well as the reproductive isolation suggested by differences in male genitalia, we feel comfortable treating all three as separate species.

There is a form of *acutifrons* subgroup in the Sierra Nevada of California and adjacent Nevada north to Klamath Marsh, Oregon, which is extremely similar to *Bembidion acutifrons*. This form would have the name *Bembidion scenicum* Casey. We currently consider this to be a separate

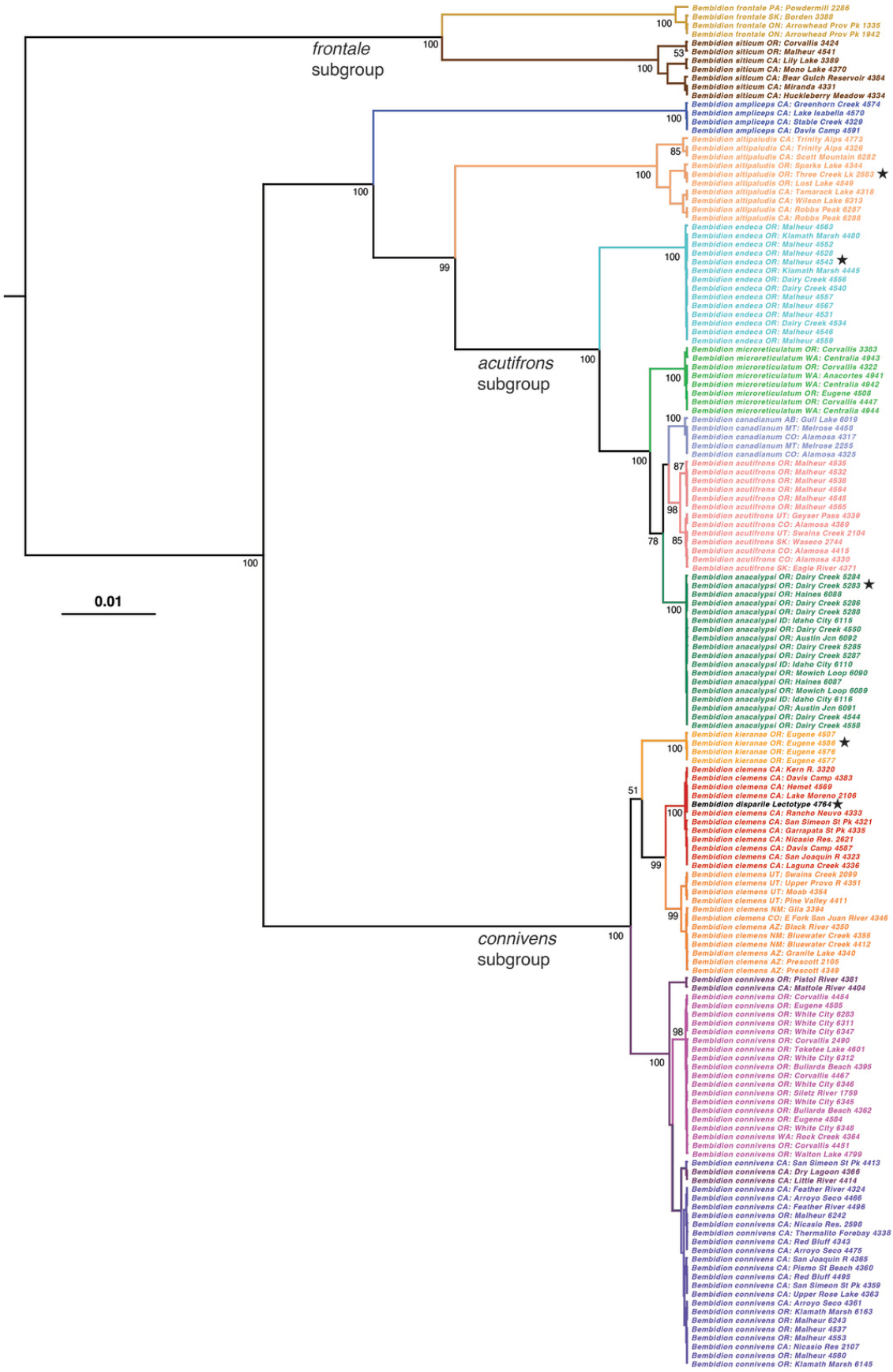

Figure 13. Maximum clade credibility tree from STACEY analysis.

Values next to nodes are posterior probabilities, expressed as a percentage; only values above 50 are shown. Primary types indicated by stars.

a

Group	epsilon values 0.0040	0.0044	0.0060	0.0102
B. frontale	40.02	40.76	44.16	50.95
B. frontale (ON+SK)	56.4	55.63	52.43	46.63
B. frontale (PA)	59.14	58.46	55.05	48.56
B. siticum	33.31	42.51	48.15	63.08
B. siticum (OR+ID)	44.76	35.05	35.59	21.02
B. siticum (CA: Lily Lk, Mono Lk)	36.27	31.94	24.88	17.93
B. siticum (CA: all but Lily Lk, Mono Lk)	25.64	20.22	19.08	11.87
B. siticum (All CA)	21.09	17.36	18.73	6.89
B. altipaludis	0.87	0.98	3.49	10.94
B. altipaludis (CA: Trinity Alps, Scott Mtn)	80.46	75.24	76.9	73.96
B. altipaludis (CA: Robbs Peak)	27.78	30.7	21.03	11.6
B. altipaludis (CA: Wilson Lk, Tamarack Lk)	9.63	9.43	6.5	2.79
B. altipaludis (OR: Lost Lk, Crater Lk, Three Ck Lk)	13.69	14.07	11.9	6.7
B. altipaludis (OR: Sparks Lk)	6.67	7.8	6.11	3.87
B. altipaludis (All but Trinity Alps nor Scott Mtn)	34.82	30.58	40.52	51.16
B. altipaludis (All OR)	32.54	33.33	27.29	17.86
B. endeca	93.52	94.28	94.6	96.21
B. canadianum	45.71	46.92	56.05	70.33
B. canadianum (CO)	51.45	50.3	41.69	27.45
B. canadianum (MT+AB)	51.08	50.11	41.41	27.47
B. acutifrons	28.79	31.45	47.87	67.15
B. acutifrons (Malheur)	65.81	62.47	46.48	27.73
B. acutifrons (East of Oregon)	62.74	60.09	45.05	27.12
B. microreticulatum	96.27	95.99	97	97.75
B. anacalypsi	95.75	96.01	96.55	97.13
B. ampliceps	97.46	97.73	97.85	98.15
B. kieranae	97.98	98.2	98.22	98.52
B. clemens	0	0	0.02	0.08
B. clemens clemens	71.93	73.82	77.72	85.44
B. clemens disparile	88.87	88.3	90.75	94.03
B. connivens	2.79	5.97	12.4	39.24
B. connivens (northern form)	85.77	79.58	72.17	48.74
B. connivens (southern form + central coastal)	30.58	24.92	31.83	28.45
B. connivens (southern form)	8.65	4.32	6.6	3.22

b

Group	epsilon values 0.0040	0.0044	0.0060	0.0102
B. frontale + B. siticum	0	0	0	0
B. acutifrons + anacalypsi	0	0.01	0.03	0.30
B. acutifrons + anacalypsi + canadianum	0	0	0	0.11
B. acutifrons + anacalypsi + canadianum + microret.	0	0	0	0

Figure 14. SPEEDEMON results.

Posterior probabilities of groups ("clusters"), expressed as percentages. A group consists of a population or group of populations, as specified. Posterior probabilities are shown for four different values of epsilon, from 0.0040 through 0.0102. **a**: Results for groups considered species or within species in our classification. **b**: Results for groups considered to consist of multiple species in our classification.

species, based on differences in the female genitalia and genomic sequencing, but further research is needed to confirm this distinction.

Bembidion ampliceps

This taxon is very distinctive in numerous morphological characters (Fig. 19a) as well as in DNA sequences (Figs 8–11); all DNA-based methods strongly support it being a single, separate species (Fig. 16).

Bembidion kieranae

This species is distinctive morphologically, including in male genitalia, and in most genes (Fig. 16). The only gene in which it is not separated from both *Bembidion connivens* and *B. clemens* is COI (Fig. 9). There is strong support for its status as a separate species in all three DNA-based species delimitation analyses (Figs 13–15).

a

Group	Tree 1	Tree 2	Tree 3
B. frontale	0.392	0.248 2sp	0.213 sit
B. siticum	0.107 3sp	0.098	0.067 2sp
B. altipaludis	0.198	0.102 2sp	0.038 2sp
B. endeca	0.672	0.057 can	0.056 mic
B. canadianum	0.291 acu	0.189 ana	0.158
B. acutifrons	0.309 ana	0.230 can	0.125
B. anacalypsi	0.425 acu	0.205 can	0.169
B. microreticulatum	0.292	0.263 can	0.194 acu
B. ampliceps	0.850	0.045 alt	0.023 cle
B. kieranae	0.528	0.258 con	0.193 cle
B. clemens	0.216	0.117 con	0.091 2sp
B. connivens	0.159	0.083 cle	0.066 kie

b

Group	Tree 1	Tree 2	Tree 3	Matching Tree #
B. frontale + B siticum	0.061 4sp	0.050	0.047 5sp	2
B. acutifrons + anacalypsi	0.338 can	0.121 mic	0.068 1sp	7
B. acutifrons + anacalypsi + canadianum	0.271 mic	0.089 1sp	0.037	27
B. acutifrons + anacalypsi + canadianum + microret.	0.170 1sp	0.081 end	0.051 2sp	56

Figure 15. DELINEATE results.

The three columns "Tree 1" through "Tree 3" list the constrained probabilities of the three trees for each analysis with the highest probabilities, in order from most probable to least probable. Each row represents one DELINEATE analysis in which the indicated group (the set of populations we are classifying as species or sets of species) is specified as unidentified, but all other populations have species designations following the classification we present in this paper. Black cells indicate the tree that treats that group as it is treated in our classification; for example, Tree 1 in the *B. frontale* analysis infers all *B. frontale* specimens as a single, distinct species, as it is treated in our classification. However, in the *B. siticum* analysis, it is the second most probable tree (Tree 2) that treats *B. siticum* as a single, distinct species, not the most probable tree (Tree 1). Black cells with probabilities over 0.50 have text in white; those with probabilities less than 0.50 have text in gray. Red cells indicate that tree treats the group as belonging to a different species than we treat it in our classification; for example, in the *B. canadianum* analysis, Tree 1 treats the *B. canadianum* specimens as belonging within *B. acutifrons*. (In red cells, the text after the probability consists of the first three letters of the specific epithet of the taxon in which those populations are grouped.) Pale blue cells indicate that the tree considers the group to consist of a one ("1sp"), two ("2sp"), or three ("3sp") distinct species, in contrast to the classification. For example, Tree 1 in the *B. siticum* analysis infers *B. siticum* to consist of three distinct species, different from all others in the study. The white cell indicates the group is considered a mix of specimens that belong to other identified species and specimens belong to separate, novel species. In the Matching Tree # column, the number of the tree that matches the classification used in this paper is indicated; for example, for *B. acutifrons* + *B. anacalypsi* analysis, the seventh most probable tree is the one that matches the classification, with *B. acutifrons* and *B. anacalypsi* as separate species distinct from each other and from other species. **a**: Results for groups considered species in our classification. **b**: Results for groups considered to consist of multiple species in our classification.

Bembidion connivens

The populations treated here as this species show notable geographic variation in both morphological and DNA traits. Specimens from the Willamette Valley in Oregon and northward consistently differ from specimens from the Central Valley and Bay Area of California and southward at 8 sites in 28S (Fig. 9), a divergence that is unprecedented within a species of *Bembidion*. Topo also shows a separation between the two areas (Fig. 11), based on about 20 inconsistent sites. There are no consistent differences between the northern form and the southern form in COI, CAD, MSP, or wg (Figs 9–11). There are also differences in microsculpture: the northern populations lack microsculpture on the pronotal disc, and have mostly isodiametric microsculpture on the elytra (e.g., specimens 4584, 4454, and 4799 in Fig. 44), whereas the southern populations have evident microsculpture across the entire pronotum and slightly transverse sculpticells on the elytra (e.g., specimens 4343 and 4466 in Fig. 44). These differences in DNA and microsculpture led Maddison (2012) to consider the northern and southern forms to belong to different species (*B. elizabethae* and *B. connivens*, respectively), even though no differences in male genitalia were evident.

However, subsequent sampling revealed two geographic regions containing intermediates. The population in southern Oregon at White City (represented by specimens 6312 and 6345 in Fig. 44) have 28S sequences matching the northern form, but have microsculpture matching the southern form (Fig. 44). Of the seven specimens sequenced from White City, one has Topo matching the typical form found in the northern population, one matches the southern population, and five are heterozygotes at most sites in Topo at which the two forms in general differ. Coastal populations in northern California and southern Oregon (shown in triangles in Fig. 44) are intermediate between the two main regions, having seven of the eight sites of 28S matching those of the northern populations, but one matching the southern populations. In Topo, these central coastal populations are more similar to other southern pop-

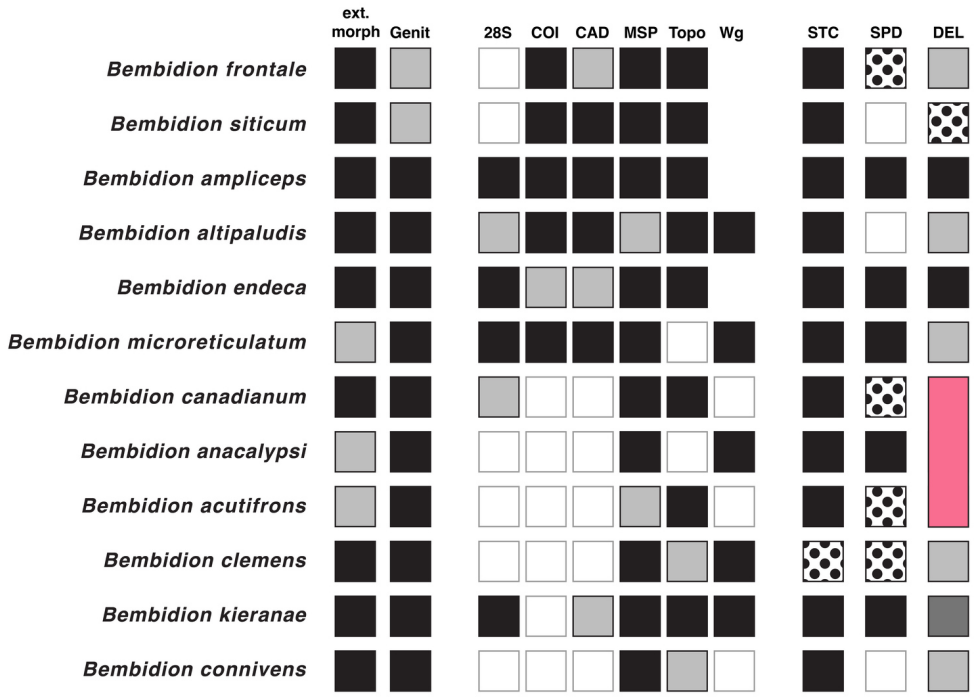

Figure 16. Summary diagram of species delimitation analyses using morphological and DNA sequence data.

The first two columns of squares show a rough estimate as to how morphologically distinct various species are; the first column indicates distinctiveness of external characters, and the second column of male genitalia. Black squares designate species that are strongly morphologically distinct (as judged by DRM), with obviously distinctive features; gray squares indicate those species with a lesser level of morphological distinctiveness, but still enough to be confident they belong to lineages worthy of species rank (especially, in a few cases, when one considers geographic distributions). In the columns labeled with gene names a square is black if in the maximum likelihood gene tree that species is monophyletic, and gray if the species is a grade distinct from all other species. The absence of some squares for the gene wg indicate the species was represented by fewer than three individuals. The column labeled "STC" shows the results from the STACEY analysis. A square is black or dotted if that group has a posterior probability ⩾ 98%; it is dotted if that group consists of two subgroups each of which have posterior probabilities ⩾98%, otherwise the square is black. The column labeled "SPD" shows the results from the SPEEDEMON analysis (Fig. 14). A square is black if that species is strongly supported (posterior probability >90%) for all examined values of epsilon; a square is dotted if that species is supported by lower posterior probabilities but all clades within that group are supported as separate species with posterior probabilities > 50%. The column labeled "DEL" shows the results from the DELINEATE analysis (Fig. 15). A square is black or gray if the tree with the maximum constrained probability has that group inferred as a distinct, single species; the square is black if probability is > 0.65, dark gray if the probability is 0.50–0.65, and light gray if the probability is <0.50. A square is dotted if the maximum constrained probability tree infers that group consists of multiple species. The pink rectangle indicates those three species are considered as a single species in the single-species DELINEATE analysis.

ulations. Structurally, beetles from the central coast vary, and include intermediates between the northern and southern forms. The discordance among 28S, Topo, and morphological data, with intermediates in southern Oregon and northernmost California, suggests recent or on-going gene flow between north and south. The STACEY (Fig. 13) and SPEEDEMON (Fig. 14) analyses suggest the northern form is a separate lineage, but do not support the southern or central coastal populations as separate species. The DELINEATE analysis (Fig. 15) supports *B. connivens* as a whole as being a single species. These results, combined with the lack of observed genitalic differences, provide justification for treating the northern or "*elizabethae*" form as belonging to *B. connivens*, and not a separate species.

Bembidion clemens

There are two forms of *Bembidion clemens*, here treated as subspecies: *B. clemens clemens* in Arizona, Nevada, Idaho, and eastern Oregon, east to Colorado and New Mexico, and *B. clemens disparile*, known only from California (Fig. 42). They differ consistently in 28S (Fig. 9), CAD (Fig. 10), and Topo (Fig. 11), leading to their estimation as two genetically separate lineages by STACEY (Fig. 13), with SPEEDEMON favoring two separate species (Fig. 14). In contrast, DELINEATE infers that both forms belong to the same species (Fig. 15). We could find no differences in male genitalia, and thus no intrinsic barriers to reproduction. The only detected consistent morphological differences are in color, with *B. clemens clemens* having paler appendages and consistently having a spot on each elytron, and *B. clemens disparile* having darker appendages and often lacking elytral spots. Less consistent are differences in pronotal width and elytral striae: most specimens of *B. clemens disparile* have a narrower prothorax and smaller punctures in the elytral striae than specimens of *B. clemens clemens*.

However, we have no DNA sequences of populations close to where the two color forms approach each other; the closest sequenced specimens (a *B. clemens clemens* from Granite Lake, Arizona, and a *B. clemens disparile* from Hemet, California) are 400 km apart. The known geographic ranges of the two forms of *B. clemens* approach each other in eastern California and western Nevada (Fig. 42). There is a series of 12 specimens from 11 mi NW Beatty, Nye County, Nevada in CSCA that are typical of *Bembidion clemens clemens*: pale appendages, with large, bright preapical spots on the elytra. There is a large series of over 100 specimens of *B. clemens disparile* in CUIC from 115 km away, at Buckhorn Springs, Deep Springs Valley, Inyo County, California. These specimens have dark legs and antennae, and small

fainter preapical spots on the elytra. There is a 300 m elevational discrepancy between these two populations, with the Nevada locality at about 1200 m and the California locality at about 1500 m.

With current evidence we feel it most appropriate to consider these two forms as belonging to a single species. As we could detect no differences in male genitalia, we predict that in geographically intermediate areas there will be evidence of gene flow between the two forms. Additional sampling and DNA data from populations near the California/Nevada or California/Arizona borders may help resolve whether these two forms occur sympatrically and maintain their genetic distinctiveness, and thus should each be given the rank of species, or whether there are signs of significant gene flow, and thus should continue to be considered conspecific.

Comparison of Species Delimitation Methods

The three methods we used for species delimitation based upon the multispecies coalescent, STACEY, SPEEDEMON, and DELINEATE, each yielded unique species inferences. Our criteria for choosing species boundaries based upon the genetically independent reproductive communities STACEY inferred (Fig. 13) yielded results very similar to our synthetic inference (Fig. 16). SPEEDEMON, in contrast, inferred more species than we did, and DELINEATE fewer (Fig. 16).

Our synthetic method for determining species boundaries utilized morphological variation, especially of characters potentially involved in intrinsic barriers to reproduction, as well as the DNA analyses, combined with information about geographic distributions, including whether forms coexist in the same habitat. Our synthesis thus had an advantage over the three multispecies coalescent methods by themselves, as the latter did not consider the morphological and geographic data. For example, in inferring that *Bembidion canadianum* and *B. acutifrons* belong to the same species, DELINEATE did not consider that the two forms are microsympatric and have significant morphological differences and distinction in 28S, MSP, and Topo in sympatry.

The multispecies coalescent methods may also be presuming less variation in evolutionary model than we presumed in our synthetic inference. For example, the DNA sequence variation in *Bembidion connivens* (e.g., as partially summarized by branch lengths in Fig. 8) is on par with that within the trio of *B. acutifrons*, *B. anacalypsi*, and *B. microreticulatum* (Fig. 8). We treat the former as one species but the latter as three, based in part on patterns of genitalic variation. However, in our DELINEATE analysis, the decision about the *acutifrons* subgroup was predicated on the classification of the remaining species, including having all *Bembidion connivens* lumped into one species. DELINEATE presumably used the extensive variation in *B. connivens* to sway its inference toward lumping the *acutifrons* subgroup species together.

The DELINEATE analysis was also hampered by the procedure we used in allowing only one of our species to be left unidentified and tested at any one time. If we had more species of absolutely certain status, and fewer species whose status we wanted to test, we could have avoided presuming that uncertain species were settled and certain, and we would have had additional certain species for DELINEATE to use for its speciation completion model inference. Nonetheless, the DELINEATE analysis at least allowed a test of the internal consistency of our methods: if DELINEATE differed in our inference of a species boundary even after presuming all other species fit our classification, then that suggests a possible internal inconsistency in the criteria we used to create the classification, or it indicates the evolutionary properties of the beetles varies across the phylogeny. When there was such a difference, as for the *acutifrons* subgroup, it forced us to examine carefully our conclusions and clarify the evidence we were using to overrule DELINEATE's inference.

Most, but not all, groups of bembidiines have DNA sequences showing coalescence of most genes within species to a much greater extent than in *Trepanedoris*. For example, in the subgenus *Pseudoperyphus* (D. R. Maddison, 2008), the *aenulum* subgroup of subgenus *Odontium* (D. R. Maddison & Arnold, 2009), subgenus *Liocosmius* (D. R. Maddison & Cooper, 2014), the *breve* group of subgenus *Plataphus* (Sproul & Maddison, 2017a), and the *levigatum* group of subgenus *Hydrium* (D. R. Maddison, 2020), as well as the related genus *Lionepha* (D. R. Maddison & Sproul, 2020), specimens from each species appear as a clade in most genes examined, including nuclear protein-coding genes. A few groups, such as the *Bembidion transversale* group, do not show this pattern, and instead show species intermingled in studied gene trees (D. R. Maddison, 2020), as is the case for many *Trepanedoris*. It is likely that multispecies coalescent methods such as SPEEDEMON and DELINEATE, even though they don't consider morphological or geographic data, would be more successful in most other groups of bembidiines. DELINEATE has already proven successful for the genus *Lionepha*, as outlined in A Complete Worked Example: *Lionepha* (https://jeetsukumaran.github.io/delineate/workflow1.html).

Taxonomic Treatment

Trepanedoris is a member of the *Bembidion* series (D. R. Maddison, 2012) of the genus *Bembidion*. The subgenus is characterized as follows. Head with engraved frontal furrows which converge anteriorly onto the clypeus; mentum in most species with prominent transverse ridge, and with anterior lateral region complete, triangular; mentum tooth triangular. Posterior angle of pronotum with a posterolateral carina; hind margin of pronotum not abruptly sinuate and notched. Each elytron possessing a crista clavicularis (see D. R. Maddison, 2012, p. 547); lateral bead ending at humerus, not angulate and prolonged onto base; elytral striae punctate, in almost all specimens only the first stria reaches the elytral apex (some specimens in the *acutifrons* subgroup have a very faint second stria also reaching the

apex). Discal setae ed3 and ed5 in elytral interval, not attached to the third stria. Mesoventral process without subapical setae. Metaventral process unmargined. Apex of last visible abdominal sternite with two setae in males, four setae in females. Apices of each paramere normally with three setae, but some individuals have two or four setae on one of the parameres. Internal sac of male genitalia with a small flagellum. Body length 2.2–3.9 mm.

The 12 species of *Bembidion (Trepanedoris) connivens* group considered here are:

- *frontale* subgroup
  - *B. frontale* (LeConte)
  - *B. siticum* Casey
- *B. altipaludis* Maddison, n. sp.
- *acutifrons* subgroup
  - *B. endeca* Maddison & Sproul, n. sp.
  - *B. canadianum* Casey
  - *B. acutifrons* LeConte
  - *B. microreticulatum* Hatch
  - *B. anacalypsi* Mendez & Maddison, n. sp.
- *B. ampliceps* Casey
- *connivens* subgroup
  - *B. kieranae* Maddison & Sproul, n. sp.
  - *B. clemens* Casey
    - *B. clemens clemens* Casey
    - *B. clemens disparile* Casey
  - *B. connivens* (LeConte)

Identification of *Trepanedoris* to species

Trepanedoris specimens are not easy to identify to species, as they are small, with subtle or difficult-to-observe morphological differences, and enough within-species variation to obscure easy differentiation. A sufficiently powerful microscope with effective lighting (either bright, diffuse light or that produced by a ring light) is necessary to be able to discern some of the key features, including presence of microsculpture sculpticells or the structure of the frontal furrow. *Bembidion altipaludis*, *B. endeca*, and *B. canadianum* are difficult to distinguish from each other using external structure, as are *B. acutifrons*, *B. microreticulatum*, and *B. anacalypsi*. There are a few species that are easy to recognize. For example, if the specimen has preapical pale spots on the elytra, and has clearly evident microsculpture on the elytral disc, then it can be one of only two species: if it is a member of the *fortestriatum* group, it will be *Bembidion concretum* Casey; if it is a member of the *connivens* group, *B. connivens*. For the most part, however, species are hard to distinguish.

We provide a series of diagnostic figures (Figs 17–19) and the following dichotomous key to aid in identification. Examination of the male genitalia (Figs 20–22) will sometimes be necessary. Comparison with known geographic ranges can also provide clues, but these small, often cryptic beetles are poorly enough collected that their known geographic ranges likely do not represent their full distributions.

The first couplet in the following key separates the two sister clades that comprise *Trepanedoris*, the *fortestriatum* group and the *connivens* group, but the key does not consider the *fortestriatum* group further. This difficult group of at least five species in North America will be covered in a later paper.

The key also does not include five or six species in the *connivens* group of which we are aware: *Bembidion scenicum* Casey, *B. remotum* Casey, and four undescribed species. *Bembidion scenicum* is known only from California and regions of Oregon and Nevada within 100 km of the California border, and is very similar to *B. acutifrons*; although we currently consider it a distinct species, further research is needed. Using the key, specimens of *B. scenicum* would be identified as *B. acutifrons;* thus, identification of members of the *acutifrons* subgroup from California and nearby areas should be treated with suspicion. *B. remotum* is from southern California, and has some similarities to *B. clemens disparile*, but is not closely related and is morphologically quite distinctive (it is larger, has a different body form, pale legs, and evident elytral spots). The four undescribed species are known only from northern California and southernmost Oregon within five kilometers of the California border. Specimens of *B. remotum* and the four undescribed species would likely be keyed to *B. clemens* or *B. kieranae*, but examination of male genitalia and diagnoses provided below should reveal that those specimens do not belong to *B. clemens* or *B. kieranae*.

The key thus covers all members of the *connivens* group outside of California and adjacent regions of Oregon and Nevada, and when used with caution, some specimens within California.

Key to adults of most North American species of *Trepanedoris*

1. Frontal furrows less diverging posteriorly, with posterior end of furrow more medially located, distant from the posterior supraorbital seta (Figs 27a, 26a). Anterior margin of labrum with evident medial bump (Fig. 29a). Male genitalia with a sharply hooked apex … ***fortestriatum* group** (not covered further)

- Frontal furrows more diverging posteriorly, with posterior end of furrow wrapping around the back of the eyes, or at least very close to the posterior supraorbital seta (Fig. 27b–f, 26b–i). Anterior margin of labrum with or without medial bump. Male genitalia with simple apex, without a hook (Figs 20–22). … ***connivens* group, 2**

2. Pronotum with large, longitudinally stretched punctures or large wrinkles in anterior transverse impression (Fig. 32a, b). Mentum without transverse ridge in front of the two medial setae (Fig. 30a). Lacking microsculpture on the dorsal surface. Small (length 2.2–2.7 mm) (Fig. 5a, b) … ***frontale* subgroup, 3**

- Pronotum anterior transverse impression with faint wrinkles (Fig. 32c, e) or smooth (Fig. 32d, f). Mentum

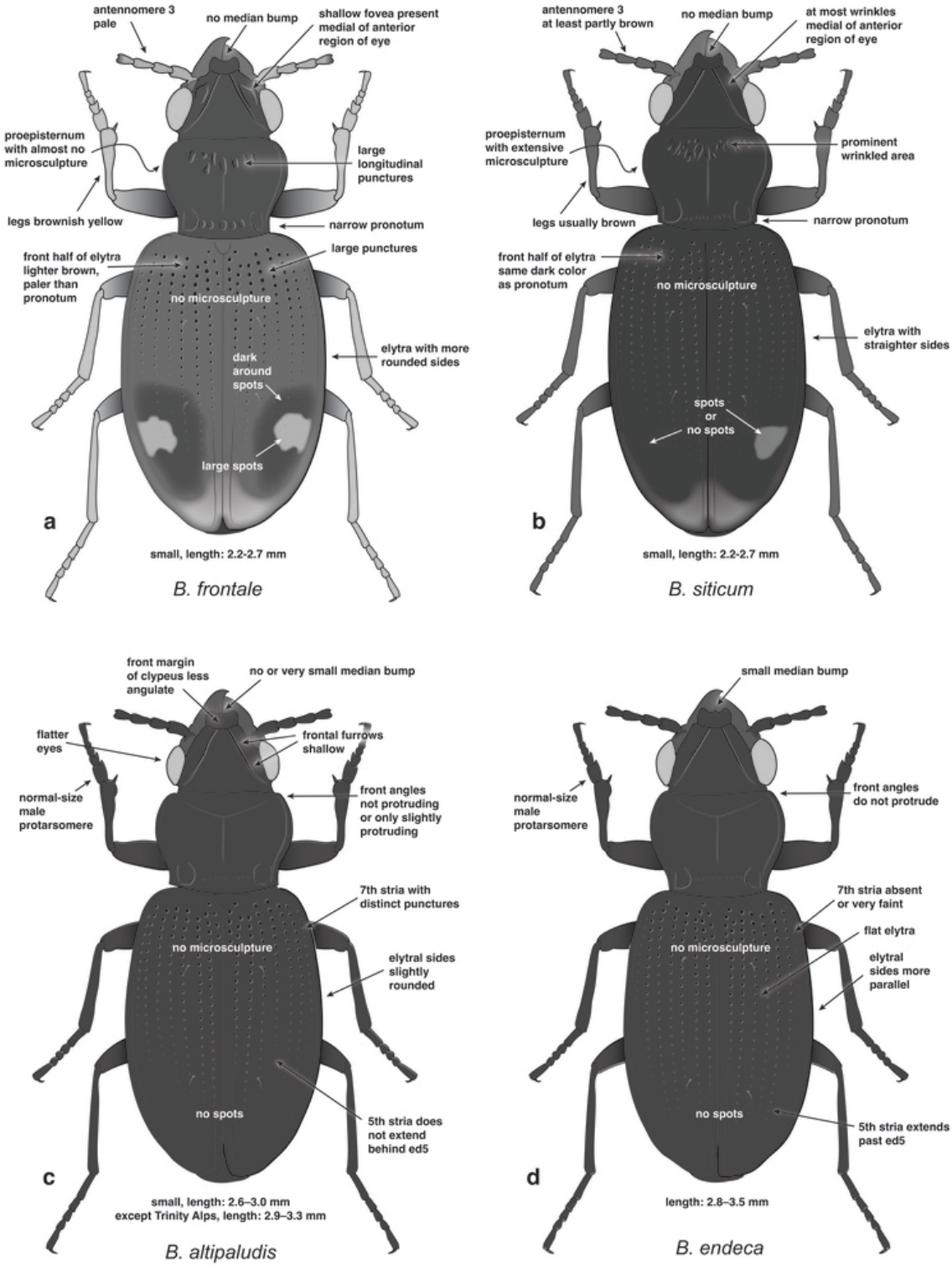

Figure 17. Diagrams of diagnostic features of *Trepanedoris* species. Apical seven antennomeres omitted, as well as most setae.

a: *Bembidion frontale*. **b**: *B. siticum*. **c**: *B. altipaludis*. **d**: *B. endeca*.

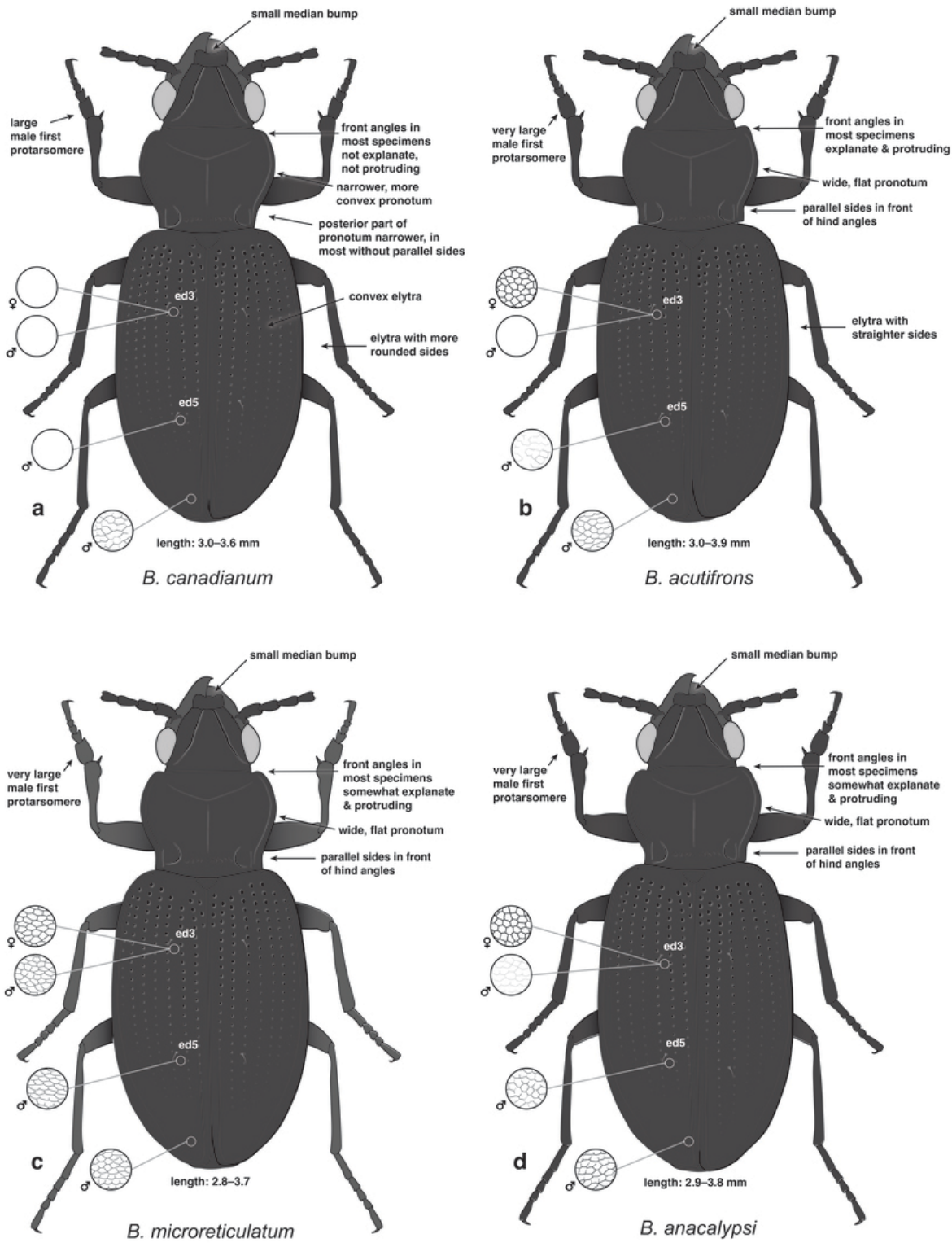

Figure 18. Diagrams of diagnostic features of *Trepanedoris* species. Apical seven antennomeres omitted, as well as most setae.

a: *Bembidion canadianum*. **b**: *B. acutifrons*. **c**: *B. microreticulatum*. **d**: *B. anacalypsi*.

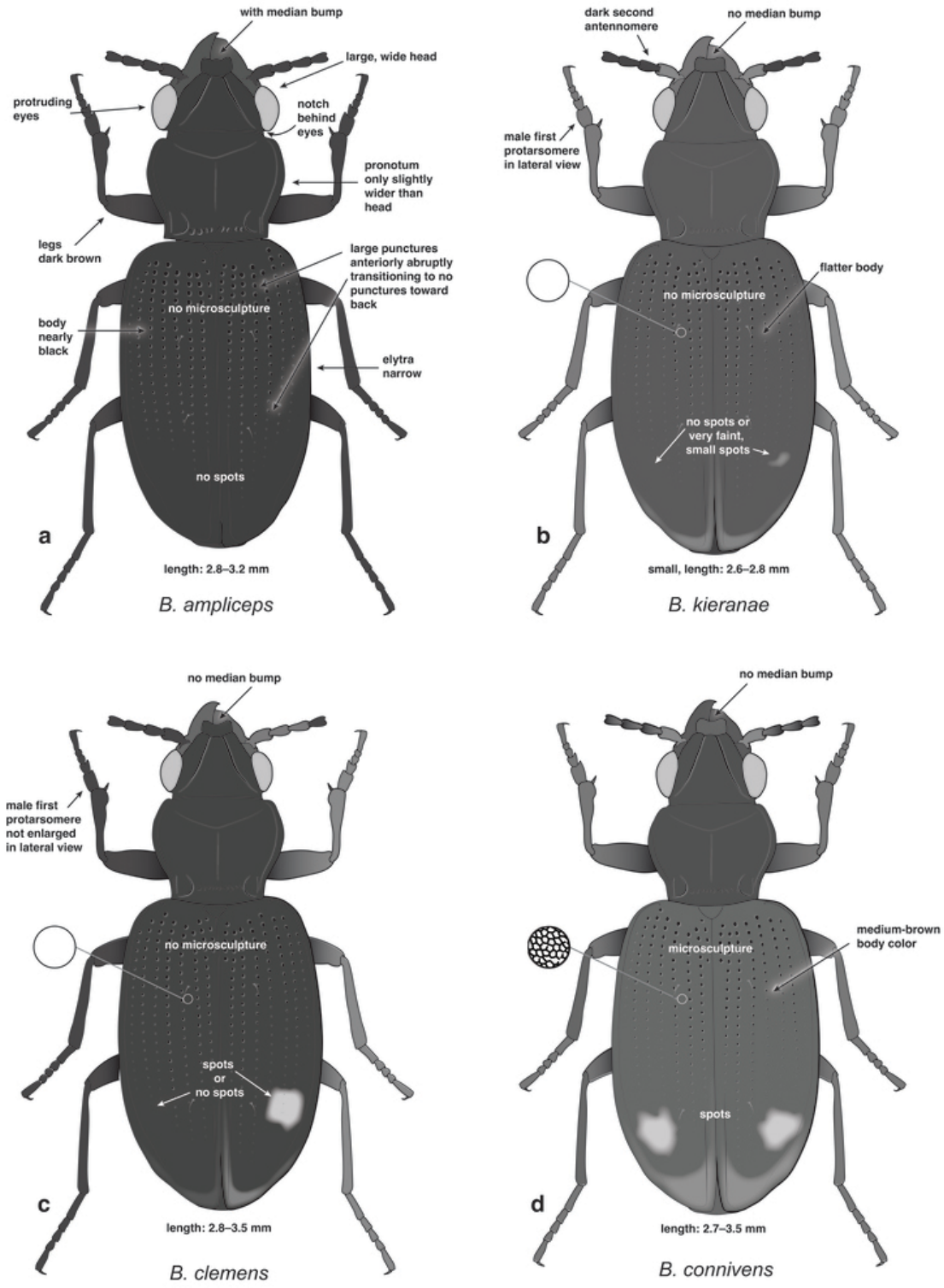

Figure 19. Diagrams of diagnostic features of *Trepanedoris* species. Apical seven antennomeres omitted, as well as most setae.

a: *Bembidion ampliceps*. **b**: *B. kieranae*. **d**: *B. clemens*. **d**: *B. connivens*.

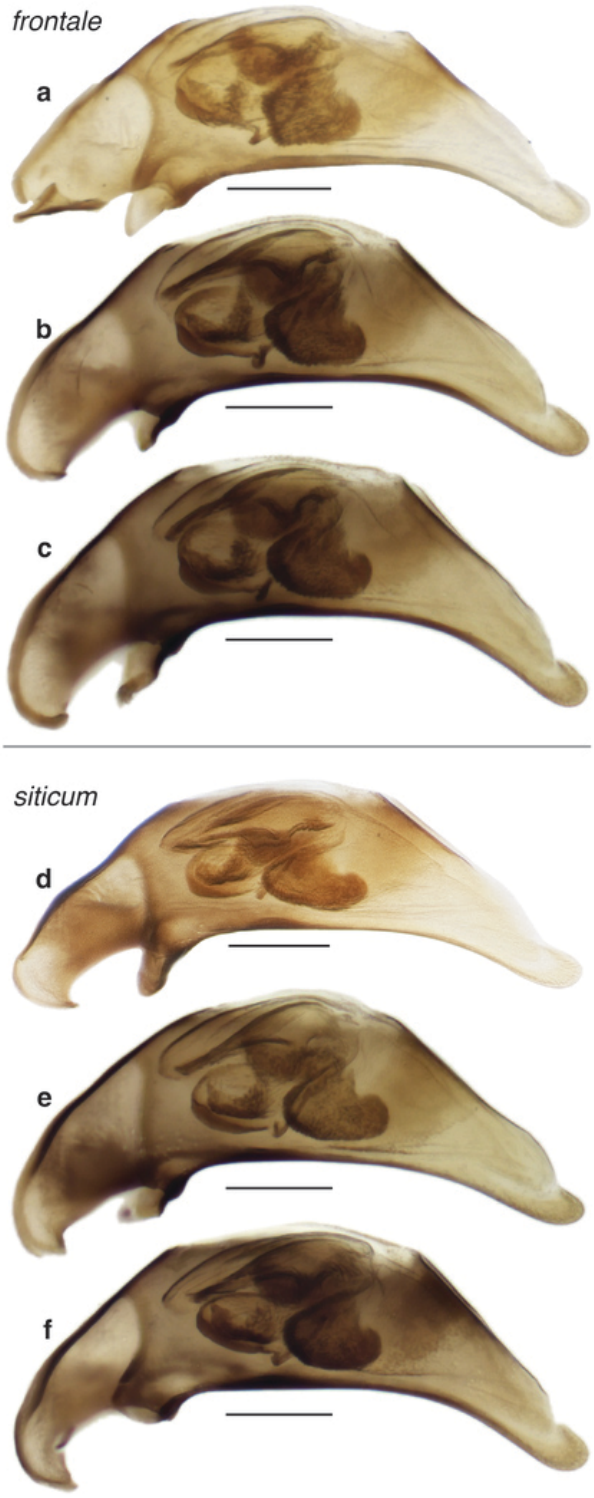

Figure 20. Aedeagus of male genitalia of *Trepanedoris* of the *frontale* subgroup, left lateral view.

a: *Bembidion frontale* (Canada: Ontario: Arrowhead Provincial Park; DNA1335). **b**: *B. frontale* (USA: Vermont: Chittenden Co., mouth of Winooski River). **d**: *B. frontale* (USA: New Hampshire: Carroll Co., Saco River at North Conway). **d**: *B. siticum* (USA: California: El Dorado Co., Lily Lake; voucher DNA3389). **e**: *B. siticum* (USA: Washington: Whitman Co., Rock Lake). **f**: *B. siticum* (USA: California: Tehama Co., Wilson Lake). Scale bars 0.1 mm.

with transverse ridge in front of the two medial setae (Fig. 30b–d). Dorsal surface with or without microsculpture. Most specimens larger bodied (length 2.6–3.9 mm) ... **4**

3. Anterior transverse impression of pronotum with punctures deep and distinct (Fig. 32a). Proepisternum without microsculpture anteriorly and laterally (Fig. 31c, e); at most with faint microsculpture near coxa. Paler, with legs and at least the basal three antennomeres a pale yellow-brown; elytra paler brown in the anterior half, contrasting against a dark ring around the preapical spot (Fig. 5a). Most specimens with a shallow fovea medial of anterior portion of eye, between frontal furrow and eye (Fig. 31a, arrow). Figures 5a, 17a ... ***B. frontale***

- Anterior transverse impression of pronotum without distinct punctures, instead having more of a wrinkled appearance (Fig. 32b). Proepisternum with well-engraved microsculpture throughout (Fig. 31d, f). Darker, with legs brown in most specimens and at least antennomere 3 brown; elytra uniformly dark brown except for the preapical spot. (Fig. 5b). Most specimens without a shallow fovea medial of anterior portion of eye, between frontal furrow and eye, although in some specimens that area is wrinkled (Fig. 31b). Known only from the Rocky Mountains and west. Figures 5b, 17b ... ***B. siticum***

4. Elytra entirely black or brown, without pre-apical spots ... **5**

- Elytra with pre-apical spots ranging in intensity from very faint to prominent ... **12**

5. Apex of elytra with evident microsculpture sculpticells (Fig. 34b, c), including some complete sculpticells. Elytral disc with or without microsculpture. Male protarsomere 1 large and wide, appearing swollen (Fig. 33c–e) ... **6**

- Apex of elytra with no trace of microsculpture sculpticells (Fig. 34a); very shiny. Elytral disc with no microsculpture. Male protarsomere 1 not as large (Fig. 33a, b, f–k) ... **9**

6. Pronotum more convex, more constricted posteriorly (Fig. 6a), in most specimens with anterior corners with less explanate margins and not extended as far forward (Fig. 32d, arrow), although a few specimens similar to Fig. 32e; with lateral margins in many specimens not parallel in front of hind angles. No microsculpture on elytral disc around ed3 and ed5 in females or males. Male protarsomere 1 not as swollen (Fig. 33c). Figures 6a, 18a ... ***B. canadianum***

- Pronotum flatter, less constricted posteriorly, in most specimens with lateral margins parallel or nearly so for a short region in front of hind angles (Fig. 6b–d); anterior corners of pronotum with explanate margins and extended anteriorly (Fig. 32e, f). Females with strong microsculpture on elytral disc with deeply engraved sculpticells, and thus with a matte luster, contrasting sharply against the shiny pronotum; males with varied microsculpture. Male

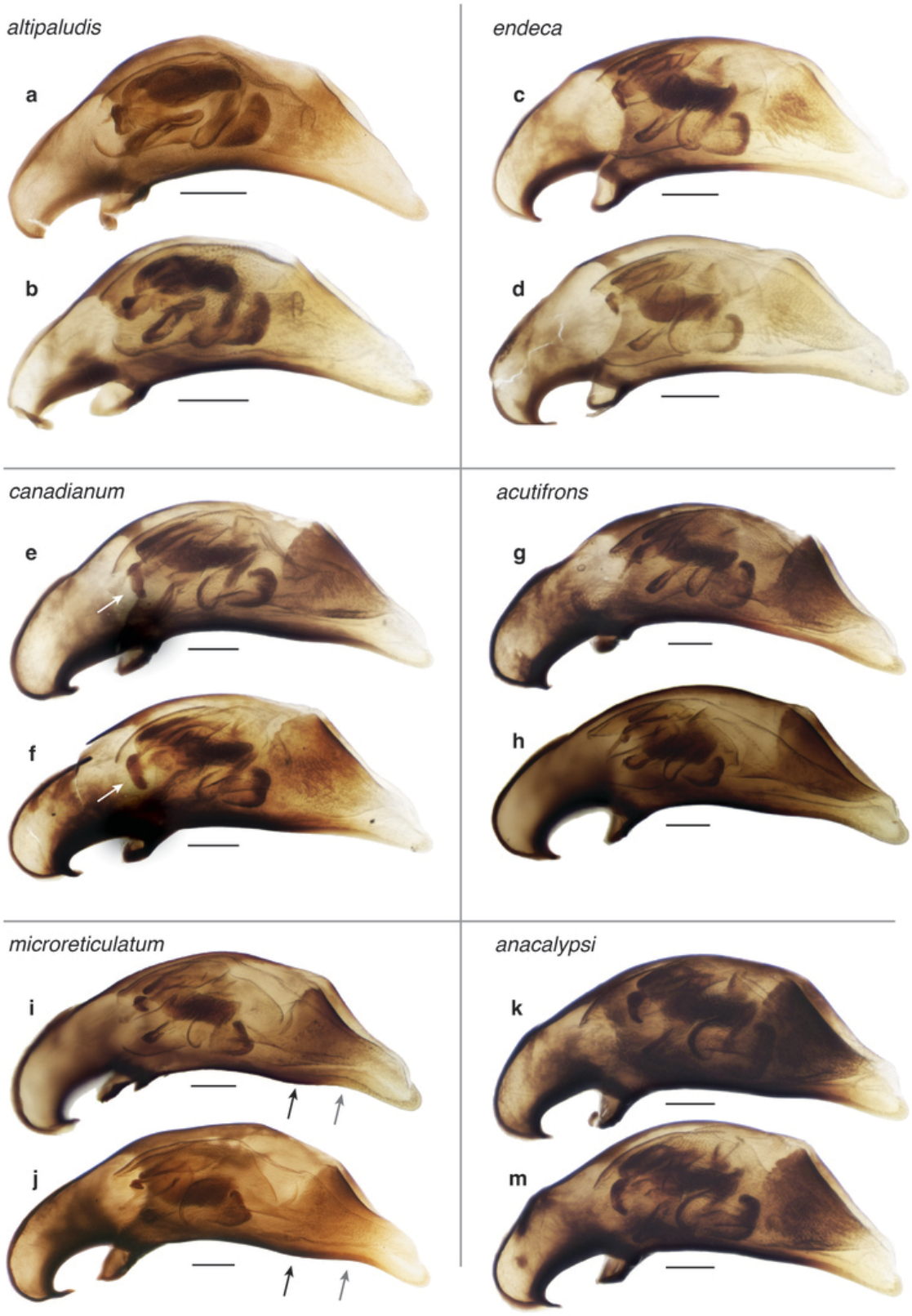

Figure 21. Aedeagus of male genitalia of *Trepanedoris* of the *acutifrons* subgroup and *Bembidion altipaludis*. left lateral view.

a: *Bembidion altipaludis* (USA: California: Sierra Co., lower Tamarack Lake; voucher DNA4318). **b**: *B. altipaludis* (USA: Oregon: Klamath Co., Munson Creek, Crater Lake NP; voucher DNA4987). **c**: *B. endeca* (USA: Oregon: Harney Co., Steens Mountain Loop Road, Malheur NWR; voucher DNA4546). **d**: *B. endeca* (USA: Oregon: Klamath Co., Klamath Marsh NWR; voucher DNA4445). **e**: *B. canadianum* (USA: Colorado: Alamosa Co., Alamosa NWR; voucher DNA4317). **f**: *B. canadianum* (USA: Montana: Beaverhead Co., Melrose, Big Hole River; voucher DNA1445). **g**: *B. acutifrons* (USA: Oregon: Harney Co., Marshall Pond, Malheur NWR; voucher DNA4538). **h**: *B. acutifrons* (USA: Colorado: Alamosa Co., Alamosa NWR; voucher DNA4330). **i**: *B. microreticulatum* (USA: Washington: Lewis Co., Centralia; voucher DNA4943. **j**: *B. microreticulatum* (USA: Oregon: Benton Co., Corvallis; voucher DNA3383). **k**: *B. anacalypsi* (USA: Oregon: Harney Co., Dairy Creek; voucher DNA4550). **m**: *B. anacalypsi* (USA: Oregon: Harney Co., Dairy Creek; voucher DNA4544). Scale bars 0.1 mm.

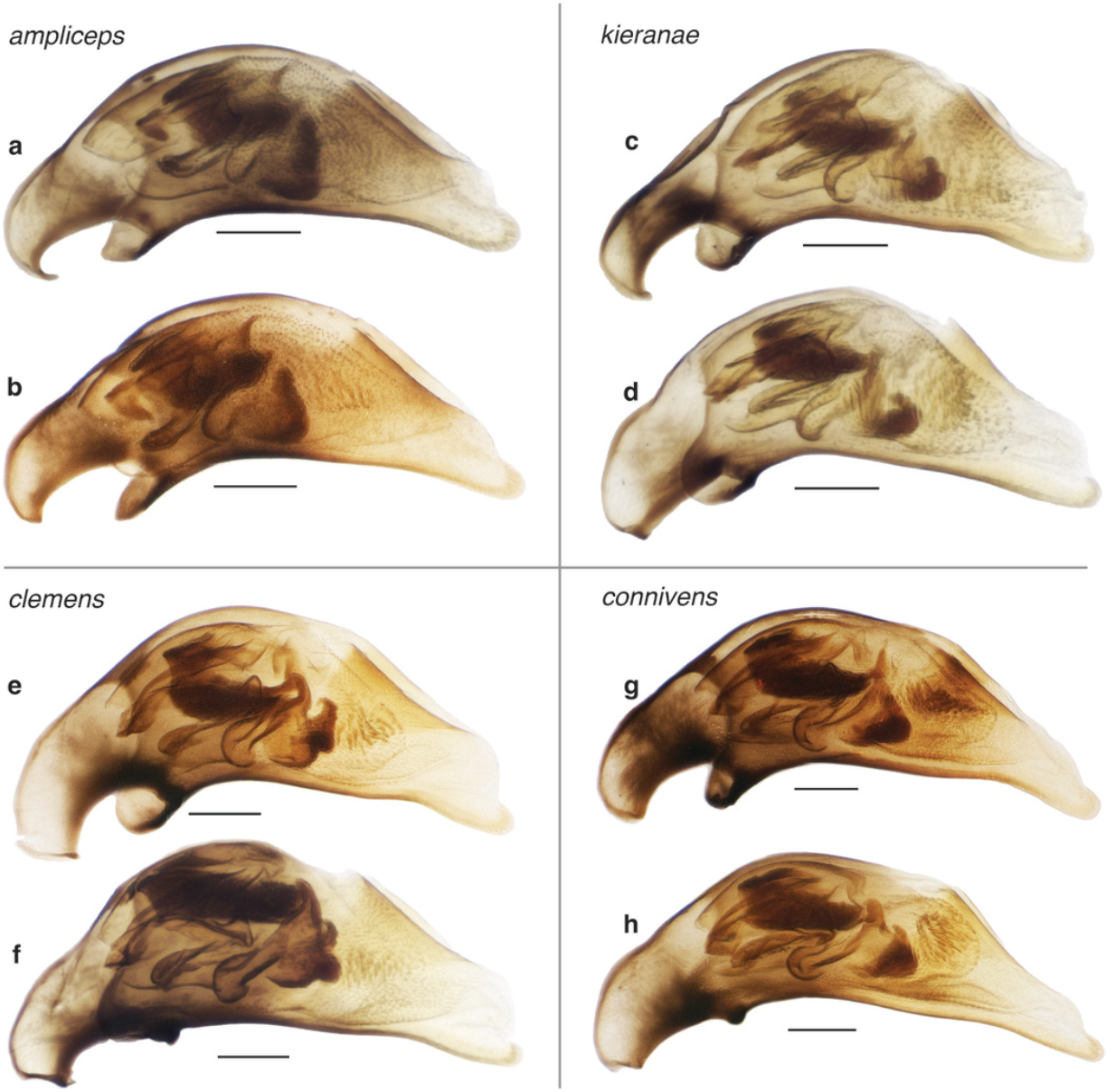

Figure 22. Aedeagus of male genitalia of *Trepanedoris* of the *connivens* subgroup and *Bembidion ampliceps*, left lateral view.

a: *Bembidion ampliceps* (USA: California: Kern Co., Lake Isabella at mouth of French Gulch; voucher DNA4570). **b**: *B. ampliceps* (USA: California: Kern Co., Stable Creek at Sawmill Rd.; voucher DNA4329). **c**: *B. kieranae* (USA: Oregon: Lane Co., SW Eugene; voucher DNA4507). **d**: *B. kieranae* (USA: Oregon: Lane Co., SW Eugene; voucher DNA4577). **e**: *B. clemens clemens* (USA: New Mexico: Taos Co., Rio Grande; voucher DNA4345). **f**: *B. clemens disparile* (USA: California: Kern Co., Davis Camp, Sequoia NF; voucher DNA4590). **g**: *B. connivens*, southern form (USA: California: Tehama Co., Red Bluff; voucher DNA4343). **h**: *B. connivens*, northern form (USA: Oregon: Lincoln Co., Siletz River E of Kernville; voucher DNA1759). Scale bars 0.1 mm.

protarsomere 1 larger, more swollen (Fig. 33d, e). ... **7**

7. Males with evident and complete sculpticells on elytral disc around both seta ed3 and ed5 (Fig. 35e, f) (although the few males known from near Lake of the Woods and Chiloquin, OR, have weak sculpticells). Male genitalia with notably sinuate ventral margin of median lobe (Fig. 21i, j), and with small spikes in internal sac near apex (Fig. 23e); sclerite 1 (Fig. 24d–f) more horizontally oriented, and thus appearing smaller in lateral view, and sclerite 2 relatively faint. Known only from western BC, WA, and OR, from the eastern slope of the Cascades and westward. Figures 6c, 18c ... ***B. microreticulatum***

- Males with weak, incomplete, or absent sculpticells on elytral disc around ed3 (Fig. 35a, c) (one male from Creston, BC, with complete sculpticells around ed3), but in some specimens with complete sculpticells around ed5 (Fig. 35d). Male genitalia with less sinuate margin of median lobe (Fig. 21g, h, k, m); spikes absent, or if present on the internal sac near apex, much larger (Fig. 23f). East of the Cascades and east of the Sierra Nevada ... **8**

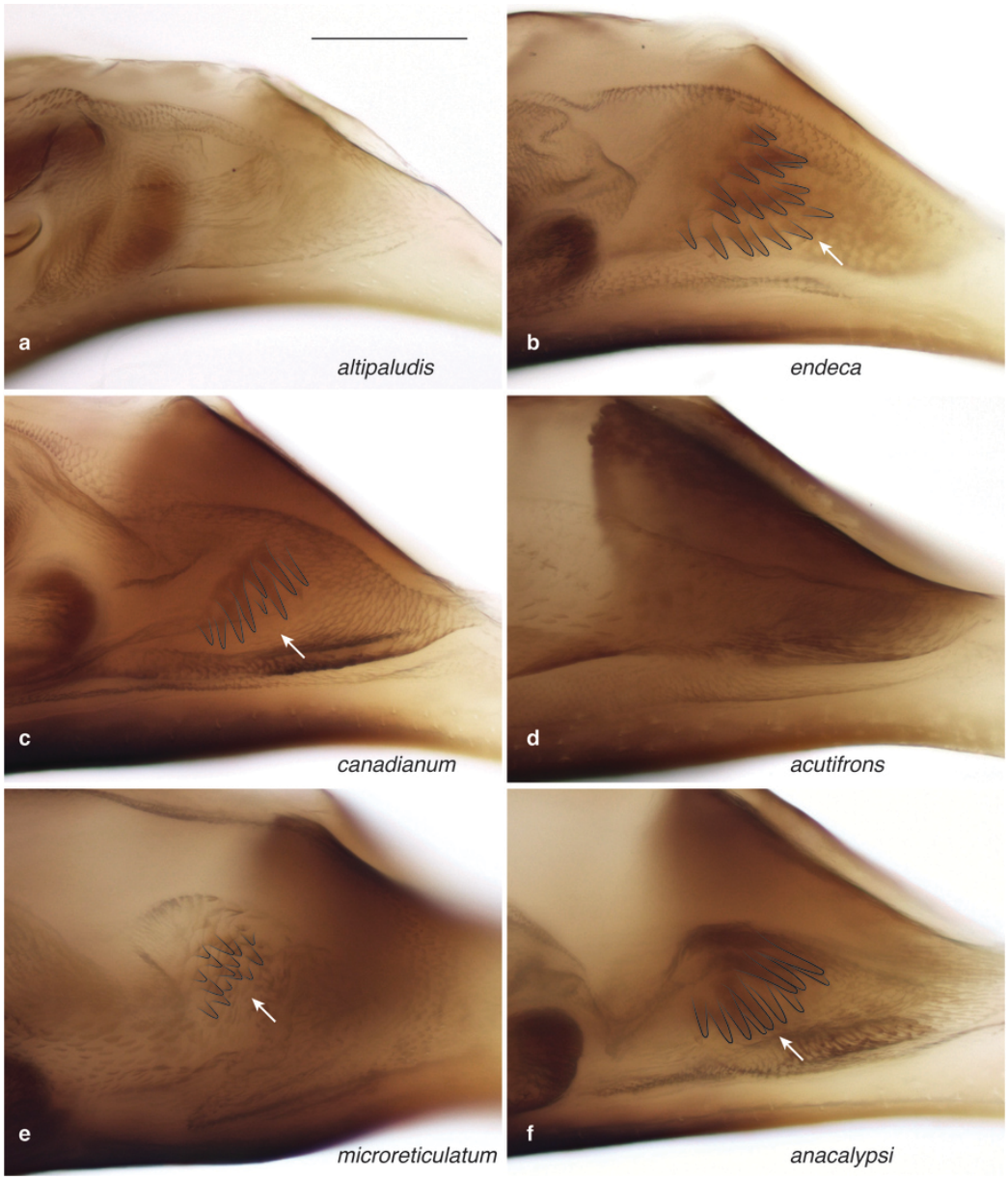

Figure 23. Close-up of apical region of the aedeagus in the *acutifrons* subgroup and *Bembidion altipaludis*.

Left lateral view. **a**: *Bembidion altipaludis* (voucher DNA4318). **b**: *B. endeca* (voucher DNA4556). **c**: *B. canadianum* (voucher DNA4317). **d**: *B. acutifrons* (voucher DNA4330). **e**: *B. microreticulatum* (voucher DNA4494). **f**: *B. anacalypsi* (holotype, voucher DNA5283). Arrows indicate spikes on internal sac; spikes outlined in thin black lines for clarity. All images at same scale; scale bar 0.1 mm.

8. Male genitalia with dense cluster of large spikes on internal sac near apex of median lobe (Fig. 23f); sclerite 1 (Fig. 24g-i) quite large in lateral view, and thus smaller, and sclerite 2 relatively faint and diffuse. Males with some faint sculpticells on elytral disc around ed3 (Fig. 35c). Front angles of prothorax in most specimens less protruding, and less explanate (Fig. 32e). Figures 6d, 18d ... ***B. anacalypsi***

- Male genitalia without cluster of spikes on internal sac (Fig. 23d); sclerite 1 (Fig. 24a–c) quite small, but sclerite 2 thin, elongate, and relatively distinct. Most males with no evident sculpticells on elytral disc around ed3 (Fig. 35a) (one male observed from Creston, BC, with evident sculpticells). Front angles of prothorax in most specimens more protruding, and more explanate (Fig. 32f). Figures 6b, 18b ... ***B. acutifrons***

9. Head (including eyes) relatively wide, almost as wide as pronotum (Fig. 7a); elytra narrow: HW/PW>0.92; HW/EW>0.62. Eyes protruding, especially posteriorly, where the protrusion results in an incised notch between the eye and the head capsule (Fig. 27d). Anterior edge of clypeus with medial region distinctly posterior of lateral region (Fig. 29d). Frontal furrows deeply incised (Fig. 26g). Elytra striae in anterior half with punctures large, abruptly transitioning behind ed5 to very small punctures (Fig. 7a). Body color nearly black, legs dark brown, antenna dark brown with antennomere 1 in some specimens slightly paler. Figures 7a, 19a ... ***B. ampliceps***

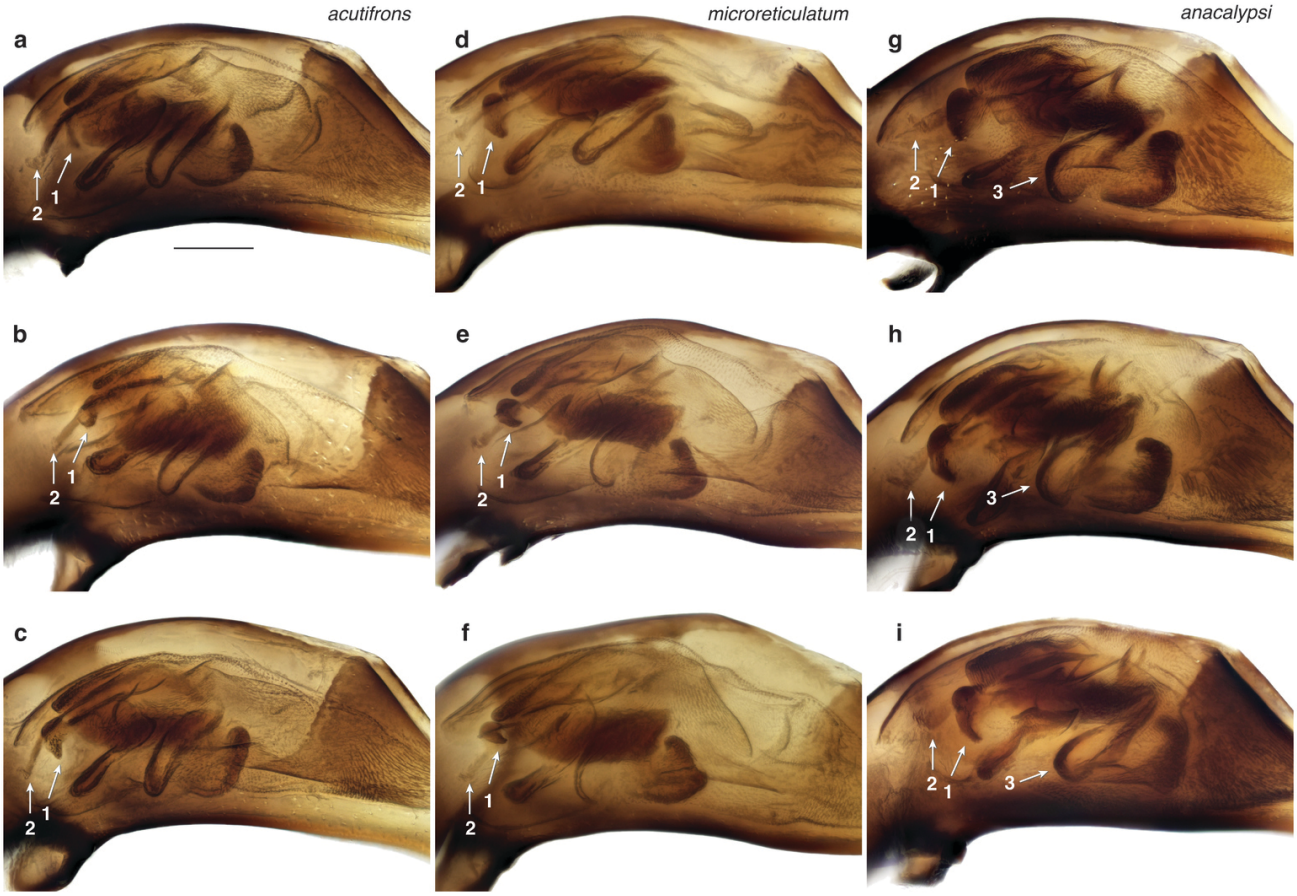

Figure 24. Close-up of the aedeagus in three species in the *acutifrons* subgroup.

Left lateral view. **a**: *Bembidion acutifrons* (USA: Utah: San Juan Co., Geyser Pass Rd nr Horse Ck, La Sal Mtns; voucher V101668). **b**: *B. acutifrons* (USA: Colorado: Alamosa Co., Alamosa NWR; voucher DNA4330). **c**: *Bembidion acutifrons* (USA: Utah: Garfield Co., Panguitch Lake; voucher V101770). **d**: *B. microreticulatum* (USA: Oregon: Klamath Co., 12 mi N Chiloquin; voucher V101623). **e**: *B. microreticulatum* (USA: Washington: Lewis Co., Fort Borst Lake, Centralia; voucher DNA4943). **f**: *B. microreticulatum* (USA: Oregon: Benton Co., Corvallis; voucher DNA3383). **g**: *B. anacalypsi* (USA: Oregon: Baker Co., 4.4 km NW Haines; voucher DNA6088). **h**: *B. anacalypsi* (USA: Oregon: Harney Co., Dairy Creek; voucher DNA4544). **i**: *B. anacalypsi* (USA: Oregon: Harney Co., Dairy Creek; voucher DNA4550). All images at same scale; scale bar 0.1 mm.

- Head not as wide, especially relative to pronotum and elytra (Figs 5c, d; 7b, c): HW/PW<0.90; HW/EW<0.60. Eyes not as protruding, without incised notch behind them (Fig. 27b, c, e, f). Anterior region of clypeus varied (Fig. 29c, e, f). Frontal furrows deeply or shallowly incised. Elytral punctures smaller (Figs 5c, d, 7b, c). Body color brown or dark brown, legs yellowish brown to dark brown, antenna with antennomere 1 yellowish brown to dark brown ... **10**

10. Mentum with central transverse ridge less well developed, sometimes interrupted medially, or less distinct; in most specimens with a medial longitudinal ridge that protrudes forward onto the mental tooth (Fig. 30b). Frontal furrows less deeply engraved than any other *Trepanedoris* (Fig. 26b); fovea in which anterior supraorbital seta sits is larger, and in most specimens elongate. Anterior margin of clypeus almost transverse, not strongly angulate (Fig. 29e). Eyes in most specimens less convex (Fig. 26b). Male genitalia with no spikes on internal sac near apex of median lobe (Fig. 23a). Small, length 2.6–3.0 mm throughout most of its range; 2.9–3.3 mm in Trinity Alps of California. Figures 5c, 17c ... ***B. altipaludis***

- Mentum with central transverse ridge complete and distinct, and without a medial longitudinal ridge (Fig. 30d). Frontal furrows more deeply engraved (Fig. 26c); fovea in which anterior supraorbital seta sits in most specimens smaller, circular. Eyes in most specimens more convex (Fig. 26c, h, i). Male genitalia with large spikes on internal sac near apex of median lobe (Figs 23b, 25b, c, d). Anterior margin of clypeus with medial region notably posterior of lateral regions, and strongly angulate (Fig. 29c, f) ... **11**

11. Labrum with evident medial bump (Fig. 29f). Prothorax wider relative to elytra (PW/EW>0.69). Legs medium to dark brown; apical half of elytral epipleura of most specimens dark brown. Oregon and California. Figures 5d, 17d ... ***B. endeca***

- Labrum with anterior edge concave, with no medial bump (Fig. 29c). Prothorax narrower relative to elytra (PW/EW<0.69). Legs pale yellowish brown to dark brown; apical half of elytral epipleura yellowish brown to dark brown ... **13**

12. Elytral disc with clearly visible microsculpture (Fig. 36b); pronotum with microsculpture evident at least laterally. Figures 7d, 19d. ... ***B. connivens***

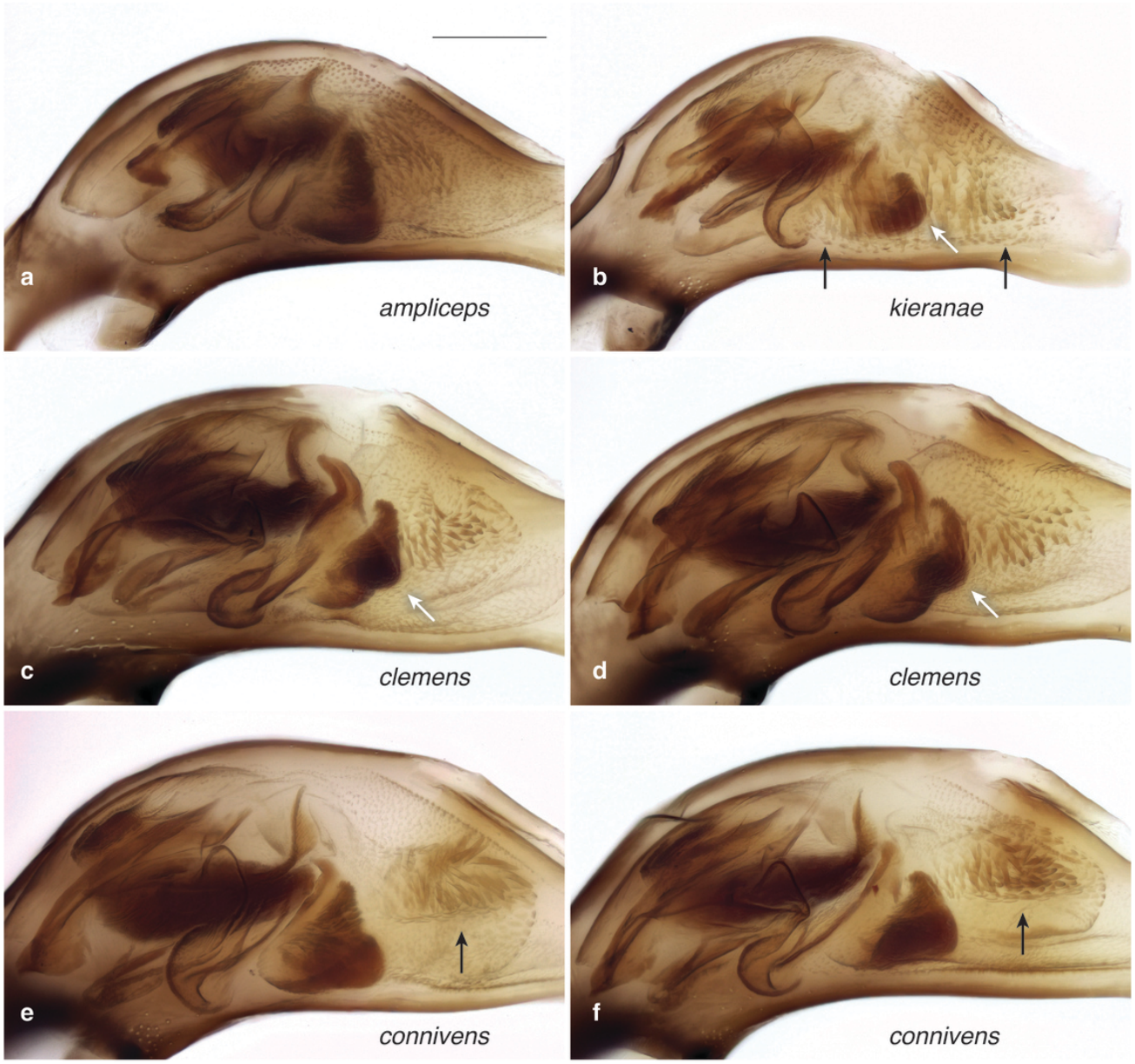

Figure 25. Close-up of the aedeagus in the *connivens* subgroup and *Bembidion ampliceps*.

a: *Bembidion ampliceps* (USA: California: Kern Co., Lake Isabella at mouth of French Gulch; voucher DNA4570). **b**: *B. kieranae* (USA: Oregon: Lane Co., SW Eugene; voucher DNA4507); arrows indicate boundaries of the field of large spikes. **c**: *B. clemens clemens* (USA: New Mexico: Cibola Co., Bluewater Creek, Zuni Mtns; voucher DNA4410); arrow indicates distinctly shaped subapical brush. **d**: *B. clemens disparile* (USA: California: Merced Co., San Joaquin R., Great Valley Grasslands SP; voucher DNA4323); arrow indicates distinctly shaped subapical brush. **e**: *B. connivens*, southern form (USA: California: Monterey Co., Arroyo Seco Campground, Los Padres NF; voucher DNA4466); arrow indicates dense field of large spikes. **f**: *B. connivens*, central coast form (USA: Oregon: Curry Co., Pistol River State Park; voucher DNA4381); arrow indicates dense field of large spikes. All images at same scale; scale bar 0.1 mm.

- Elytral with no visible microsculpture (Fig. 36a); pronotum with no visible microsculpture ... **13**

13. Male protarsomere 1 relatively wider, appearing swollen with more rounded sides (Fig. 33g), and inflated dorsally (Fig. 33i). Body relatively flat. Antennomeres 2–11 dark brown. Preapical spots when present very small and faint. Small, 2.6–2.8mm. Male genitalia with a smaller, simple subapical brush (Figs 22c,d; 25b, white arrow), and with the subapical spikes within the internal sac large and distributed over a wide area (Fig. 25b, black arrows). Western Oregon. Figures 7b, 19b ... ***B. kieranae***

- Male protarsomere 1 relatively narrower, with more parallel sides (Fig. 33h), and less inflated dorsally (Fig. 33j). Body more convex. Antennomeres 2–4 varied, from pale brown to dark brown. Preapical spots on elytra from absent to very small and faint to large and distinct. Most specimens larger, 2.8–3.5 mm. Male genitalia with a larger subapical brush that is distinctly shaped, with a central bulge abruptly tapering to narrow dorsal and ventral projections (Figs 22e,f; 25c,d, white arrows), and with the subapical spikes within the internal sac smaller, denser, and more localized (Fig. 25c,d). California,

Figure 26. Dorsal view of heads.

a: *Bembidion fortestriatum*; arrows indicate posterior end of frontal furrows. **b**: *B. altipaludis*. **c**: *B. endeca*. **d**: *B. canadianum*. **e**: *B. acutifrons*. **f**: *B. anacalypsi*. **g**: *B. ampliceps*. **h**: *B. kieranae*. **i**: *B. clemens*. Scale bars 0.1 mm.

eastern Oregon, Idaho, Nevada, Arizona, Utah, Colorado, and New Mexico. Figures 7c, 19c ... ***B. clemens***

Species accounts

The diagnoses and descriptions that follow are written presuming the characteristics of the *connivens* group of *Trepanedoris*, and thus those characteristics are not mentioned.

Bembidion frontale (LeConte, 1847) (Figs 5a, 17a, 20a–c, 31a,e, 32a, 37)

Ochthedromus frontalis LeConte, 1847, p. 462. Syntype(s) in MCZ (type number 26893). Type locality: Detroit, Wayne County, Michigan, USA. (More information and photographs at https://mczbase.mcz.harvard.edu/guid/MCZ:Ent:28693.)

Diagnosis and description. The combination of very small size, pale appendages (including a pale antennomere 3), the dark pronotum contrasting against the pale front half of the elytra, prominent pale elytral spots surrounded by a dark ring, strikingly narrow pronotum, with large, irregular punctures on the front of the pronotum, and lack of microsculpture on most of the proepisternum are distinctive (Figs 5a, 17a).

Elytra paler brown in the anterior half, contrasting against a dark ring around the preapical spot (Fig. 5a), and in most specimens contrasting against the pronotum, which is generally much darker; legs and at least the basal three antennomeres a pale yellow-brown. Frontal furrows deeply engraved, wrapping around the back of the eye. Most specimens with a shallow fovea medial of anterior portion of eye, between frontal furrow and eye (Fig. 31a, arrow). Labrum without median bump. Mentum without transverse ridge. Pronotum very narrow, barely wider than the head, with narrow lateral explanate area in the anterior half; anterior transverse impression of pronotum with punctures deep and distinct (Fig. 32a). Proepisternum shiny, without microsculpture laterally, at most with faint microsculpture

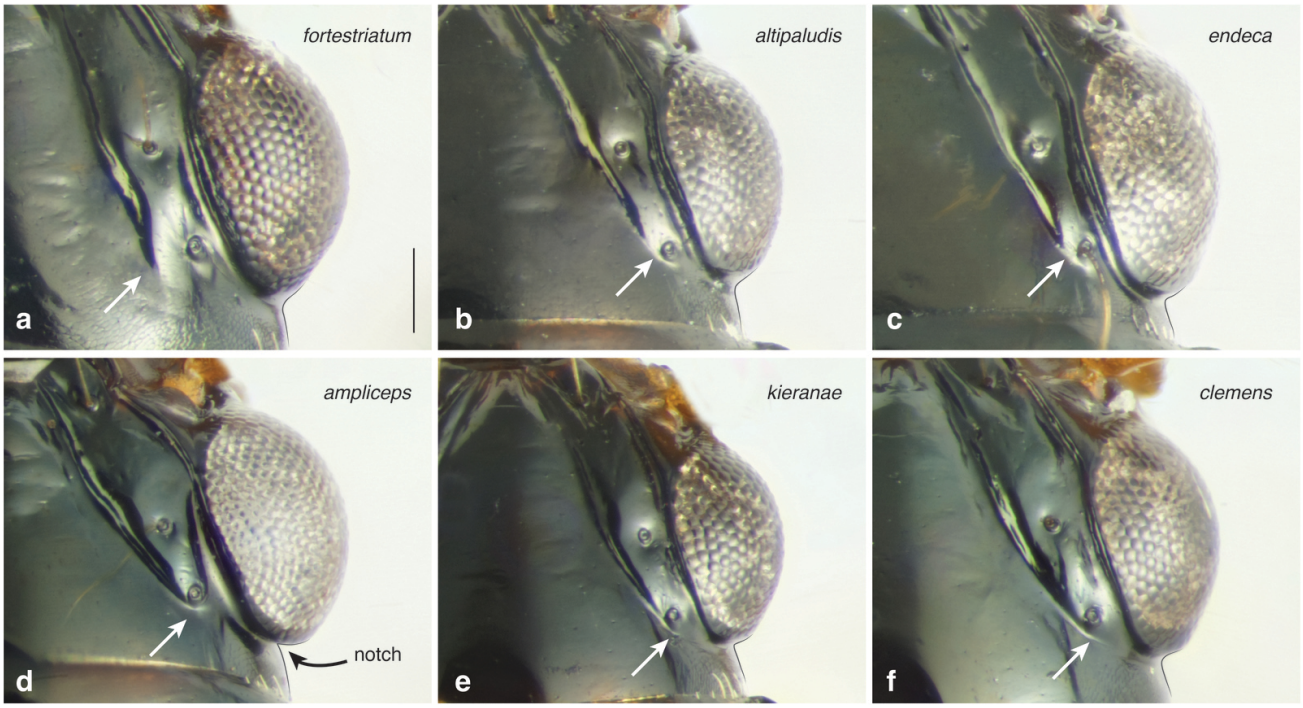

Figure 27. Dorsal view of right posterior corner of heads.

a: *Bembidion fortestriatum*. **b**: *B. altipaludis*. **c**: *B. endeca*. **d**: *B. ampliceps*. **e**: *B. kieranae*. **f**: *B. clemens*. White arrows mark end of frontal furrows. Edge of head capsule outlined in thin black lines behind the eyes for greater clarity; antennae graphically removed in a few images. All images at same scale; scale bar 0.1 mm.

near coxa (Fig. 31c, E). Protarsomere 1 of male narrow, only slightly wider than second protarsomere. Elytral striae with large, deep, well-separated punctures in the anterior half; seventh stria with deep and distinct punctures in the anterior third. Microsculpture lacking on pronotum and elytra. With full flight wings as far as known. Aedeagus with arcuate ventral margin and long, extended, narrow apex (Fig. 20a–c). Body length 2.2–2.7 mm.

Comparison with similar species. This species along with *B. siticum* are the smallest *Bembidion* in North America. They form a clade (the *frontale* subgroup) with specimens characterized by small size, the anterior transverse impression of the prothorax wrinkled or with notable punctures, a mentum lacking the transverse ridge present in all other *Trepanedoris* (Fig. 35a), lack of a median bump on the labrum (Fig. 29b), lack of dorsal microsculpture, and (in most specimens) preapical spots on the elytra, in addition to other characters typical of the *connivens* group. Most easily distinguished from *B. siticum* by the lack of microsculpture over most of the proepisternum (Fig. 31c, e), which is therefore shinier; the front regions of the pronotum being less wrinkled and instead with more distinct, elongate punctures; having larger elytral punctures; and being overall paler in color, especially antennomere 3 and front half of the elytra. Against Lindroth (1963), the male genitalia of *B. frontale* and *B. siticum* are very similar to one another in details of the internal sac (Fig. 20).

Variation. One specimen from Concord, NH (CSCA), has the anterior transverse impression of the prothorax wrinkled, similar to that typical for *B. siticum*, rather than the distinct, separated punctures of other *B. frontale*. In all other ways, however, this specimen has the characteristics of typical *B. frontale*.

Distributions. From British Columbia east to Nova Scotia in Canada, and from Idaho eastward to Maine, south to Missouri and Virginia (Fig. 37; Bousquet, 2012).

Habitat. Most commonly found on dark, damp, organic soil covered in leaf litter around pools of water or streams in the shade of trees, in forests or near their edges.

Bembidion siticum Casey, 1918 (Figs 5b, 17b, 20d–f, 29b, 30a, 31b,d,f, 32b, 37)

Bembidion siticum Casey, 1918, p. 157. Lectotype male in NMNH (type number 36803), designated by Lindroth (1975, p. 122), examined. Type locality: Gualala River, Mendocino County, California.

Bembidion adolescens Casey, 1918, p. 158. Lectotype female in NMNH (type number 37071), designated by Lindroth (1975, p. 122), examined. Type locality: Booneville, Mendocino County, California.

Diagnosis and description. The combination of very small size, darker appendages in most specimens (including a darker antennomere 3 with at least the central region being brown), the pronotum and elytra being of similar tones, the front of the pronotum with prominent wrinkles, and presence of clear microsculpture throughout the proepisternum are distinctive (Figs 5b, 17b).

Elytra uniformly dark brown except for the preapical spot, if present (Fig. 5b); in most specimens with legs and antennae brown, including at least antennomere 3. Frontal furrows deeply engraved, wrapping around the back of the

Figure 28. Head of *Bembidion altipaludis* and *B. canadianum* showing variation in eye size.

a: *Bembidion altipaludis* from the Cascades of Oregon (USA: Oregon: Deschutes Co., Three Creek Meadow). **b**: *B. altipaludis* from the Trinity Alps (USA: California: Trinity Co., Canyon Creek). **c**: *B. canadianum* (USA: Montana: Beaverhead Co., Melrose, Big Hole River). **d**: *B. canadianum* (USA: Colorado: Alamosa Co., Alamosa NWR). Scale bars 0.1 mm.

eye. Most specimens without a single shallow fovea medial of anterior portion of eye, between frontal furrow and eye, although in some specimens that area is wrinkled (Fig. 31b). Labrum without median bump (Fig. 29b). Mentum without transverse ridge (Fig. 30a). Pronotum narrow, with wider lateral explanate area in the anterior half; transverse impression of pronotum without distinct punctures, instead having more of a wrinkled appearance (Fig. 32b). Proepisternum with well-engraved microsculpture throughout, with sculpticells forming a semicircular pattern centered on the procoxae (Fig. 31d, f). Protarsomere 1 of male narrow, only slightly wider than second protarsomere. Elytral striae with distinct punctures in anterior half; seventh stria with distinct punctures in the anterior third. Microsculpture lacking on pronotum and elytra. With full flight wings as far as known. Aedeagus with ventral margin in many specimens less arcuate and with blunter, thicker apex than in *B. frontale* (Fig. 20d–f). Body length 2.2–2.7 mm.

Comparison with similar species. Most similar to *Bembidion frontale*; see under that species for a discussion of differences. Darker specimens of *B. siticum* can readily be identified, as all specimens of the *frontale* subgroup we have seen with dark appendages and lacking elytral spots are *B. siticum*; paler specimens of *B. siticum* can most readily be identified by the distinctive microsculpture on the proepisternum (Fig. 31d, f).

Variation. One male, from Meeks Bay, Eldorado Co., California (CSCA), has only the faintest suggestion of wrinkles in the anterior impression of the pronotum, which is effectively smooth. In genitalia and mentum structure it matches the characteristics of the *frontale* subgroup, and in all other characters, including proepisternal microsculpture, it matches *Bembidion siticum* in particular.

Distributions. Known only from the Rocky Mountains and westward, from Montana and Idaho south to Nevada and west to British Columbia and California (Fig. 37).

Habitat. Found in many different environments between 20 and 3000 m in elevation, including marsh, pond, silt or sand creek and river shores, and wet meadows, shaded or unshaded, often but not always with extensive vegetation of sedges, grasses, and other plants (e.g., Fig. 1d). It occurs around the shores of Mono Lake, California, and is therefore salt tolerant, but most habitats show no evidence of high salinity.

Bembidion altipaludis Maddison, n. sp. (Figs 5c, 17c, 21a,b, 23a, 26b, 27b, 28a,b, 29e, 33a, 38)

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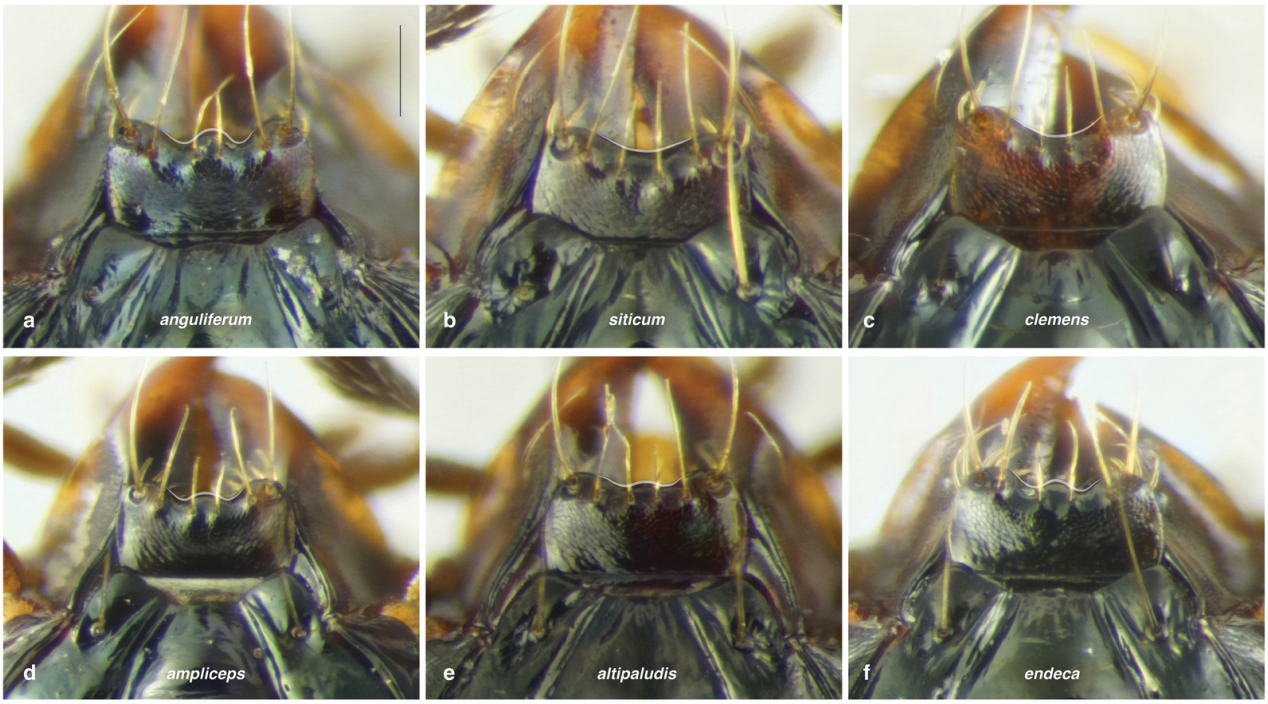

Figure 29. Labrum and clypeus variation within *Trepanedoris*.

a: *Bembidion anguliferum* (LeConte), a member of the *fortestriatum* group. **b**: *B. siticum*. **c**: *B. clemens*. **d**: *B. ampliceps*. **e**: *B. altipaludis*. **f**: *B. endeca*. Central region of anterior edge of labrum outlined for greater clarity. All images at same scale; scale bar 0.1 mm.

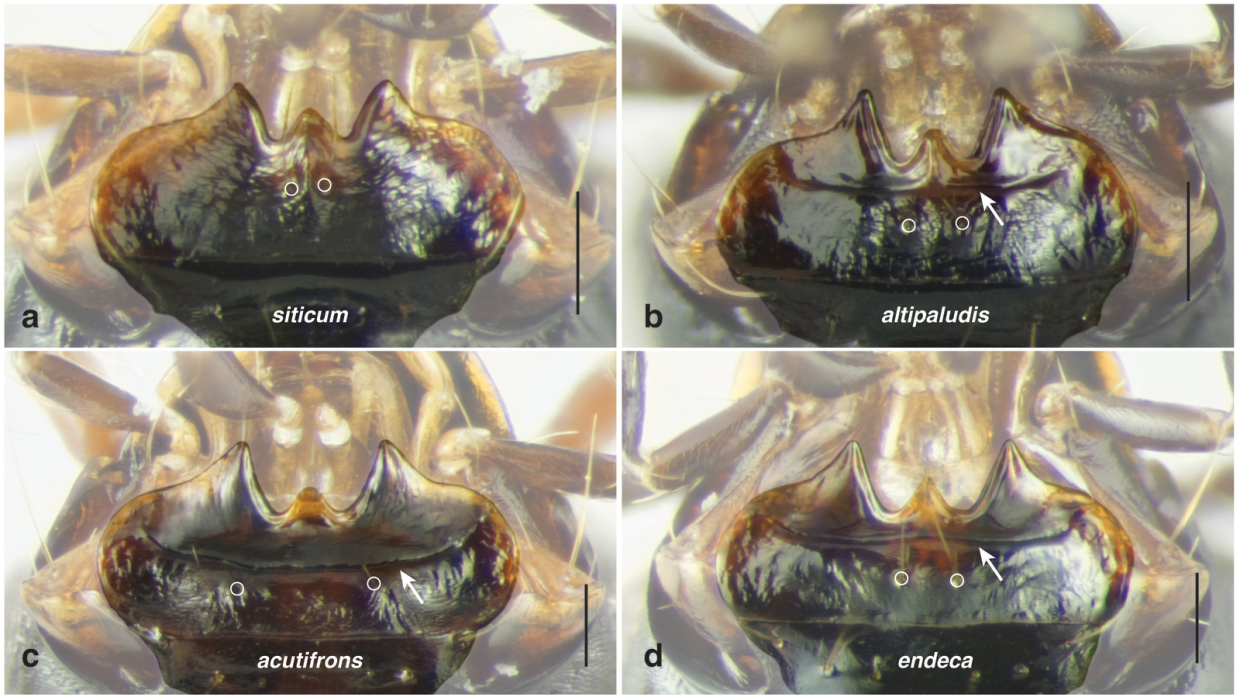

Figure 30. Variation in the mentum within *Trepanedoris*.

a: *Bembidion siticum*. **b**: *B. altipaludis*. **c**: *B. acutifrons*. **d**: *B. endeca*. White circles mark pore punctures of setae. Parts other than the mentum graphically faded. Arrows mark transverse ridge. Scale bars 0.1 mm.

Holotype male here designated, deposited in OSAC, labeled "USA: Oregon: Deschutes Co., Three Creek Lake, 1985m, 44.1018°N 121.6211°W, 1.vii.2010. DRM 10.065. D.R. Maddison", "David R. Maddison DNA2583 DNA Voucher" [printed on pale green paper], and "HOLOTYPE Bembidion altipaludis Maddison" [partly handwritten on red paper], and "Oregon State Arthropod Collection OSAC_0002000009 [matrix code]" [printed on both sides of white paper]. Genitalia mounted in Euparal on small card (with DNA2583 written on it) beneath specimen; ex-

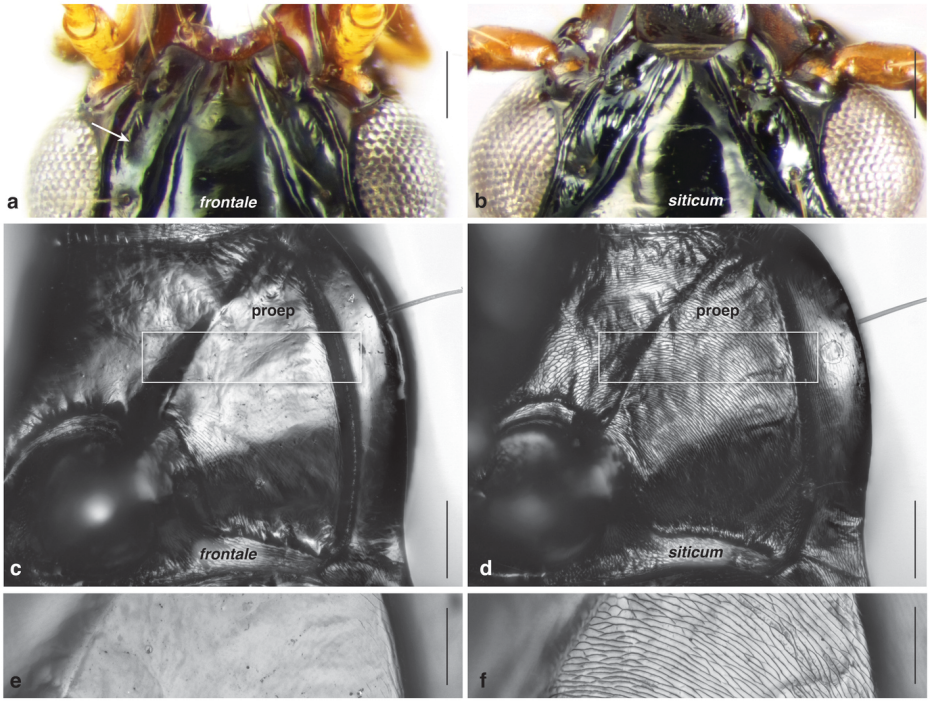

Figure 31. Heads and prothoraces of the *frontale* subgroup.

a and **b**: Dorsal surface of anterior portion of head; scale bars 0.1 mm. **c** and **d**: Lateral view of left side of prothorax showing the proepisternum ("proep"); scale bars 0.1 mm. **e** and **f**: Close-up of proepisternum, showing the region marked with a white rectangle in **c** and **d**; scale bars 0.05 mm. **a**, **c**, and **e**: *Bembidion frontale*; **b**, **d**, and **f**: *Bembidion siticum*. Arrow in **a** indicates the shallow fovea present in *B. frontale*.

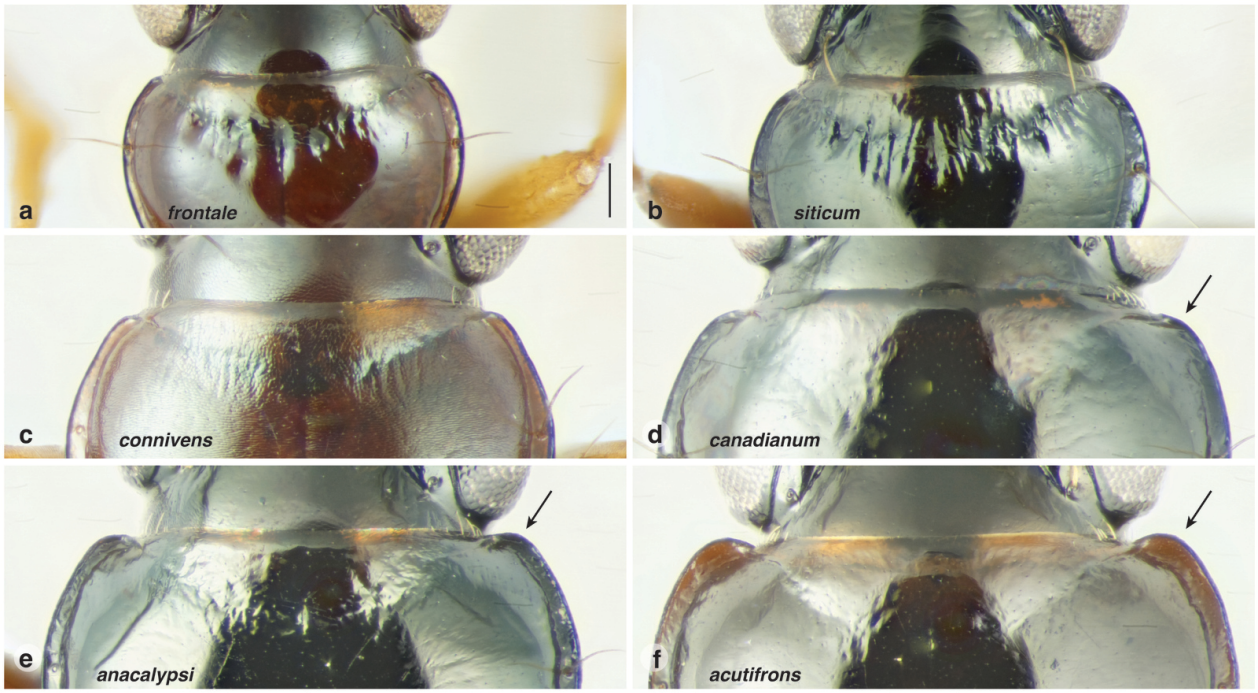

Figure 32. Variation in the anterior portion of the pronotum within *Trepanedoris*.

a: *Bembidion frontale*, showing large, longitudinally elongated punctures in the anterior impression. **b**: *B. siticum*, showing the prominent wrinkles in the anterior impression. **c**: *B. connivens*. **d**: *B. canadianum*, showing the relatively flat anterior lateral corners (arrow). **e**: *B. anacalypsi*, showing the anterior lateral corners slightly projecting forward (arrow). **f**: *B. acutifrons*, showing the anterior lateral corners notably projecting forward (arrow). All images at same scale; scale bar 0.1 mm.

tracted DNA stored separately in OSAC. GenBank accession numbers for DNA sequences of the holotype are PV292156, PV291986, PV288163, PV288009, PV287855, and PV287745. Type locality: Three Creek Lake, 44.1018°N 121.6211°W, Deschutes County, Oregon, USA.

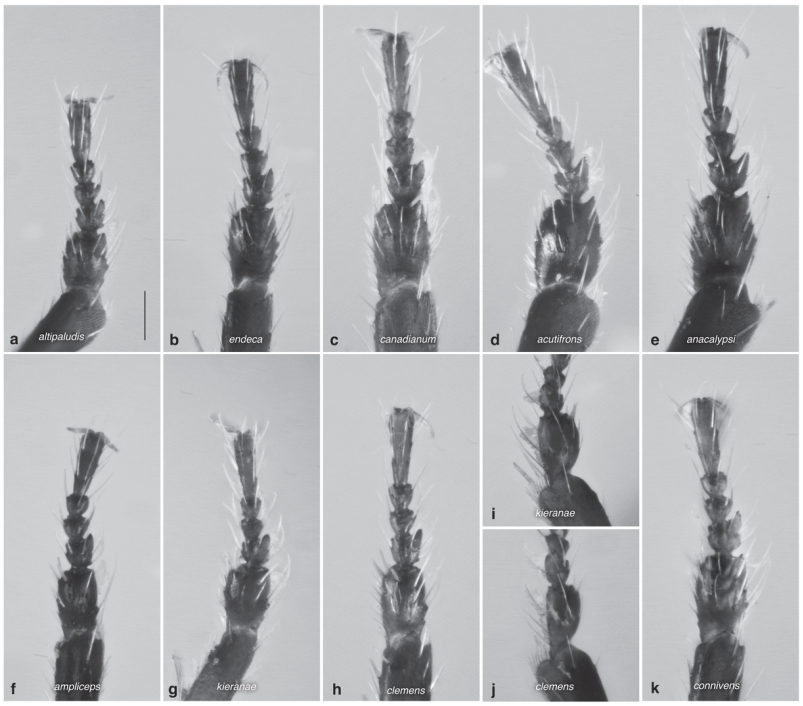

Figure 33. Variation in male protarsomeres across *Trepanedoris*.

Dorsal view of left male protarsus (**a**–**h**, **k**) and left lateral view of right male protarsus (**j**, **k**). **a**: *Bembidion altipaludis*. **b:** *B. endeca*. **c**: *B. canadianum*. **d**: *B. acutifrons*. **e**: *B. anacalypsi*. **f**: *B. ampliceps*. **g**: *B. kieranae*. **h**: *B. clemens*. **i**: *B. kieranae*. **j**: *B. clemens*. **k**: *B. connivens*. All images at same scale; scale bar 0.1 mm.

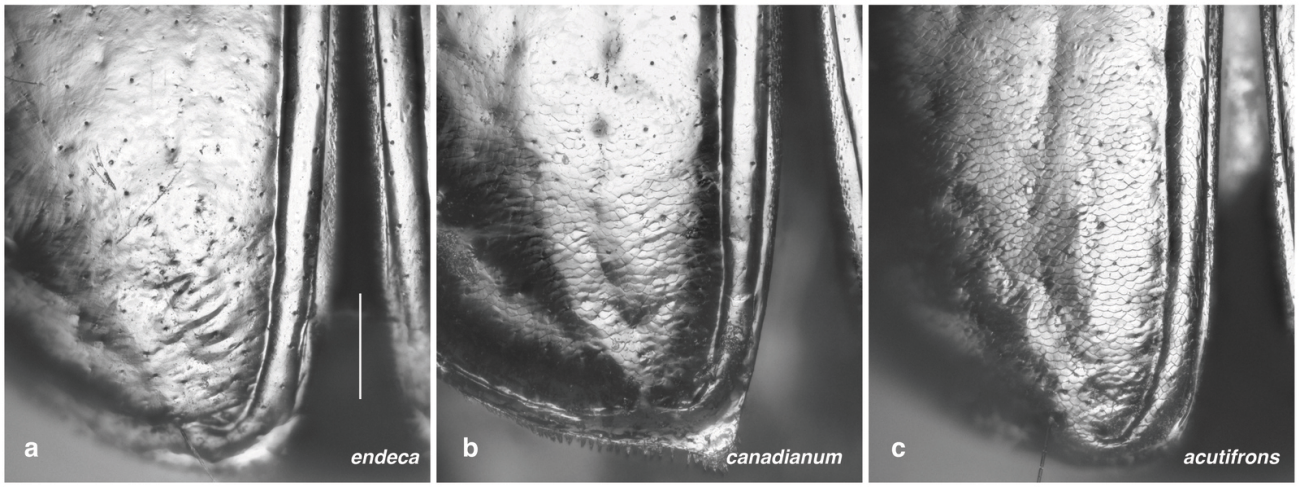

Figure 34. Variation in microsculpture of the elytral apex in males of the *acutifrons* subgroup.

a: *Bembidion endeca*. **b**: *B. canadianum*. **c**: *B. acutifrons*. All images at same scale; scale bar 0.1 mm.

Paratypes. All 257 specimens designated as paratypes are from Oregon, and are deposited in OSAC, NHMUK, BYUC, CAS, CMNH, CNC, CSCA, CUIC, EMEC, MCZ, MNHN, NMNH, and MZLU. They are from the following localities: Clackamas Co., Mt. Hood, Government Camp (3, OSAC); Wasco Co., Bear Springs (39, OSAC, NHMUK, CAS, CMNH, CNC, CSCA, CUIC, EMEC, MCZ, MNHN, NMNH, MZLU); Linn Co., Clear Lake (1, OSAC); Linn Co., Lost Lake, 1220m 44.4315°N 121.9117°W (31, OSAC, BYUC, EMEC, MNHN, MZLU); Linn Co., Lost Lake, 1200m 44.4319°N 121.9103°W (1, OSAC); Linn Co., Lost Lake, 1216m, 44.4352°N 121.906°W (1, OSAC); Linn Co., Lost Lake, 1218m 44.4345°N 121.9074°W (8, OSAC); Linn Co., Pacific Crest Trailhead on route 20, 1460m 44.4236°N 121.8539°W (3, OSAC); Jefferson Co., 15 mi NW Sisters, Blue Lake (4, JRLC); Jefferson Co., Suttle Lake. 4300 feet (1, CMNH); Deschutes Co., Elk Lake (9, OSAC); Deschutes Co., N Sparks Lake, along hwy 372, 1669m 44.0285°N 121.7356°W (9, OSAC); Deschutes Co., Sisters, McKenzie Pass, 6000 ft (6, CMNH); Deschutes Co., Soda Creek at hwy 372, 1665m 44.0263°N 121.7253°W (5, OSAC); Deschutes Co., Three Creek Lake, 1985m 44.1018°N 121.6211°W (13, OSAC, CAS, CNC); Deschutes Co., Three Creek Meadow, 1920m 44.1152°N 121.6257°W (5, OSAC); Deschutes Co., Three Creek

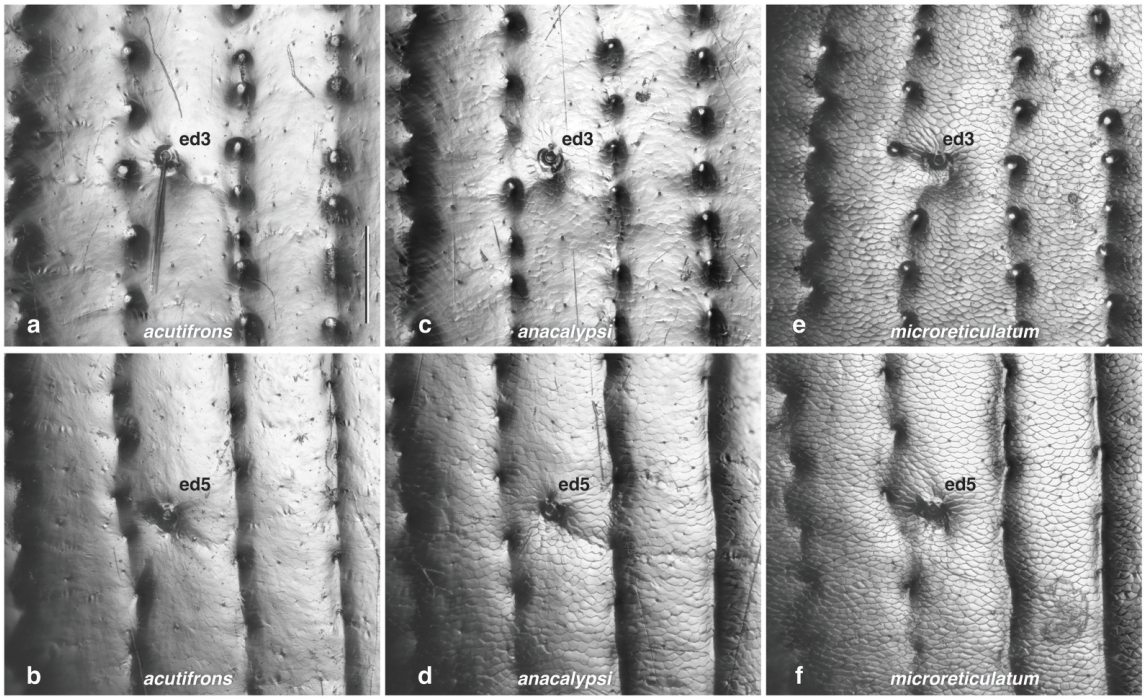

Figure 35. Variation in microsculpture on the elytral disc around the anterior discal setae in males of the *acutifrons* subgroup.

a–**c**: Microsculpture around the anterior discal seta, ed3; **d**–**f**: Microsculpture around the posterior discal seta, ed5. **a, b**: *Bembidion acutifrons*. **c, d**: *B. anacalypsi*. **e, f**: *B. microreticulatum*. All images at same scale; scale bar 0.1 mm.

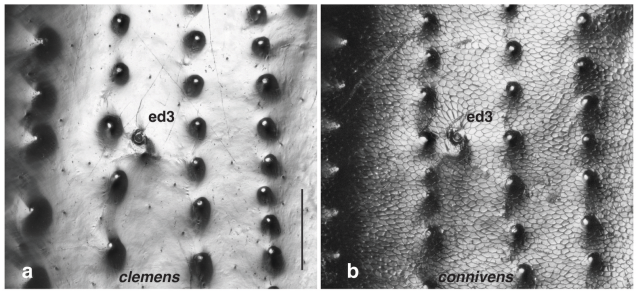

Figure 36. Microsculpture on the elytral disc around seta ed3 in two males of the *connivens* subgroup.

a: *Bembidion clemens disparile* (USA: California: Kern Co., Davis Camp, Sequoia NF). **b**: *B. connivens* (USA: California: Tehama Co., Red Bluff). Both to same scale; scale bar 0.1 mm.

Meadow, 1925m 44.1153°N 121.6263°W (12, OSAC); Deschutes Co., Three Creek near lake outlet, 1988m, 44.1015°N 121.6217°W (6, OSAC, BYUC); Deschutes Co., Meadow SE of Todd Lake, 1859m, 44.0228°N 121.6823°W (8, OSAC); Klamath Co., Castle Crest Trailhead, Crater Lake NP, 42.8923°N 122.1312°W, 1970m (1, OSAC); Klamath Co., Hwy 140, 3 km W. Ft Klamath Jct (1, CNC); Klamath Co., Lake of the Woods (2, OSAC); Klamath Co., Munson Creek, Crater Lake NP, 1984m 42.8986°N 122.1344°W (53, OSAC, NMNH); Klamath Co., Munson Creek, Crater Lake NP, 42.8987°N 122.1343°W, 1981m (43, OSAC, NMNH).

Additional specimens. 88 specimens not designated as paratypes were examined from California, from the following localities: Siskiyou Co. (1, NMNH); Siskiyou Co., Medicine Lk (2, NMNH); Siskiyou Co., Scott Mountain Summit, 1643m 41.2776°N 122.6991°W (5, OSAC); Siskiyou Co., Sisson (1, NMNH); Shasta Co., Lassen N. P., Emerald Lk (1, CUIC); Tehama Co., 7 mi NW Mineral, 6000 ft (1, UNHC); Tehama Co., Wilson Lake, 1600m 40.3425°N 121.4350°W (1, OSAC); Butte Co., 5 mi. NE Butte Meadows, Cherry Hill Cpgd (2, CSCA); Plumas Co., 6 mi N.W Chester on Benner Ck (2, NMNH); Plumas Co., Beaver Lk (1, NMNH); Plumas Co., Graeagle (3, CSCA); Sierra Co., lower Tamarack Lake, 2045m, 39.6103°N 120.6564°W (2, OSAC); Sierra Co., Snag Lake (19, CMNH, CSCA); Sierra Co., Yuba Pass (22, CSCA); El Dorado Co. unnamed lake, 1 km WxNW Robbs Peak Dam 1652m. 38.9430°N,120.3780°W (20, EMEC, OSAC); Trinity Co., Canyon Creek, 1199m, 40.9223°N 123.0253°W (1, OSAC); Trinity Co., Upper Canyon Cr. Meadows, 1418m, 40.9405°N 123.0183°W, 17.vi.2014. J.S. Sproul (4, OSAC)

Derivation of specific epithet. Derived from the Latin words “altus”, meaning “high”, and “paludis”, meaning “marsh”, referring to the habitat of this higher-elevation species. It is to be treated as a noun in apposition.

Diagnosis and description. The small size, dark color, convex body, relatively flat eyes, relatively shallow frontal furrows, lack of microsculpture on the pronotum and elytra, even at the elytral apex, relatively small male protarsomeres, and longitudinal ridge on the mentum tooth are distinctive (Figs 5C, 17c).

Body black to dark brown, without elytral spots; antennae dark brown, legs dark brown or with reddish brown tibiae. Eyes relatively flat (Figs 26b, 27b, 28a,b), less protruding than all *Trepanedoris* other than some specimens of *B. endeca* and *B. canadianum* (e.g., Fig. 28d); without incised notch behind the eyes. Frontal furrows more shallowly en-

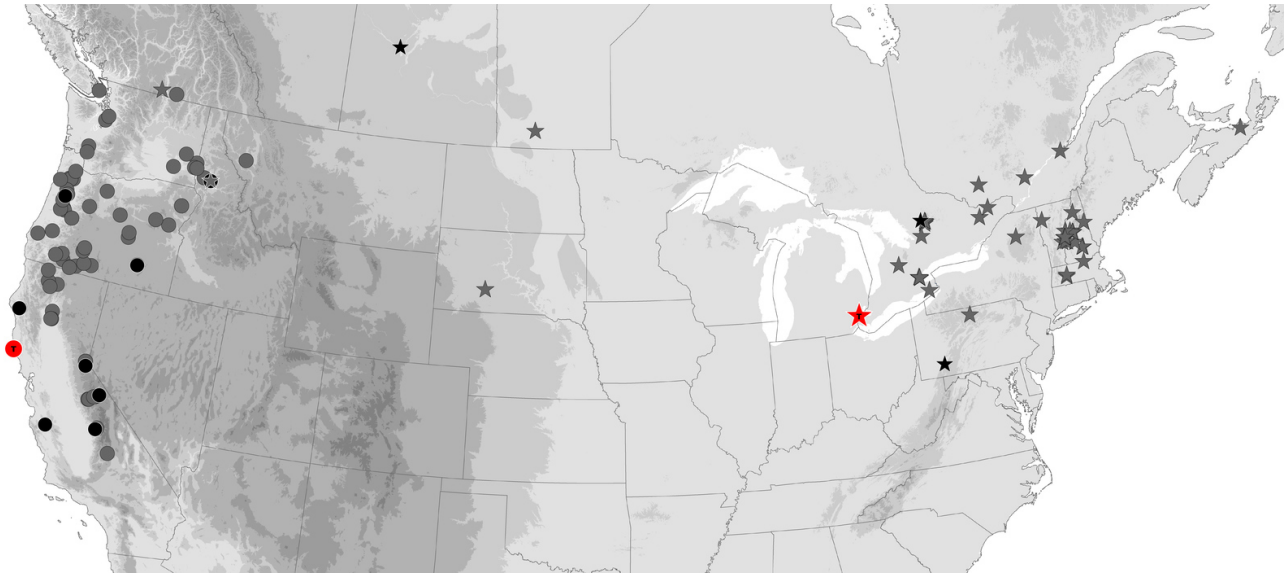

Figure 37. Geographic distribution of specimens examined of *Bembidion frontale* (stars) and *B. siticum* (circles).

Black symbols indicate those for which DNA data is available; gray symbols are other localities. Larger red symbols with a T indicate type localities.

graved than other *Trepanedoris*, especially on the clypeus and behind the anterior supraorbital seta, where the furrow barely reaches the posterior supraorbital seta (Fig. 27b); furrows on front margin of the clypeus further apart than most other *Trepanedoris*, with anterior margin of clypeus straighter, less angulate (Fig. 29e). Fovea in which anterior supraorbital seta sits is unusually large and elongate. Bump on anterior margin of labrum absent or very small (Fig. 29e). Mentum with transverse ridge not as distinct as in other *Trepanedoris*, in some specimens interrupted medially, or less distinct, in most specimens connected to a longitudinal ridge on the tooth itself (Fig. 30b). Pronotum relatively narrow (PW/EW=0.68–0.69), less constricted toward the back (PWh/PW=0.751–0.794), lateral margins sinuate; lateral margins near hind angle not parallel; lateral margin evenly explanate toward anterior margin, which is at most slightly protruding; anterior surface of pronotum smooth or with very faint wrinkles. Protarsomere 1 of male of normal size for a *Bembidion*, both in length and width (Fig. 33a). Elytra with slightly rounded sides; striae with punctures in anterior half; fifth stria with no trace of punctures behind the posterior discal seta ed5; in most specimens with clear, distinct punctures in anterior third of stria seven. Dorsal surface of prothorax and elytra (including apex) without visible microsculpture. With full flight wings as far as known. Aedeagus (Fig. 21a,b) with ventral surface evenly and slightly arcuate; without spikes on internal sac near apex (Fig. 23a). Body length 2.6–3.3 mm.

Comparison with similar species. This is the smallest of the unspotted species of *Trepanedoris* with dark legs; it is most easily confused with *Bembidon endeca* and *B. canadianum*. As with both of those, males and females both lack microsculpture on the elytral disc. In contrast to *B. endeca*, specimens are smaller, the elytra are more rounded, the seventh stria in most is more distinct but the fifth stria ends earlier. In contrast to *B. canadianum*, specimens are smaller, less convex, and there are no traces of microsculpture near the elytral apex. *Bembidion altipaludis* lacks the spikes in the internal sac near the aedeagal apex (Fig. 23a) that are possessed by both *B. endeca* (Fig. 23b) and *B. canadianum* (Fig. 23c). From members of the *B. acutifrons* subgroup other than *B. endeca* and *B. canadianum*, females of *B. altipaludis* are easy to distinguish because of the lack of elytral microsculpture (females of members of the *B. acutifrons* subgroup other than *B. endeca* and *B. canadianum* have strongly engraved elytral microsculpture and thus elytra that are very matte in appearance), and males by the normal-sized protarsomeres (Fig. 33a) in contrast to swollen protarsomeres (Fig. 33d, e).

Variation. Specimens in the Trinity Alps of California are larger than elsewhere: body length is 2.6–3.0 mm throughout most of its range, but 2.9–3.3 mm in the Trinity Alps. The specimens from the Trinity Alps also have flatter eyes than those from elsewhere (e.g., Fig. 28b).

Distributions. Known from Oregon and California, from 1200–2000m in elevation (Fig. 38). As the northernmost locality is on the slopes of Mt Hood in northern Oregon, the species may also occur in the Cascades of southern Washington. It may also be in westernmost Nevada in the Sierra Nevada.

Habitat. Found in dark, damp, organic soil in wet meadows and marshy edges of lakes at relatively high elevation (e.g., Fig. 1e).

Notes. There are many specimens of this species in Melville Hatch's collection (OSAC), some of which were identified by Hatch as *Bembidion ampliceps* Casey.

Bembidion endeca Maddison & Sproul, n. sp. (Figs 4, 5d, 17d, 21c,d, 23b, 26c, 27c, 29f, 30d, 33b, 34a, 38)

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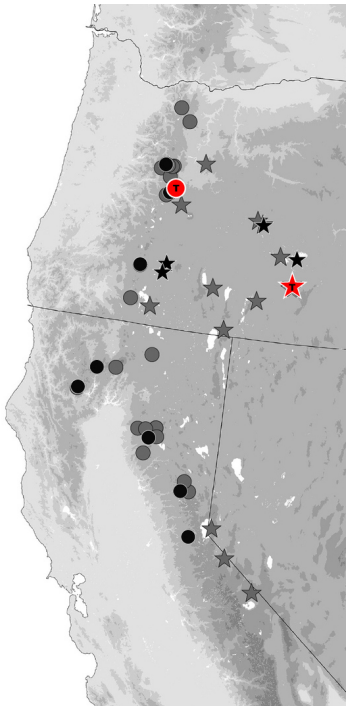

Figure 38. Geographic distribution of specimens examined of *Bembidion altipaludis* (circles) and *B. endeca* (stars).

Black symbols indicate those for which DNA data is available; gray symbols are other localities. Larger red symbols with a T indicate type localities.

Holotype male here designated, deposited in OSAC, labeled "USA: Oregon: Harney Co., Bridge Creek Canal pond, Malheur NWR, 1267m 42.8653°N 118.8793°W, 16.v.2015. DRM 15.050. Maddison, Sproul, & Z499 Class", "David R. Maddison DNA4543 DNA Voucher" [printed on pale green paper], "HOLOTYPE Bembidion endeca Maddison & Sproul" [partly handwritten on red paper], and "Oregon State Arthropod Collection OSAC_0002000010 [matrix code]" [printed on both sides of white paper]. Genitalia mounted in Euparal on small card (with DNA4543 written on it) beneath specimen; extracted DNA stored separately in OSAC. GenBank accession numbers for DNA sequences of the holotype are PV292173, PV292003, PV288180, PV288025, PV287872, and PV287752. Type locality: Bridge Creek Canal pond, Malheur National Wildlife Refuge, 42.8653°N 118.8793°W, Harney County, Oregon, USA.

Paratypes. 70 specimens from Oregon are designated as paratypes, and are deposited in OSAC, BYUC, CAS, CMNH, CNC, CUIC, EMEC, MCZ, MNHN, MZLU, NHMUK, and NMNH. They are from the following localities: Jefferson Co., Utopia Marsh, T12S R14e S28 (2, OSAC); Harney Co., Marshall Pond, Malheur NWR, 1247m 43.2663°N 118.8446°W (1, OSAC); Harney Co., Bridge Creek Canal pond, Malheur NWR, 1267m 42.8653°N 118.8793°W (26, OSAC, BYUC, CAS, CMNH, CNC, EMEC, MCZ, MNHN, MZLU, NHMUK, NMNH); Harney Co., Steens Mountain Loop Road, Malheur NWR, 1281m 42.8181°N 118.8748°W (1, OSAC); Harney Co., Malheur NWR, Fivemile Rd, 1271m 42.8934°N 118.8916°W (4, OSAC, BYUC); Harney Co., Dairy Creek, 1370m, 43.7306°N 119.6527°W (1, OSAC); Harney Co., Dairy Creek, 1375m 43.7181°N 119.6377°W (9, OSAC); Harney Co., Dairy Creek, 1375m 43.7181°N 119.6377°W(5, OSAC); Harney Co., Dairy Creek, 1375m 43.7182°N 119.6377°W (5, OSAC); Harney Co., Dairy Creek, 43.7184°N 119.6376°W, 1375m, 1.vi.2024 (6, OSAC, CUIC); Harney Co., French Glen (1, OSAC); Harney Co., N shore Harney Lake, T26S R291/2E Sec35NE1/4 (1, JRLC); Harney Co., Nicoll Creek, Ochoco NF, 43.7759°N 119.7736°W, 1550m (1, OSAC); Klamath Co., Klamath Marsh NWR, Williamson R, 1382m 42.965°N 121.5805°W (1, OSAC); Klamath Co., Klamath Marsh NWR, Wocus Bay, 1374m 42.8257°N 121.6445°W (1, OSAC); USA: Oregon: Klamath Co., Upper Klamath Lake (1, OSAC); Lake Co., Lakeview, Willow Creek Camp (1, CNC); Lake Co., Paisley (1, CNC); Lake Co., Rattlesnake Draw, Rock Creek Canyon, Hart Mountain NAR, 5330 ft (2, CMNH)

Additional specimens. 13 specimens not designated as paratypes were examined from the following localities: USA: Oregon: Deschutes Co., Deschutes River, Sunriver, 43.8633°N 121.4524°W, 1261m (1, OSAC); USA: California: Alpine Co., Monitor Pass (6, CSCA); USA: California: Mono Co., Bodie (4, NMNH); USA: Nevada: Douglas Co., Lake Tahoe, Glenbrook (2, OSAC).

Derivation of specific epithet. This species is named after the eleven members of the research group of nine undergraduate students, along with DRM and JSS, that participated in the Discovering Insect Species course and formed the team we called "The Trepaneleven". "Εντεκα" or "endeca" is the Greek word for eleven; it is a noun in apposition.

Diagnosis and description. A dark, spotless, relatively parallel-sided species (Figs 5d, 17d) without microsculpture on the pronotum and elytra, even at the elytral apex, the seventh stria absent or very faint but with a relatively long fifth stria, with male protarsomeres of normal width but slightly elongate, and lacking a longitudinal ridge on the mentum tooth.

Body black to dark brown, antennae and legs medium to dark brown; apical half of elytral epipleura of most specimens dark brown. Body relatively parallel-sided. Eyes of some specimens relatively flat; without incised notch behind the eyes. Frontal furrows deeply engraved, nearly touching posterior supraorbital seta (Fig. 27c). Fovea in which anterior supraorbital seta sits in most specimens small and circular. Anterior margin of clypeus with medial region notably posterior of lateral regions, and strongly angulate (Fig. 29f). Labrum with evident medial bump (Fig. 29f). Mentum with distinct transverse ridge, without longitudinal ridge on tooth (Fig. 30d). Pronotum relatively wide (PW/EW=0.69–0.73), moderately constricted toward the back (PWh/PW=0.728–0.770); lateral margins less sinuate; lateral margins near hind angle not parallel; lateral margin evenly explanate toward anterior margin, which is at most slightly protruding; anterior surface of pronotum smooth, with no punctures or wrinkles. Protarsomere 1 of male slightly longer than typical for *Bembidion* (Fig. 33b). Elytral striae with distinct but small punctures in the anterior half; fifth stria with punctures extending behind the posterior discal seta ed5; seventh stria almost absent in most specimens, with at most barely discernable punctures, although

some specimens have a few distinct punctures. Dorsal surface of prothorax and elytra (including apex, Fig. 34a) without visible microsculpture. Flight wings full length or short. Aedeagus with ventral surface in most specimens evenly arcuate, relatively straight (Fig. 21c,d); with a cluster of well-spaced, medium length spikes on internal sac near apex (Fig. 23b). Body length 2.8–3.5 mm.

Comparison with similar species. Very similar to *Bembidion altipaludis*; for distinguishing characteristics, see the treatment of that species. Also very similar to *B. canadianum*, from which it is most easily distinguished by the slightly less convex body, especially the elytra, which are almost flat (most easily seen in lateral view), in contrast to the elytra of *B. canadianum* which are longitudinally gently rounded throughout in lateral view; a pronotum that is less constricted in the back relative to the maximum width (PWh/PW ranges between 0.728–0.770 in *B. endeca*, 0.700–0.729 in *B. canadianum*); and the lack of microsculpture at the elytral apex; *B. endeca* in general has darker legs, whereas many *B. canadianum* have browner or reddish-brown legs, contrasting against the darker body color. From *B. acutifrons*, *B. microreticulatum*, and *B. anacalypsi*, females of *B. endeca* are easy to distinguish because of the lack of elytral microsculpture (females of members of the *B. acutifrons* subgroup other than *B. endeca* and *B. canadianum* have strongly engraved elytral microsculpture throughout the elytra and thus the elytra are very matte in appearance), and males by the narrower protarsomeres (Fig. 33b).

Variation. The length of flight wings varies within this species. Three specimens examined from Klamath Marsh and nearby have full flight wings, but examined specimens from Dairy Creek have wings about half the length of an elytron, from Malheur National Wildlife Refuge about one third the length of an elytron, and one from Brodie, California about one quarter.

Distributions. Found in two disjunct areas (Fig. 38): central Oregon (between 1250–1850m), and the eastern edge of the central Sierra Nevada in California and adjacent Nevada (between 1900–2550m).

Habitat. Found on damp, organic soil at the base of grasses and sedges in marshy regions, but not close to the water's edge (Fig. 1a, b, d). At the type locality found between 1 and 3 m from the water, and at Dairy Creek found at least 1.5 m from the water's edge.

Notes. A specimen from Upper Klamath Lake, Oregon (OSAC) was identified by Lindroth in 1958 as *B. canadianum*.

Bembidion canadianum Casey, 1924 (Figs 6a, 18a, 21e,f, 23c, 26d, 28c,d, 32d, 33c, 34b, 39)

Bembidion canadianum Casey, 1924, p. 43. Lectotype female in NMNH (type number 37083), designated by Lindroth (1975, p. 122), examined. Type locality Edmonton, Alberta, Canada.

Diagnosis and description. Adults of this species (Figs 6a, 18a) are dark and unspotted, with the prothorax and elytra quite convex, the pronotum laterally rounded and constricted toward the back, with the front angles of pronotum not notably prolonged anteriorly, the elytra are parallel-sided, male protarsomere 1 is not strikingly enlarged, and both sexes have no microsculpture on the elytral disk, but have visible microsculpture near the elytral apex.

Body dark brown, without elytral spots; antennae dark brown or with antennomere 1 and bases of antennomeres 2 and 3 as light as pale reddish brown; legs brown or reddish brown, lighter in color than the body. Prothorax and elytra notably convex, relatively parallel sided. Eyes moderate in size to flat (Fig. 28c,d); without incised notch behind the eyes. Frontal furrows deeply engraved, wrapping around back of eyes. Labrum with small medial bump (Fig. 29f). Mentum with distinct transverse ridge, but without longitudinal ridge on tooth. Pronotum with lateral margins very rounded, and strongly constricted toward the back such that the width at the hind margin is much narrower than the maximum width (PWh/PW = 0.700–0.729); most specimens with anterior corners with less explanate margins and not extended as far forward at front angle (Fig. 32d, arrow), although a few specimens similar to Fig. 32e; most specimens with posterior part of lateral margins not parallel to each other; anterior surface of pronotum smooth, with no punctures or wrinkles. Protarsomere 1 of male large, slightly swollen basally (Fig. 33c). Elytral striae with distinct but small punctures in the anterior two-thirds; fifth stria with punctures extending behind the posterior discal seta ed5; seventh stria with very small but distinct punctures. Dorsal surface of prothorax and elytral disc without visible microsculpture, but elytral apex with microsculpture (Fig. 34b). Wings short, about three-quarters the length of an elytron, in observed specimens (from Alberta, Colorado, and Montana). Ventral margin of aedeagus relatively straight (Fig. 21e,f); sclerite 1 (white arrow in Fig. 21e, f) large, vertically oriented and thus the majority of it is evident from the left view; with a cluster of moderately large spikes on internal sac near apex (Fig. 23c). Body length 3.0–3.6 mm.

Comparison with similar species. Similar to other dark, shiny *Trepanedoris* species, including *Bembidion altipaludis*, and *B. endeca*; see those species treatments for differentiating characteristics. From *Bembidion kieranae* distinguished by the larger size, more convex body, darker body, notably larger male protarsomere 1 (compare Fig. 33c to Fig. 33g), presence of a median bump on the labrum, and presence of elytral microsculpture near the elytral apex; the male genitalia are also different in many details (compare Fig. 21e,f to Fig. 22c,d), including the much larger patch of spikes in the aedeagus of *B. kieranae* (Fig. 25b).

Variation. There is variation in eye size within this species (Fig. 28c,d), including within populations; for example, there are small- and large-eyed individuals around one pond in the Alamosa National Wildlife Refuge in Colorado.

Distributions. British Columbia east to Quebec, and south to Colorado in the west (Fig. 39). We have seen specimens from British Columbia, Alberta, and Manitoba, as well as Montana and Colorado, from 240–2300m in elevation. In addition, Lindroth (1963) reports the species from Saskatchewan, Ontario, and Québec.

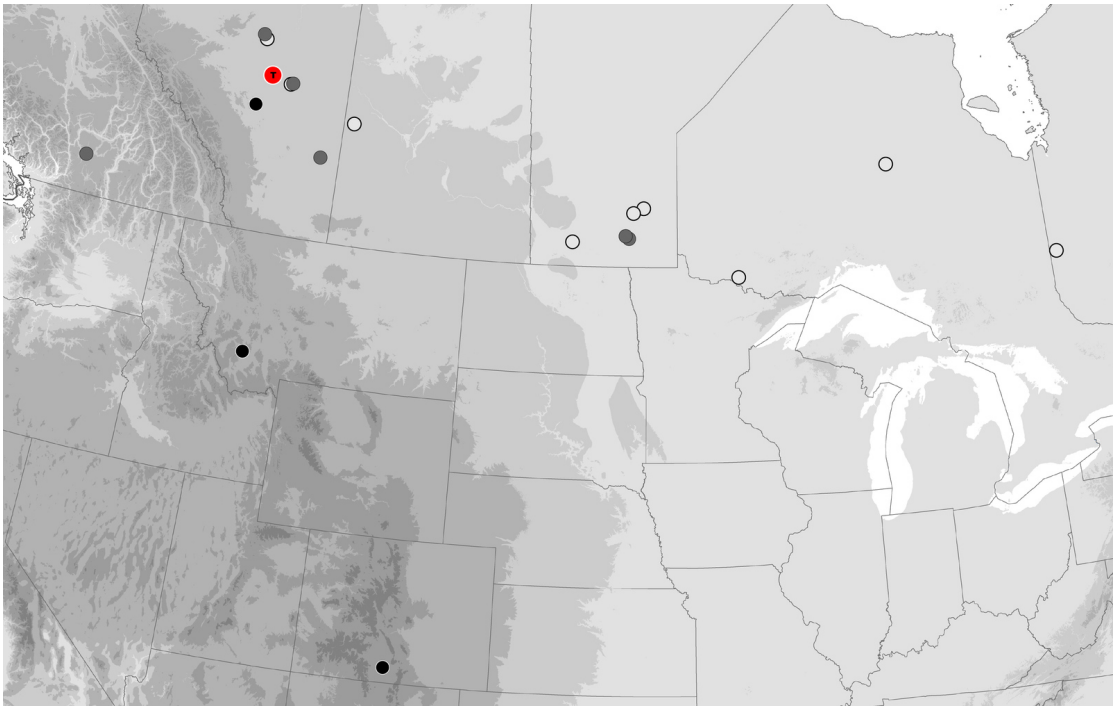

Figure 39. Geographic distribution of *Bembidion canadianum*.

Black circles indicate those for which DNA data is available; darker gray are other localities for specimens we have examined; pale gray are localities from Lindroth (1963) based upon specimens we have not examined. Larger red circle with a T indicates the type locality.

Habitat. We have collected it at three localities. Near the Big Hole River in Melrose, MT, it was found on dark, damp, organic soil with sparse grasses in a depression in the partial shade of large cottonwood trees; among sedges on dark organic soil covered with dead sedge leaves around the shore of a small pond in the Alamosa National Wildlife Refuge; and at the base of vegetation on damp soil above the shore of Gull Lake, several meters from the water.

Bembidion acutifrons LeConte, 1879 (Figs 6b, 18b, 21g,h, 23d, 24a–c, 26e, 30c, 32f, 33d, 34c, 35a,b, 40)

Bembidium acutifrons LeConte, 1879, p. 509. Holotype male in MCZ (type number 5553), examined. Type locality: Alamosa, Colorado, USA.

Diagnosis and description. Among the species considered, this and the following two species form a distinct trio of large, dark, unspotted species with wide, flat pronota often having the lateral margins of the posterior angle parallel to one another, males having very large protarsomere 1, and females with deeply engraved elytral microsculpture causing the matte luster of the elytra to contrast strikingly against the dark, shiny pronotum. *Bembidion acutifrons* itself (Figs 6b, 18b) is distinguished by the lack of microsculpture on the elytral disc of males around anterior discal seta ed3, and the lack of spikes in the internal sac of the aedeagus near its apex (Fig. 23d).

Body dark brown to almost black, without spots on elytra; antennae mostly dark brown, with some specimens having antennomere 1 and the bases of antennomeres 2 and 3 reddish brown; legs dark brown to reddish brown. Body broad and relatively flat. Eyes of normal size; without incised notch behind the eyes. Frontal furrows deeply engraved, wrapping around back of eyes. Labrum with small medial bump. Mentum with distinct transverse ridge, but without longitudinal ridge on tooth. Pronotum flat and wide, especially in the anterior half, where the front angles protrude in most specimens (Fig. 32f); anterior surface of pronotum smooth or with very faint wrinkles. Protarsomere 1 of male very large, distinctly swollen (Fig. 33d). Elytral striae with distinct but small punctures in the anterior two-thirds to three quarters; fifth stria with punctures extending behind the posterior discal seta ed5; seventh stria with very small but distinct punctures. Dorsal surface of prothorax without microsculpture. Elytral disc of males without microsculpture around ed3 (Fig. 35a, b) (although one male from Creston, BC, has complete sculpticells around ed3), but with deeply engraved microsculpture in females, giving their surface a matte luster, contrasting sharply against the shiny pronotum. Elytral apex with visible microsculpture in both sexes (Fig. 34b). With full-length flight wings in examined specimens. Ventral margin of aedeagus evenly curved (Fig. 21g) or only slightly sinuate (Fig. 21h); sclerite 1 (Fig. 24a–c) small and tapered, horizontally oriented and thus appearing even smaller in left lateral view; a thin dark sclerotized region (sclerite 2, Fig. 24a–c) extends from sclerite 1 toward the front (base) of the aedeagus; without spikes on internal sac near apex (Fig. 23d), or at most with three or four very small spikes. Body length 3.0–3.9 mm.

Comparison with similar species. Most easily confused with *Bembidion microreticulatum* and *B. anacalypsi*. From *B. microreticulatum*, males can be distinguished by the lack of microsculpture on the elytral disc, the lack of small spikes

on the internal sac near the aedeagal apex, and the notably less sinuate ventral margin of the aedeagus. From *B. anacalypsi*, males are distinguished by the absence of microsculpture on the elytral disc and the lack of large spikes on the internal sac near the aedeagal apex. In addition, sclerite 1 is small and more horizontally positioned in *B. acutifrons*, and sclerite 2 is thin, long, and distinct (Fig. 24a–c).

There is another form of the *acutifrons* subgroup, *B. scenicum,* which is apparently distinct from *B. acutifrons*. It matches *B. acutifrons* in all described morphological features, and would be identified as such. It is only easily distinguishable from *B. acutifrons* in characteristics of the female genitalia and by using genomic data. It is known from Klamath Marsh in southern Oregon, as well as the Sierra Nevada of California and adjacent Nevada. Research is needed to clarify the status of this form; it will be treated in a subsequent paper.

B. acutifrons might also be hard to distinguish from other dark, spotless species, including *B. canadianum*, *B. endeca*, and *B. altipaludis*; to separate specimens, see the treatments of those species.

Variation. None noted in morphological features; DNA sequences indicate the population from Malheur National Wildlife Refuge is distinctive genetically (e.g., Fig. 13).

Distributions. We have examined specimens from British Columbia south to Nevada, and east to Colorado and Saskatchewan (Fig. 40), from 560–3020m in elevation. In the literature it is reported north to Alaska and Nunavut, and east to Manitoba and South Dakota (Bousquet, 2012).

Habitat. Found at bases of sedges, grasses, and *Typha* in marshy areas, either close to the water or up to at least 3m away from the water's edge (Fig. 1b). In the La Sal Mountains of Utah it was found abundantly on damp sand and silt soil in a depression around an old fire ring in a clearing in an aspen forest, without standing water nearby.

Bembidion microreticulatum Hatch (Figs 6c, 18c, 21i, j, 23e, 24d–f, 35e,f, 40)

Bembidion microreticulatum Hatch, 1950, p. 105. Holotype male in NMNH, examined. Type locality: Stickney Lake, Snohomish County, Washington, USA.

Diagnosis and description. As with *Bembidion acutifrons* and *B. anacalypsi*, *B. microreticulatum* adults are large, dark, and unspotted with wide, flat pronota often having the lateral margins of the posterior angle parallel to one another, males having very large protarsomere 1, and females with deeply engraved elytral microsculpture causing the matte luster of the elytra to contrast strikingly against the dark, shiny pronotum; *Bembidion microreticulatum* itself (Figs 6c, 18c) is distinguished by the well-engraved microsculpture on the elytral disc of males around ed3, and the short spikes in the internal sac of the aedeagus towards its apex (Fig. 23e).

Brown to dark brown, in most specimens with the elytra slightly paler than the pronotum; without spots on elytra; antennae dark brown except for antennomere 1, which is pale reddish brown in many specimens; legs dark brown to pale reddish brown. The northern specimens are in general paler than the southernmost ones. Eyes of normal size; without incised notch behind the eyes. Frontal furrows deeply engraved, wrapping around back of eyes. Labrum with small medial bump. Mentum with distinct transverse ridge, but without longitudinal ridge on tooth. Pronotum narrower than *B. acutifrons*, with front angles less protruding; anterior surface of pronotum smooth or with very faint wrinkles. Protarsomere 1 of male very large, appearing distinctly swollen (as in Fig. 33d). Elytral striae with distinct but small punctures in the anterior half; fifth stria with punctures in most specimens not extending behind the posterior discal seta ed5; seventh stria in most specimens effaced, although a few specimens have very small punctures. Most of dorsal surface of prothorax without microsculpture (some specimens have sculpticells around the edges of the pronotum). Elytral disc of most males and females with clearly defined microsculpture (Fig. 35e, f), giving their surface a matte luster, contrasting sharply against the shiny pronotum. The few males known from the southern end of the range (near Lake of the Woods and Chiloquin, Oregon) have relatively effaced sculpticells. With full-length flight wings in examined specimens except those from Lake of the Woods in southern Oregon; specimens from there have shorter wings, about the length of an elytron, likely rendering them incapable of flight. Aedeagus well sclerotized and dark; ventral margin of aedeagus quite sinuate, with a thickened region (black arrows in Fig. 21i, j) followed by a preapical indentation (gray arrows in Fig. 21i, j), with apex quite narrow; sclerite 1 (Fig. 24d–f) small, horizontally oriented and thus appearing even smaller in the left view; with small spikes on internal sac near apex (Fig. 23e). Body length 2.8–3.7 mm.

Comparison with similar species. To distinguish this species from *B. altipaludis*, *B. endeca*, *B. canadianum*, and *B. acutifrons*, see the discussions under those species. From *B. anacalypsi*, most easily distinguished by the more deeply engraved elytral microsculpture in males, and shorter and fewer spikes on the internal sac of the aedeagus near its apex. In addition, sclerite 1 is smaller (Fig. 24d–f).

Variation. Northern males have more deeply engraved elytral microsculpture, and appear somewhat paler.

Distributions. Known only from British Columbia, Washington, and Oregon, from the from the Pacific Coast to the eastern slopes of the Cascades (Fig. 40). Most specimens are from sea level to 500m, but the specimens from southern Oregon are from about 1500m.

Habitat. Found on marshy shores of ponds and lakes. At Fort Borst Lake in Centralia, Washington, several specimens were found at the bases of sedges and grasses in a partly shaded region along the upper bank of the lake. They are common in marshes in the San Juan and Gulf Islands between Vancouver Island and the mainland (JRB); some of the marshes in which they have been found have some seawater influence, suggesting a tolerance for saline water (James Bergdahl, pers. comm. 2023).

Notes. Considered a synonym of *Bembidion acutifrons* by Lindroth (1963).

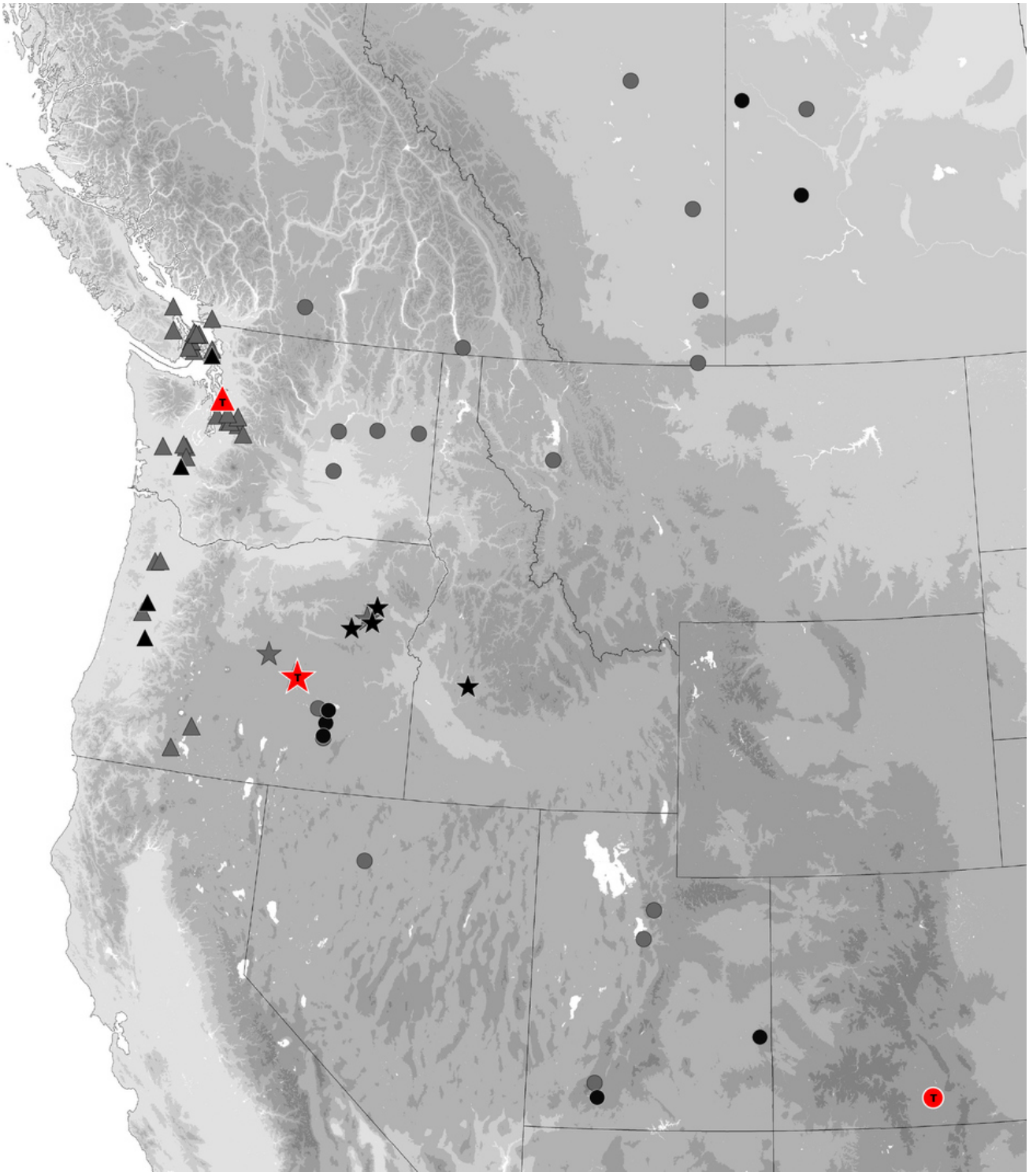

Figure 40. Geographic distribution of specimens examined of *Bembidion acutifrons* (circles), *B. microreticulatum* (triangles), and *B. anacalypsi* (stars).

Black symbols indicate those for which DNA data is available; gray symbols are other localities. Larger red symbols with a T indicate type localities.

Bembidion anacalypsi Mendez & Maddison, n. sp.
(Figs 6d, 18d, 21k,m, 23f, 24g–i, 26f, 32e, 33e, 35c,d, 40)

urn:lsid:zoobank.org:act:DA86CF21-73EB-46A5-975F-446C4624BA9C

Holotype male here designated, deposited in OSAC, labeled "USA: Oregon: Harney Co., Dairy Creek, 1375m 43.7182°N 119.6377°W, 14.v.2018. DRM 18.015. Maddison, Boyd, Mendez", "David R. Maddison DNA5283 DNA Voucher" [printed on pale green paper], and "HOLOTYPE Bembidion anacalypsi Mendez & Maddison" [partly handwritten on red paper], and "Oregon State Arthropod Collection OSAC_0002000011 [matrix code]" [printed on both

sides of white paper]. Genitalia mounted in Euparal on small card (with DNA5283 written on it) beneath specimen; extracted DNA stored separately in OSAC. GenBank accession numbers for DNA sequences of the holotype are PV292210, PV292040, PV288217, PV288062, PV287909, and PV287782. Type locality: Dairy Creek, 43.7182°N 119.6377°W, Harney County, Oregon, USA.

Paratypes. 120 specimens from Oregon and Idaho are designated as paratypes, and are deposited in OSAC, BYUC, CAS, CMNH, CNC, CUIC, EMEC, MCZ, MNHN, MZLU, NHMUK, and NMNH. They are from the following localities: Oregon: Baker Co., 4.4 km NW Haines, 44.9379°N 117.9821°W (10, OSAC); Oregon: Baker Co., Deer Creek at highway 6, Mowich Loop, 1237m 44.6929°N 118.0657°W (12, OSAC, NHMUK); Oregon: Baker Co., Sumpter (5, CNC); Oregon: Grant Co., Austin House, jct Hwys 26 & 27, 1005 m (1, CNC); Oregon: Grant Co., Pond near Bridge Creek, Austin Junction, 1285m 44.5719°N 118.5059°W (17, OSAC, CUIC); Oregon: Crook Co., Ochoco NF pond S of NF-16, 44.0242° N 120.3433°W, 1572m (6, OSAC); Oregon: Harney Co., Dairy Creek, 1370m, 43.7306°N 119.6527°W (2, OSAC); Oregon: Harney Co., Dairy Creek, 1375m 43.7178°N 119.6357°W (3, OSAC); Oregon: Harney Co., Dairy Creek, 1375m 43.7182°N 119.6377°W (29, OSAC, CAS, CMNH, EMEC, MNHN, MCZ, MZLU, NMNH); Oregon: Harney Co., Dairy Creek, 43.7184°N 119.6376°W, 1375m (24, OSAC, BYUC, EMEC); Idaho: Boise Co., 2 mi E Idaho City, 1210m 43.8281°N 115.7991°W (9, OSAC); Idaho: Boise Co., 2 mi E Idaho City, 1210m 43.8282°N 115.7990°W (2, OSAC).

Derivation of specific epithet. From the Greek word "anakalypsi", a noun meaning "discovery". This species is so named for several reasons. The first known specimens were collected during one of the field trips of the Discovering Insect Species course, a key element of which was introducing students to the process of discovery. The name also commemorates the moment when one of the students and authors, Danielle Mendez, discovered the large genitalic spikes that confirmed the distinctiveness of this species. Finally, the name expresses the personal growth and self-discovery she experienced in working on this project as an undergraduate in the Maddison Lab, in response to the challenges of the project, and the relationships built with lab mates.

Diagnosis and description. As with *Bembidion acutifrons* and *B. microreticulatum*, *B. anacalypsi* adults are large, dark, and unspotted with males having wide, flat pronota in most specimens having the lateral margins of the posterior angle parallel to one another, males with very large protarsomere 1, and females with deeply engraved elytral microsculpture causing the matte luster of the elytra to contrast strikingly against the dark, shiny pronotum; *Bembidion anacalypsi* itself (Figs 6d, 18d) is distinguished by the traces of microsculpture on the elytral disc of males around discal seta ed3, and a dense patch of long spikes in the internal sac of the aedeagus towards its apex (Fig. 23f).

Body dark brown, without spots on elytra; antennae dark brown, except for antennomere 1, which is dark reddish brown in some specimens; legs dark brown or dark reddish brown. Eyes of normal size; without incised notch behind the eyes. Frontal furrows deeply engraved, wrapping around back of eyes. Labrum with small medial bump. Mentum with distinct transverse ridge, but without longitudinal ridge on tooth. Pronotum narrower than in *B. acutifrons*, with front angles less protruding; anterior surface of pronotum smooth or with very faint wrinkles. Protarsomere 1 of male very large, appearing distinctly swollen (Fig. 33e). Elytral striae with distinct but small punctures in the anterior two-thirds to three quarters; fifth stria with punctures extending behind the posterior discal seta ed5; seventh stria with very small punctures, in some specimens almost effaced. Dorsal surface of prothorax without microsculpture. Elytral disc of males with weakly engraved microsculpture (Fig. 35c), especially around the posterior discal seta (Fig. 35d), but with deeply engraved microsculpture in females, giving their surface a matte luster, contrasting sharply against the shiny pronotum. Length of flight wings variable. Aedeagus well sclerotized and dark; ventral surface of aedeagus at most slightly sinuate (Fig. 21k, m); sclerite 1 (Fig. 24g–i) large, vertically oriented and thus the majority of it is evident in left lateral view; sclerite 3 (Fig. 24g–i) relatively wide, and with a thick anterior margin; with a dense patch of very large spikes on internal sac near apex (Fig. 23f). Body length 2.9–3.8 mm.

Comparison with similar species. Characteristics to distinguish this species from other dark, spotless species, including *B. altipaludis*, *B. endeca*, *B. canadianum*, *B. acutifrons*, and *B. microreticulatum*, are outlined in the discussions of those species. In addition, sclerite 1 in the aedeagus appears large in lateral view (Fig. 24g–i), and sclerite 3 is relatively circular with a thick anterior margin.

Variation. Length of flight wings varies from full length at the type locality and Mowich Loop, to shorter, likely too short for flight, from all other examined localities.

Distributions. Known only from eastern Oregon and western Idaho (Fig. 40), between 1000 and 1600m elevation.

Habitat. Found on damp, organic soil at the base of grasses, sedges, or *Typha* in wet meadows and marshy regions (Fig. 1d). At the type locality found at least 1.5 m from the water's edge, with most specimens found in a dried-up backchannel on damp soil and leaf litter at the base of small willows, and on damp soil in a wet meadow far from water at Mowich Loop, Oregon. In other places found close to the water's edge (for example, around a small pond near Bridge Creek at Austin Junction, Oregon, and near Idaho City, Idaho).

Bembidion ampliceps Casey, 1918 (Figs 7a, 19a, 22a,b, 25a, 26g, 27d, 29d, 33f, 41)

Bembidion ampliceps Casey, 1918, p. 161. Holotype male in NMNH (type number 37084), examined. Type locality: Gilroy Hot Springs, Santa Clara County, California, USA.

Diagnosis and description. One of the more distinctive *Trepanedoris*, with its narrow, parallel-sided, nearly black body, head including the bulging eyes almost as wide as the pronotum, eyes with a distinctive notch behind them, dark brown appendages, and lack of microsculpture on the shiny dorsal surface, and the larger punctures in the front half

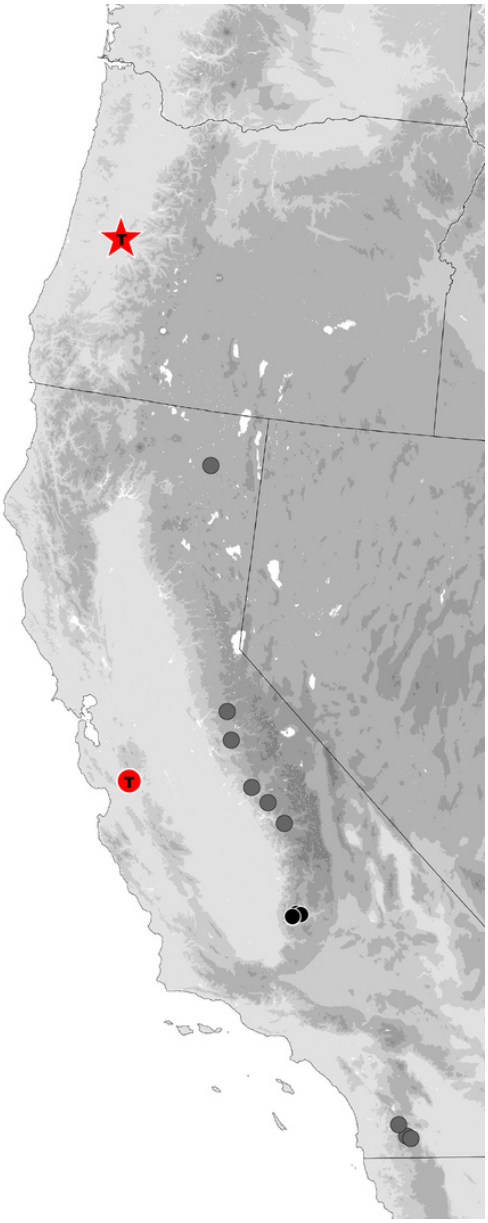

Figure 41. Geographic distribution of specimens examined of *Bembidion ampliceps* (circles) and *B. kieranae* (star).

Black symbols indicate those for which DNA data is available; gray symbols are other localities. Larger red symbols with a T indicate type localities.

of the elytra abruptly transitioning to very small punctures around posterior discal seta ed5 (Figs 7a, 19a).

Body color nearly black, legs dark brown, antenna dark brown with antennomere 1 in some specimens slightly paler. Head including eyes wide, almost as wide as the narrow pronotum (HW/PW>0.92); elytra narrow (HW/EW>0.62). Eyes protruding, larger than other described *Trepanedoris*; incised notch behind the eyes (Fig. 27d). Frontal furrows deeply engraved, wrapping around back of eyes. Anterior edge of clypeus with medial region distinctly posterior of lateral region (Fig. 29d). Labrum with medial bump. Mentum with distinct transverse ridge, but without longitudinal ridge on tooth. Pronotum narrow, not wide in front, and thus lateral margins less sinuate than most other species; lateral margins with explanate area sharply delimited, wide, and of approximately equal width throughout; hind angles nearly a right angle; anterior surface of pronotum smooth, without punctures or wrinkles. Protarsomere 1 of male narrow, only slightly wider than second protarsomere (Fig. 33f). Elytra striae in anterior half with punctures large, abruptly transitioning behind ed5 to very small punctures (Figs 7a, 19a). Seventh stria short, about half the width of elytron, but with relatively large punctures. Dorsal surface without microsculpture and thus very shiny. With full-length flight wings, as far as known. Aedeagus (Fig. 22a, b) with relatively evenly arcuate ventral margin. Body length 2.8–3.2 mm.

Comparison with similar species. Most similar to *Bembidion kieranae* and *B. clemens disparile*. From both distinguished by darker color, especially the much darker legs, the bulging eyes with a distinct notch behind them, narrow body, medial bump on the labrum, and the large punctures in the anterior half of the elytra abruptly transitioning to very small punctures around discal seta ed5.

Variation. None observed.

Distributions. We have only seen specimens from California (Fig. 41). Lindroth (1963) reports it from Oregon, apparently based upon material in Hatch's collection (OSAC), but we could find no specimens in Hatch's collection of this species from Oregon. Known localities range in elevation from 350–1670m.

Habitat. We have found *Bembidion ampliceps* on the clay and sand shores of two creeks in Kern County (Fig. 1f), as well as on the vegetation covered sand and organic matter shores of Lake Isabella at the mouth of French Creek. Based upon these observations and other locality labels, it appears to be typically associated with small creeks (e.g., Gilroy Hot Springs, Upper Rush Creek in Modoc County, and North Fork of the Tuolumne River near Pinecrest).

Notes. *Bembidion ampliceps* is a name used for other species of *Trepanedoris* in various collections, especially for *Bembidion altipaludis*. The specimens called "*Bembidion* sp nr *ampliceps* Nr-1" and "*Bembidion* sp nr *ampliceps* Nr-2" in Maddison (1985) are all *Bembidion clemens clemens*.

Bembidion kieranae Maddison & Sproul, n. sp. (Figs 7b, 19b, 22c,d, 25b, 27e, 26h, 33g,i, 41)

urn:lsid:zoobank.org:act:E8F62822-1A96-4366-8EF3-B0CFC8A5424B

Holotype male here designated, deposited in OSAC, labeled "USA: Oregon: Lane Co., SW Eugene, 288m 44.0031°N 123.1299°W, 5.vi.2015. DRM 15.069. Maddison, Kieran, Mendez, Zeleznik", "David R. Maddison DNA4586 DNA Voucher" [printed on pale green paper], and "HOLOTYPE Bembidion kieranae Maddison & Sproul" [partly handwritten on red paper], and "Oregon State Arthropod Collection OSAC_0002000012 [matrix code]" [printed on both sides of white paper]. Genitalia mounted in Euparal on small card (with DNA4586 written on it) beneath specimen; extracted DNA stored separately in OSAC. GenBank accession numbers for DNA sequences of the holotype are PV292295, PV292125, PV288302, PV288151, PV287994,

and PV287842. Type locality: Eugene, 44.0031°N 123.1299°W, Lane County, Oregon, USA.

Paratypes. 7 specimens are designated as paratypes and deposited in OSAC and EMEC. They are from the following nearby localities in Oregon: Lane Co., SW Eugene, 287m, 44.0031°N 123.1299°W (1, OSAC); Lane Co., SW Eugene, 288m, 44.0031°N 123.1299°W (5, OSAC, EMEC); Lane Co., SW Eugene, 248m, 44.0029°N 123.1330°W (1, OSAC)

Derivation of specific epithet. This species is named after Shannon R.C. Kieran (now Blair), a student in the Discovering Insect Species class, who found the first known specimen at her parents' property, and who participated in the collection of most of the type series.

Diagnosis and description. A small, relatively flat species without elytral spots or at most very faint spots, without a patch of prominent wrinkles or punctures in the anterior region of the pronotum, without microsculpture on the dorsal surface, lacking an incised notch behind the eyes, with pale legs but with only antennomere 1 pale (Figs 7b, 19b); with a distinctive male aedeagus with, among other details, a large patch of spikes (Fig. 22c,d).

Body dark brown with apices of elytra slightly paler; some specimens have faint preapical spots. Legs pale yellowish brown; antennomere 1 pale yellowish brown, although darker toward apex; second and later antennomeres mostly darker brown. Eyes of normal size, without incised notch behind the eyes. Frontal furrows deeply engraved, wrapping around back of eyes. Labrum without medial bump. Mentum with distinct transverse ridge, but without longitudinal ridge on tooth. Anterior surface of pronotum smooth, without punctures or wrinkles. Protarsomere 1 of male of normal width for a *Bembidion* (Fig. 33g), although appearing swollen when viewed from the side (Fig. 33i). Punctures of elytral striae of relatively uniform size in front of discal seta ed5, of decreasing size behind that point; seventh stria with distinct punctures in the anterior half; fifth stria punctate from front of elytra to behind ed5. Dorsal surface without microsculpture. With full-length flight wings, as far as known. Aedeagus with ventral margin at most slightly sinuate (Fig. 22c,d); with a smaller, simple subapical brush (white arrow in Fig. 25b), and with large subapical spikes that are distributed over a wide area (black arrows in Fig. 25b). Body length 2.6–2.8 mm.

Comparison with similar species. Most likely to be confused with *Bembidion clemens disparile* and *B. ampliceps*. From *B. clemens* most easily distinguished by the smaller size, flatter body, male protarsomere appearing swollen in lateral view, and distinctive aedeagus. For characteristics distinguishing it from *B. ampliceps*, see the treatment of that species.

Variation. Some specimens have very faint preapical spots, whereas others lack such spots.

Distributions. Only known from around two ponds 250 m apart in Eugene, Oregon, between 250–290m in elevation (Fig. 41). The first specimen was collected at the type locality on 25 April 2015, when the pond was filled with water. Five specimens were collected on 5 June 2015, four from around the same type-locality pond as the first specimen, and one from a pond 250 m away. At the time of this second collecting event, the type-locality pond had no standing water, and was filled with tall grasses (Fig. 1c). In late April, late May, and early October 2023 attempts were made to find additional specimens at the type locality, with no success. In May 2025 another attempt was made, and two additional specimens were found. The species is likely more widespread, perhaps at the base of thick vegetation around vernal pools. The lack of other known specimens may be a result of the beetles being overlooked because of their small size, their cryptic microhabitat, and general lack of collecting of very small carabids around vernal pools in Oregon.

Habitat. At the type locality, seven of the eight known specimens were found around a vernal pool on an open, unshaded hillside (Fig. 1c). The pond is fed by a seep above the pond about 5m away. Other *Bembidion* species around the pond included *Bembidion siticum*, *B. connivens*, *B. microreticulatum*, *B. (Lindrochthus) delectum* Casey, *B. (Trechonepha) iridescens* (LeConte), and *B. (Notaphus) coloradense* Hayward; the harpaline *Bradycellus californicus* (LeConte) was abundant. At least four of the specimens of *Bembidion kieranae* were found around the base of *Juncus patens* E.Mey in layers of the plants' dead leaves. The nearby pond at which one specimen was found was longer-lasting and was partly shaded by trees. Seven of the eight specimens were found during daylight hours, and only one after dark, even though at least six person-hours were spent searching at dusk and after dark using headlamps.

Bembidion clemens Casey, 1918 (Figs 7c, 19c, 22e,f, 25c,d, 26i, 27f, 29c, 33h,j, 36a, 42)

Bembidion clemens Casey, 1918, p. 159. Lectotype female in NMNH (type number 37080), designated by Erwin (1984, p. 168), examined. Type locality: Provo, Utah County, Utah, USA.

Bembidion invidiosum Casey, 1918, p. 162. Lectotype male in NMNH (type number 37081) , designated by Erwin (1984, p. 169), examined. Type locality: road between Fort Wingate and Jemez Springs, Sandoval County, New Mexico, USA.

Bembidion disparile Casey, 1918, p. 161. Lectotype male in NMNH (type number 37074), examined. GenBank accession numbers for DNA sequences of the lectotype are PV292283, PV292113, PV288290, PV288136, PV287982, and PV287831. Type locality: Santa Barbara, Santa Barbara County, California, USA.

Bembidion vapidum Casey, 1918, p. 160. Lectotype male in NMNH (type number 37073), examined. Type locality: Mount Diablo, Contra Costa County, California, USA.

Diagnosis and description. A variable species distinguished by the lack of microsculpture on the dorsal surface, lack of prominent wrinkles or punctures on the anterior half of the pronotum, normal sized eyes, head and elytra of normal width (Figs 7c, 19c), and a distinctive subapical brush in the male aedeagus (Fig. 25c, d, white arrows). For much of its range east of California and south of Idaho and Montana, it is the only known *connivens* group with pale preapical spots on the elytra and without dorsal microsculpture.

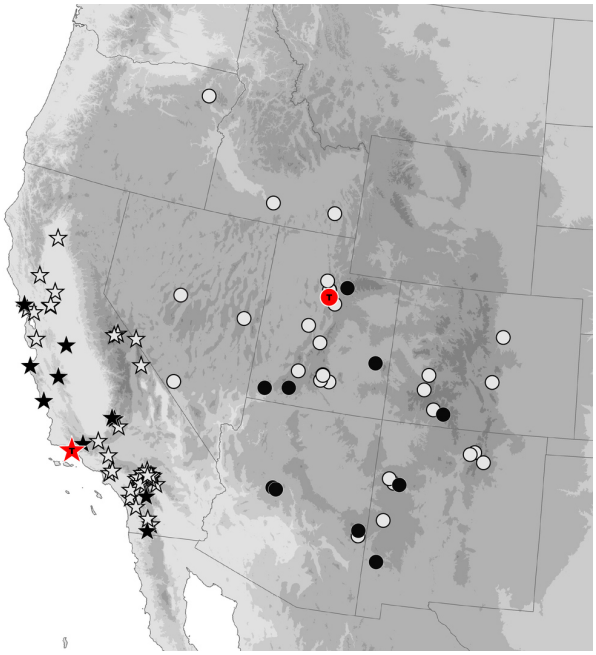

Figure 42. Geographic distribution of specimens examined of *Bembidion clemens*.

Circles indicate specimens of *B. clemens clemens*, based upon DNA sequences and color of appendages (black circles) or color of appendages only (white circles). Stars indicate specimens of *B. clemens disparile*, based upon DNA sequences and color of appendages (black stars) or color of appendages only (white stars). Larger red symbols with a T indicate type localities. The large circle is the type locality of *Bembidion clemens* Casey, and the large star is the type locality of the name *Bembidion disparile* Casey. There is in addition a doubtful record of *B. clemens disparile* from Dallas, Texas (see text).

Body very dark brown, in specimens east of California with front half of elytra slightly paler, a reddish brown; preapical spots on elytra from absent to very small and faint to large and distinct (as in Fig. 7c); antennomeres 2–4 variable in color, from pale brown mostly east of California) to dark brown (California); legs also variable in color, from pale brownish yellow in the east (e.g., Fig. 7c) to brown in California. Eyes of normal size, without incised notch behind the eyes. Frontal furrows deeply engraved, wrapping around back of eyes. Labrum without medial bump. Mentum with distinct transverse ridge, but without longitudinal ridge on tooth. Anterior surface of pronotum smooth or with very faint wrinkles. Protarsomere 1 of male relatively narrow (Fig. 33h), not swollen dorsally (Fig. 33j). Seventh elytral stria with distinct punctures. Dorsal surface without microsculpture. With full-length flight wings, as far as known. Aedeagus with a notably sinuate ventral margin (Fig. 22e, f), and with a large, distinctly shaped subapical brush with a large central bulge contrasting against narrow dorsal and ventral projections (Fig. 25c, d, white arrows), and with the subapical spikes within the internal sac smaller, denser, and more localized (Fig. 25c, d). Body length 2.8–3.5 mm.

Comparison with similar species. Most easily confused with *Bembidion kieranae* and *B. connivens*. For separation from *B. kieranae*, see that account of that species. From *Bembidion connivens* readily distinguished by the lack of microsculpture on the dorsal surface.

Variation. Specimens from east of the Sierra Nevada and Cascade Mountains (Nevada, Arizona, Utah, and eastward) are paler, with pale yellowish-brown legs and the elytral spots pale and distinct; they also have slightly wider prothoraces (PW/EW=0.652–0.670, with seven of the eight measured specimens ⩾0.660), and in most specimens larger elytral punctures. Specimens from California are darker, with legs in most specimens darker brown, antennomere 2 at least in part darker brown, and with the elytral spots absent or smaller and darker. Specimens of the California form also have slightly narrower prothoraces (PW/EW=0.617–0.659, with seven of the nine measured specimens ⩽0.654), and in most specimens smaller punctures in the elytral striae (most noticeable by comparing seventh striae). These different forms are distinctive in sequences of 28S, CAD, and Topo, but as no differences in male genitalia could be detected and as the potential contact zone around the borders of California with Nevada and Arizona have not been adequately sampled, we treat them as belonging to the same species (see discussion above, under "Implications for Species Boundaries"). However, because of the clear differences in color and DNA, we feel it useful to give them different names and so treat them as two subspecies. To the eastern form belongs the lectotype of *Bembidion clemens* Casey and *B. invidiosum* Casey, and thus the form takes the name *Bembidion clemens clemens*. To the western form belongs the lectotypes of *Bembidion disparile* Casey and *B. vapidum* Casey, and as first revisers we choose the name *B. clemens disparile* for the name of this form. The lectotype of *Bembidion disparile* Casey is extremely teneral and thus structurally distorted; its morphological characteristics were not evident sufficiently to place it, even to species. However, the DNA sequences clearly indicate that it belongs to the western form of *B. clemens* (Figs 8-11).

Distributions. We have seen specimens from California and Oregon east to Colorado and New Mexico (Fig. 42). *Bembidion clemens clemens* is known from 1000–2450m in elevation, and *B. clemens disparile* from 5–3025m. There are two specimens in CSCA, originally in Kenneth W. Cooper's collection, labeled "Texas, Dallas. KWC 67e27". "67e27" is Cooper's code for the date 27 May 1967. Those two specimens exactly match *Bembidion clemens disparile* in color and pronotal width. This is far outside the known distribution of that form, and far east of any other known *B. clemens*; we view those specimens as likely mislabeled.

Habitat. This species is not particularly associated with marshes, instead being found in a wide variety of habitats. Most captures of *Bembidion clemens clemens* have been associated with running water. For example, we found it common under *Populus* leaf litter on damp, dark soil in the shade of large trees along Granite Creek in Prescott, Arizona; under dead grass leaves on dark, organic silt on the shores of the Rio Grande in northern New Mexico; among sand and leaf litter on the shores of a small, mostly shaded creek in Pine Valley, Utah. We have found *Bembidion clemens disparile* in more diverse habitats. For example, it is common on the marshy shores of Lake Moreno in southern California; under leaf litter on damp sand under willows on the shore of San Simeon Creek in San Luis Obispo County;

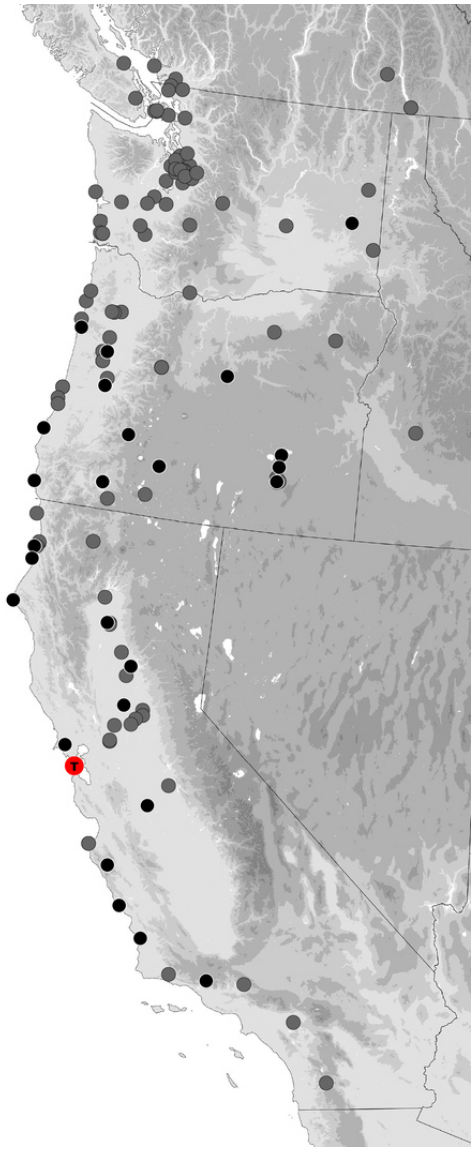

Figure 43. Geographic distribution of specimens examined of *Bembidion connivens*.

Black circles indicate those for which DNA data is available; darker gray are other localities. The large red circle with a T is the type locality.

on damp silt under dead *Typha* leaves around the backwater of the San Joaquin River.

Notes. This is the species called "*Bembidion* sp nr *ampliceps* Nr-1" and "*Bembidion* sp nr *ampliceps* Nr-2" in Maddison (1985). The species called "*Bembidion (Trepanedoris)* sp. 'Lake Moreno'" in Sproul and Maddison (2017b) is *Bembidion clemens disparile*.

Bembidion connivens (LeConte, 1852) (Figs 7d, 19d, 22g,h, 25e,f, 32c, 33k, 36b, 43, 44)

Ochthedromus connivens LeConte, 1852, p. 188. Two syntypes in MCZ (type number 5556), examined. Type locality: San Francisco, San Francisco County, California, USA.

Bembidion digressum Casey, 1918, p. 155. Lectotype female in NMNH (type number 37075), designated by Lindroth (1975, p. 122), examined. Type locality: Saint Helena, Napa County, California, USA.

Bembidion elizabethae Hatch, 1950, p. 104. Holotype in NMNH, examined. Type locality: Licten Springs, King County, Washington, USA.

Diagnosis and description. One of the more distinctive species of *Trepanedoris*, the only one with preapical spots on the elytra and evident microsculpture throughout the elytral disc (Figs 7d, 19d).

A relatively pale species, with a medium-brown body, the pronotum often slightly darker than the elytra, and with preapical spots on the elytra ranging from very faint to prominent. Legs yellowish brown, the tibiae sometimes darker. Antennomere 1 yellowish brown, the second and more apical antennomeres darker brown. Eyes without incised notch behind the eyes. Frontal furrows deeply engraved, wrapping around back of eyes. Labrum without medial bump. Mentum with distinct transverse ridge, but without longitudinal ridge on tooth. Anterior surface of pronotum smooth or with very faint wrinkles. Protarsomere 1 of male of normal width for a *Bembidion* (Fig. 33k). Seventh stria of elytra either with very faint punctures or almost effaced. Dorsal surface of head and prothorax with microsculpture, although mostly effaced in some northern specimens. Elytral disc with microsculpture over the entire surface, giving the surface a matte luster, especially in southern females. With full-length flight wings, as far as known. Aedeagus (Fig. 22g,h) with a less-sinuate ventral margin than *Bembidion clemens*, and with a denser patch of spikes in the apical regions (Fig. 25e,f, arrows). Body length 2.7–3.5 mm.

Comparison with similar species. A distinctive species unlikely to be confused with others except possibly *Bembidion clemens*; for differences, see the account of that species.

Variation. This species varies in microsculpture and DNA sequences (Fig. 44). See under "Implications for Species Boundaries", above, for a more complete discussion.

Distributions. We have seen specimens from British Columbia south to California (Fig. 43), from 0–1560m in elevation. It has also been reported from Montana and New Mexico (Bousquet, 2012); the latter is likely in error, and probably refers to specimens of *Bembidion clemens clemens*.

Habitat. As with *Bembidion clemens*, this species is found in a wide variety of habitats, and is not restricted to marshes. It was very common under dead reeds on the clay and silt banks of a small pond at Arroyo Seco in Monterey County; at the base of small *Juncus* on dark sand mixed with organic matter at the edge of an estuarian marsh at Carmel State Beach; on the marshy shores of Lost Lake in the Cascades of Oregon (Fig. 1e); on damp soil among grasses and herbs on the edge of a vernal pool in Corvallis, Oregon; at the base of *Typha* in ditches along roadsides; under leaf litter around temporary puddles of water in a forest clearing near Kernville, Oregon.

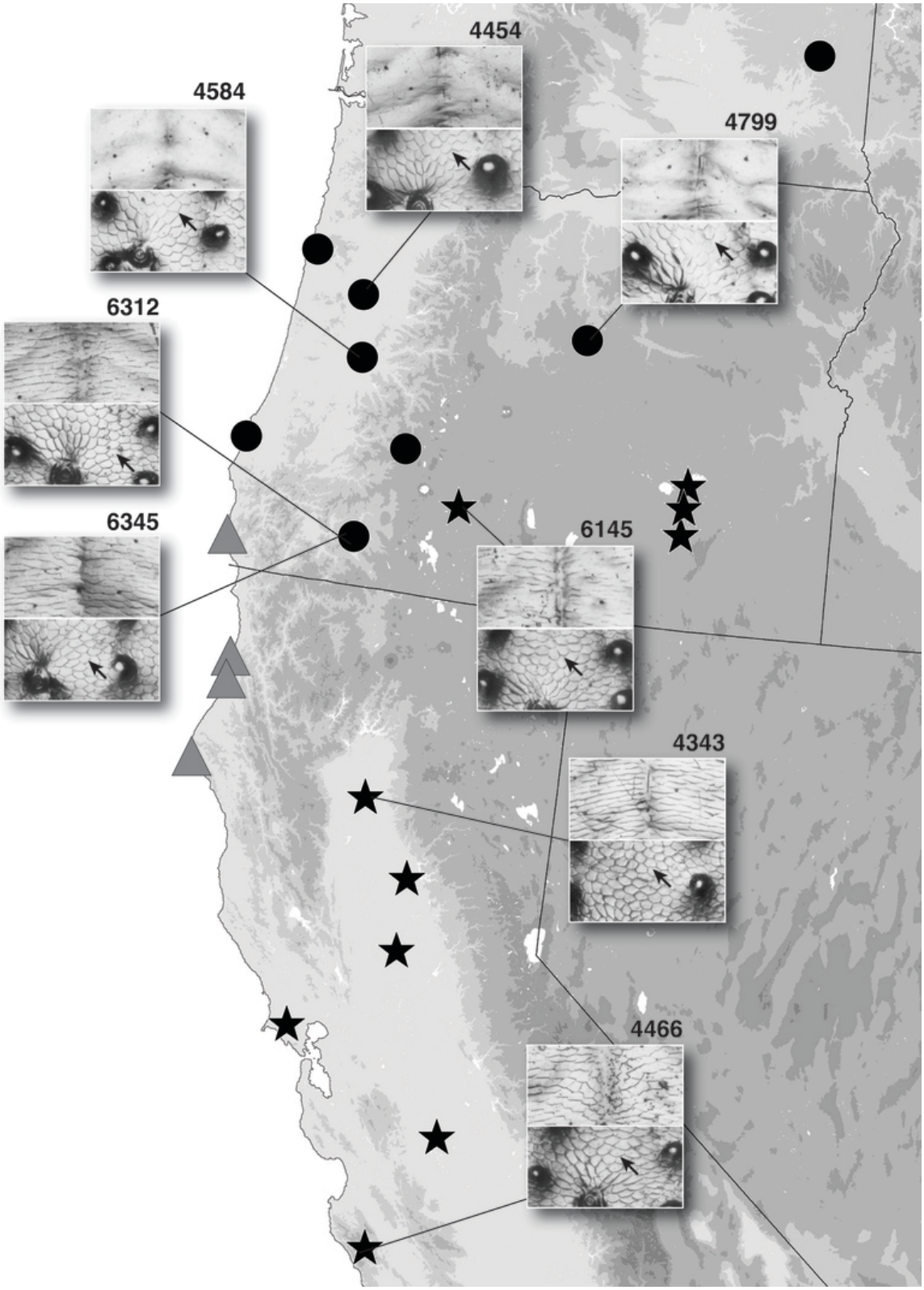

Figure 44. Geographic variation within *Bembidion connivens* specimens whose DNA was sequenced.

Stars are localities showing the "southern" form of 28S, circles are localities with the "northern" form, and triangles the intermediate, "central coastal" form. The inset photographs show the pronotal microsculpture (top) and elytral microsculpture (bottom) of eight of the specimens. DNA voucher code numbers are shown above each inset. Arrows show the regions to compare when judging whether sculpticells are isodiametric (e.g., 4454 and 4799) or transversely stretched (e.g., 4343 and 4466).

Conclusions

Through examination of morphological characters, DNA sequences, and geographic distribution, we have inferred that there are twelve species in the *connivens* group of *Bembidion* subgenus *Trepanedoris* outside of California and immediately adjacent areas of Oregon and Nevada, four of which we describe as new species. The characteristics of

the species so delimited are documented, and we have presented various tools that should prove useful to help identify specimens. The integrated approach we used combined visual examination of morphological features with multiple Bayesian multispecies coalescent analyses of seven genes to help infer reproductive communities or species, allowing us to compare different coalescent-based methods. We find that SPEEDEMON tended to split species more than our integrated approach, whereas DELINEATE tended to lump more than synthetic inference.

In addition to a more complete study of the California fauna, future work in the groups of *Trepanedoris* we examined could focus on increasing geographic sampling of specimens to test some of our decisions about species delimitation. Regions of particular interest include the area in between the two subspecies of *Bembidion clemens*, and the areas between pairs of the trio of *Bembidion acutifrons*, *B. microreticulatum*, and *B. anacalypsi*. Sampling between the two disjunct regions in which *Bembidion endeca* has been found would allow understanding of their divergence, as would sequencing DNA of the populations from the Sierra Nevada. The diversity within *Bembidion frontale*, *B. siticum*, and *B. altipaludis* could also be more thoroughly explored. Increasing the number of loci studied through genomic sequencing would also enable useful tests of our hypotheses.

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Data Availability

DNA sequences have been deposited in GenBank with accession numbers PV287742–PV288302, PV291974–PV292125, and PV292144–PV292295.

Supporting information

Supplementary tables, as well as a file containing matrices of gene sequences for each specimen as well as inferred trees, are deposited in Dryad at https://doi.org/10.5061/dryad.9cnp5hqx1.

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References

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M. A., Rambaut, A., & Drummond, A. J. (2014). BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLoS Computational Biology*, *10*, e1003537. https://doi.org/10.1371/journal.pcbi.1003537

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., De Maio, N., Matschiner, M., Mendes, F. K., Müller, N. F., Ogilvie, H. A., du Plessis, L., Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., … Drummond, A. J. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *15*, e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Bousquet, Y. (2012). Catalogue of Geadephaga (Coleoptera, Adephaga) of America, north of Mexico. *Zookeys*, *245*, 1–1722. https://doi.org/10.3897/zookeys.245.3416

Casey, T. L. (1918). A review of the North American Bembidiinae. *Memoirs on the Coleoptera*, *8*, 1–223.

Casey, T. L. (1924). Additions to the known Coleoptera of North America. *Memoirs on the Coleoptera*, *11*, 1–347. https://doi.org/10.5962/bhl.title.15993

Douglas, J., & Bouckaert, R. (2022). Quantitatively defining species boundaries with more efficiency and more biological realism. *Communications Biology*, *5*, 755. https://doi.org/10.1038/s42003-022-03723-z

Douglas, J., Jiménez-Silva, C. L., & Bouckaert, R. (2022). StarBeast3: Adaptive Parallelized Bayesian Inference under the Multispecies Coalescent. *Systematic Biology*, *71*, 901–916. https://doi.org/10.1093/sysbio/syac010

Erwin, T. L. (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. http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=8876114

Flouri, T., Jiao, X., Rannala, B., & Yang, Z. (2018). Species tree inference with BPP using genomic sequences and the multispecies coalescent. *Molecular Biology and Evolution*, *35*, 2585–2593. https://doi.org/10.1093/molbev/msy147

Green, P. (1999). *Phrap* (0.990329) [Computer software]. http://phrap.org

Green, P., & Ewing, B. (2002). *Phred* (0.020425c) [Computer software]. http://phrap.org

Hatch, M. H. (1950). Studies on the Coleoptera of the Pacific Northwest. II. Carabidae: Bembidiini. *Pan Pacific Entomologist*, *26*.

Jones, G. (2017). Algorithmic improvements to species delimitation and phylogeny estimation under the multispecies coalescent. *Journal of Mathematical Biology*, *74*, 447–467. https://doi.org/10.1007/s00285-016-1034-0

Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution*, *30*, 772–780. https://doi.org/10.1093/molbev/mst010

LeConte, J. L. (1847). 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*, 355–464. https://doi.org/10.5962/bhl.title.9701

LeConte, J. L. (1852). Descriptions of new species of Coleoptera, from California. *Annals of the Lyceum of Natural History of New York*, *5*, 185–216. https://doi.org/10.1111/j.1749-6632.1852.tb00123.x

LeConte, J. L. (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, C. H. (1963). The ground-beetles (Carabidae, excl. Cicindelinae) of Canada and Alaska. Part 3. *Opuscula Entomologica Supplementum*, *XXIV*, 201–408.

Lindroth, C. H. (1975). Designation of holotypes and lectotypes among ground beetles (Coleoptera, Carabidae) described by Thomas L. Casey. *The Coleopterists Bulletin*, *29*, 109–147. https://doi.org/10.5962/p.372770

Löbl, I., & Löbl, D. (2017). *Catalogue of Palaearctic Coleoptera, Volume I. Archostemata-Myxophaga-Adephaga*. Brill. https://doi.org/10.1163/9789004330290_003

Maddison, D. R. (1985). Chromosomal diversity and evolution in the ground beetle genus *Bembidion* and related taxa (Coleoptera: Carabidae: Trechitae). *Genetica*, *66*, 93–114. https://doi.org/10.1007/BF00139715

Maddison, D. R. (1993). Systematics of the Holarctic beetle subgenus *Bracteon* and related *Bembidion* (Coleoptera: Carabidae). *Bulletin of the Museum of Comparative Zoology*, *153*, 143–299.

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

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

Maddison, D. R. (2020). Shards, sequences, and shorelines: two new species of *Bembidion* from North America (Coleoptera, Carabidae). *Zookeys*, *1007*, 85–128. https://doi.org/10.3897/zookeys.1007.60012

Maddison, D. R. (2023). A new subgenus of *Bembidion* Latreille from México and Guatemala, with descriptions of two new species (Coleoptera: Carabidae). *Proceedings of the California Academy of Sciences*, *67*, 433–450.

Maddison, D. R., & Arnold, E. (2009). A review of the *Bembidion (Odontium) aenulum* subgroup (Coleoptera: Carabidae), with description of a new species. *Zootaxa*, *2214*, 45–61. https://doi.org/10.11646/zootaxa.2214.1.3

Maddison, D. R., & Cooper, K. W. (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. https://doi.org/10.1111/zoj.12188

Maddison, D. R., Kanda, K., Boyd, O. F., Faille, A., Porch, N., Erwin, T. L., & Roig-Juñent, S. (2019). Phylogeny of the beetle supertribe Trechitae (Coleoptera: Carabidae): Unexpected clades, isolated lineages, and morphological convergence. *Molecular Phylogenetics and Evolution*, *132*, 151–176. https://doi.org/10.1016/j.ympev.2018.11.006

Maddison, D. R., & Maddison, W. P. (2023a). *Chromaseq: a Mesquite package for analyzing sequence chromatograms* (1.60) [Computer software]. https://chromaseq.mesquiteproject.org

Maddison, D. R., & Maddison, W. P. (2023b). *Zephyr: a Mesquite package for interacting with external phylogeny inference programs* (3.31) [Computer software]. https://zephyr.mesquiteproject.org

Maddison, D. R., & Maruyama, M. (2019). Phylogenetic relationships and convergent evolution of ocean-shore ground beetles (Coleoptera: Carabidae: Trechinae: *Bembidion* and relatives). *Systematic Entomology*, *44*, 39–60. https://doi.org/10.1111/syen.12307

Maddison, D. R., & Smith, A. O. (2026). The Joy of Discovery. *Bulletin of the Society of Systematic Biologists*, *5*. https://doi.org/10.18061/bssb.6995

Maddison, D. R., & Sproul, J. S. (2020). Species delimitation, classical taxonomy and genome skimming: a review of the ground beetle genus *Lionepha* (Coleoptera: Carabidae). *Zoological Journal of the Linnean Society*, *189*, 1313–1358. https://doi.org/10.1093/zoolinnean/zlz167

Maddison, W. P., & Maddison, D. R. (2023). *Mesquite: a modular system for evolutionary analysis* (3.81) [Computer software]. https://www.mesquiteproject.org

Maddison, W. P., & Maddison, D. R. (2025). *Mesquite: a modular system for evolutionary analysis* (4.02) [Computer software]. https://www.mesquiteproject.org

Maddison, W. P., & Whitton, J. (2023). The species as a reproductive community emerging from the past. *Bulletin of the Society of Systematic Biologists*, *2*, 1–35. https://doi.org/10.18061/bssb.v2i1.9358

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, *37*, 1530–1534. https://doi.org/10.1093/molbev/msaa015

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*, 901–904. https://doi.org/10.1093/sysbio/syy032

Sproul, J. S., & Maddison, D. R. (2017a). Cryptic species in the mountaintops: species delimitation and taxonomy of the *Bembidion breve* species group (Carabidae: Coleoptera) aided by genomic architecture of a century-old type specimen. *Zoological Journal of the Linnean Society*, *183*, 556–583. https://doi.org/10.1093/zoolinnean/zlx076

Sproul, J. S., & Maddison, D. R. (2017b). Sequencing historical specimens: successful preparation of small specimens with low amounts of degraded DNA. *Molecular Ecology Resources*, *17*, 1183–1201. https://doi.org/10.1111/1755-0998.12660

Sukumaran, J., Holder, M. T., & Knowles, L. L. (2021). Incorporating the speciation process into species delimitation. *PLoS Computational Biology*, *17*, e1008924. https://doi.org/10.1371/journal.pcbi.1008924