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Phylogeny of *Bembidion* and related ground beetles (Coleoptera: Carabidae: Trechinae: Bembidiini: Bembidiina)

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ABSTRACT

The phylogeny of the large genus *Bembidion* and related genera is inferred from four nuclear protein-coding genes (CAD, *wingless*, arginine kinase, and topoisomerase I), ribosomal DNA (28S and 18S), and the mitochondrial gene cytochrome oxidase I (COI). 230 of the more than 1200 species of *Bembidion* are sampled, as well as 26 species of five related genera, and 14 outgroups. Nuclear copies (numts) of COI were found sparsely scattered through sampled species. The resulting phylogeny, based upon individual gene analyses and combined analyses using maximum likelihood and parsimony, is very well supported at most nodes.

Additional analyses explored the evidence, and corroborate the phylogeny. Seven analyses, each with one of the seven genes removed from the combined matrix, were also conducted, and yielded maximum likelihood bootstrap trees sharing over 92% of their nodes with the original, well-resolved bootstrap trees based on the complete set of seven genes. All key nodes were present in all seven analyses missing a single gene, indicating that support for these nodes comes from at least two genes. In addition, the inferred maximum likelihood tree based on the combined matrix is well-behaved and self-predicting, in that simulated evolution of sequences on the inferred tree under the inferred model of evolution yields a matrix from which all but one of the model tree's clades are recovered with bootstrap value >50, suggesting that internal branches in the tree may be of a length to yield sequences sufficient to allow their inference. All likelihood analyses were conducted under both a proportion-invariable plus gamma site-to-site rate variation model, as well as a simpler gamma model. The choice of model did not have a major effect on inferred phylogenies or their bootstrap values.

The inferred phylogeny shows that *Bembidarenas* is not closely related to Bembidiina, and *Phrypeus* is likely distant as well; the remaining genera of Bembidiina form a monophyletic group. *Lionepha*, formerly considered a subgenus of *Bembidion*, is shown to be outside of the clade of *Asaphidion* + *Bembidion*, and is separated as its own genus. *B.* (*Phyla*) *obtusum* is quite isolated within *Bembidion*, and there is some evidence that the remaining *Bembidion* form a clade.

Within *Bembidion*, there are three large clades that are well-supported, the *Bembidion*, *Odontium*, and *Ocydromus* Series. The *Bembidion* Series contains *Bembidion* (*s. str.*), *Notaphus*, *Furcacampa*, *Emphanes*, *Trepanedoris*, *Diplocampa*, and related Holarctic species; all species from South America, Australia, New Zealand; and most species from southern Africa and Madagascar. All species in South America, except for members of *Notaphus* and *Nothocys*, form a clade, the *Antiperyphanes* Complex, which has independently radiated into body forms and niches occupied by multiple, independent Northern-Hemisphere forms. All species from New Zealand, including *Zecillenus*, and Australian species formerly placed in *Ananotaphus* together form a clade. *Bembidion* (*s. str.*) and *Cyclolopha* are in a clade with the Old World, Southern Hemisphere lineages *Notaphocampa*, *Sloanephila*, and *Omotaphus*. The large subgenus *Notaphus* appears to have originated in South America, with all Northern Hemisphere *Notaphus* arising from within a south-temperate grade. All major variation in frontal furrows on the head is contained within the *Bembidion* Series. The *Odontium* Series contains subgenera *Hirmoplataphus* and *Hydriomicrus*, which together are the sister clade of *Odontium*, *Bracteon*, *Ochthedromus*, *Pseudoperyphus*, and *Microserrullula*. The very large *Ocydromus* Series, dominant in the Holarctic region, includes the *Ocydromus* Complex, with many subgenera, including *Hypsipezum* and *Leuchydrium*; the phylogeny within this group is notably at odds with the current classification. Also included in the *Ocydromus* Series are *Nepha* and *Bembidionetolitzkya*, as well as the *Princidium* Complex, in which the intertidal *B.* (*Cillenus*) *laterale* falls.

Outside these three series are a number of smaller groups, including the *Plataphus* Complex (containing *Blepharoplataphus*, *Plataphus*, the latter including *Plataphodes*); the *Hydrium* Complex (*Metallina*,

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Chlorodium, and *Hydrium*, which contains *Eurytrachelus*), whose sister group might be subgenus *Andrewesa*; *Trechonepha* and *Liocosmius*, which might be sisters; and *B.* (*Melomalus*) *planatum*, which is not close to *Plataphus*. There is some evidence that these groups plus the *Ocydromus* and *Odontium* Series form a clade.

A few enigmatic groups were harder to place. The sister group of the pair *Philochthus* plus *Philochthemphanes* might be *B. wickhami*; *Eupetedromus* is well outside the three major series and not related to *Notaphus*; the high-elevation Asian group *Hoquedela* is a very isolated lineage.

Notaphiellus is removed from synonymy with *Nothocys*, and placed in synonymy with *Notaphus*; *Plataphodes* is synonymized with *Plataphus*, as *Plataphus* is paraphyletic otherwise; *Eurytrachelus* is synonymized with *Hydrium*. A new subgenus, *Lindrochthus*, is described to house the distinctive *B. wickhami*.

The implications of the inferred phylogeny for some morphological characters used in Bembidiina systematics are explored, and some of the most widely used (e.g., location of discal seta ed3 on the elytron, and shape of the shoulder) are shown to be notably homoplastic. For example, the location of elytral seta ed3 has undergone at least nine transitions between two states.

1. Introduction

In the early 1970s, while exploring the garden of my backyard in Burlington, Ontario, Canada, I found a small beetle running on the soil. It was about 3 mm long, shiny black with yellowish spots; I was fascinated by its elegant form, quick movements, and intricacy of its structure for something so small. The beetle was a *Bembidion quadrimaculatum* Linnaeus, type species of the worldwide genus *Bembidion* Latreille, although it would take a few years before I was to learn its identity. When I did I was even more intrigued by this genus: although nearly 0.1% of all known species on Earth is a *Bembidion*, and the genus is common in habitats often visited by people, they are unknown to most humans. Among systematic entomologists, this large branch of the tree of life is also mysterious, as it has no explicit phylogenetic hypothesis about its broad structure.

Bembidion is one of the commonest and most diverse groups of small predators on shores of bodies of water in temperate regions of the world. This genus, with over 1200 described (Lorenz, 2005) and many undescribed species, is most diverse in the Holarctic region, but there are also centers of diversity in temperate South America (Argentina, Chile, and northward in the Andes) and New Zealand. Adults range in size from 2 to 9 mm in length, with the majority between 3 and 6 mm.

Most species live along banks of running (Fig. 1A) or standing (Fig. 1B) waters, where they feed on arthropods, including adult insects emerging from the water (Hering, 1998; Hering and Plachter, 1997; Paetzold et al., 2005; personal observations). Numerous species, including *Bembidion quadrimaculatum*, live far from water, in open fields (Fig. 1C). A few species occur along the edge of permanent snowfields (Fig. 1D); I have watched adults walk on the snow at night, and feed on torpid insects trapped on the cold ice.

Species of *Bembidion* are diverse in form (Figs. 2–5), and they possess abundant morphological variation that taxonomists use to distinguish species. Species vary in form of the prothorax and elytra, microsculpture, color pattern, mouthparts, male genitalia, and other characters. With this wealth of morphological characters, one might expect that phylogeny of the group has been well-studied, and there have been a few phylogenetic studies of individual subgenera (e.g., Bonavita and Vigna Taglianti, 2010; Maddison, 1993, 2008; Sasakawa, 2007). But there has been no formal phylogenetic study of morphological variation in *Bembidion* as a whole, or even large clades, although narrative, non-cladistic arguments have been presented about possible broad-scale patterns in the phylogeny and geographic movements of lineages.

For example, Jeannel (1962) suggests that the large subgenus *Notaphus* originated in South America, and that North American species of the group may have originated from a single "pulse" northward; Toledano (2005, 2008b) extends this proposal further, and suggests that many Northern Hemisphere subgenera, or perhaps the entire subtribe of Bembidiina, have a Gondwanan origin. Although the biogeographic hypotheses have been moderately explicit, the phylogenetic hypotheses have been less so. This is perhaps not surprising given the extreme diversity of the group, and the complexity of patterns of variation of morphological characters.

The boundaries of *Bembidion* are not well defined, in part as there are no apparent synamorphies for its members. To the followers of Netolitzky (1942, 1943) and Lindroth (1963, 1980), *Bembidion* consists of all members of the supertribe Trechitae that have a reduced apical palpomere, a brush sclerite in the endophallus of the male genitalia, male foretarsomeres with adhesive setae arranged in rows, and that also do not possess the apomorphies of other taxa such as subtribe Xystosomina or the genus *Asaphidion*. The character states defining *Bembidion* are either plesiomorphic within Trechitae (e.g., male adhesive setal characteristics), or derived states for groups broader than just *Bembidion* (palpomere size, brush sclerite).

In addition to a lack of knowledge about their cladistic structure, there have been two schools of thought about the most functional classification to use. In contrast to Netolitzky's and Lindroth's broad concept of *Bembidion*, Jeannel (1941) split the French fauna of this group into 15 genera. That philosophy proved difficult to apply to other faunas. A lack of clarity as to how the rest of the world's Bembidiina related to the fauna of France left numerous species outside of France unplaced (including many in North America), many other species placed into French "genera" based upon weak evidence (Toledano, 2002), or relegated by default into many separate genera (Basilewsky, 1972; Jeannel, 1962). Without a well-understood phylogeny to reign in the chaos, many of these groups have since been moved back into *Bembidion* (e.g., by Hurka, 1996; Toledano, 2002), and an increasing majority of workers now treat most species of subtribe Bembidiina as members of a large, inclusive genus *Bembidion* (e.g., Kryzhanovskij et al., 1995; Lorenz, 2005; Maddison, 1993; Marggi et al., 2003; Ortuño and Toribio, 2005; Toledano, 2008b).

In addition to *Bembidion*, there are about seven smaller genera considered to belong to the subtribe Bembidiina. These include four genera containing 20–50 species each (*Ocys* Gistel, *Asaphidion* Gozis, *Sinechostictus* Motschulsky, and *Amerizus* Chaudoir; Fig. 6B–I), and three genera containing only 1–2 species each (*Orzolina* Machado, *Caecidium* Uéno, and *Sakagutia* Uéno). Two genera that share morphological similarities with Bembidiina, *Phrypeus* Casey (Fig. 6J) and *Bembidarenas* Erwin (Fig. 6K and L), have recently been removed from Bembidiina (Erwin et al., 2010; Maddison and Ober, 2011), based in part on the molecular data presented here.

Studies to date of the structure of adult and larval *Bembidion* suggest that patterns of morphological variation are complex, with

Fig. 1. Typical habitats occupied by *Bembidion*. (A) Shore of river at USA: Washington: Whatcom Co., 1.4 mi S of Deming, Nooksack River, 70 m. Habitat of about 12 species of *Bembidion* of the subgenera *Bracteon*, *Odontium*, *Ocydromus*, *Plataphus*, *Plataphodes*, *Hydrium*, *Notaphus*, and *Liocosmius*. (B) Shore of pond at Spain: Madrid: Embalse de Valmayor, near Galapagar, 850 m. Habitat of about 10 species of *Bembidion* of the subgenera *Bembidion*, *Testedium*, *Notaphus*, *Trepanes*, *Philochthus*, *Emphanes*. (C) Open field at Canada: Alberta: Rock Lake, 1400 m. Habitat of four species of *Bembidion* of the subgenera *Bembidion*, *Hydrium*, *Metallina*, and *Ocydromus*, as well as *Asaphidion yukonense*. (D) Edges of snowfields (foreground) at Canada: British Columbia: Downtown Road, 50.5303°N 122.2712°W, 2000 m. Habitat of three species of the "*Plataphodes* group" of the subgenus *Plataphus*. Photographs reprinted with permission, copyright David Maddison, released under a Creative Commons CC-BY 3.0 license.

no obvious apomorphies that would allow one to propose large clades within the genus. There are also few morphological hints about relationships of smaller bembidiine genera to *Bembidion* itself. Although more detailed morphological studies, especially of male genitalia (Lindroth, 1963), may provide evidence about the phylogeny, I suggest that the most fruitful next step to understanding the history of this diverse group is a study of the wealth of characters provided by DNA sequences.

To that end, I here present the results of a molecular phylogenetic study of 256 species of Bembidiina, representing a sample of worldwide diversity. The seven genes examined reveal large, decisively supported clades, some surprising, and a well-resolved phylogeny.

2. Materials and methods

2.1. Taxon sampling, identification, and classification

230 species of *Bembidion*, 26 species of Bembidiina other than *Bembidion*, and 14 species of outgroups were sequenced (Tables 1–3; locality data in Supplementary content Tables S1–S3). Outgroups were chosen based upon the results of Maddison and Ober (2011), who found that pogonines, zolines, or anillines contain the likely sister group to Bembidiina. *Bembidion* species were sampled from 23 countries, with (for logistical reasons) an emphasis on those living in North America (142 species), and fewer from Central and South America (30), Europe and northern Africa (33), Asia (10), southern Africa and Madagascar (4), and Australia and New Zealand (11). All vouchers are deposited in the author's DNA voucher collection at the Oregon State Arthropod Collection, except for the five holotypes sequenced, which are in their type depositories.

Identifying *Bembidion* to species can be challenging because of lack of modern revisionary works in some faunas. The resources used to identify specimens are listed in Tables 1–3. Some of the species sequenced could not be identified with confidence or are likely undescribed. Names with "*cf.*" in them indicate that it is unclear whether or not the specimen belongs to that species; names with "sp. nr." indicate that the specimen belongs to a different (perhaps unnamed) species that is close to the one named. For example, there are at least two species now contained within current concepts of *Bembidion curtulatum* Casey; as I am uncertain as to whether the one included in this paper is the true *B. curtulatum*, it is called "*B. cf. curtulatum*"; *B. cf. cognatum* may or may not be *B. cognatum*, but if not, it is closely related. On the other hand, "*B.* sp. nr. *chilense* Solier" is distinct from *B. chilense*, and may be undescribed. Species listed as simply "sp. 1" or "sp. 2" are either undescribed species or belong to groups needing revision, and thus are currently unidentifiable. Some of the species studied are not recognized as distinct in the literature; my unpublished observations based upon morphological and molecular sequence data indicate that they are distinct. In particular: (1) *B. scintillans* Bates is a species distinct from *B. aratum* LeConte, and not synonymous as stated by Erwin (1984); (2) *B. elizabethae* Hatch is a species distinct from *B. connivens* (LeConte), contrary to the claim by Lindroth (1963). In addition, *B. innocuum* Casey is a senior synonym of *B. marinianum* Casey, and thus is the valid name.

One of the taxa used in the analysis, labeled "*Bembidion* Chimera", is represented by some sequences from *B. quadrulum*

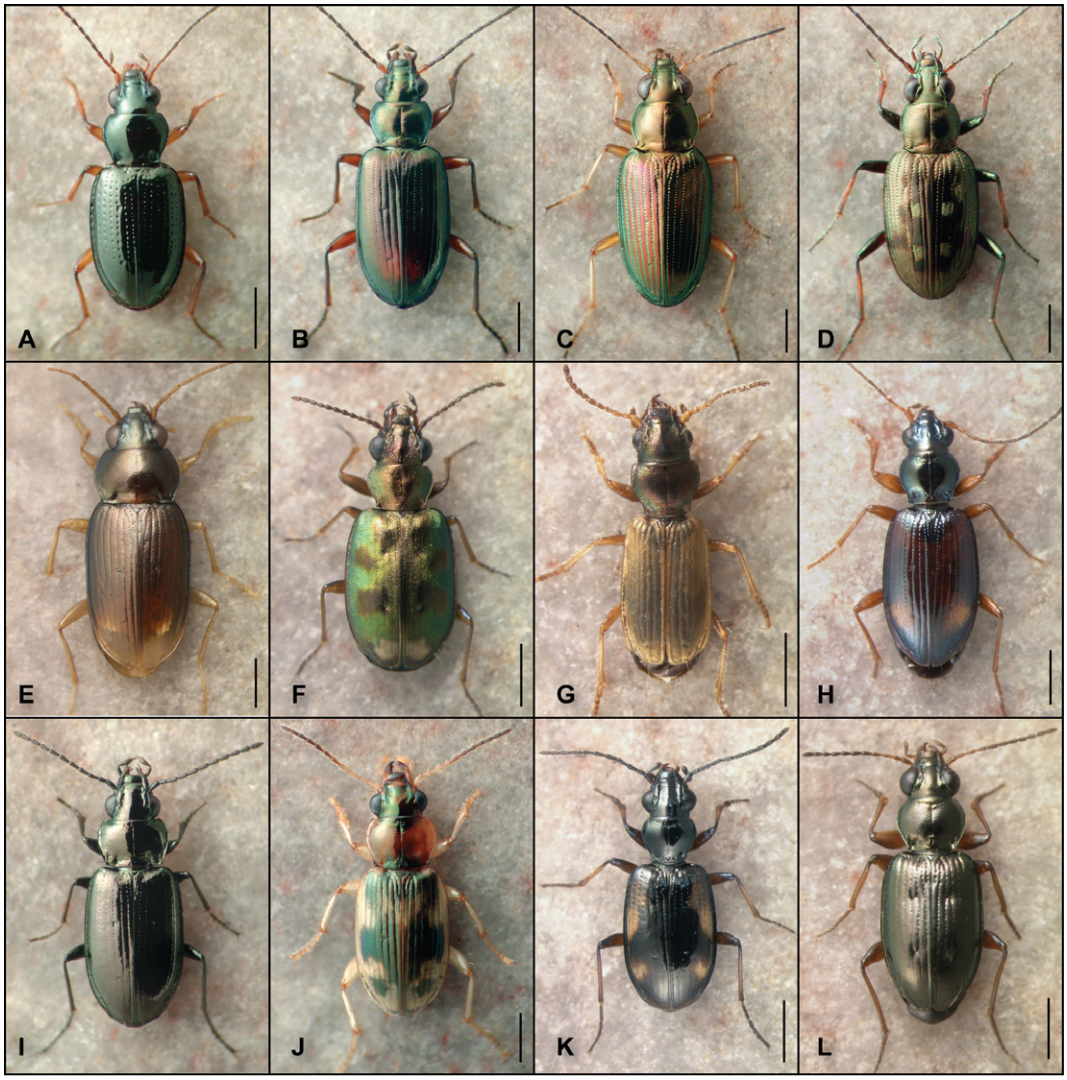

Fig. 2. Adults of *Bembidion* of the *Odontium* Series (A–E), *Ocydromus* Series (F–K), and subgenus *Hoquedela* (L). Scale bar is 1 mm. (A) *B.* (*Hydriomicrus*) *brevistriatum* (USA: California: Salinas River). (B) *B.* (*Pseudoperyphus*) *rufotinctum* (USA: Vermont: Quechee Gorge). (C) *B.* (*Odontium*) *confusum* (USA: Iowa: Manchester). (D) *B.* (*Bracteon*) *inaequale* (USA: West Virginia: Hambleton). (E) *B.* (*Microserrullula*) *xanthacrum* (India: Nedungadu). (F) *B.* (*Testedium*) *laetum* (Spain: Madrid: Galapagar). (G) *B.* (*Cillenus*) *laterale* (Spain: Boiro). (H) *B.* (*Ocydromus*) *scopulinum* (Canada: Ontario: Burnel). (I) *B.* (*Testediolum*) *commotum* (USA: California: Frog Lake). (J) *B.* (*Leuchydrium*) *tigrinum* (USA: Oregon: Charleston). (K) *B.* (*Nepha*) *callosum subconvexum* (Spain: Avila: Las Navas del Marques). (L) *Bembidion* (*Hoquedela*) sp. 1 (China: Yunnan Prov.: Gaoligong Shan). Photographs reprinted with permission, copyright David Maddison, released under a Creative Commons CC-BY 3.0 license.

LeConte and some from a *B. concolor* (Kirby) individual. The latter specimen was originally thought to be a *B. quadrulum*, but additional examination and sequencing after the analyses were complete indicate that it is a small, aberrant *B. concolor*. As the relationships within *Hirmoplataphus* are not a subject of this study, and as there are five other members of this subgenus in the study, this chimera of closely related *Hirmoplataphus* species, well-nested within the subgenus, should not affect relationships between subgenera investigated here.

The specimen of *Amerizus* (*Amerizus*) sp. sequenced (voucher DNA1481) is a larva, which was identified to species by matching its cytochrome oxidase I sequence to that of an adult *Amerizus* from the same locality (the sequence from the adult has been deposited in GenBank as accession JQ277270). Chromatograms of adult and larval COI reveal identical sequences except for 10 bases in which one has a double peak and the other has just one of those peaks. A maximum likelihood analysis of COI (conducted in the same way as described below under Phylogenetic Analysis) shows the adult and larva grouping together in their own clade with 100% bootstrap support. As there is only one known *Amerizus* species in the Abajo Mountains of Utah (D.H. Kavanaugh, pers. comm. 2011), and as no other non-*Bembidion* Bembidiina are known from that area, I conclude that the larva does belong to the same *Amerizus* (*Amerizus*) species.

The classification of species into subgenera and genera in Bembidiina differs from author to author. The classification used in this paper is an amalgam of those in Lindroth (1963, 1980), Lorenz (2005), Ortuño and Toribio (2005), Marggi et al. (2003), and Toledano (2000), among others, with some modifications as a result of the present work. The classification presented in Tables 1–3 reflects some changes required by my results, but others will be necessary as more work is done to correlate clades discovered here with morphological characters and type species of subgenera. In some classifications of *Bembidion*, the informal rank of "series" and "complex" have been used for taxa that group subgenera together. In the classification used here, series are clades that consist of multiple complexes, which are themselves composed of multiple subgenera. As the phylogeny of *Bembidion*

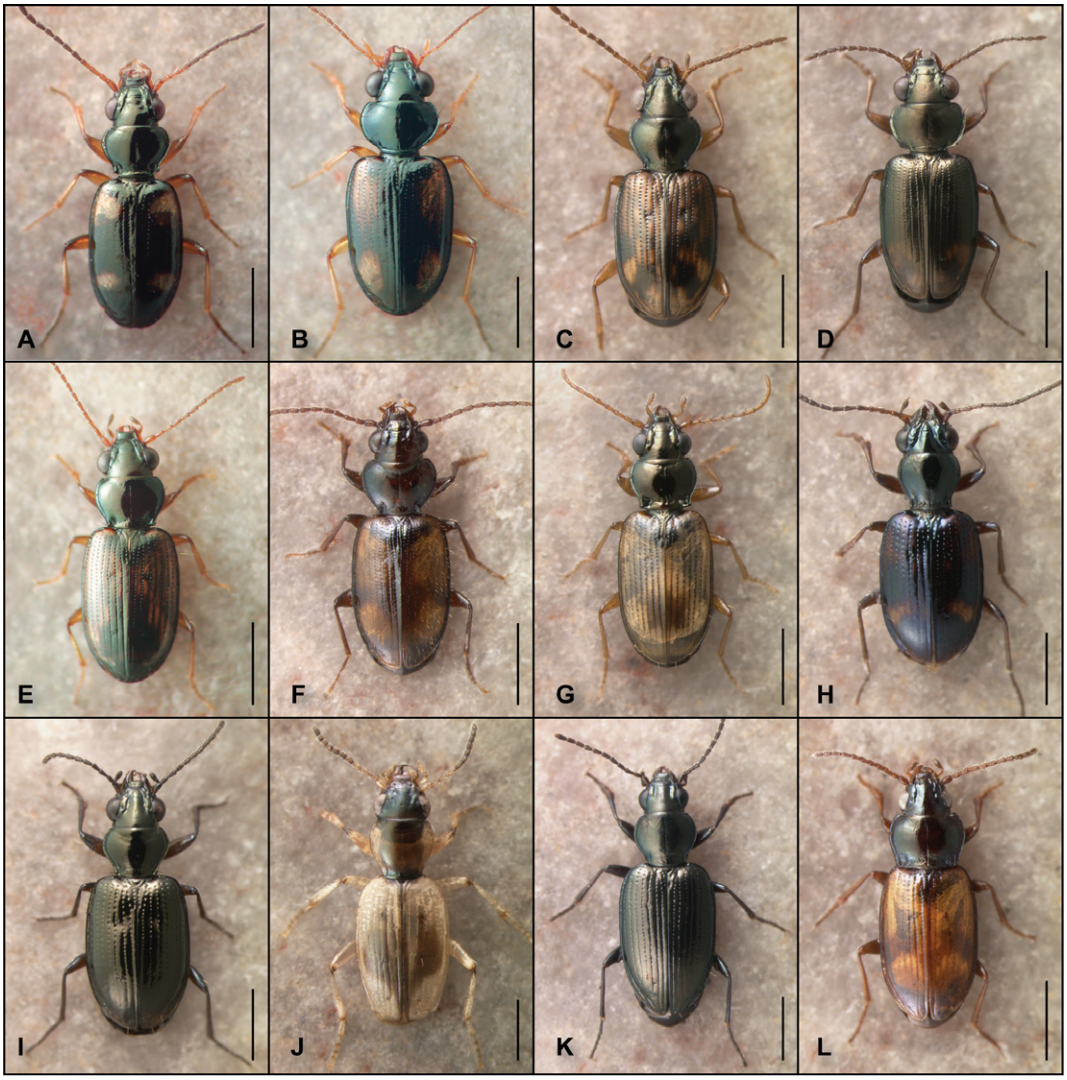

Fig. 3. Adults of *Bembidion* of the *Bembidion* Series. Scale bar is 1 mm. (A) *Bembidion* (*Bembidion*) *quadrimaculatum* (USA: Massachusetts: Cambridge). (B) *B.* (*Cyclolopha*) *poculare* (USA: Arizona: Santa Rita Mtns,). (C) *B.* (*Omotaphus*) sp. 2, (Madagascar: Fianarantsoa: Ranomafana National Park). (D) *B.* (*Sloanephila*) *jacksoniense* (Australia: Queensland: Brigalow Res. Station). (E) *B.* (*Furcacampa*) *impotens* (USA: Texas: Pontotoc). (F) *B.* (*Neobembidion*) *constricticolle* (USA: Arizona: Willcox Playa). (G) *B.* (*Notaphemphanes*) *ephippium* (Spain: Albacete: Villa de Chinchilla). (H) *B.* (*Trepanes*) *articulatum* (Czech Republic: Bohemia: Lány). (I) *B.* (*Australoemphanes*) *ateradustum* (Australia: Victoria: Kororoit Creek). (J) *B.* (*Zecillenus*) sp. 1 (New Zealand: Foxton Beach, Manuwatu). (K) *B.* (*Zemetallina*) *anchonoderum* (New Zealand: near Havelock North, Tukituki River). (L) *B.* (*Peryphodes*) *salinarium* (Canada: Saskatchewan: Chaplin Lake). Photographs reprinted with permission, copyright David Maddison, released under a Creative Commons CC-BY 3.0 license.

becomes better understand, additional well-defined clades will need to be named between the level of subgenus and genus.

2.2. DNA extraction, amplification, and sequencing

Abbreviations for genes used in this paper are: 28S or 28S rDNA: 28S ribosomal DNA; 18S or 18S rDNA: 18S ribosomal DNA; COI: cytochrome oxidase I; *wg*: *wingless*; CAD: carbamoylphosphate synthetase domain of the rudimentary gene; ArgK: arginine kinase; Topo: topoisomerase I.

Fragments for these genes were amplified using the Polymerase Chain Reaction on either an MJ Research PTC-150 Minicycler or an Eppendorf Mastercycler Thermal Cycler, using either Eppendorf Hotmaster Taq or TaKaRa Ex Taq and the basic protocols recommended by the manufacturers. Primers and details of the cycling reactions used are given in the Appendix. The amplified products were then cleaned, quantified, and sequenced at the University of Arizona's Genomic and Technology Core Facility using either a 3730 or 3730 XL Applied Biosystems automatic sequencer.

Assembly of multiple chromatograms for each gene fragment and initial base calls were made with Phred (Green and Ewing, 2002) and Phrap (Green, 1999) as orchestrated by Mesquite's Chromaseq package (Maddison and Maddison, 2009a,b) with subsequent modifications by Chromaseq and manual inspection. Multiple peaks at a single position in multiple reads were coded using IUPAC ambiguity codes.

Two sequences proved especially problematic to obtain. All four specimens of *Bembidion spinolai* sequenced for 28S rDNA, including voucher 2017 included in the analysis, showed evidence of multiple copies of 28S, with length differences between the copies yielding sections of chromatograms too complex to determine the sequence unambiguously. 18S rDNA for *Bembidion affine* also had a region that could not be sequenced, although the reasons for the lack of clarity were not evident. As a result, for both 28S in *B. spinolai* and 18S for *B. affine*, some internal sections of the sequences were removed from both the sequences submitted to GenBank and those used in the analyses.

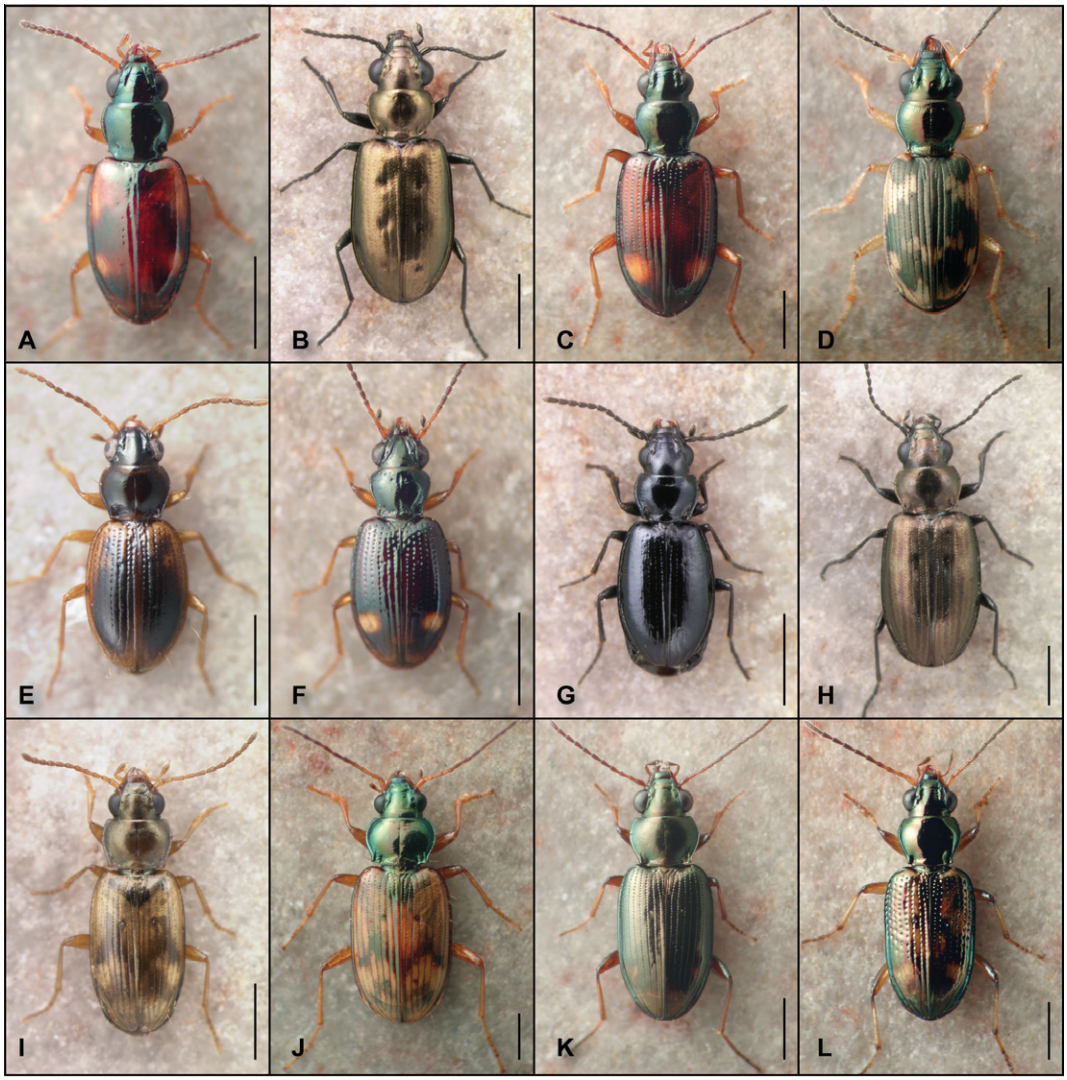

Fig. 4. Adults of *Bembidion* of the *Bembidion* Series (cont'd), including members of the *Antiperyphanes* Complex (A–D), and the *Notaphus* Complex (H–L). Scale bar is 1 mm. (A) *Bembidion* (*Nothonepha*) sp.nr. *lonae* (Chile: Reg. IX: Rio Allipén). (B) *B.* (*Notholopha*) *sexfoveolatum* (Chile: Reg. IX: Malalcahuello). (C) *B.* (*Antiperyphanes*) *spinolai* (Chile: Reg. IX: Rio Allipén). (D) *B.* (*Plocamoperyphus*) *mandibulare* (Chile: Chiloé: Cucao). (E) *B.* (*Semicampa*) *semicinctum* (Canada: Ontario: Dwight). (F) *B.* (*Trepanedoris*) *frontale* (USA: New Hampshire: North Conway). (G) *B.* (*Nothocys*) *anthracinum* (Chile: Reg. Met.: La Parva). (H) *B.* (*Notaphus*) *cupreostriatum* (Chile: Reg. IX: Malalcahuello). (I) *B.* (*Notaphus*) *cillenoides* (Argentina: Medoza: Salinas del Diamante). (J) *B.* (*Notaphus*) *dorsale* (Canada: Alberta: Taber). (K) *B.* (*Notaphus*) *rapidum* (USA: Texas: Pontotoc). (L) *B.* (*Notaphus*) *scintillans* (USA: New Mexico: Gila). Photographs reprinted with permission, copyright David Maddison, released under a Creative Commons CC-BY 3.0 license.

The new sequences obtained have been deposited in GenBank with accession numbers JN170134 through JN171559, and JN982212 through JN982227.

2.3. Sequence alignment

Alignment was not difficult for any of the protein-coding genes. There were no insertion or deletions (indels) evident in the sampled CAD, topoisomerase, or COI sequences. In ArgK, no deletions were observed, and the only insertions were two 60-nucleotide introns within the outgroup genus *Serranillus*; these were excluded from analyses. *Wingless*, in contrast, showed multiple indels, but each of these were small, restricted to very few taxa, and for the most part separated from one another along the length of the sequence. There were 6 inserted nucleotides (two amino acids) in three species of subgenus *Odontium*: *B. aenulum*, *B. paraenulum*, and *B. coxendix*; three inserted nucleotides in the same place in *Bembidion* (*Zemetallina*) *parviceps*; six inserted nucleotides in a different region in the two species of subgenus *Omotaphus* sampled. These inserted nucleotides were all excluded from analyses. In addition, some insertions were present in members of the outgroup subtribe Anillina: there were two three-nucleotide deletions in *Geocharidius*, and two different three-nucleotide deletions in *Typhlocaris*.

In contrast, the two ribosomal genes showed a rich history of insertions and deletions, which complicated alignment. 28S was first subjected to multiple sequence alignment in Opal (Wheeler and Kececioglu, 2007), using default parameter values; the alignment was then manually adjusted to fix only obvious flaws, with taxon names hidden to avoid bias. The majority of insertion and deletion events evident were in the far outgroups, including *Bembidarenas* and *Phrypeus;* there were relatively few insertion or deletions within *Bembidion*, *Asaphidion*, *Amerizus*, *Lionepha*, *Ocys*, or *Sinechostictus*. Sites were excluded if they were ambiguously aligned within *Bembidion* or the near outgroups (*Asaphidion*, *Amerizus*, *Ocys*, *Lionepha*, and *Sinechostictus*), as judged mentally. The sites thus removed were contiguous stretches of four or more sites containing mostly gaps (with 10% or fewer taxa having data in

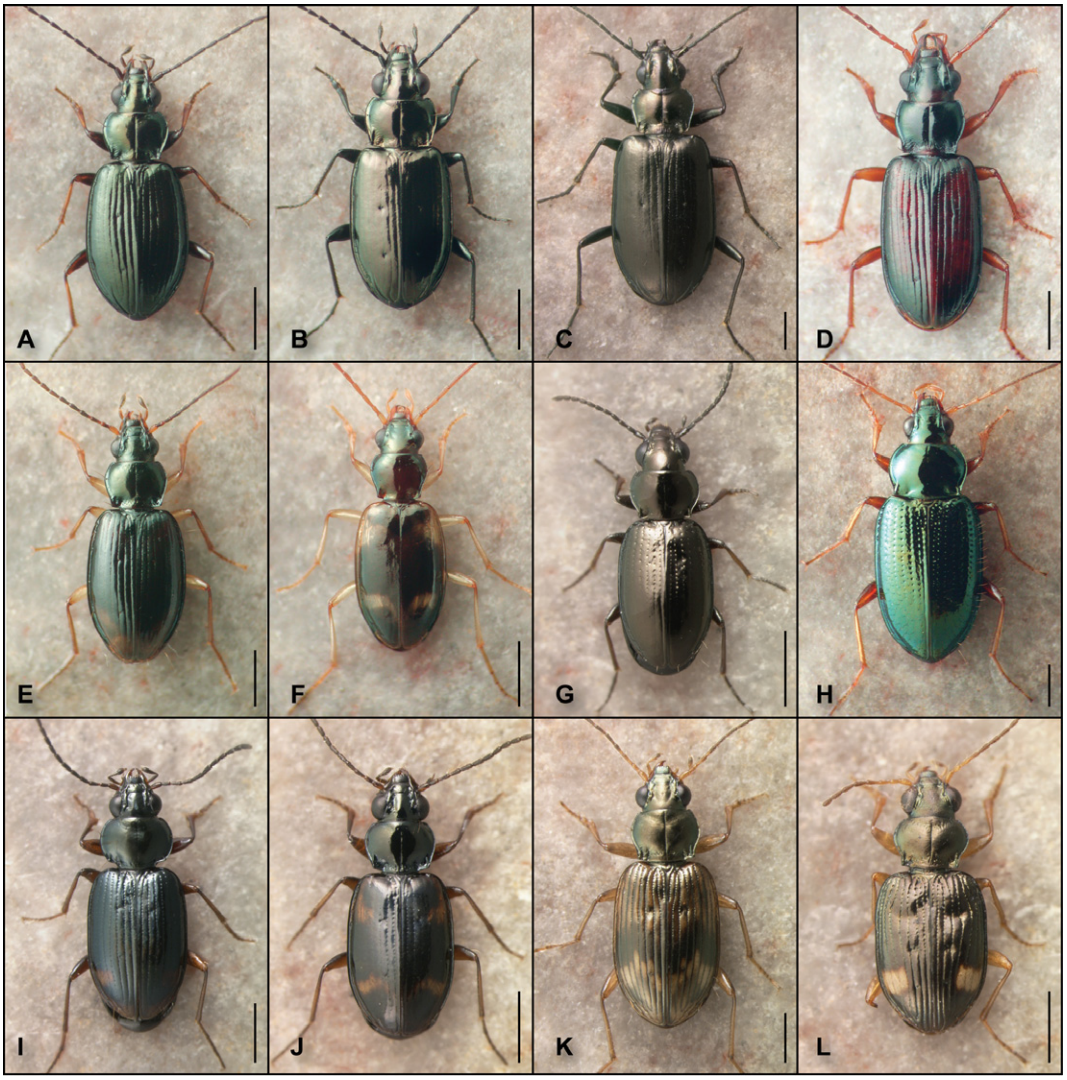

Fig. 5. Adults of *Bembidion* not placed to Series. Scale bar is 1 mm. (A) *Bembidion* (*Plataphus*) *simplex* (USA: West Virginia: N Fork Cherry River). (B) *B.* (*Plataphus*) *breve* (USA: California: Frog Lake). (C) *B.* (*Melomalus*) *planatum* (USA: Alaska: Healy). (D) *B.* (*Trichoplataphus*) *rolandi* (USA: Virginia: Glasgow). (E) *B.* (*Trechonepha*) *iridescens* (USA: California: Prefumo Canyon). (F) *B.* (*Liocosmius*) *festivum* (USA: California: Cache Creek). (G) *B.* (*Metallina*) *dyschrinum* (USA: Washington: Blue Mountains). (H) *B.* (*Hydrium*) *levigatum* (USA: Texas: Utley). (I) *B.* (*Philochthus*) *biguttatum* (Czech Republic: Bohemia: Cernozice pr. Hradec). (J) *B.* (*Lindrochthus*) *wickhami* (USA: California: Mt Tamalpais). (K) *B.* (*Eupetedromus*) *variegatum* (USA: Vermont: Burlington). (L) *B.* (*Andrewesa*) *cf. incisum* (China: Yunnan: Gongshan Co.: Dulongjiang Township). Photographs reprinted with permission, copyright David Maddison, released under a Creative Commons CC-BY 3.0 license.

those sites). Embedded within some of these stretches were one or two sites containing data, which were also removed. The regions at each end of the alignment, where more than 25% of the taxa lacked sequenced data, were also removed. In total, the regions that were kept and analyzed contained most of the data (88%) in the original matrix. The same methods were used to align 18S as for 28S, with the addition of consideration of secondary structure for determining primary homology, based upon a secondary structure model of 18S from *Bembidion chalceum* (Cannone et al., 2002; see http://www.rna.ccbb.utexas.edu/SIM/4D/Coleoptera_2009/, accessed 21 July 2011). Bases participating in each conserved stem region were considered homologous throughout the taxa; this caused a very small alteration of alignment from that inferred by Opal. As with 28S, most insertion and deletion events were within the far outgroups plus *Bembidarenas* and *Phrypeus*, rather than *Bembidion* and near relatives. Regions of ambiguous alignment were excluded from analyses. The regions that remained and that were analyzed contained only small (1–4 nucleotide) insertions and deletions within *Bembidion* and near relatives.

2.4. Phylogeny inference

Models of nucleotide evolution where chosen with the aid of ModelTest version 3.7 (Posada, 2005). For all genes, the model chosen by the Akaike Information Criterion (AIC) was a General Time Reversible rate matrix with a proportion of sites being invariable and the remainder following a gamma distribution (the GTR + I + Γ model).

Models of amino acid evolution were chosen with the aid of ProtTest version 2.4 (Abascal et al., 2005). The model chosen by AIC for CAD, *wingless*, and topoisomerase was JTT + I + Γ; for arginine kinase Dayhoff + I + Γ.

Bayesian analyses were attempted, but not completed, as 300,000,000 generations was not sufficient for convergence under the various models and MCMC conditions explored.

Maximum likelihood and parsimony analyses were conducted on multiple nucleotide matrices. In addition to seven individual gene matrices, three matrices were formed by concatenating genes: (1) a combined matrix of all seven genes (“AllData”), (2) a

Fig. 6. Adults of *Bembidion* (*Phyla*) *obtusum* (A) and Bembidiina other than *Bembidion* (B–I), and two genera traditionally placed in Bembidiina (J–L). Scale bar is 1 mm. (A) *Bembidion* (*Phyla*) *obtusum* (Canada: Ontario: Burlington). (B) *Asaphidion curtum* (USA: Massachusetts: Cambridge). (C) *Asaphidion yukonense* (Canada: Alberta: Rock Lake). (D) *Amerizus wingatei* (USA: North Carolina: Mt Mitchell). (E) *Lionepha osculans* (USA: Oregon: Goodman Creek). (F) *Lionepha disjuncta* (Canada: British Columbia: Summit Creek). (G) *Sinechostictus elongatus* (Spain: Madrid: Rio Cofio). (H) *Sinechostictus* (*Pseudolimnaeum*) sp. 2 (China: Yunnan Prov.: Gaoligong Shan). (I) *Ocys harpaloides* (Spain: Beuda). (J) *Phrypeus ricksecker*i (USA: California: Jedidiah Smith Redwood St Pk). (K) *Bembidarenas reicheellum* (Chile: Reg. X: Chaihuin). (L) *Bembidarenas setiventre* (Chile: Reg. X: Chaihuin). Photographs reprinted with permission, copyright David Maddison, released under a Creative Commons CC-BY 3.0 license.

combined matrix of the four nuclear protein-coding genes (“NucProt”), and (3) a combined matrix of the two ribosomal genes and the one mitochondrial gene (“RiboMito”). The seven-gene matrix has 12% missing sequences (228 sequences missing of the 1890 possible), the NucProt matrix has 6% missing sequences (63 of 1080), and the RiboMito matrix has 20% missing genes (165 of 810). For some particular questions matrices of other combinations of genes were also analyzed; these analyses are discussed below. In addition, some analyses were conducted on the amino acids (translated from the nucleotides) in a combined matrix of the four nuclear protein-coding genes. Multigene matrices were partitioned by gene, with each gene allowed to have independent parameter values for the model of evolution.

Maximum likelihood (ML) analyses were conducted using RAxML version 7.04 (Stamatakis, 2006). For each matrix of individual genes, 2000 search replicates were conducted to find the maximum likelihood trees, and 2000 non-parametric bootstrap replicates were used to calculate bootstrap values for groups of interest, which are reported as maximum likelihood bootstrap percentages (MLB). For multigene matrices, the numbers of search and bootstrap replicates were 1000. For analyses of amino acids, uncertain amino acids (caused by ambiguity in the underlying nucleotides) were converted to missing data before analysis.

Although an $I + \Gamma$ model of site-to-site rate variation was chosen by AIC for the likelihood analyses, a simpler Γ model was also explored. There has been concern expressed about the use of an $I + \Gamma$ model, with some authors favoring the Γ model because of estimation difficulties caused by interaction between the proportion of invariable sites and the gamma distribution (Yang, 2006; A. Stamatakis in RAxML 7.0.4 manual). Whether this might cause problems for estimation of the phylogeny is unclear. I explored the effects of including or not including the proportion of invariable sites by analyzing all matrices with a $I + \Gamma$ model, and separately with a Γ model. Comparisons were made in clade composition and bootstrap values of the bootstrap trees that resulted from the two analyses, using the Average of Values Associated with Nodes feature of Mesquite (Maddison and Maddison, 2011). For each of the CAD and AllData matrices, five additional $I + \Gamma$ bootstrap analyses were conducted, as well as five additional Γ bootstrap analyses, each starting from different random-number

Table 1
Sampling of *Bembidion* species. The ID column indicates how the voucher specimens were identified: if there is a number, then the specimen was identified by me using the reference listed at the end of this table; if there is a single letter, then the specimen is the holotype of the species ("H"), or I compared the specimen to the primary type or syntypes ("T") or paratypes or cotypes ("P") of the species, or I compared the specimen to specimens identified by an expert in the fauna ("S"), along with the relevant literature; if there is a two-letter code, then the specimen was identified by one of the following experts: JZ: Juan Zaballos; KD: Konjev Desender; LT: Luca Toledano; MB: Martin Baehr; MH: Matthias Hartmann; PB: Paolo Bonavita; PJ: Peter Johns; YB: Yves Bousquet. The taxon labeled "*B.* Chimera" is composed of two specimens, sampled for different genes; specimen 2258 is a *Bembidion quadrulum* LeConte, but specimen 1341 is a *Bembidion concolor* (Kirby); see text for further details. In the remaining columns, four-digit numbers are D.R. Maddison DNA voucher numbers; further information on the specimens is given in the Supplementary content. Where two numbers are listed, the sequence was formed by combining data from the two specimens. Other entries are GenBank numbers of previously published sequences from Maddison (2008), Maddison and Arnold (2009), Ober and Maddison (2008), Maddison and Swanson (2010), Maddison and Ober (2011), and Hildebrandt and Maddison (2011).

	ID	CAD	wg	ArgK	Topo	28S	18S	COI
***Odontium* Series**								
Hydriomicrus Complex								
Subgenus *Hirmoplataphus* Lindroth								
B. concolor (Kirby)	7	EF649387	EF649472	EF648693	EU677644	EF648833	EF648611	EF649110
B. humboldtense Blaisdell	7	1167	2267	2267	2267	2267	1167	1167
B. nigrum Say	7	2269	1162	2269	2269	1162		1162
B. Chimera	7	2258	1341	2258		1341		1341
B. recticolle LeConte	7	2045	2265	2265	2045	2045		2265
B. salebratum (LeConte)	7	1318	1318	1318		1318		1318
Subgenus *Hydriomicrus* Casey								
B. brevistriatum Hayward	T	1442	1442	1442		1442		1422
B. californicum Hayward	T	EF649386	EF649471	EF648692	1451	EF648832	EF648610	EF649109
B. innocuum Casey	T	1361	1361	1361		1361		1361
B. quadratulum Notman	7	1317	1317	1317		1317		1317
B. semistriatum (Haldeman)	7	1804	1804	1804	1804	1804	1804	1804
Odontium Complex								
Subgenus *Odontium* LeConte								
B. aenulum Hayward	12	GQ424221	GQ424283	1681	1681	GQ424265	1681	1681
B. bowditchii LeConte	7	EF649391	EF649476	EF648697	0814	EF648839	EF648615	EF649113
B. confusum Hayward	7	EF649393	EF649478	EF648699	0877	EF648840	EF648617	EF649115
B. coxendix Say	7	EF649394	EF649481	EF648700	1805	EF648837	EF648618	EF649116
B. durangoense Bates	T	0818	0818	0818	0818	0818		0818
B. paraenulum Maddison	H	GQ424226	GQ424288	1856	1856	GQ424282	1856	1856
B. persimile A. Morawitz	S	1288	1288	1288	1288	1288	1288	1288
B. striatum (Fabricius)	S	1687	1687	1687		1687		
Subgenus *Bracteon* Bedel								
B. alaskense Lindroth	10	0842	0842	0842		0842		
B. argenteolum Ahrens	10	1129	1129	1129		1129		
B. balli Lindroth	10	EF649389	EF649474	EF648695	1967	EF648838	EF648613	EF649111
B. carinula Chaudoir	10	1276	1276	1276	1276	1276	1276	1276
B. foveum Motschulsky	10	0817	0817	0817		0817	0817	
B. inaequale Say	10	EU677546	EU677673	2316	EU677648	EU677693	2316	2316
B. lapponicum Zetterstedt	10	EF649395	EF649479	EF648701	1604	EF648841	EF648619	EF649118
B. levettei carrianum Casey	10	EF649396	EF649480	EF648702	0108	EF648842	EF648620	EF649119
B. litorale (Olivier)	10	1704	1704	1704		1217		
B. punctatostriatum Say	10	0813	0813	0813		0813		
Subgenus *Ochthedromus* LeConte								
B. americanum Dejean	7	EF649388	EF649473	EF648694	1688	EF648834	EF648612	EF649117
B. bifossulatum (LeConte)	T	EF649390	EF649475	EF648696	1323	EF648835	EF648614	EF649112
B. cheyennense Casey	T	EF649392	EF649477	EF648698	1691	EF648836	EF648616	EF649114
Subgenus *Pseudoperyphus* Hatch								
B. antiquum Dejean	11	EF649403	EF649490	EF648709	1236	EF648851	EF648626	EF649128
B. arenobilis Maddison	H	EF649418	EF649526	EF648724	1852	EF648986	EF648638	EF649167
B. bellorum Maddison	H	EF649423	EF649536	EF648729	1902	EF648869	EF648640	EF649177
B. chalceum Dejean	11	EF649431	EF649548	EF648737	EU677650	EF648892	EF648647	EF649200
B. honestum Say	11	EF649437	EF649581	EF648743	1659	EF649024	EF648654	EF649240
B. integrum Casey	11	EF649445	EF649609	EF648751	EU677646	EF649056	EF648659	EF649272
B. louisella Maddison	H	EF649456	EF649653	EF648762	1250	EF648924	EF648677	EF649337
B. rothfelsi Maddison	H	EF649465	EF649668	EF648771	1543	EF648960	EF648685	EF649383
B. rufotinctum Chaudoir	11	EF649470	EF649676	EF648776	EU677652	EF649108	EF648691	EF649349
Subgenus *Microserrullula* Netolitzky								
B. xanthacrum Chaudoir	MH	1360	1360	1360	1360	1360	1360	1360
***Ocydromus* Series**								
Princidium Complex								
Subgenus *Princidium* Motschulsky								
B. punctulatum Drapiez	LT	1713	1713	1713	1713	1713	1713	1713
Subgenus *Testedium* Motschulsky								
B. flavoposticatum Jacquelin du Val	14	1342	1342	1342		1342		
B. laetum Brullé	14	1424	1424	1424	1424	1424	1424	1424

(*continued on next page*)

Table 1 (*continued*)

	ID	CAD	wg	ArgK	Topo	28S	18S	COI
Subgenus *Paraprincidium* Netolitzky								
B. ruficolle (Panzer)	9	1701	1701	1701	1701	1701	1701	1701
Subgenus *Cillenus* Samouelle								
B. laterale (Samouelle)	9	1965	GU556031	1965	1965	GU556082	0602	1965
Ocydromus Complex								
Subgenus *Ocydromus* Clairville								
B. modestum (Fabricius)	LT	1755	1755	1755	1755	1755		1755
B. scopulinum (Kirby)	7	1282	1282	1282	1282	1282		1282
Subgenus *Peryphus* Dejean								
B. actuosum Casey	7	2103	2103	2103	2103	2103		2103
B. obscurellum obscurellum (Motschulsky)	7	1814	1814	1814		1814		
B. plagiatum (Zimmerman)	7	EU677542	EU677670	EU677516	EU677641	EU677687	2427	1745
B. rupicola (Kirby)	7	2059	2059	2059		2059		
B. sejunctum semiaureum Fall	7	1817	1817	1817	1817	GU454738	1817	GU454768
B. tetracolum Say	7	1929	1929	1929	1929	1929	1929	1929
Subgenus *Terminophanes* Müller-Motzfeld								
B. mckinleyi carneum Lindroth	7	1606	1606	1606	1606	1606	1606	1606
Subgenus *Ocyturanes* Müller-Motzfeld								
B. dudichi Csiki	PB	2146	2146	2146		2146		
Subgenus *Asioperyphus* Vysoky								
B. bimaculatum (Kirby)	7	1281	1281	1281	1281	1281	1281	1281
B. lenae Csiki	7	2379	2379	2379	2379	2379	2379	2379
Subgenus *Peryphanes* Jeannel								
B. maroccanum Antoine	PB	2492	2492	2492	2492	2492		2492
B. platynoides Hayward	7	2074	2074	2074	2074	2074	2074	2074
B. stephensi Crotch	7	1758	1758	1758		1758		
B. texanum Chaudoir	7	1940	1940	1940	1940	1940		1940
Subgenus *Testediolum* Ganglbauer								
B. commotum Casey	7	2136	2136	2136	2136	GU454737	2136	GU454767
B. nebraskense LeConte	7	2501	1300	2501		2501		
Subgenus *Euperyphus* Jeannel								
B. ripicola L. Dufour	PB	2061	2061	2061	2061	2061		2061
B. transversale group								
B. lugubre LeConte	T	1712	1712	1712	1712	1712	1712	1712
B. mexicanum Dejean	T	2192	2192	2192	2192	GU454739	2192	GU454769
B. pernotum Casey	T	2483	2483	2483	2483	2483		2483
B. perspicuum (LeConte)	T	1120	1120	1120	1120	GU454740	1120	GU454770
B. sarpedon Casey	T	2484	2484	2484	2484	2484		2484
B. transversale Dejean	T	EU677541	EU677667	2157	EU677639	EU677688	2157	GU454797
Subgenus *Hypsipezum* Alluaud								
B. (*Hypsipezum*) sp. 1		1304	1304	1304	1304	2391	1304	1304
Subgenus *Leuchydrium* Casey								
B. tigrinum LeConte	S	1958	1958	1958	1958	1958	1958	1958
Ocydromus Complex *incertae sedis*								
B. grapii Gyllenhal	7	1928	1928	1928	1928	1928	1928	1928
B. vernale Bates	3	2421	2421	2421	2421	2421		2421
Other subgenera in *Ocydromus* Series								
Subgenus *Nepha* Motschulsky								
B. callosum subconnexum deMonte	14	1327	1327	1327	1327	1327	1327	1327
B. genei illigeri Netolitzky	2	1484	1484	1484	1484	1484	1484	1484
Subgenus *Bembidionetolitzkya* Strand								
B. astrabadense (Mannerheim)	MH	1435	1435	1435	1435	1435	1435	1435
B. geniculatum Heer	LT	1756	1756	1756	1756	1756	1756	1756
B. liangi Toledano and Schmidt	20	1716	1716	1716	1716	1716	1716	1716
***Bembidion* Series**								
Bembidion Complex								
Subgenus *Bembidion* Latreille								
B. mutatum Gemminger and Harold	7	2015	2015	2015		2015		
B. pedicellatum LeConte	7	1441	1441	1441	1441	1441		1441
B. quadrimaculatum dubitans (LeConte)	7	0676	0676	0676	0676	0676	0676	0676
B. quadripustulatum Audinet-Serville	14	1931	1931	1931		1931		
B. sciakyi sciakyi Toledano	16	2071	2071	2071	2071	2071		2071
Subgenus *Cyclolopha* Casey								
B. poculare Bates	15	1339	1339	1339	1339	1339	1339	1339
B. sphaeroderum Bates	15	2509	2509	2509	2509	2509		2509

Table 1 (*continued*)

	ID	CAD	wg	ArgK	Topo	28S	18S	COI
Subgenus *Notaphocampa* Netolitzky								
B. foveolatum Dejean	PB	1751	1751	1751	1751	1751		1751
B. niloticum Dejean	MH	1749	1749	1749	1749	1749	1749	1749
B. riverinae Sloane	18	1402	1402	1402	1402	1402		1402
Subgenus *Omotaphus* Netolitzky								
B. (*Omotaphus*) sp. 1		1428	1428	1428	1428	1428	1428	1428
B. (*Omotaphus*) sp. 2		1744	1744	1744		1744		1744
Subgenus *Sloanephila* Netolitzky								
B. jacksoniense Guérin-Méneville	18	1994	1403	1403	1994	1403	1403	1994
Ananotaphus Complex								
Subgenus *Ananotaphus* Netolitzky								
B. errans Blackburn	18	1741	1741	1741	1741	1741	1741	1741
Subgenus *Australoemphanes* Toledano								
B. ateradustum Liebherr	18	1700	1700	1700		1700		
Subgenus *Gondwanabembidion* Toledano								
B. proprium Blackburn	18	1740	1740	1740	1740	1740	1740	1740
Subgenus *Zecillenus* Lindroth								
B. (*Zecillenus*) sp. 1		0595	GU556072	0595	0595	0595	GU556153	0595
Subgenus *Zemetallina* Lindroth								
B. anchonoderum Bates	8	1322	1322	1322	1322	1322	1322	1322
B. hokitikense Bates	8	1439	1439	1439		1439		
B. parviceps Bates	8	1427	1427	1427	1427	1427	1427	1427
Subgenus *Zeplataphus* Lindroth								
B. maorinum Bates	8	1412	1412	1412	1412	1412		1412
B. tairuense Bates	8	0607	0607	0607	0607	GU556089	0607	0607
Antiperyphanes Complex								
Subgenus *Antiperyphanes* Jeannel								
B. caoduroi Toledano	19	1987	1987	1987	1987	1987	1987	1987
B. chilense Solier	5	1466	1466	1466	1466	1466		1466
B. sp. nr. *chilense* Solier		0714	GU556037	2317	2317	0714	0714	0714
B. spinolai Solier	T	2217	2217	2217	2016	2017		2217
Subgenus *Antiperyphus* Jeannel								
B. hirtipes (Jeannel)	T	2335	2335	2335	2335	2335		2335
B. rufoplagiatum Germain	T	1452	1452	1452	2207	1452	1452+2207	1452
Subgenus *Nothonepha* Jeannel								
B. sp. nr. *lonae* Jensen-Haarup	T	1457	1457	1457	1457	1457		1457
B. lonae Jensen-Haarup	T	1321	1321	1321	1321	1321	1321	1321
Subgenus *Pacmophena* Jeannel								
B. melanopodum Solier	T	2307	2307	2307	2307	2307	2307	2307
B. scitulum Erichson	5	1347	1347	1347	1347	1347		1347
Subgenus *Notholopha* Jeannel								
B. rugosellum (Jeannel)	LT	1348	1348	1348	1348	1348	1348	1348
B. sexfoveatum Germain	T	2208	2208	2208	2208	2208	2208	2208
B. (*Notholopha*) sp. 1		2046	2046	2046		2046		
Subgenus *Ecuadion* Moret and Toledano								
B. rawlinsi Moret and Toledano	13	1462	1462	1462	1462	1462		1462
B. rogersi Bates	3	2414	2414	2414	2414	2414	2414	2414
Subgenus *Plocamoperyphus* Jeannel								
B. mandibulare Solier	5	EU677545	EU677669	2203	EU677643	EU677689	2203	2203
Furcacampa Complex								
Subgenus *Furcacampa* Netolitzky								
B. affine Say	7	1443	1443	1443	1443	1443	1443	1443
B. cf. cognatum Dejean	S	1406	1406	1406	2023	1406	1406	1406
B. impotens Casey	7	1455	1455	1455	1455	1455		1455
B. mimus Hayward	7	2086	2086	2086	2086	2086		2086
B. versicolor (LeConte)	7	1422	1422	1422	1422	1422	1422	1422
Subgenus *Neobembidion* Bousquet								
B. constricticolle Hayward	7	2399	2399	2399	2399	2399	2399	2399
Diplocampa Complex								
Subgenus *Diplocampa* Bedel								
B. assimile Gyllenhal	14	1421	1421	1421	1421	1421	1421	1421
B. transparens (Gebler)	7	1943	1943	1943	1943	1943		1943
Subgenus *Semicampa* Netolitzky								
B. muscicola Hayward	7	1409	1409	1409	1409	1409	1409	1409
B. roosevelti Pic	7	2050	2050	2050		2050		
B. semicinctum Notman	7	2283	1328	2283	1328	1328		1328

(*continued on next page*)

Table 1 (*continued*)

	ID	CAD	wg	ArgK	Topo	28S	18S	COI
Other Subgenera in *Bembidion* Series								
Subgenus *Notaphus* Dejean								
Southern *Hemisphere* Grade (includes *Notaphiellus* Jeannel)								
B. solieri Gemminger and Harold	LT	1464	1464	1464	1464	1464	1464	1464
B. cupreostriatum Germain	T	2245	2245	2245	2245	2245		2245
B. calverti Germain	T	2007	2007	2007		2007		
B. cillenoides Jensen-Haarup	T	1999	1999	1999		1999		
B. convergens C. Berg	LT	1453	1453	1453		1453		
B. posticale Germain	T	2003	2003	2003		2003		
B. sp. nr. *ugartei* Toledano	5	1420	1420	1420	1420	1420	1420	1420
B. (*Notaphus*) sp. 1		2020	2020	2020	2020	2020		2020
Northern *Hemisphere* Clade								
B. aeneicolle (LeConte)	7	1816	1816	1816		1816		1816
B. castor Lindroth	7	2043	2043	2043	2043	2043		2043
B. coloradense Hayward	T	2138	2138	2138	2138	2138		2138
B. contractum Say	7	1467	1467	1467	1467	1467		1467
B. cordatum (LeConte)	7	1801	1801	1801		1801		
B. dejectum Casey	7	1932	1932	1932		1932		1932
B. dorsale Say	7	1895	1895	1895	1895	1895	1895	1895
B. flohri Bates	7	1753	1753	1753	1753	1753	1753	1753
B. idoneum Casey	T	1815	1815	1815		1815		
B. indistinctum Dejean	7	1998	1998	1998		1998		
B. insulatum (LeConte)	7	0444	GU556030	0444		0444		
B. oberthueri Hayward	7	1358	1358	1358		1358		
B. obtusangulum LeConte	7	2051	2051	2051		2051		
B. patruele Dejean	7	1473	1473	1473		1473		1473
B. umbratum (LeConte)	7	1757	EU677671+1757	1757	EU677640	EU677691	1757	2166
B. varium Olivier	14	1359	1359	1359	1359	1359	1359	1359
B. versutum LeConte	7	1715	1715	1715		1715		
B. rapidum group								
B. aratum (LeConte)	T	2284	1405	2284	2284	1405	1405	2137
B. nubiculosum Chaudoir	S	2044	2044	2044		2044		
B. rapidum (LeConte)	7	EU677543	EU677668	EU677518	EU677642	EU677690	1754	1754
B. scintillans Bates	T	1305	1305	1305	1305	1305	1305	1305
B. sparsum Bates	3	2009	2009	2009	2009	2009		2009
Subgenus *Nothocys* Jeannel								
B. anthracinum Germain	T	2228	2228	2228	2228	2228	2228	2228
B. marginatum Solier	T	1416	1416	1416		1416		
B. (*Nothocys*) sp. 1		1460	1460	1460	1460	1460	1460	1460
B. (*Nothocys*) sp. 2		1433	1433	1433		1433		
Subgenus *Trepanedoris* Netolitzky								
B. concretum group								
B. concretum Casey	7	2041	2041	2041	2041	2041		2041
B. fortestriatum (Motschulsky)	7	2098	2098	2098	2098	2098	2098	2098
B. pseudocautum Lindroth	7	1436	1436	1436	1436	1436	1436	1436
B. connivens group								
B. canadianum Casey	7	1445	1445	1445	1445	1445		1445
B. clemens Casey	T	2105	2105	2105	2105	2105		2105
B. connivens (LeConte)	T	2107	2107	2107	2107	2107		2107
B. elizabethae Hatch	T	1759	1759	1759	1759	1759	1759	1759
B. frontale (LeConte)	7	2286	2286	2286	2286	2286	2286	2286
B. siticum Casey	7	1429	1429	1429	1429	1429		1429
Subgenus *Notaphemphanes* Netolitzky								
B. ephippium (Marsham)	14	1425	1425	1425	1425	1425	1425	1425
Subgenus *Peryphodes* Casey								
B. ephippigerum (LeConte)	7	1927	1927	1927		1927		
B. salinarium Casey	7	1444	1444	1444	1444	1444	1444	1444
Subgenus *Trepanes* Motschulsky								
B. articulatum (Panzer)	LT	2049	2049	2049	2049	2049		2049
B. octomaculatum (Goeze)	14	1440	1440	1440	1440	1440	1440	1440
Subgenus *Emphanes* Motschulsky								
B. diligens Casey	7	1334	1334	1334		1334		1334
B. transversum G. Müller	14	1415	1415	1415		1415		
B. vile (LeConte)	7	1463	1463	1463	1463	1463	1463	1463
Unplaced to Series								
Plataphus Complex								
Subgenus *Blepharoplataphus* Netolitzky								
B. hastii Sahlberg	9	1703	1703	1703	1703	1703	1703	1703
Subgenus *Plataphus* Motschulsky								
B. basicorne Notman	7	1911	1911	1911	1911	1911	1911	1911

Table 1 (*continued*)

	ID	CAD	wg	ArgK	Topo	28S	18S	COI
B. cf. curtulatum Casey	T	2144	2144	2144	2144	2144		2144
B. gebleri turbatum Casey	7	1417	1417	1417	1417	1417	1417	1417
B. gordoni Lindroth	7	2358	2358	2358	2358	2358		2358
B. rufinum Lindroth	7	1434	1434	1434		1434		
B. rusticum rusticum Casey	7	1302	1302	1302		1302		
B. simplex Hayward	7	1921	1921	1921	1921	1921	1921	1921
B. stillaguamish Hatch	7	1438	1438	1438	1438	1438	1438	1438
"*Plataphodes*" group								
B. breve (Motschulsky)	7	1930	1930	1930	1930	1930	1930	1930
B. complanulum (Mannerheim)	7	2083	2083	2083	2083	2083		2083
B. farrarae Hatch	7	2084	2084	2084	2084	2084		2084
B. haruspex Casey	7	1476	1476	1476		1476		
B. kuprianovii Mannerheim	7	2101	2101	2101	2101	2101	2101	2101
B. quadrifoveolatum Mannerheim	7	1356	1356	1356		1356		
Hydrium Complex								
Subgenus *Hydrium* LeConte								
B. interventor Lindroth	7	1131	1131	1131	1131	1131	1131	1131
B. levigatum Say	7	1693	1693	1693	1693	GU5560083	1693	1693
B. nitidum (Kirby)	7	1941	1941	1941	1941	1941		
B. obliquulum LeConte	7	1132	1299	1132		1299		
Subgenus *Metallina* Motschulsky								
B. dyschirinum LeConte	7	0896	GU556029	0896	0896	0896		0896
B. lampros (Herbst)	LT	1727	1727	1727		1727		
B. properans (Stephens)	7	1279	1279	2315	2315	1279	1279	2315
Subgenus *Chlorodium* Motschulsky								
B. luridicorne Solsky	LT	1964	1964	1964	1964	1964	1964	1964
Philochthus Complex								
Subgenus *Philochthemphanes* Netolitzky								
B. cf. exquisitum Andrewes	17	2069	2069	2069	2069	2069	2069	2069
Subgenus *Philochthus* Stephens								
B. biguttatum (Fabricius)	LT	1747	1747	1966	1966	1966	1966	1966
B. escherichi Ganglbauer	14	1969	1969	1969	1969	1969	1969	1969
B. guttula (Fabricius)	14	1404	1404	1404		1404		
B. lunulatum (Geoffroy)	LT	1724	1724	1724	1724	1724	1724	1724
B. mannerheimii C.R. Sahlberg	LT	1746	1746	1746		1746		
Unplaced to Series or Complex								
Subgenus *Hoquedela* Müller-Motzfeld								
B. cf. csikii Jedlicka	21	0916	GU556028	0916	0916	0916	0916	0916
B. (*Hoquedela*) sp. 1		2068	2068	2068	2068	2068	2068	2068
Subgenus *Lindrochthus*, n. subg.								
B. wickhami Hayward	4	2280	2280	2280	2280	2280	2280	2280
Subgenus *Eupetedromus* Netolitzky								
B. dentellum (Thunberg)	LT	1714	1714	1714	1714	1714	1714	1714
B. graciliforme Hayward	7	1330	1330	1330		1330		
B. immaturum Lindroth	7	1510	1510	1510	1510	1510	1510	1510
B. incrematum LeConte	7	1411	1411	1411		1411	1411	
B. variegatum Say	7	1469	1469	1469	1469	1469	1469	1469
Subgenus *Trechonepha* Casey								
B. iridescens (LeConte)	7	1431	1431	2076	1431	1431	1431	1431
B. trechiforme (LeConte)	7	2271	2271	2271	2271	2271	2271	2271
Subgenus *Liocosmius* Casey								
B. festivum Casey	T	2078	2078	2000	2078	2000	2078	2000
B. horni Hayward	7	1408	1408	1408	1408	1408	1408	2123
B. mundum (LeConte)	7	2080	2080	2080	2080	2080	2080	2080
Subgenus *Melomalus* Casey								
B. planatum (LeConte)	7	0601	GU556035	0601	1386	GU556086	1386	1386
Subgenus *Trichoplataphus* Netolitzky								
B. mimekara Toledano & Schmidt	22	1366	1366	1366	1366	JF800053	1366	JF800057
B. grandiceps Hayward	7	1689	1689	1689	1689	JF800047		JF800059
B. planum (Haldeman)	7	1423	1423	1423	1423	JF800048	1423	JF800067
B. rolandi Fall	7	1319	1319	1319	1319	JF800045		JF800072
Subgenus *Andrewesa* Netolitzky								
B. cf. incisum Andrewes	23	2067	2067	2067	2067	2067	2067	2067
Subgenus *Phyla* Motschulsky								
B. obtusum Audinet-Serville	7	0895	0895	0895	0895	0895	0895	0895

References for identification: (1) Antoine (1955), (2) Bonavita and Vigna Taglianti (2010), (3) Erwin (1982), (4) Erwin and Kavanaugh (1981), (5) Jeannel (1962), (6) Jørum and Mahler (1985), (7) Lindroth (1963), (8) Lindroth (1976), (9) Lindroth (1985), (10) Maddison (1993), (11) Maddison (2008), (12) Maddison and Arnold (2009), (13) Moret and Toledano (2002), (14) Ortuño and Toribio (2005), (15) Perrault (1982), (16) Toledano (1999), (17) Toledano (2000), (18) Toledano (2005), (19) Toledano (2008b), (20) Toledano and Schmidt (2008), (21) Toledano and Sciaky (1998), (22) Toledano and Schmidt (2010), (23) Schmidt (2010).

Table 2
Sampling of Bembidiina other than *Bembidion*. See legend of Table 1 for more details.

	ID	CAD	wg	ArgK	Topo	28S	18S	COI
Amerizus Chaudoir								
Subgenus *Amerizus*								
Amerizus spectabilis (Mannerheim)	7	2082	2082	2082	2082	2082	2082	2082
Amerizus wingatei (Bland)	7	1566	1566	1566	1566	1566	1566	1566
Amerizus (*Amerizus*) sp.		1481	GU556024	1481		GU556074		1481
Subgenus *Tiruka* Andrewes								
Amerizus (*Tiruka*) sp.		2066	2066	2066	2066	2066	2066	2066
Asaphidion Gozis								
Asaphidion alaskanum Wickham	7	0585	GU556026	0585	0585	GU556076	0585	0585
Asaphidion cf. championi Andrewes	MH	2010	2010	2010		2010		
Asaphidion curtum (Heyden)	6	0267	GU556027	0267	0267	GU556078	AF002792	0267
Asaphidion granulatum Andrewes	MH	2012	2012	2012		2012		
Asaphidion cf. griseum Andrewes	MH	2011	2011	2011	2011	2011	2011	2011
Asaphidion indicum (Chaudoir)	MH	1343	1343	1343		1343		
Asaphidion rossii (Schaum)	1	1344	1344	1344	1344	1344		1344
Asaphidion yukonense Wickham	7	EU677540	EU677666	EU677515	EU677638	1897	1897	1897
Lionepha Casey								
Lionepha casta (Casey)	4	1400	1400	1400	1400	1400		1400
Lionepha disjuncta (Lindroth)	4	1896	1896	1896	1896	1896	1896	1896
Lionepha erasa (LeConte)	4	1320	1161	1161	1320	1320	1320	1320
Lionepha osculans (Casey)	4	1401	1401	1401	1401	1401	1401	1401
Ocys Stephens								
Ocys harpaloides (Audinet-Serville)	KD	0569	GU556048	0569		GU556103	0569	
Ocys quinquestriatus (Gyllenhal)	KD	1077	1077	1077	1077	1077	1077	1077
Sinechostictus Motschulsky								
Subgenus *Sinechostictus*								
Sinechostictus cribrum (Jacquelin du Val)	PB	1183	1183	1183		1183		
Sinechostictus dahlii (Dejean)	PB	1396	1396	1396	1396	1396		1396
Sinechostictus elongatus (Dejean)	14	1349	1349	1349	1349	1349	1349	1349
Sinechostictus solarii (G. Müller)	PB	0603	GU556060	1397	1397	1397	0603	1397
Subgenus *Pseudolimnaeum* Kraatz								
Sinechostictus alesmetana Toledano	LT	2368	2368	2368	2368	2368		
Sinechostictus (*Pseudolimnaeum*) sp. 2 (*exaratus* group)		2248	2248	2248		2248		
Sinechostictus (*Pseudolimnaeum*) sp. 3 (*exaratus* group)		1399	1399	1399	1399	1399	1399	1399
Sinechostictus (*Pseudolimnaeum*) sp. 4		1398	1398	1398		1398		

Table 3
Sampling of outgroups. See legend of Table 1 for more details.

	ID	CAD	wg	ArgK	Topo	28S	18S	COI
Pogonini								
Diplochaetus planatus (G.H. Horn)	YB	1959	AF437938	1959	1959	AF438060	AF002789	1959
Pogonus chalceus (Marsham)	S	1711	GU556057	0679	1711	GU556114	GU556144	1711
Sirdenus grayii (Wollaston)	S	EU677539+1777	EU677665	1777	EU677637	EU677685	1777	1777
Thalassotrechus barbarae (G.H. Horn)	S	1919	GU556065	1919	1919	GU556124	1919	1919
Zolini								
Merizodus angusticollis Solier	5	0453	GU556045	0453		GU556099	AF012487	
Merizodus sp. nr. *catapileanus* Jeannel	5	2199	2199	2199	2199	2199	2199	2199
Oopterus helmsi (Sharp)	PJ	0354	GU556073	0354	0354	GU556132	AF002787	0354
Sloaneana lamingtonensis Baehr	MB	2312	2312	2312	2312	2312	2312	2312
Bembidiini: Anillina								
Geocharidius sp.		1763	1763	1763	1763	1763	1763	1763
Serranillus sp.		2309	2309	2309	2309	GU556116	GU556145	2309
Typhlocharis armata Coiffait	JZ	1718	1718	1718	1718	GU556130	GU556152	1718
Trechitae *incertae sedis*								
Bembidarenas reicheellum (Csiki)	T	2213	2213	2213	2213	2213	2213	2213
Bembidarenas setiventre Nègre	P	2214	2214	2214	2214	2226	2214	2214
Phrypeus rickseckeri (Hayward)	7	0776	GU556056	0776	2341	GU556113	2341+0776	2341

seeds. These were compared pairwise (one I + Γ analysis to a randomly chosen Γ analysis) to see if differences observed in the initial comparisons were robust to bootstrap sampling variation.

Most-parsimonious trees (MPTs) were sought using PAUP* (Swofford, 2002). To search for most parsimonious trees, 2000 replicates were conducted, each beginning with a starting tree formed with the random addition sequence option, with each replicate saving no more than 25 trees. For parsimony bootstrap analyses in PAUP*, 1000 bootstrap replicates were examined, each of which used a heuristic search with four replicates, each beginning with a starting tree formed by the random addition sequence option, with TBR branch rearrangement, with each replicate saving no more

than 25 trees; the estimated bootstrap values are reported as parsimony bootstrap percentages (PB).

Because of the tendency in some analyses for long-branched outgroups (including anillines and zolines) to move within *Bembidion*, an additional suite of analyses was done that included only the species of *Asaphidion* and *Bembidion*. These analyses were performed in the same way as analyses of the full matrices, except that only 1000 bootstrap replicates or optimal tree searches were conducted, and RAxML version 7.2.6 was used. The resulting trees were rooted between *Asaphidion* and *Bembidion*.

To explore the contribution of individual genes to the multigene analyses, ML bootstrap analyses were conducted on seven multigene matrices, each formed by removing a different, single gene from the AllData matrix. The ML bootstrap analyses on each exactly matched those for the AllData analyses, except that RAxML version 7.2.6 was used rather than 7.04.

2.5. Simulation studies

A simulation study was conducted to see if the inferred model of character evolution and phylogeny are "self-predicting", that is, they are such that, combined, they would predict sequences of the length examined were sufficient to correctly infer the phylogeny. If the character evolution model and phylogeny failed the test, this would be disturbing, and would decrease our confidence in the inferred phylogeny (Hillis, 1996; Maddison et al., 1999). The test might fail, for example, if the tree had long, separated branches that might result in long-branch attraction for sequences that evolved along the branches of the phylogeny, or if the internal branches were short enough to have accumulated too few evolutionary changes to leave traces of their existence. The simulation study began with the maximum likelihood tree from the combined data matrix of all genes, inferred under the GTR + I + Γ model. For

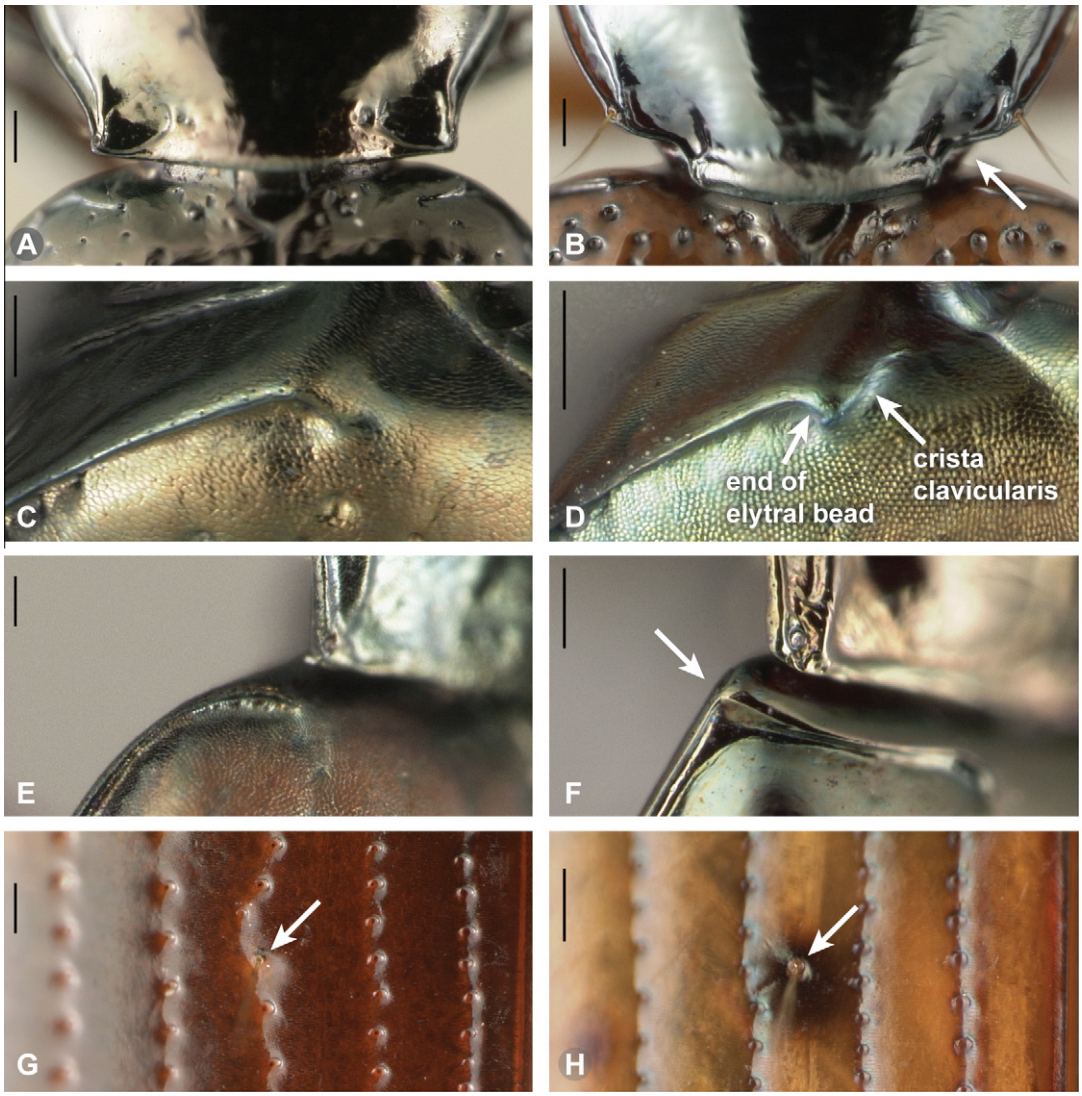

Fig. 7. Four characters traditionally used to classify the major groups of *Bembidion*. Scale bar 0.1 mm. (A and B) shape of the posterior edge of the pronotum, whether (A) more or less straight, or (B) notched (marked by arrow) such that base of the pronotum at the center is distinctly and abruptly more posteriad of the hind angles. (A) *Bembidion* (*Metallina*) *dyschrinum*, USA: Washington: Blue Mountains; (B) *B.* (*Philochthus*) *lunulatum*, Czech Republic: Bohemia: Revnicov. (C and D) front region of elytron, between the end of the elytral bead and the base of the elytron, whether (C) simple, or (D) with a ridge, termed a crista clavicularis. (C) *B.* (*Pseudoperyphus*) *antiquum*, USA: Missouri: Big River; (D) *B.* (*Notaphocampa*) *foveolatum*, Republic of South Africa: Mpumalanga: Kruger National Park. (E and F) groove at shoulder of elytron, whether (E) gently curved, or (F) abruptly angled (marked by arrow). (E) *B.* (*Melomalus*) *planatum*, USA: Colorado: Beaver Creek at Gunnison River; (F) *B.* (*Hydrium*) *nitidum*, Canada: Ontario: Listowel. (G and H) position of discal setae on elytra, whether (G) situated in a stria (in this species, the striae are rows of punctures; base of seta marked by arrow), or (H) situated between striae, in an interval (base of seta marked by arrow). (G) *B.* (*Ocydromus*) *lugubre*, USA: New Mexico: San Juan; (H) *B.* (*Notaphus*) *dorsale*, Canada: Alberta: Taber.

each gene, the following steps were used to create a simulated matrix: species were removed from the tree if no sequences of that gene were obtained for that species; branch lengths of the tree, plus parameter values for the GTR + I + Γ model, were inferred using PAUP's lscore command, on the pruned tree, and based on the observed sequences; a matrix of the same length as the observed matrix for that gene was simulated up the branches of the pruned tree (now with gene-specific branch lengths) using the fully specified GTR + I + Γ model in Mesquite's Genesis package. The simulated matrices for all genes were concatenated into one matrix, and a maximum likelihood bootstrap analysis with 1000 replicates was conducted in RAxML using the same procedure as was used for the observed matrix.

2.6. Morphological methods

Methods of specimen preparation for morphological work, raising of larvae, and terms used, are given in Maddison (1993, 2008).

Adults from each species sequenced were scored for four morphological characters that have been used to classify *Bembidion* subgenera into series and complexes: (1) shape of the hind margin of the pronotum, whether more or less straight (Fig. 7A) or whether sinuate and notched with a concavity (Fig. 7B, arrow), such that the hind angle bearing the seta is notably forward of the central portion of the hind margin; (2) presence of a crista clavicularis (Fig. 7D), a ridge extending from the anterior end of the elytral bead (the ridge beside the groove along the elytral edge) toward the articulation of the elytron with the mesothorax; (3) shape of the elytral bead at the shoulder, whether rounded (Fig. 7E), sinuate (with an anteriorly directed concavity, not pictured), or angulate (Fig. 7F, arrow); (4) whether the discal setae, near the center of an elytron, are positioned in a stria (Fig. 7G), near a stria but not in it, or in the interval between striae (Fig. 7H). For the last character, only the front discal seta (ed3) was scored.

The states for the last two characters were mapped onto the maximum likelihood bootstrap trees for the AllData matrix using parsimony in Mesquite (Maddison and Maddison, 2011) to yield an estimate of the minimum number of evolutionary changes.

3. Results

3.1. Inferred phylogeny

The tree with highest likelihood found for the combined matrix of all seven genes under a GTR + I + Γ model is presented in Fig. 8. Eighty percent (178 of 222) of the clades in that tree are also present in the most parsimonious trees and in the maximum likelihood tree under a GTR + Γ model; the 44 clades that are *not* present in optimal trees from all three analyses are marked in Fig. 8 by an open circle on basally pale branches. Many of these regions of uncertainty are within subgenera or complexes that I have a

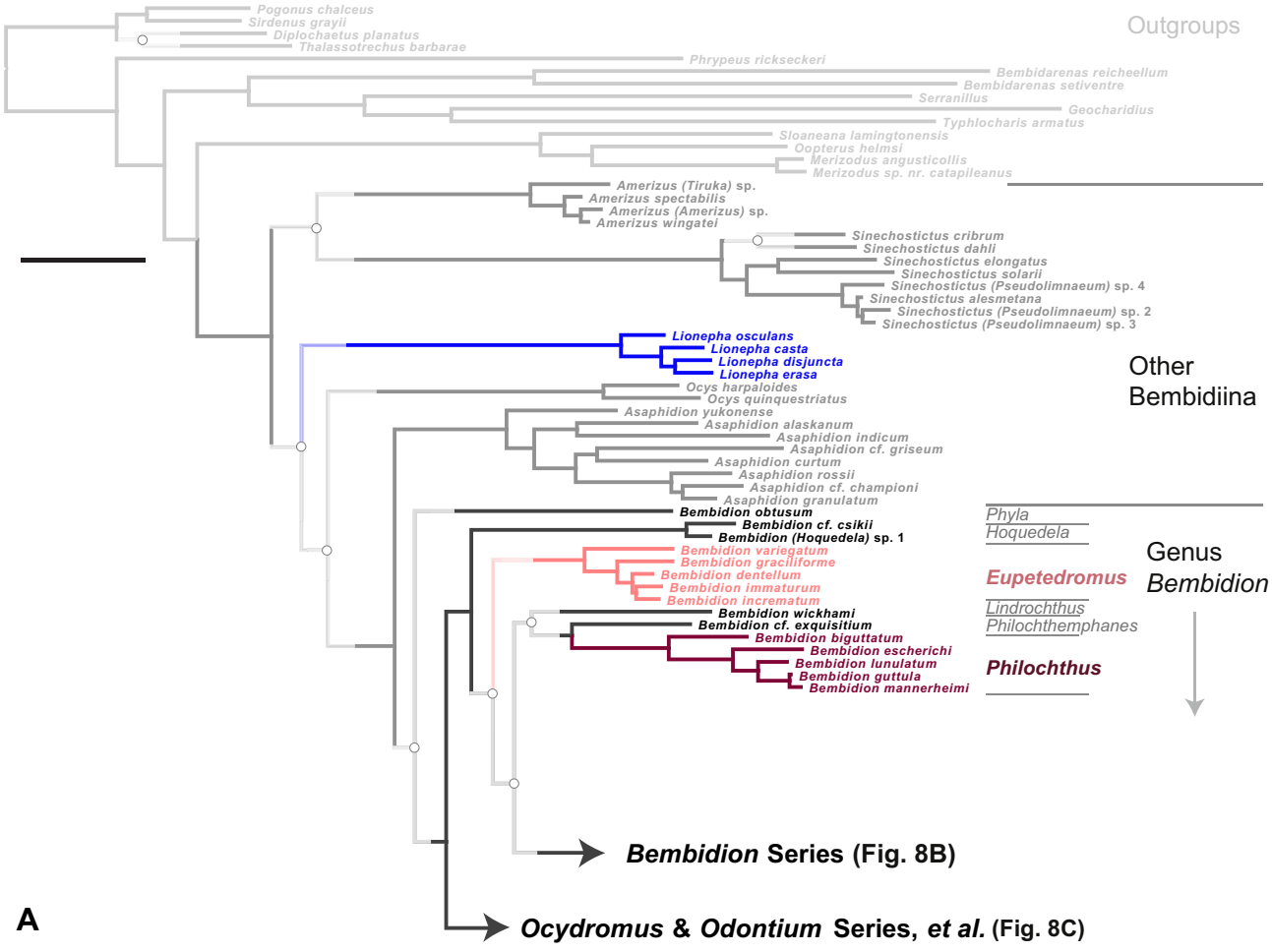

Fig. 8. Maximum likelihood tree for all seven genes combined under the I + Γ model site-to-site rate variation model. Clades that are basally pale and marked with an open circle are absent from either the maximum likelihood tree under the Γ model or maximum parsimony trees; all other clades are present in all three analyses (i.e., ML trees under I + Γ and Γ models, and maximum parsimony trees). Most labels on the right side are supra-specific taxa (subgenera, complexes, and series) within the genus *Bembidion;* the color scheme for these taxa is used throughout Figs. 8–10, 15). Scale bar: 0.1 substitutions per site, as reconstructed by RAxML.

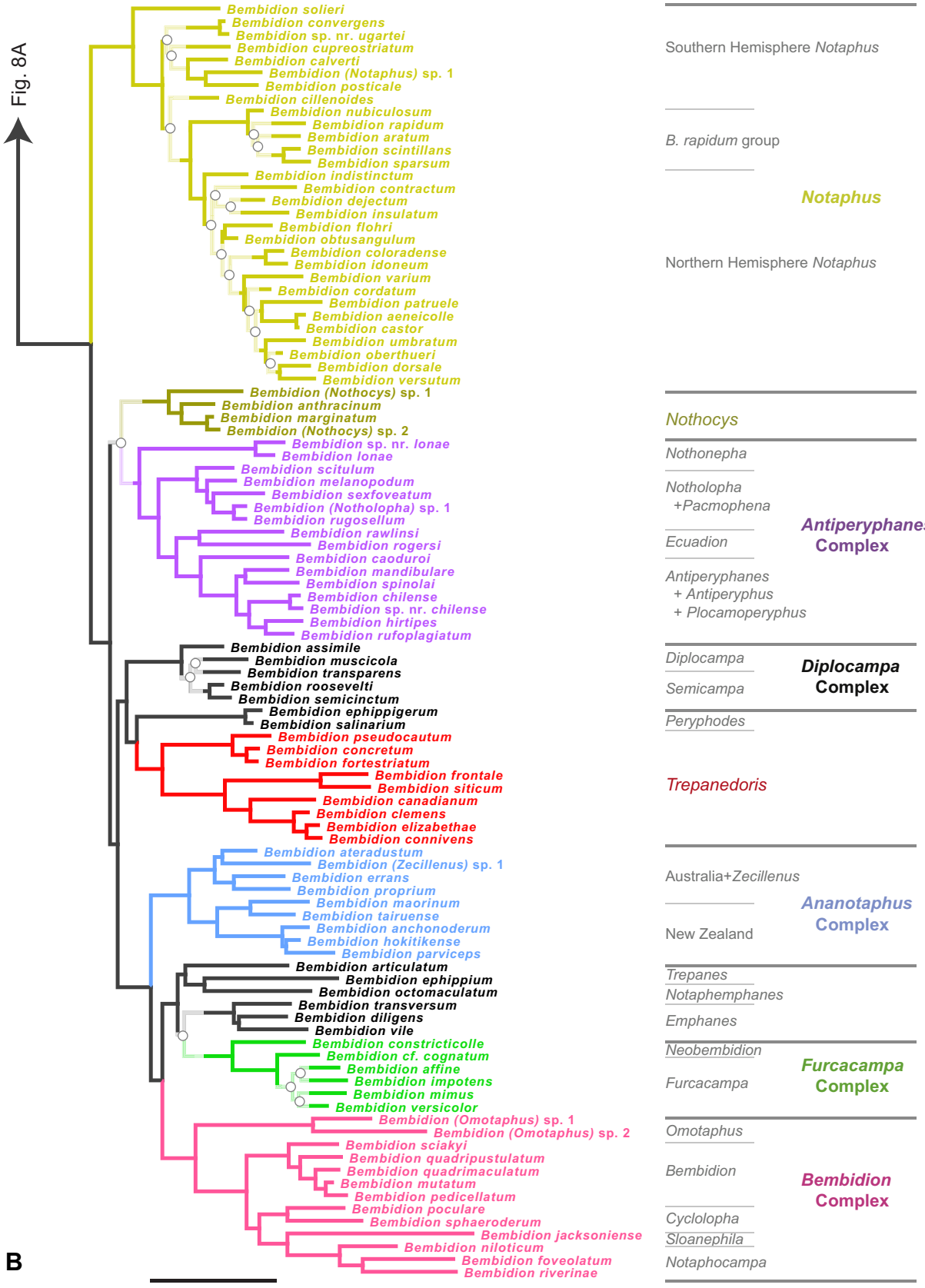

Fig. 8 (*continued*)

sampled densely, such as *Notaphus* (Fig. 8B) and the *Odontium* Complex (Fig. 8C). But there are three regions of deeper nodes that also vary between these analyses: (1) the relationships of Bembidiina genera (Fig. 8A); the relationship of *Asaphidion* and *Bembidion obtusum* to the rest of *Bembidion* (Fig. 8A); the potential near-relatives of the *Bembidion* Series (Fig. 8A); the relationship of the *Hydrium*

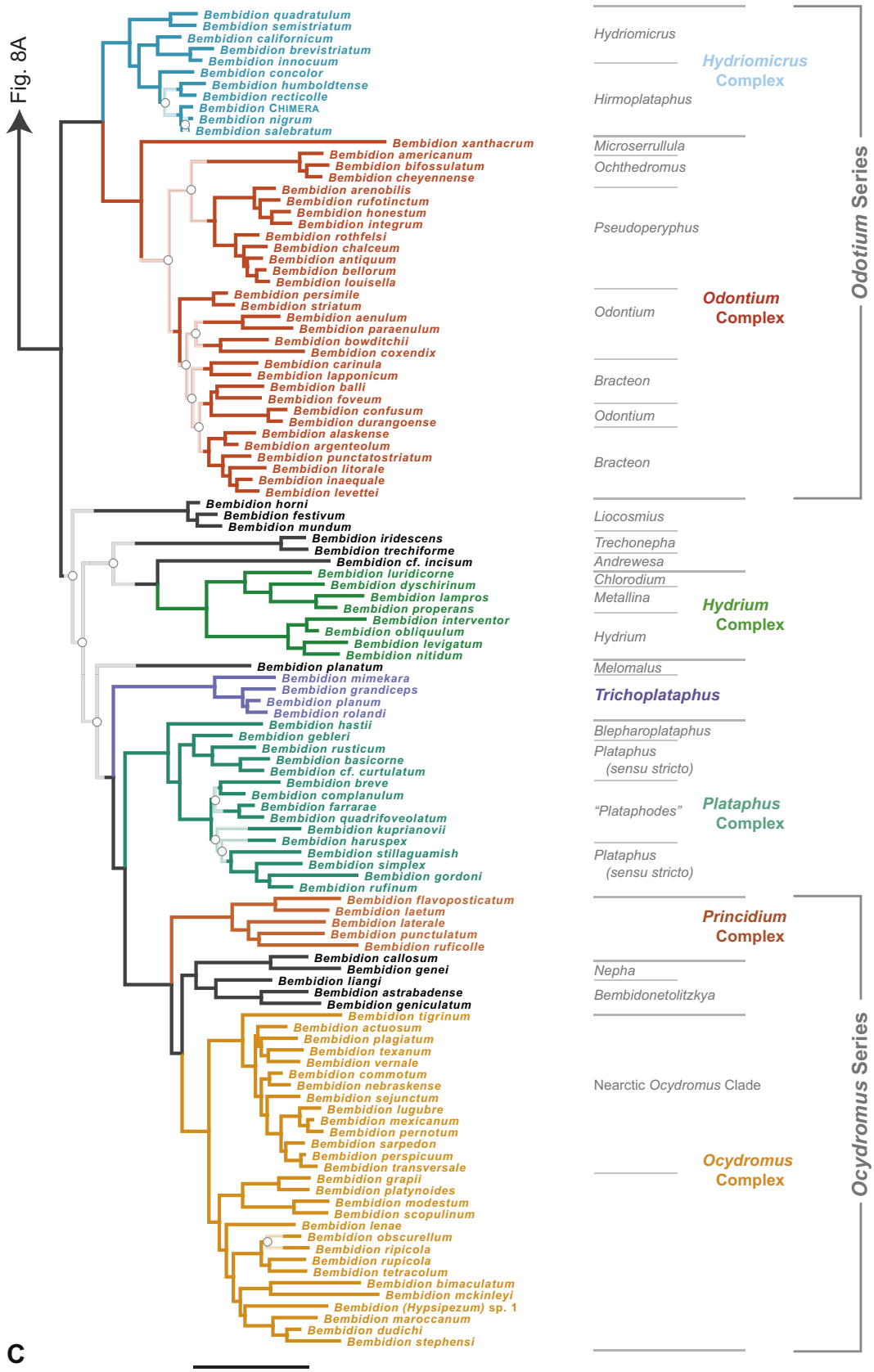

Fig. 8 (*continued*)

Complex and a few other groups to the *Ocydromus* Series and *Plataphus* Complex (Fig. 8C). Only two of these 44 inconsistent clades are present in the ML bootstrap tree with MLB ⩾75 (Fig. 9), those being the *Eupetedromus* + *B. wickhami* + *Philochthemphanes* + *Philochthus* + *Bembidion* Series clade (Fig. 8A) and the *Ochthedromus* + *Pseudoperyphus* clade (Fig. 8C).

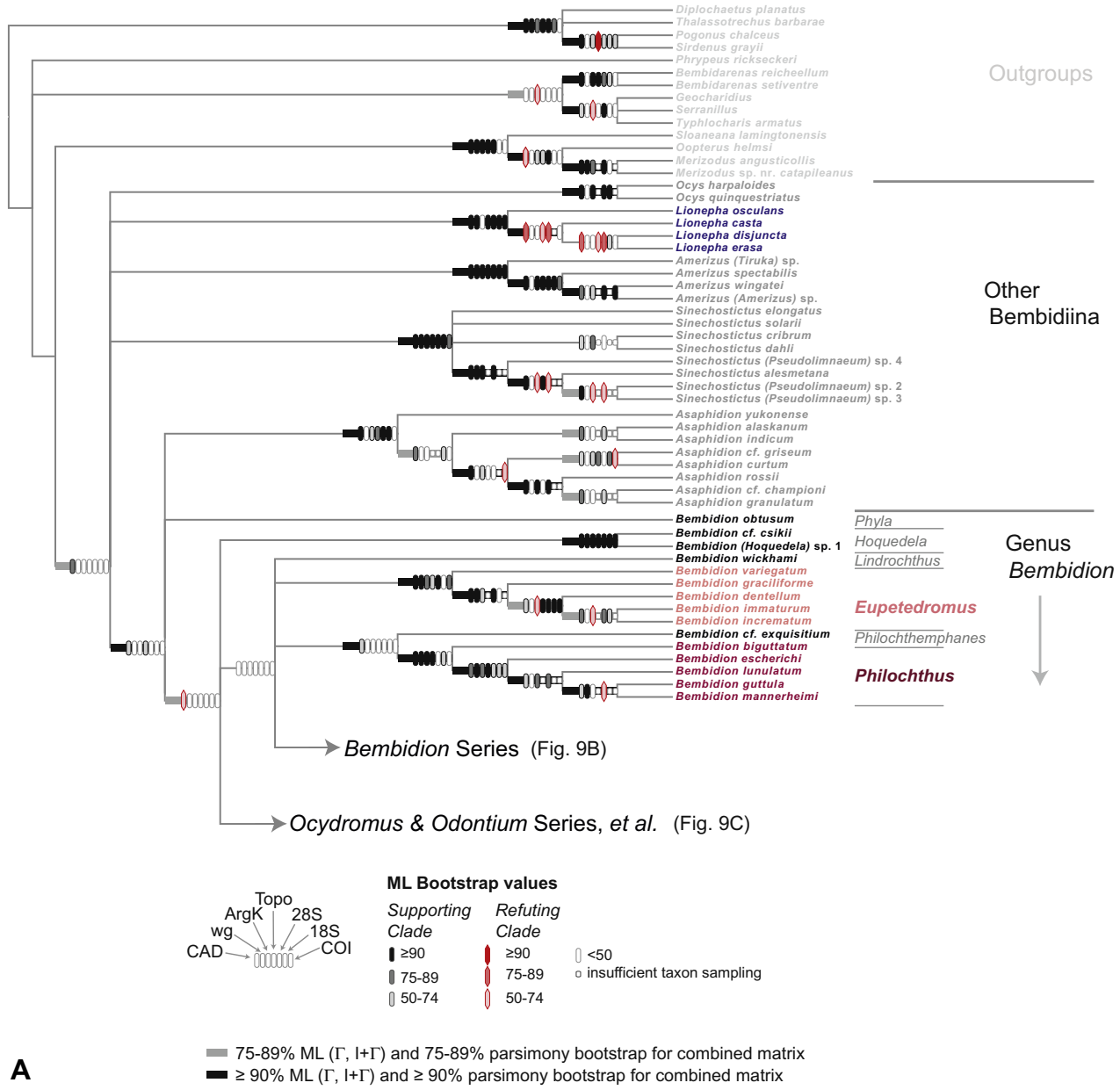

Fig. 9. Maximum likelihood bootstrap tree for all seven genes combined. Only branches with bootstrap value ⩾75 are shown. Branches have thick horizontal bars if those clades are present in ML bootstrap trees using the I + Γ site-to-site rate variation model, ML bootstrap trees using the Γ rate variation model, and in parsimony bootstrap trees, all at the 75% (gray) or 90% (black) level; the three bootstrap trees are presented individually in Supplementary content Figs. S1–S3. The seven small vertical bars on each branch indicate support in favor (gray to black) or against (pink to red) that clade in the ML bootstrap trees for each of the seven genes analyzed individually.

The ML bootstrap analysis of the AllData matrix shows most clades supported by MLB ⩾75 (Fig. 9). Fig. 9 also shows the parsimony bootstrap support for the clades as well as ML bootstrap support for individual genes. Support for or against notable clades and hypotheses in the ML (I + Γ) and parsimony analyses is summarized in Tables 4 and 5.

For those clades or hypotheses listed in Table 5 that are present in ML trees or MPTs from multiple genes, but not present in trees from other genes, I view the evidence in their favor as stronger than the evidence against, as while there was consistency in the trees in their favor, there was no observed consistency in the contradictory trees. For example, for hypothesis 5.11, the existence of a *Ocydromus* Series + *Plataphus* Complex + *Trichoplataphus* clade, there are three analyses that show this clade (CAD ML, 28S parsimony, and 18S ML), but all other analyses and other genes either show the tree unresolved, or show contradictory clades. However, the contradictory clades differ from gene to gene: in *wingless*, the ML tree shows the *Ocydromus* Series belonging to a clade with the genus *Lionepha*, the tribe Zolini, and the subgenera *Trechonepha* and *Melomalus*, to the exclusion of the *Plataphus* Complex and *Trichoplataphus*; the ArgK ML tree shows the *Ocydromus* Series in a clade with the genera *Asaphidion*, *Ocys*, and *Lionepha*, the subtribe Anillina, the *Hydrium* Complex, *Plataphus* Complex, etc., but without *Trichoplataphus*; and so on. That is, the presence of check marks in Table 5 in two or more genes shows consistency of the results across multiple genes, but the presence of x's in two or more genes was not noted to indicate a consistent, contradictory hypothesis.

Most of the subgenera, complexes, and series are monophyletic in the combined data analyses as well as many of the individual gene analyses; this is visually evident by adjacency of like-colored

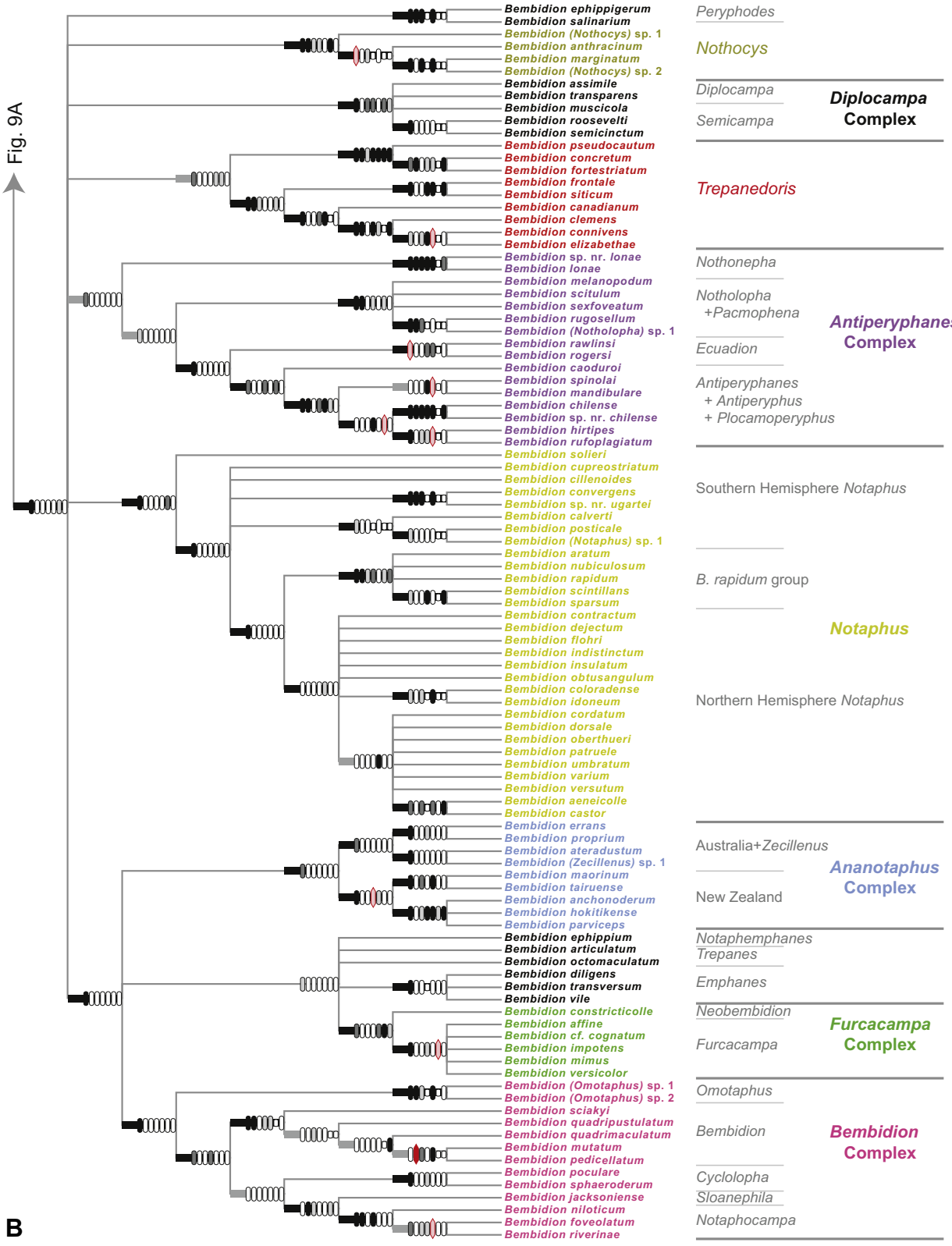

Fig. 9 (*continued*)

taxa and branches in Figs. 8 and 9 for the combined data, and in Fig. 10 for the individual gene analyses (see the Supplementary content Figs. S4–S7 for more detailed images of individual gene phylogenies).

3.2. Effect of site-to-site rate variation models

The model used for site-to-site rate variation had minimal effect on the inferred phylogeny in maximum likelihood analyses. The

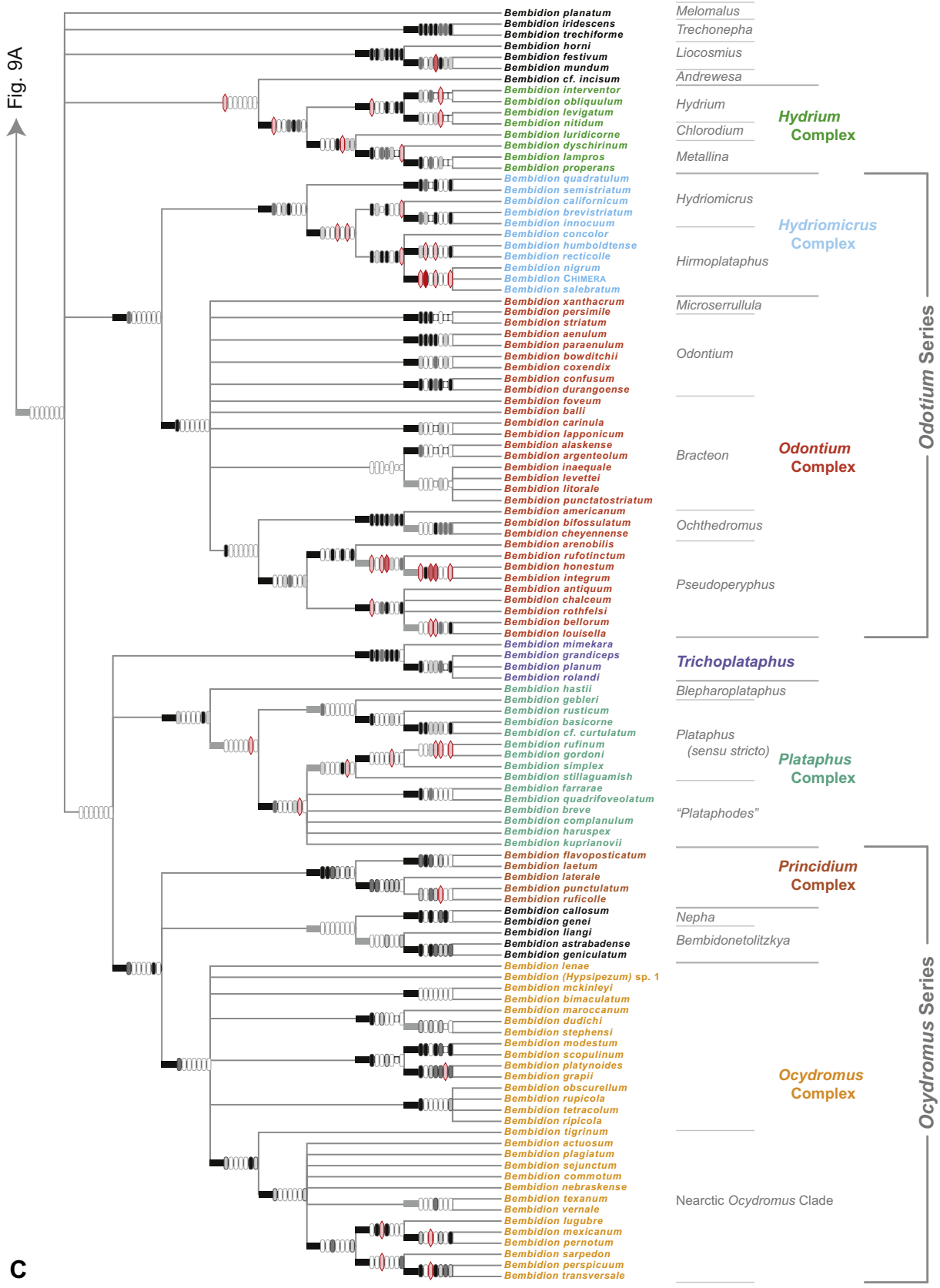

Fig. 9 (*continued*)

bootstrap trees inferred under the two models shared 94–98% of their clades (Table 6). Clades that were present in the I + Γ trees but absent from Γ trees had low bootstrap values, at most 51-66%, depending upon the matrix. Thus, all of the clades in the analyses with bootstrap values ⩾67% are robust to changes in the site-to-site rate variation model. The difference between

bootstrap values of clades present in both analyses was at most 18 percentage points (Table 6), with most clades having bootstrap values that differ by less than 5 percentage points (Figs. 11 and 12). Variation of this estimated difference in bootstrap values between I + Γ and Γ analyses was slight (e.g., stars around points 1, 2, and 3 in Fig. 12). Optimal likelihood trees from the combined, seven-gene matrix were similar between the I + Γ and Γ analyses, sharing 91% of their clades. Optimal likelihood trees from single-gene analyses

Table 4
Support for and against various clades or hypotheses based on simultaneous analyses of multiple genes. ML: Maximum likelihood analysis; P: parsimony analysis. Numbers in main body indicate the bootstrap support expressed as a percentage; check marks indicate that the clade is present in the optimal (maximum likelihood or most parsimonious) trees but with bootstrap value below 50; x indicates that a contradictory clade was present in the optimal (maximum likelihood or most parsimonious) trees but with bootstrap value below 50; negative values indicate bootstrap support for a contradictory clade. Boxes in gray to black indicate support for the clade; boxes in pink to red indicate support against that clade, with darker colors indicating stronger support. Blank boxes indicate no support for or against the clade because of lack of resolution in the inferred trees. *Abbreviations*: "inc." = "including", "exc." = "excluding". Trees all rooted at Pogonini. (For interpretation to colors in this table, the reader is referred to the web version of this paper.)

		7 genes		NucProt, 4 genes		RiboMito, 3 genes	
		ML	P	ML	P	ML	P
	Boundary of Bembidiina, non-*Bembidion* genera						
4.1	Bembidiina (exclusive of *Bembidarenas*, *Phrypeus*)	98	90	97	89	-50	✓
4.2	Bembidiina (inc. *Bembidarenas*)	-95	-78	-90	-62	-50	x
4.3	Bembidiina (inc. *Phrypeus*)	-82	-69	-66	-60	-50	x
	Boundaries of *Bembidion*, *Lionepha* placement						
4.4	*Asaphidion* + all *Bembidion* other than *Lionepha* (*B. obtusum* optional, can include other Bembidiina)	99	99	93	94	71	✓
4.5	*Lionepha* within non-*Bembidion* clade or grade [1]	99	99	93	94	71	✓
4.6	*Bembidion* (exc. *Lionepha*, inc. *B. obtusum*)	✓	-62	-66	-79	✓	x
4.7	*Bembidion* (exc. *Lionepha*, exc. *B. obtusum*)	94	80	88	89	x	x
	Major structure within *Bembidion*						
4.8	*Bembidion* Series	100	100	99	100	88	70
4.9	*Ocydromus* Series	100	100	97	99	97	86
4.10	*Odontium* Series	99	94	99	86	57	✓
4.11	*Ocydromus*, *Odontium* Series + *Hydrium*, *Plataphus* Complexes + *Trichoplataphus*, *Melomalus*, *Liocosmius*, *Trechonepha*, *Andrewesa*	95	82	94	85	x	x
4.12	As 4.11 but with the only non-*Bembidion* in the analysis being *Asaphidion*	97	89	98	92	x	x
4.13	*Ocydromus* Series + *Plataphus* Complex + *Trichoplataphus*	82	53	✓	x	63	51
	***Bembidion* Series**						
4.14	*Bembidion* Complex	99	98	98	98	✓	x
4.15	*Bembidion* + *Cyclolopha* + *Notaphocampa* + *Sloanephila*	100	99	100	100	✓	✓
4.16	*Ananotaphus* Complex	100	100	99	100	✓	x
4.17	*Ananotaphus* (*sensu* Darlington) + *Zecillenus*	100	100	97	99	95	80
4.18	*Zeplataphus* + *Zemetallina*	100	100	100	99	69	✓
4.19	*Furcacampa* + *Neobembidion*	100	100	99	95	99	98
4.20	*Bembidion* Complex + *Ananotaphus* Complex + *Furcacampa* Complex + Notaphemphanes + *Trepanes* + *Emphanes*	100	96	98	90	x	x
4.21	*Notaphus*	100	100	100	100	✓	x
4.22	*Nothocys*	100	100	100	100	✓	✓
4.23	*Notaphus* + *Nothocys*	-68	x	x	x	x	x
4.24	*B. rapidum* species group	100	100	100	100	✓	✓
4.25	N. Hemisphere *Notaphus*	98	94	62	56	58	62
4.26	N. Hemisphere *Notaphus* + *B. rapidum* group	98	99	99	99	x	x
4.27	Paraphyly of S. American *Notaphus*	99	98	98	98	✓	

Table 4 (*continued*)

		7 genes		NucProt, 4 genes		RiboMito, 3 genes	
		ML	P	ML	P	ML	P
	***Bembidion* Series (continued)**						
4.28	*Antiperyphanes* Complex	98	88	96	75	✓	✓
4.29	*Diplocampa* Complex	100	100	100	100	56	✓
	***Odontium* Series**						
4.30	*Hydriomicrus* Complex	99	100	91	95	99	98
4.31	*Odontium* Complex (inc. *Microserrullula*)	99	94	99	86	68	57
4.32	*Odontium* Complex (exc. *Microserrullula*)	58	-58	-53	-55	✓	
	***Ocydromus* Series**						
4.33	*Princidium* Complex (inc. *Cillenus*)	100	100	100	100	75	79
4.34	*Ocydromus* Complex	100	99	98	99	x	x
4.35	Nearctic *Ocydromus* Clade [2]	96	98	86	92	70	71
4.36	Nearctic *Ocydromus* Clade + *B. tigrinum*	100	100	98	95	98	96
	***Plataphus* Complex**						
4.37	*Plataphus* Complex	100	100	98	99	99	95
4.38	*Plataphus* Complex + *Melomalus*	-82	-63	x	-56	-63	-51
4.39	*Plataphus* + *Plataphodes*	88	96	85	99	99	x
4.40	*Plataphus* exc. *Plataphodes*	-100	-100	-98	-100	-77	-61
4.41	*Plataphodes*+*B. stillaguamish*+*gordoni*+*rufinum*+*simplex*	100	100	98	100	77	61
4.42	*Plataphodes*	-50		-69	-58	x	-56
4.43	*Plataphus* Complex + *Trichoplataphus*	x	-63	✓	-56	-59	x
	***Hydrium* Complex and Others**						
4.44	*Hydrium* Complex	100	100	99	100	98	92
4.45	*B. levigatum* within *"Eurytrachelus"* [3]	99	95	98	96	62	68
4.46	*Andrewesa* + *Hydrium* Complex	95	69	64	-66	79	61
4.47	*Trechonepha* + *Liocosmius*	x	-69	x	-74	93	81
4.48	*Philochthus* + *Philochthemphanes*	97	96	93	92	65	64

[a] *Lionepha* outside of the smallest clade containing all *Bembidion* + *Asaphidion* or *Lionepha* sister to a non-*Bembidion*.
[b] Nearctic *Ocydromus* Clade: *B. actuosum*, *B. plagiatum*, *B. texanum*, *B. vernale*, *B. sejunctum*, *B. commotum*, *B. nebraskense*, plus the *B. transversale* group.
[c] *B. levigatum* as sister to part, but not all, of "*Eurytrachelus*".

had fewer clades shared between the I + Γ tree and Γ tree. The percentage of shared clades ranged from 57% (18S) and 59% (ArgK) to 78% (Topo) and 87% (CAD). This does not indicate as much disparity caused by model differences as one might at first suppose, as very near-optimal trees differ from the optimal ones by a large amount as well. For example, the optimal ArgK I + Γ tree (ln likelihood = −14301.60) shares only 59% of its clades with the second best ArgK I + Γ tree (ln likelihood = −14302.57) found in a different search replicate, the same percentage of clades shared between the best I + Γ and best Γ tree.

3.3. Simulation studies

Of the 268 clades present in the optimal likelihood tree used as the model for the simulation study (i.e., the tree shown in Fig. 8), all but one clade were inferred correctly in the maximum likelihood bootstrap tree based upon the simulated data. Only the clade of *Trepanedoris* + *Peryphodes* was missed in the 50% bootstrap tree, as it was present in only 44.6% of the bootstrap replicates. Thus, the tree is self-predicting, at least in this single test.

3.4. Nuclear copies of COI

Several chromatograms obtained for COI show distinct double peaks at isolated sites, indicating that there are two nucleotides present in the underlying amplified sequence at each of those sites. This suggests that there is a heterogeneous pool of COI sequences in the sample. In addition to the mitochondrial copy of COI, it is likely that the other copy or copies are in the nucleus, that is, they are "numts" (Thalmann et al., 2004).

Among COI sequences acquired for this work, evidence for numts is found in multiple species, scattered throughout sampled species. Outside of *Bembidion*, numts are apparently present in *Sinechostictus* (*Pseudolimnaeum*) sp. 3, *Amerizus* (*Amerizus*) sp.,

Table 5
Support for various groups based on analyses of individual genes. Dashes indicate no support for or against the clade because of insufficient taxon sampling for that gene. "±" indicates that the group is a clade in the optimal tree if the placements of *Trechonepha* and *Andrewesa* are ignored, otherwise it is not a clade. See the legend of Table 4 for more explanation.

		CAD		*wg*		ArgK		Topo		28S		18S		COI	
	number of taxa	*270*		*270*		*270*		*207*		*270*		*159*		*216*	
		ML	P	ML	P	ML	P	ML	P	ML	P	ML	P	ML	P
	Boundary of Bembidiina, non-*Bembidion* genera														
5.1	Bembidiina (exclusive of *Bembidarenas*, *Phrypeus*)	77	65	x	x	x	x	✓	✓		x	x		x	x
5.2	Bembidiina (inc. *Bembidarenas*)	-59	-61	x	-68	x	x	✓	x		-69	x		x	x
5.3	Bembidiina (inc. *Phrypeus*)	53	-61	x	x	x	x	x	x		-69	✓		x	x
	Boundaries of *Bembidion*, placement of *Lionepha*														
5.4	*Asaphidion* + all *Bembidion* other than *Lionepha* (*B. obtusum* optional, can include other Bembidiina)	57	83	x	x	x	x	59	72	x	x	✓	✓	x	
5.5	*Lionepha* within non-*Bembidion* clade or grade	57	83	x	50	✓	✓	59	72	x	✓	54	✓	✓	
5.6	*Bembidion* (exc. *Lionepha,* inc. *B. obtusum*)	-66	-86	x	x	x	x	x		x	x	x		x	
5.7	*Bembidion* (exc. *Lionepha*, exc. *B. obtusum*)	-57	-83	x	x	x	x	x	-56	x	x	x	x	x	
	Major structure within *Bembidion*														
5.8	*Bembidion* Series	90	97	✓	✓	x	x	x	x	x	✓	51	58	x	x
5.9	*Ocydromus* Series	89	90	x	x	x	✓	x		✓	✓	93	96	x	x
5.10	*Odontium* Series	83	84	✓	73	x	x	✓		✓		x		x	
5.11	*Ocydromus, Odontium* Series + *Hydrium, Plataphus* Complexes + *Trichoplataphus, Melomalus, Liocosmius, Trechonepha, Andrewesa*	x	x	x	x	x	x	x	x	x	x	x	x	x	
5.12	As 5.11 but with the only non-*Bembidion* in the analysis being *Asaphidion*	✓	✓	67	66	x	x	±	✓	x		x	x	x	x
5.13	*Ocydromus* Series + *Plataphus* Complex + *Trichoplataphus*	✓		x	x	x	x	x		x	✓	✓		x	
	***Bembidion* Series**														
5.14	*Bembidion* Complex	91	70	✓	64	x		x		x	x	x		x	
5.15	*Bembidion* + *Cyclolopha* + *Notaphocampa* + *Sloanephila*	81	78	✓	90	✓	✓	82	88	✓	x	x		x	
5.16	*Ananotaphus* Complex	81	92	✓	60	x	x	x	x	x	x	x		x	
5.17	*Ananotaphus (sensu* Darlington) + *Zecillenus*	81	87	✓	87	x	x	x	x	x	✓	x		✓	✓
5.18	*Zeplataphus* + *Zemetallina*	99	100	✓	51	✓	✓	-56	-60	✓	64	✓		x	
5.19	*Furcacampa* + *Neobembidion*	88	88	✓	91	✓	x	x		85	81	97	96	51	56
5.20	*Bembidion* Complex + *Ananotaphus* Complex + *Furcacampa* Complex + Notaphemphanes + *Trepanes* + *Emphanes*	92	84	✓	x	✓	x	x	x	x		x		x	
5.21	*Notaphus*	90	95	✓	89	x	x	x	x	✓		77	72	x	x
5.22	*Nothocys*	99	100	94	95	51	52	58	65	x		97	84	x	
5.23	*Notaphus* + *Nothocys*			x	x	x	x	x	x	x		✓		x	x
5.24	*B. rapidum* species group	99	100	99	100	69	96	83	82	x	x	60	58	82	75
5.25	N. Hemisphere *Notaphus*	x		x	x	✓	x	x	x	52	53	x		✓	
5.26	N. Hemisphere *Notaphus* + *B. rapidum* group	96	94	✓	✓	x	x	✓	✓	✓	✓	✓	✓	x	
5.27	Paraphyly of S. American *Notaphus*	99	99	✓	✓	x		x	x	✓	✓	51	57	x	
5.28	*Antiperyphanes* Complex	84	79	x	x	✓	✓	✓	55	x	x	x		x	x
5.29	*Diplocampa* Complex	99	100	✓	57	88	97	89	95	x		76	70	51	70
	***Odontium* Series**														
5.30	*Hydriomicrus* Complex	80	73	x	✓	x	x	68	72	90	91	✓		x	
5.31	*Odontium* Complex (inc. *Microserrullula*)	92	85	✓	76	x	x	✓		✓	✓	✓		x	
5.32	*Odontium* Complex (exc. *Microserrullula*)	63	81	x	x	x	x	x	✓	x		x		x	

Table 5 (*continued*)

		CAD		*wg*		ArgK		Topo		28S		18S		COI	
	number of taxa	*270*		*270*		*270*		*207*		*270*		*159*		*216*	
		ML	P	ML	P	ML	P	ML	P	ML	P	ML	P	ML	P
	***Ocydromus* Series**														
5.33	*Princidium* Complex (inc. *Cillenus*)	96	89	96	96	87	91	62	62	✓	✓	61	55	x	✓
5.34	*Ocydromus* Complex	80	73	✓	✓	x	x	✓		x	x	x		x	
5.35	Nearctic *Ocydromus* Clade	68	73	✓	✓	x		x	x	x	x	x	55	67	88
5.36	Nearctic *Ocydromus* Clade + *B. tigrinum*	64		✓	53	x	x	✓	62	✓	✓	98	99	64	
	***Plataphus* Complex**														
5.37	*Plataphus* Complex	55		✓	✓	✓	58	x		✓	✓	99	99	58	✓
5.38	*Plataphus* Complex + *Melomalus*	x		x	x	x	x	x		x	x	x		x	
5.39	*Plataphus* + *Plataphodes*	✓		✓	✓	x		✓	51	✓	✓	x		x	x
5.40	*Plataphus* exc. *Plataphodes*	-77	-54	x	-51	x	x	x		-61	-66	-56	-51	x	x
5.41	*Plataphodes+B. stillaguamish+B. gordoni+B. rufinum+B. simplex*	77	54	✓	51	✓	✓	✓		61	66	x		✓	✓
5.42	*Plataphodes*	x		x	x	x		x		x	-54	x		✓	
5.43	*Plataphus* Complex + *Trichoplataphus*	x		✓	✓	x	x	x		x		x		x	
	***Hydrium* Complex and Others**														
5.44	*Hydrium* Complex	-65	53	✓		✓	✓	86	85	98	98	85	79	✓	x
5.45	*B. levigatum* within *"Eurytrachelus"*		86	✓	✓	✓	✓	64				-	-	-	-
5.46	*Andrewesa* + *Hydrium* Complex	-66		✓	54	x	x	x	x	x		x	x	x	x
5.47	*Trechonepha* + *Liocosmius*	52	60	x	x	x		x	x	83	88	x		✓	
5.48	*Philochthus* + *Philochthemphanes*	73	69	✓	✓	x	x	x	x	x		✓		✓	

Table 6
Comparison of maximum-likelihood bootstrap trees between analyses using a Γ rate variation model and an I + Γ rate variation model. Trees showing clades with bootstrap values ⩾ 50% were compared. For example, for the CAD matrix, the 50% bootstrap tree from the I + Γ analysis had 178 clades, the Γ tree had 173 clades, 171 of which were shared between the two analyses. Thus, 7 of the clades in the I + Γ tree were not present in the Γ tree, and 2 clades in the Γ tree were not present in the I + Γ tree. The maximum bootstrap value of the 7 clades that were present in the I + Γ tree but not the I + Γ tree was 54.2. Among the clades that are present in both trees, the average value of the absolute difference in bootstrap values is 1.66 percentage points. The maximum difference in the bootstrap value for I + Γ tree minus that for the Γ tree is 12.6 percentage points, and the minimum is −16.9 percentage points; thus, there is one clade for which the bootstrap percentage for the I + Γ tree is 12.6 more than for the Γ tree, and another for which the bootstrap percentage for the Γ tree is 16.9 more than the I + Γ tree. The majority of differences are much less than these extremes (see Figs. 13 and 14).

Matrix	Number clades in I + Γ tree	Number clades in Γ tree	Number clades in common	Maximum MLB of missing clade	Average absolute MLB difference	Maximum MLB difference	Minimum MLB difference
CAD	178	173	171	54.2	1.66	12.6	−16.9
wg	123	118	118	55.2	1.61	7.9	−6.5
ArgK	106	110	103	66.1	1.85	18.0	−10.6
Topo	104	101	99	58.9	1.80	9.5	−7.3
28S	113	111	111	52.6	0.77	2.7	−2.8
18S	66	65	64	51.4	1.62	5.3	−13.0
COI	69	67	65	56.8	1.79	11.1	−8.2
All Data	223	222	216	60.9	1.19	7.2	−14.5
			Minimum	51.4	0.77	2.7	−2.8
			Maximum	66.1	1.85	18.0	−16.9

Geocharidius, and *Merizodus* sp. nr. *catapileanus*. Within *Bembidion*, numts were found in the subgenera *Peryphus* (*B. sejunctum*), *Bembidionetolitzkya* (*B. geniculatum*), *Hydriomicrus* (*B. brevistriatum*), *Notaphus* (*B. dejectum* and *B. contractum*), *Zemetallina* (*B. parviceps*), *Notaphemphanes* (*B. ephippium*), and *Hoquedela* (*B. cf. csikii*). Numts may be present in other taxa, but if so, they were not sufficiently amplified to be obvious.

3.5. Morphological results

Two of the morphological characters examined, shape of the hind margin of the pronotum and presence of a crista clavicularis, proved difficult to score, as the characters were not binary, but entailed complex, continuous variation, with minimal gaps between states. The notch on the hind margin of the pronotum varied from obvious, and large, as in Fig. 7B, to absent, as in Fig. 7A, with a broad spectrum in between. Some species (e.g., *B.* (*Testedium*) *laetum* and *B.* (*Nothonepha*) sp. nr. *lonae*) have a slight notch, others a hint of a notch (e.g., *B.* (*Liocosmius*) *mundum*, *B.* (*Trepanes*) *octomaculatum*); others have modified prothoraces (e.g., *B.* (*Nepha*) spp.), or were lacking setae to mark the hind angle (e.g., *B.* (*Zeplataphus*) *maorinum*), or have prothoraces so constricted as to allow no room for a notch (e.g., most *Ananotaphus* Complex members), such that coding the character was fraught with complexity. Similarly, the crista clavicularis ranged from absent (e.g., in subgenus *Plataphus*), to being a distinct, complete fold, extending from the end of the

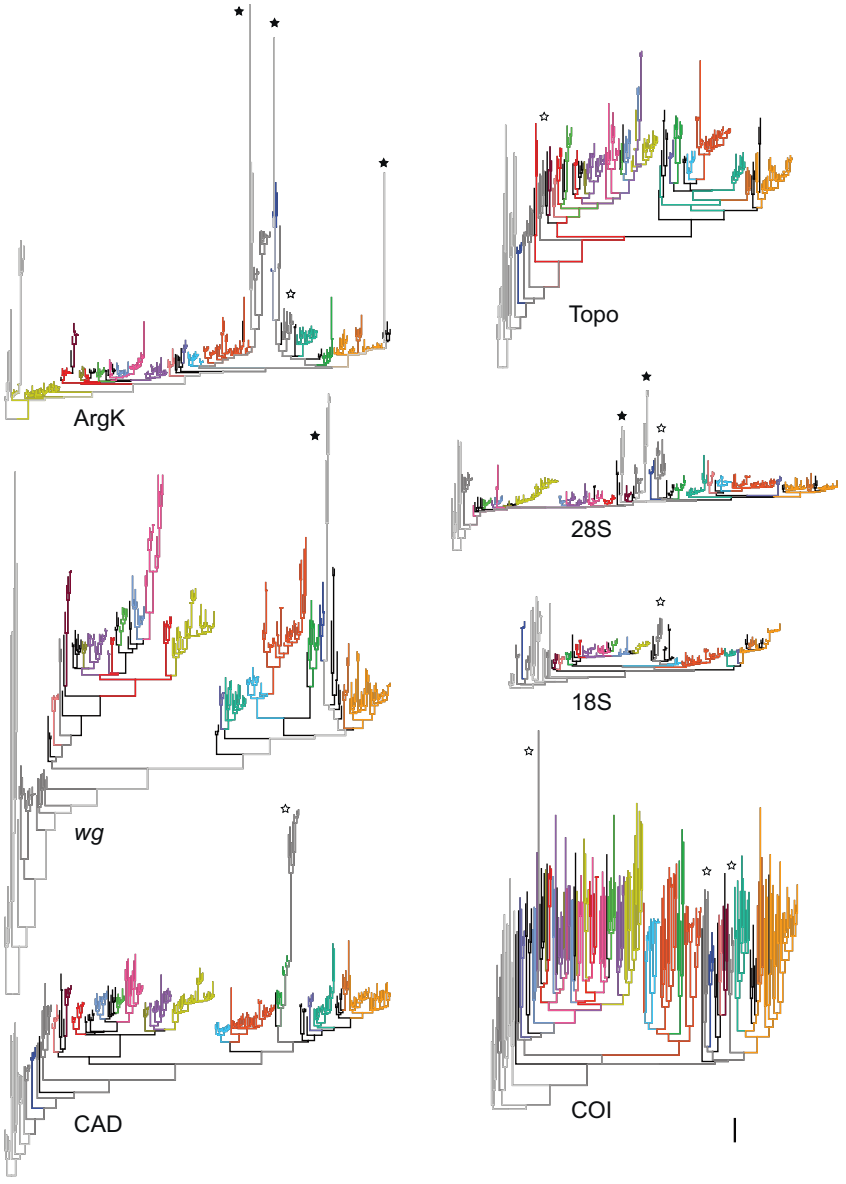

Fig. 10. Maximum likelihood trees under the I + Γ model for individual genes. Color of branches matches those in Fig. 8. All genes are to the same scale, with branch lengths as reconstructed by RAxML. Scale bar: 0.1 substitutions per site. Stars indicate non-*Bembidion* placed within *Bembidion* as here defined; open stars are members of Bembidiina, and black stars are non-Bembidiina. Complete illustrations of these trees, with taxon names, are in the Supplementary content.

elytral bead to the articulation point of the elytron with the mesothorax (e.g., in *Bembidion s. str.*). But there were many intermediates, of different forms. In some species, there is a short ridge that extends about half the distance from a denticle at the end of the elytral bead to the articulation; in others, it extends about a quarter the distance; in yet others, there is miniscule ridge; and in others, there are states in-between. It varied in both length, and in depth; in some, the ridge was very low, barely visible. In *Semicampa*, there is variation within species. For example, in *B. semicinctum*, some specimens have a distinct, complete, but faint and very low ridge; in other specimens, the ridge is effaced except for a very short region near the end of the elytral bead.

Because of the difficulty of coding, the shape of the hind margin of the prothorax and nature of the crista clavicularis were not recorded for all taxa, and were only scored across a selection of species sufficient to indicate that they are highly homoplastic. A pronotum with a sinuate and notched hind margin has arisen in *Philochthus* (with a similar prothorax shape in *B. wickhami* being possibly homologous), the *Bembidion* Complex (*Cyclolopha* and *Sloanephila*), and outside *Bembidion* in *Ocys harpaloides*. Sinuate and notched hind margins on narrower prothoraces (which therefore have, by necessity, narrower hind margins and shorter notches) have independently arisen at least twice elsewhere in the *Bembidion* Series (e.g., *B.* (*Nothonepha*) sp. nr. *lonae*, *Bembidion s. str.*), and in the outgroup *Bembidarenas setiventre*. A distinct fold that might be termed a crista clavicularis has an even more scattered distribution. For example, it is present in some but not all members of the *Ocydromus* Complex (e.g., *B.* (*Ocydromus*) *vernale* but not *B.* (*Ocydromus*) *mexicanum*), the *Princidium* Complex (e.g., *B.* (*Testedium*) *laetum* but not *B.* (*Paraprincidium*) *ruficolle*), the *Antiperyphanes* Complex (e.g., *B.* (*Antiperyphus*) *rufoplagiatum* but not *B.* (*Nothonepha*) *lonae*). The complexity of variation in the crista clavicularis has also been noted by Bonavita and Vigna Taglianti (2010).

The position of seta ed3 was scored on all 270 species (Supplementary content Table S4), but it too showed continuous variation. It varies from fully within a stria to being in the center of the interval between striae, and states in between. Toledano (2005) noted the difficulty in scoring this character, in part as the position can

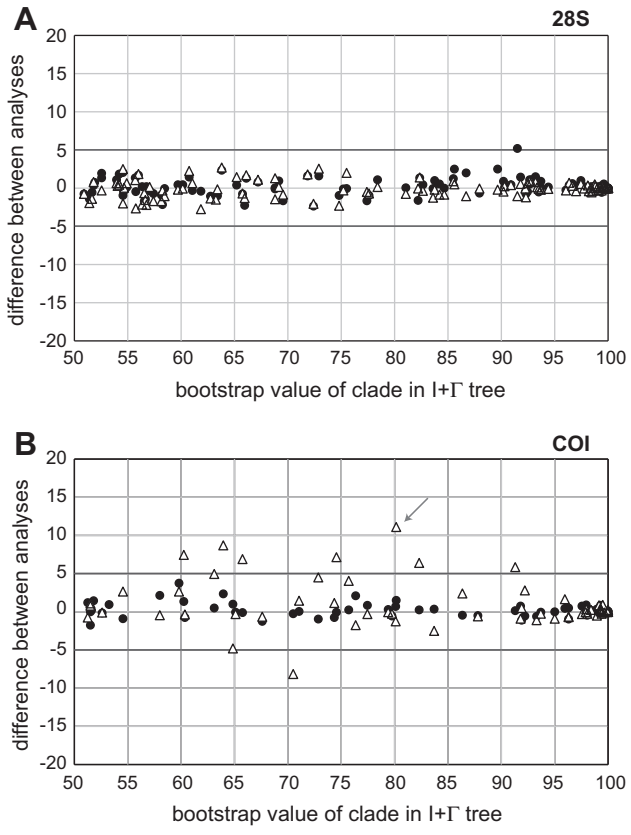

Fig. 11. Differences in percentage bootstrap values between analyses for both (A) 28S and (B) COI. Each open triangle represents a clade that is present in both maximum likelihood bootstrap tree using an I + Γ model and the maximum likelihood bootstrap tree using a Γ model. The x-axis position of an open triangle is the bootstrap value for that clade in the I + Γ tree, and the *y*-axis position is the bootstrap value in the I + Γ tree *minus* the bootstrap value in the Γ tree. Thus, the clade represented by the triangle indicated by the arrow in (B), which is the *B. honestum* + *B. integrum* + *B. rufotinctum* clade of the subgenus *Pseudoperyphus*, has a bootstrap value of 80% in the I + Γ bootstrap tree, and this value is 11% higher than the bootstrap value for the same clade in the Γ bootstrap tree. Clades with symbols below 0 have higher bootstrap support in the Γ bootstrap tree than the I + Γ bootstrap tree. Closed circles are from a similar comparison, but in which the two trees being compared are from independent I + Γ bootstrap analyses starting with different seeds. An equivalent plot for CAD is shown in Fig. 12A; among the other genes, *wingless* and topoisomerase have plots similar to that shown here for COI, arginine kinase has a plot similar to that shown for CAD in Fig. 12A, and 18S has a plot with less scatter in the I + Γ/Γ comparisons than COI, but more so than 28S.

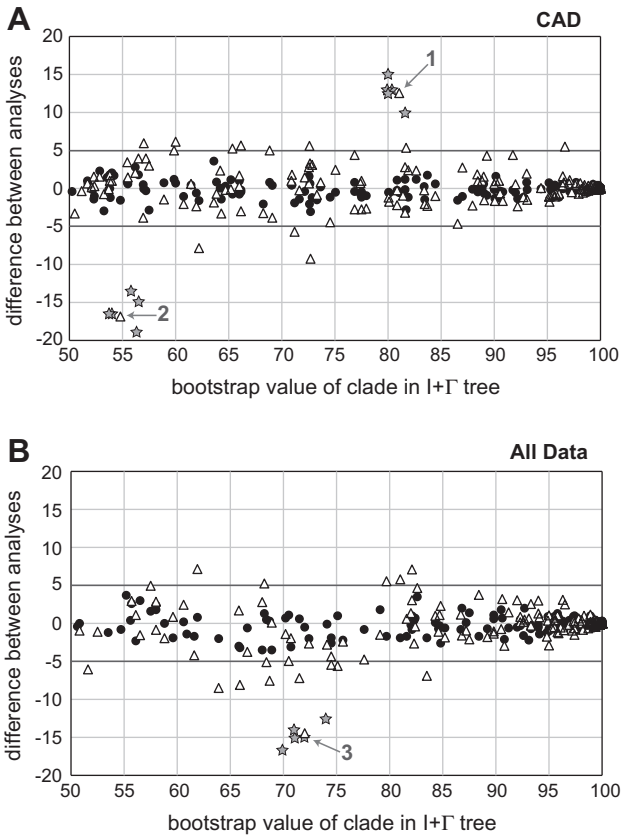

Fig. 12. Differences in percentage bootstrap values between analyses for both (A) CAD and (B) all 7 genes combined. See caption of Fig. 11 for more explanation. In (A), the gray stars around clades 1 (*Lionepha disjuncta* + *L. osculans*) and 2 (*Oopterus* + *Sloaneana*) show sampling variation around those points, as inferred from 5 additional paired analyses, i.e., each star represents a novel I + Γ bootstrap run and a novel Γ bootstrap run, all from different seeds. In (B), the gray stars around clade 3 (*Diplochaetus* + *Thalassotrechus*) similarly show sampling variation around that point.

appear different depending upon whether one looks at the dorsal or ventral surface of the elytron, and in part because of variation within species (and between the two elytra of single specimens). This character was scored as fully in the third stria, near the third stria (within two diameters of the pore around the seta from the stria), or in the interval between striae. For purposes of estimating number of transitions on the tree, the "near stria" scores were converted to missing data so as to produce a conservative estimate of evolutionary changes. The estimated number of changes in this character (that is, from ed3 fully in the interval to fully in the stria, or *vice versa*) is at least 9 (Fig. 13A).

The shape of the elytral bead at the shoulder, whether angulate or not, has states that are somewhat more distinctive. Some species (e.g., *Bembidon* (*Bracteon*) *alaskense*) have shoulders that were only slightly angulate, but most species had shoulders that were either smoothly rounded or distinctly angulate. For purposes of estimating the minimum frequency of origins or losses of angulation, the third state (elytral bead sinuate at shoulder) was recoded as missing data. The estimated number of changes in this character (to or from an angulate shoulder) is at least 3, with an average over bootstrap replicates of the minimum number of changes being 4.8 (Fig. 13B).

4. Discussion

4.1. Phrypeus, Bembidarenas, and the boundaries of Bembidiina

The subtribe Bembidiina has traditionally consisted of all Trechitae with small apical palpomeres that do not also have the derived characters of Tachyina, Xystosomina, Anillina, or Trechini; so defined, one might expect the group to be non-monophyletic. This sense of Bembidiina includes *Phrypeus* (Fig. 6J) and *Bembidarenas* (Fig. 6K and L), which differ in many regards from other bembidiines, and indeed adults have characteristics (such as long, parallel frontal furrows extended posteriorly) that suggest they belong nearer trechines than Bembidiina. Larvae of *Phrypeus* have characteristics suggesting they are outside of Bembidiina (Grebennikov and Maddison, 2005), a result corroborated by molecular phylogenetic analysis of Trechitae based on 18S rDNA, 28S rDNA, and the *wingless* gene (Maddison and Ober, 2011). Morphological data also suggests *Bembidarenas* does not belong with other Bembidiina. Three larvae of *Bembidarenas setiventre* from Argentina (Neuquén: Arroyo Queñi at Lago Queñi), identified by matching 28S rDNA sequences to adults (data not shown) have two claws on their legs, a plesiomorphic state in Trechitae, in contrast to the derived, single claw that is present in a group of Trechitae including Bembidiina (Grebennikov, 2008; Grebennikov and Maddison, 2005).

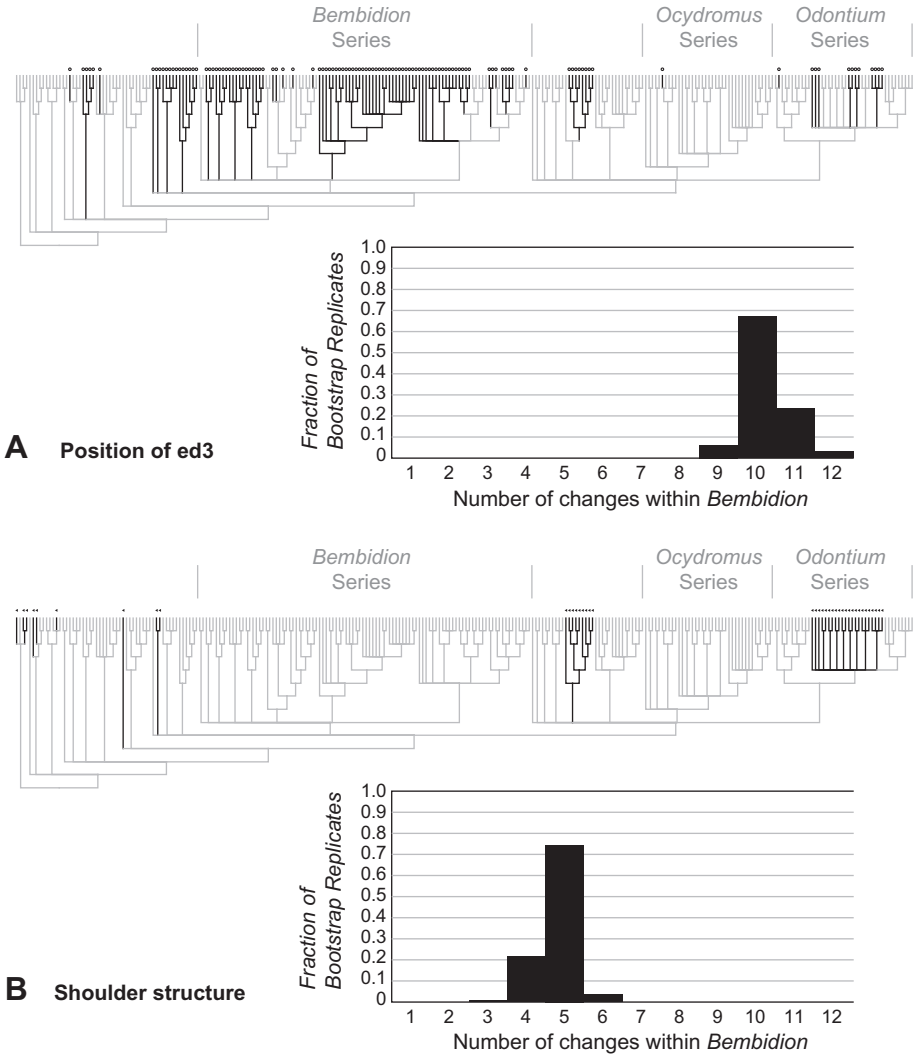

Fig. 13. Parsimony reconstructions of two morphological characters. Each character is shown mapped onto the AllData ML bootstrap tree (only showing clades of MLB ⩾90). Each chart shows the distribution of minimum number of reconstructed changes within *Bembidion* in the character over all 1000 trees from the bootstrap replicates; for this calculation, outgroup states were set to missing data. (A) Position of ed3. The black lines in the tree indicate lineages with ed3 clearly in the third interval, far from a stria. Average number of changes over the bootstrap replicates is 10.2. (B) Structure of elytral bead at shoulder, whether angulate or not. The black lines in the tree indicate lineages with the bead distinctly angulate. Average number of changes over the bootstrap replicates is 4.8.

The seven genes analyzed here provide strong evidence for monophyly of Bembidiina exclusive of *Phrypeus* and *Bembidarenas*, with an MLB of 98 and PB of 90 for the seven-gene analyses (Table 4.1). The evidence comes primarily from CAD and topoisomerase (Table 5.1), although the most-parsimonious trees of the RiboMito matrix also shows this group to be monophyletic. The five genes other than CAD and topoisomerase, when analyzed separately, provide weak evidence against monophyly.

The data also provide evidence against inclusion of both *Bembidarenas* (Table 4.2) and *Phrypeus* (Table 4.3) in Bembidiina; inclusion of these genera is contradicted by an alternative clade including other tribes or subtribes, with MLB = 95 for *Bembidarenas*, and MLB = 82 for *Phrypeus*. Three genes individually speak out against inclusion of *Bembidarenas* in Bembidiina (Table 5.2), but the individual-gene evidence for *Phrypeus* is ambiguous (Table 5.3).

4.2. Relationships of Lionepha

Lionepha (Fig. 6E and F) contains nine described species that live from California north to Alaska, east to Colorado, with a center of diversity in the Pacific Northwest. They are common, small, mostly black or dark brown beetles that live under leaf litter on moist ground in open habitats in clearings in forests, under rocks in grasslands, and on the banks of streams. They look very much like typical members of the genus *Bembidion*, in which they have been classified until now.

The genes sequenced provide strong evidence that *Lionepha* does not belong within *Bembidion*, and that it falls outside the clade containing *Bembidion* and *Asaphidion. Asaphidion* is a Holarctic genus of about 40 species containing adults with large eyes and atypical elytra (Fig. 6B and C). A clade containing *Asaphidion* plus all *Bembidion* exclusive of *Lionepha* is very well supported by the combined analyses of all seven genes, with a bootstrap value of 99% for both likelihood and parsimony analyses (Table 4.4). It is strongly supported by the nuclear protein-coding genes alone (93 MLB, 94 PB), and independently supported by the RiboMito matrix (71 MLB). For the nuclear protein-coding genes, there is signal even at the amino acid level: the maximum likelihood tree (Supplementary content Fig. S8) and most parsimonious trees show this group as a clade.

Analysis of individual genes indicates that evidence for a clade including *Asaphidion* + *Bembidion* exclusive of *Lionepha* comes from multiple sources. Although only two of the genes (topoisomerase and 18S) individually support this clade, and those only

moderately or weakly (Table 5.4), the separate signals corroborate each other. CAD supports more strongly a clade of *Asaphidion* + *Bembidion* + *Sinechostictus* (57 MLB, 83 PB), again to the exclusion of *Lionepha*.

The other four genes (*wingless*, ArgK, 28S, COI) show contradictory relationships, with optimal trees having *Lionepha* contained within *Bembidion*, although this is not supported with bootstrap values greater than 50. Notably, for all four of these genes, *Lionepha* is joined within *Bembidion* by some outgroups or non-*Bembidion* Bembidiina: in *wingless*, *Lionepha* is sister to Zolini in the ML tree (Supplementary content Fig. S4B); in ArgK, *Lionepha* is in a clade that also contains *Asaphidion*, *Ocys*, *Bembidion obtusum*, and one of the anillines (Supplementary content Fig. S5A); in 28S, *Lionepha* is in a grade with Zolini, *Amerizus*, *Ocys*, and *Asaphidion* (Supplementary content Fig. S6A); in COI, *Lionepha* is sister to *Asaphidion* (Supplementary content Fig. S7). Thus, even in these four genes, there is some signal that *Lionepha* belongs with taxa now considered outside of *Bembidion* (Table 5.5). The placement within *Bembidion* of this complex differs from gene to gene: in *wingless*, it is with the *Ocydromus* Series and a few smaller lineages; in ArgK, it is with the *Plataphus* Complex and subgenus *Trechonepha*; in 28S, it is near the base of most *Bembidion* other than the *Bembidion* Series; in COI, it is with a small group including the subgenera *Eupetedromus* and *Philochthus*. The variability of placement of *Lionepha* in optimal trees for these four genes, and its association with outgroups or non-*Bembidion* Bembidiina, suggests that inclusion within *Bembidion* of *Lionepha* in these individual gene trees is likely an artifact. In fact, when analyzed together these four genes show the same pattern exhibited by individual analyses of CAD, topoisomerase, and 18S: *Asaphidion* + *Bembidion* exclusive of *Lionepha* form a clade, although this is only weakly supported (MLB = 51).

In summary, the support for *Lionepha* falling outside of a clade of *Asaphidion* + *Bembidion* comes from multiple analyses and sources. This signal comes from at least four sources independently: individually from analyses of CAD, topoisomerase, and 18S; combined analyses of the remaining four genes (ArgK, *wg*, 28S, COI). There is also very strong support indicated by the combined analyses of all seven genes.

There are no known morphological characters that support monophyly of *Asaphidion* plus *Bembidion* exclusive of *Lionepha*. Grebennikov (1997) and Grebennikov and Maddison (2005) report a larval synapomorphy of *Asaphidion* + *Bembidion* to be the close approximation of seta FR4 and FR5 on the head, a feature that *Sinechostictus* and other trechites lack. I have studied a second or third instar larva of *Amerizus* sp. (from the Abajo Mountains in Utah, identified by DNA matching), and first instar larvae of *Lionepha casta* (raised *ex ovo* from Marys Peak, Oregon). They also have FR4 and FR5 very close together, indicating that this derived state is a synapomorphy for a larger group of bembidiines that includes at least *Asaphidion*, *Bembidion*, *Lionepha*, and *Amerizus*, and so this trait is mute with respect to the placement of *Lionepha* relative to *Bembidion*.

There are, however, molecular synapomorphies. In arginine kinase, there is one nucleotide (site 168 in the analyzed matrix) that is uniformly C in the sequenced *Bembidion* and *Asaphidion*, but is G in all remaining Bembidiina, including *Lionepha*; the outgroups vary, being either C or G. In 18S, there are 3 synapomorphies of *Asaphidion* + *Bembidion* exclusive of Lionepha (sites 518, 551, and 560), and 2 of *Ocys* + *Asaphidion* + *Bembidion* (sites 1305 and 1310).

For all of these reasons, I remove *Lionepha* from *Bembidion*. An alternative change in the classification that could be considered is to broaden the scope of *Bembidion* to include *Lionepha*, but in so doing *Asaphidion* would also need to be merged with *Bembidion*. This change, against more than a century of tradition about a group of common European carabids, would be the more drastic move, especially given the notable differences in external structure between *Asaphidion* and *Bembidion* (Maddison, 1993).

4.3. Monophyly of Bembidion, and relationships of Bembidiina genera

The evidence presented above showing that *Lionepha* does not belong within *Bembidion* also excludes *Sinechostictus*, *Ocys*, and *Amerizus* from the genus. *Ocys* (Fig. 6I) and *Amerizus* (Fig. 6D) have been excluded previously (e.g., Lorenz, 2005; Marggi et al., 2003; Toledano, 2000), based on their overall dissimilarities to *Bembidion* rather than a cladistic analysis. *Sinechostictus* (Fig. 6G and H) has traditionally been placed within or near the *Ocydromus* complex (Antoine, 1955; Jeannel, 1941), with which they share a very similar appearance (compare, for example, Fig. 2H to Fig. 6G). However, larval characteristics (Grebennikov, 1997) suggest *Sinechostictus* falls outside of the *Bembidion* + *Asaphidion* clade, and the group has been formally removed from *Bembidion* (Ortuño and Toribio, 2005), an action supported by the molecular data presented here.

With *Sinechostictus*, *Ocys*, *Amerizus*, and *Lionepha* removed, the monophyly of *Bembidion* is still not assured. Although *Bembidion* so-delimited is monophyletic in the maximum likelihood $I + \Gamma$ tree (Fig. 8), it is not supported in bootstrap analyses of the seven-gene matrix (Table 4.6), and has no individual gene support (Table 5.6). However, there is support for the monophyly of *Bembidion* exclusive of *B. obtusum* (subgenus *Phyla*), with MLB = 94 and PB = 80 for the seven-gene analysis, with this support coming primarily from the four nuclear protein coding genes (Table 4.7); individual gene analyses provides some evidence against the monophyly of *Bembidion* exclusive of *Lionepha* and *Phyla* (Table 5.7).

The subgenus *Phyla* thus appears to be a phylogenetically important group, perhaps the sister group of remaining *Bembidion*. *Phyla* (Fig. 6A) contains nine species of small, dark *Bembidion* native to the western Palearctic (Dudko, 1999; Huber and Marggi, 1997). The subgenus was considered related to subgenus *Odontium* by Jeannel (1941), in part because of the angulate shoulder margin in adults. However, my results indicate that *Phyla* is distant from *Odontium*, and worthy of much more detailed study in the future.

4.4. Relationships among genera of Bembidiina

Among the six genera of Bembidiina sampled (*Sinechostictus*, *Ocys*, *Amerizus*, *Lionepha*, *Asaphidion*, and *Bembidion*), the evidence is weak for any particular relationship among them except for the presence of the above-mentioned *Asaphidion* + *Bembidion* clade. Relationships among the other genera are unresolved in the bootstrap trees (Fig. 9A), and vary among the optimal trees from analysis to analysis (Fig. 8A).

4.5. Major structure of Bembidion

The results support several large clades that include multiple subgenera. Those clades that include multiple subgenera I will call "complexes"; those that include multiple complexes are called "series".

One of the largest clades evident within *Bembidion* is the *Bembidion* Series, which includes *Bembidion* in the strict sense, *Notaphus*, and many other subgenera from around the world (Fig. 9B). This group includes a portion of Jeannel's (1941) *Bembidion* Series and *Notaphus* Series, and corresponds more or less to Perrault's (1981) delimitation of the genus *Bembidion*. It is not the most speciose series within *Bembidion*, but it is very diverse in form and color (Figs. 3 and 4). Many species within the group have relatively small adults, less than 4.5 mm in length, although there are large species in New Zealand and temperate South America. The *Bembidion*

Series as defined here is well supported by nuclear protein-coding genes (MLB = 99 and PB = 100 for the NucProt matrix) and independently, but to a lesser extent, the ribosomal plus mitochondrial genes (MLB = 88 and PB = 70 for the RiboMito matrix), with very strong support from the seven-gene matrix (Table 4.8). Much of this support comes from CAD, but *wingless* and 18S also show the *Bembidion* Series to be monophyletic (Table 5.8).

One of the more speciose clades in *Bembidion* is the *Ocydromus* Series (Fig. 2F–K), a group of over 450 species (Lorenz, 2005), but with less evident structural diversity than the *Bembidion* Series. The group includes the *Ocydromus* and *Princidium* Complexes, as well as the subgenus *Nepha* (Fig. 2K). *Bembidionetolitzkya* also belongs in this group, as indicated by Jeannel (1941) and Vigna Taglianti (1993), not in the *Plataphus* Complex as suggested by Toledano (2008a). *Ocydromus* Series adults are in general brown or black, and are relatively large, with most species over 4.5 mm in length—included here are the largest *Bembidion*. They are most diverse in the mountains of the Palaearctic. The group is almost restricted to the Northern Hemisphere, with the only species in the Southern Hemisphere being some high-elevation *Hypsipezum* on Mount Kenya in Africa, just south of the equator, and a few other *Ocydromus* Complex in eastern and southern Africa (Paolo Bonavita, pers. comm.), and some species in the mountains of Indonesia (Luca Toledano, pers. comm.). Monophyly of the *Ocydromus* Series is well supported by individual gene analyses of CAD and 18S, with lesser evidence from topoisomerase (Table 5.9), and very strong support from multigene analyses (Table 4.9).

The *Odontium* Series, consisting of the *Hydriomicrus* Complex (Fig. 2A) and *Odontium* Complex (Fig. 2B–E), is a relatively small group restricted to the Northern Hemisphere, except for subgenus *Microserrullula*, which straddles the equator. Evidence for monophyly of this series comes from CAD, *wingless*, topoisomerase, and 28S (Table 5.10), as well as combined analyses (Table 4.10). Thus, *Hirmoplataphus* belongs in the *Odontium* Series, not the *Plataphus* Complex as suggested by Toledano (2008a).

There is relatively weak evidence that most species of *Bembidion* outside the *Bembidion* Series form a clade. In particular, the species shown in Figs. 8C and 9C, that is, *Ocydromus* Series + *Odontium* Series + *Plataphus* Complex + *Hydrium* Complex + *Trichoplataphus* + *Melomalus* + *Liocosmius* + *Trechonepha* + *Andrewesa*, may form a clade. This very large group is supported as a clade with MLB = 95 and PB = 82 for the seven-gene, AllData matrix (Table 4.11), with this support coming mostly from the nuclear protein-coding genes. However, there is no evident support from any single gene (Table 5.11). This is in part because of the tendency of some outgroups to move within this group in the individual gene analyses (e.g., see Fig. 10, and Supplementary content Figs. S4–S7). In the analysis in which the outgroups are not included, and in which the only non-*Bembidion* included is *Asaphidion*, then this group is supported independently and unambiguously by both CAD and *wingless* (Table 5.12), with the group also present in the most-parsimonious trees for topoisomerase. As the existence of this clade is in part a function of exactly where the root of *Bembidion* is placed, its presence in three genes when the only non-*Bembidion* is *Asaphidion* provides relatively weak evidence, as removing the other genera from the analysis might reduce the ability of the root of *Bembidion* to be accurately inferred. Nonetheless, the possible existence of this large group raises the interesting possibility that *Bembidion* is deeply split between two huge clades.

Another smaller group, consisting of the *Ocydromus* Series + *Plataphus* Complex + *Trichoplataphus*, is supported by weak evidence. In the AllData analyses, MLB = 82 and PB = 53, with some support seen in both the NucProt and RiboMito matrices (Table 4.13). The clade is also present in analyses from three genes (the ML tree in CAD, MPTs in 28S, and ML tree in 18S), but there are weak contradictory results (Table 5.13).

The subgenera *Eupetedromus*, *Philochthemphanes*, *Philochthus*, and *B. wickhami* appear to be outside any of the major lineages, but their exact placement is unclear, and to this group could be added *Hoquedela* (Fig. 2L). The relationships of the latter are quite uncertain, but the first four mentioned may be related to the *Bembidion* Series: they group with it supported by MLB = 82 and PB = 65 for the AllData matrix. This clade is present with MLB $\geqslant$67 in all seven analyses in which a single gene was removed from the combined matrix, indicating that support for the clade comes from at least two genes. Single-gene analyses of CAD, *wingless*, and topoisomerase support the grouping, but ArgK, 28S, and 18S present an alternative, contradictory placement: in the large clade with the *Ocydromus* Series + *Odontium* Series mentioned above. Whether they go with the *Bembidion* Series or the rest of *Bembidion* is a function of the location of the root of *Bembidion*. The existence of support for both alternatives makes me cautious to propose with confidence that *Eupetedromus*, *Philocththemphanes*, *Philochthus*, and *B. wickhami* are related to the *Bembidion* Series.

4.6. Bembidion Series: Bembidion Complex

Within the *Bembidion* Series, there is a complex of subgenera around *Bembidion* (*s. str.*) that is primarily southern in distribution. The exception is the Holarctic subgenus *Bembidion* (Fig. 3A), which is the northernmost member of the complex. *Cyclolopha* (Fig. 3B) is centered in México (Perrault, 1982), with two species in the southwest USA, and two in Guatemala. The remaining subgenera in this group are Old World species, mostly in the Southern Hemisphere, with *Omotaphus* (Fig. 3C) in Madagascar and Africa, *Notaphocampa* from Africa north and east to the Indian subcontinent, south-east Asia, and Australia, and *Sloanephila* (Fig. 3D) from Australia (Toledano, 2005). Support for this amphitropical clade comes from three separate sources: individually from CAD and *wingless* (Table 5.14), and likelihood analysis of the RiboMito matrix (Table 4.14). The combined analysis of all genes shows this clade with MLB = 99 and PB = 98.

Within the *Bembidion* complex, *Omotaphus* is sister to the remaining four subgenera. The clade consisting of the four remaining subgenera is well supported by four separate genes (Table 5.15) and the combined analyses (Table 4.15).

Some members of the *Bembidion* Complex share a sinuate hind margin of the pronotum, notched such that the lateral corners are notably forward of the medial part of the hind margin (Fig. 7B). *Cyclolopha* and *Sloanephila* have this most distinctly; *Bembidion* s. str. is similar, but with a narrower notch, and both *Notaphocampa* and *Omotaphus* are variable in that regard. The notched and sinuate hind margin of *Sloanephila* led Darlington (1962) to consider it related to *Philochthus*, a Palaearctic subgenus with a similar pronotum, and Toledano (2005) considered *Notaphocampa* and *Sloanephila* to be sisters (as strongly supported here, Fig. 9B), with that pair being sister to *Philochthus*. However, the DNA sequence data definitively places *Philochthus* outside of the *Bembidion* Series, far from *Sloanephila*, indicating that the sinuate pronotal base is independently derived in the two groups.

The Southern Hemisphere members of this group, all in the Old World, very much resemble some members of the subgenus *Notaphus*, a group with a complementary distribution. The similarities in body form and color led Jeannel (1946) to classify *Omotaphus* within *Notaphus*, and the similarity between *B.* (*Sloanephila*) *jacksoniense* (Fig. 3D), *Notaphocampa*, and *B.* (*Notaphus*) *rapidum* (Fig. 4K) is notable. Toledano (2005) suggested that *Notaphocampa* and *B.* (*Notaphus*) *rapidum* are not related, and that their shared color patterns are symplesiomorphies. My results confirm their independence, and show clearly that *Notaphus* and the Old World, Southern Hemisphere "*Notaphus*-like" *Bembidion* are only distantly related. However, it is much more likely that their similar color

patterns (metallic green, with a pale yellowish band at the back of the elytra) are a result of convergence, as *B. rapidum* and *Notaphocampa* are distantly enough related that presuming the color pattern to be symplesiomorphic would require many losses in neighboring lineages.

4.7. Bembidion Series: the Ananotaphus Complex of New Zealand and Australia

The *Bembidion* fauna of New Zealand contains 36 described, endemic species, arrayed in six subgenera (Lindroth, 1976, 1980), and include species living along bodies of water, such as gravel shores of rivers. Many (such as *B.* (*Zemetallina*) *anchonoderum*, Fig. 3K) resemble Northern Hemisphere species in similar habitats. The subgenus *Zecillenus* (Fig. 3J), which lives on the shores of estuarian streams (Larochelle and Lariviere, 2001), was originally described as a separate genus, but was recently shown to belong within *Bembidion* (Maddison and Ober, 2011) based on DNA sequences, and there is some evidence from male genitalic and pronotal structures that it belongs near the Australian subgenus *Ananotaphus* and the Hawaiian subgenus *Nesocidium* (Liebherr, 2008; Toledano, 2005). Relationships of the New Zealand species have otherwise not been closely examined.

The fauna of Australia is depauperate for such a large, temperate landmass, with only 10 endemic species (Toledano, 2005). In addition to the African/southeast Asian/Australian subgenus *Notaphocampa* and the southeast Asian/Australian *Sloanephila*, and some intertidal species similar in form to subgenus *Cillenus*, there is a group of four species, including the sampled *B. ateradustum* (Fig. 3I), *B. proprium*, and *B. errans*, previously considered to belong together in the subgenus *Ananotaphus* (Darlington, 1962). In examining the Australian fauna, Toledano (2005) concluded that the group was polyphyletic, and that three lineages were represented. He created two new subgenera, *Gondwanabembidion* and *Australoemphanes*, to house two of the species. He proposed that *B.* (*Australoemphanes*) *ateradustum* is closer to the Holarctic subgenus *Emphanes* than to the remaining Australian species.

My results suggest that species formally considered as members of *Ananotaphus* (*Ananotaphus sensu* Darlington) form a clade along with *Zecillenus* (Fig. 9B), a result supported by individual analyses of three genes (Table 5.17). In addition, multigene analyses (Table 4.17) show strong support from both the nuclear protein-coding genes (MLB = 97, PB = 99) and the RiboMito matrix (MLB = 95, PB = 80). This confirms the proposals by Toledano (2005) and Liebherr (2008) that *Ananotaphus* and *Zecillenus* are related. In addition, it is clear that the morphological diversity noted by Toledano as evidence for the polyphyly of *Ananotaphus sensu* Darlington is instead the result of lineages in an independent, Australian-New Zealand radiation converging upon similar forms living elsewhere, such as Holarctic *Emphanes*.

I recommend that *Ananotaphus*, *Zecillenus*, *Gondwanabembidion*, and *Australoemphanes* be merged into one subgenus, but, in the interests of nomenclatorial stability, do not do so until Hawaiian *Nesocidium* can be studied further. If they should also belong to this group, as Liebherr (2008) suggests, then *Nesocidium* would be the valid name. For the remainder of this paper, I will term this group "*Ananotaphus sens. lat.*".

The New Zealand subgenera *Zemetallina* and *Zeplataphus* form a clade, well supported by the combined analyses (Table 4.18) and individual analyses of four genes (Table 5.18), although a fifth gene, topoisomerase, shows bootstrap support for an alternative arrangement, that is, against monophyly. I have not sampled the subgenera *Zeactedium*, *Zeperyphus*, and *Zeperyphodes* from New Zealand, but I speculate that they are part of the same clade, based upon genitalic similarities (Lindroth, 1976).

The New Zealand clade and *Ananotaphus sens. lat.* are sister groups, forming a clade endemic to Australia and New Zealand (and, potentially, Hawaii, should *Nesocidium* belong here), the *Ananotaphus* Complex, with MLB = 100 and PB = 100 for the seven-gene analyses (Table 4.16) and support individually from CAD and *wingless* (Table 5.16).

4.8. Bembidion Series: Furcacampa Complex

One of the more taxonomically difficult groups of *Bembidion* is the Nearctic subgenus *Furcacampa*, which includes very small, mottled *Bembidion* (Fig. 3E) which live around bodies of water, including temporary pools. They are especially diverse in the southwestern USA. The frontal furrows on the dorsal surface of the head of species in this group vary from simple and shallow, to deep and anteriorly convergent. The latter group of species, centered around *B. cognatum*, has been considered a separate group of uncertain placement (Erwin, 1982), but they belong within *Furcacampa* (Fig. 9B).

The sister group of *Furcacampa* is the subgenus *Neobembidion*, a recently created subgenus housing the three species of the North American *Bembidion constricticolle* group (Fig. 3F; Bousquet and Webster, 2006). The endophallus of the male genitalia of *Neobembidion* is almost identical in structure to that of *Furcacampa*, and thus their relationship is not surprising; it may be more appropriate to consider these as two species groups within one subgenus, an action I would recommend if study of additional members of *Neobembidion* confirm the relationship proposed here.

4.9. Bembidion Series: A larger group containing the Bembidion, Ananotaphus, and Furcacampa Complexes

There is some evidence that the *Bembidion* Complex, *Ananotaphus* Complex, *Furcacampa* Complex, plus three other subgenera (*Emphanes*, *Notaphemphanes*, and *Trepanes*) form a clade. This is supported in the seven-gene analysis with MLB = 100 and PB = 96, with all of the evidence coming from nuclear protein-coding genes (Table 4.20). Most of the signal is in CAD, although there is support for this clade in the ML trees for *wg* and ArgK.

4.10. Bembidion Series: Notaphus and Nothocys

Notaphus is a large subgenus with many species, most with mottled patterns on the elytra, relatively dull luster, and slightly metallic in part (e.g., Fig. 4J). They typically inhabit open shorelines of lakes, ponds, and rivers, often at low elevation (e.g., Fig. 1A and B). It is the most widespread subgenus of *Bembidion*, especially diverse in North and South America, with some species in the Palearctic. It is one of the few groups of *Bembidion* with equatorial species at sea level (e.g., *Bembidion* (*Notaphus*) *commissum* Erichson in coastal Ecuador; Toledano, 2008b). *Notaphus* are not known from the Southern Hemisphere in the Old World, except for recently invasive species (Lindroth, 1976; Toledano, 2005).

In the past, several other subgenera of *Bembidion* have been considered closely related to *Notaphus*, including the Holarctic *Eupetedromus* (Toledano, 2002), and the South American *Notaphiellus* and *Nothocys* (Jeannel, 1962; Toledano, 2002).

Eupetedromus is a small subgenus of about a dozen species (Lorenz, 2005), living with *Notaphus* in many Holarctic habitats. Although they have elytra with mosaic patterns (Fig. 5K) similar to those of *Notaphus*, the current molecular data clearly indicates that they are not closely related, as *Eupetedromus* is outside of the *Bembidion* Series (Fig. 9A), and appear to be distinct from any major group of *Bembidion*.

Nothocys and *Notaphiellus* are two subgenera erected by Jeannel (1962) within his genus *Notaphus* which are restricted to cold-

temperate South America. They differ in minor details, and for this reason they have been recently synonymized (Toledano, 2008b). Unlike most true *Notaphus*, *Nothocys* (Fig. 4G) and *Notaphiellus* (Fig. 4H) in general lack pale mottling on the elytra, although one undescribed species from the island of Chiloe, Chile, has a small patch of yellowish spots. My results indicate that *Notaphiellus* (represented in my analyses by *B. solieri*, the type species of *Notaphiellus*, and *B. cupreostriatum*) forms a clade with *Notaphus* (Fig. 9) which is very strongly supported (MLB and PB = 100 for the seven gene analysis (Table 4.21), with support also from separate analyses of four of the genes (Table 5.21). For this reason, I remove *Notaphiellus* from synonymy with *Nothocys*, and synonymize *Notaphiellus* within *Notaphus*.

In contrast, *Nothocys* is quite distinct. Its monophyly is supported by five of the seven genes (Table 4.22), and in the seven-gene analysis has MLB and PB = 100). In almost all analyses, including of combined data, *Nothocys* is separated from *Notaphus* (Figs. 8B and 9B, Tables 4.23 and 5.23, Supplementary content Figs. S4–S7). Only the maximum likelihood tree with 18S shows *Nothocys* and *Notaphus* forming a clade; in all other analyses, including of combined data, *Nothocys* is separated from *Notaphus*. However, the evidence against a *Nothocys* + *Notaphus* clade is relatively weak, as there is no firm evidence that either is related to a third group.

In my experience, *Nothocys* live in different habitats than *Notaphus*. Most *Notaphus* live in open, sand, silt, or clay shores of bodies of water, whereas most *Nothocys* live away from water bodies, on the dark, damp soil near bogs (e.g., a species at Senda Darwin, Chiloe, Chile), or at high elevation along seeps (e.g., *B. anthracinum* at La Parva, Reg. Met., Chile), or in open, high-elevation grasslands (e.g., two species at Cuesta del Obispo, Salta, Argentina).

Within *Notaphus*, one of the most unexpected findings is the existence of a clade, the *B. rapidum* species group, that includes *B. rapidum* (Fig. 4K), *B. sparsum*, *B. nubiculosum*, *B. scintillans* (Fig. 4L), and *B. aratum*. The first three have traditionally been considered normal members of *Notaphus*, but the later two species, with a very different appearance in part because of their extremely shiny luster, have been placed in *Eupetedromus* (Erwin, 1982), although doubts have been raised about that placement (Bousquet and Webster, 2006). The evidence that *B. scintillans* and *B. aratum* do not belong to *Eupetedromus*, but instead belong to the *B. rapidum* species group of *Notaphus*, is compelling, and is moderately or strongly supported by six of the genes (Table 5.24) as well as the combined data (Table 4.24).

The structure of male genitalia also suggest that *B. aratum* is closer to *B. rapidum* than to *Eupetedromus* (Fig. 14). *Notaphus*, including *B. aratum*, possess the derived feature of an N-sclerite (Toledano, 2008b), lacking in *Eupetedromus*. In general, the structure of the sclerites of the endophallus (the complex, multilayered, dark structures in the center of each image in Fig. 14) is much more similar among members of the *B. rapidum* species group than between any of those and *Eupetedromus*, although this similarity should be viewed cautiously as no analysis has yet been done to document which of the visible traits are derived.

The center of diversity of the *B. rapidum* species group appears to be in southern North America. *Bembidion rapidum* is a widespread species, north to southern Canada, whereas the other four species occur from the southwest USA south to at least Costa Rica (*B. sparsum*). I have seen several other species of similar appearance from México that are morphologically similar to members of the *B. rapidum* group, intermediate in form between *B. sparsum* and *B. scintillans*. I predict, based upon general body form, that *B. commissum* from South America may also belong to this clade.

The remaining *Notaphus* of the Holarctic region, exclusive of the *B. rapidum group*, form a clade. This Northern Hemisphere clade of *Notaphus* is strongly supported in the seven-gene analysis (Table 4.25), with moderate support being independently present from both the nuclear protein-coding genes and the RiboMito matrix (Table 4.25), although there is only very weak support and some contradictory evidence when the genes are analyzed independently (Table 5.25). This Northern Hemisphere clade includes mottled, relatively flat *Notaphus* such as *B. varium*, *B. patruele*, and *B. castor*, as well as an array of salt-tolerant species such as the more convex and bullet-shaped members of the *B. contractum* species group, as well as dark and more parallel-sided forms such as *B. obtusangulum*. That these unusual body forms of species living in saline habitats are derived is indicated by the abundance of species in South America, outside the Northern Hemisphere clade, that are extremely similar to the North American *B. patruele* and *B. castor* in shape and color pattern.

The *B. rapidum* group plus the Northern Hemisphere clade, which include all sampled *Notaphus* from Central America and the Holarctic region, together from a clade, as indicated by high bootstrap values for multigene analyses (Table 4.26). Support is also indicated in analyses of five of the genes when analyzed independently (Table 5.26).

In contrast, the South American *Notaphus* appear to be a grade. The evidence for paraphyly of Southern Hemisphere *Notaphus* is strong, with *B. solieri* being sister group of the remainder, as indicated by an MLB = 99 and PB = 98 in the seven-gene analysis for all *Notaphus* other than *B. solieri* (Fig. 8B, Table 4.27), and support for this relationship in four of the seven genes (Table 5.27). This South American grade includes species that Jeannel (1962) included in the genus *Notaphiellus*, as well as his subgenus *Austronotaphus* (*B. convergens*, *B.* sp. nr. *ugartei*), and *Notaphus* proper (e.g., *B. calverti*). The latter species looks very much like a standard North American *Notaphus*, similar to *Bembidion castor*, but it is evidently rather distantly related. Other members of this grade are diverse in form and habitat, from dark forms like *B. cupreostriatum* (Fig. 4H), living on small streamsides in high-elevation meadows in Chile, to flat, pale species such as *B. cillenoides* (Fig. 4I), living around lower-elevation saline ponds in Argentina.

The position of *B. solieri* plus other South American *Notaphus* as a basal grade of *Notaphus* suggests that the subgenus as a whole may have originated in South America, with dispersal northward (Fig. 16). This corroborates Jeannel's (1962, p. 536) notion that *Notaphus* originated in south temperate regions, and that the large radiation of North American (and from there, Palaearctic) *Notaphus* arose as one pulse from South America.

4.11. Bembidion Series: Antiperyphanes Complex

According to Jeannel (1962), the South American fauna includes three lineages of Bembidiina, only two of which are now considered part of *Bembidion*: the *Notaphus* Series (which he considered to include *Notaphus*, *Nothocys*, *Notholopha*, and *Pacmophena*, among the subgenera treated here), and the *Peryphus* Series (including *Nothonepha*, *Antiperyphus*, *Antiperyphanes*, and *Plocamoperyphus*). His division and placement of the species of temperate South America into two Holarctic lineages is based on the traditional character of the position of a seta on the third interval of the elytron, with those adults having the seta in the interval, far removed from a stria (Fig. 7H), being considered *Notaphus*, and those with the seta in or very close to a stria (Fig. 7G) being in the *Peryphus* Series (now called the *Ocydromus* Series). Indeed, some of these Chilean and Argentian species look very much like Holarctic species (compare, for example, *B.* (*Antiperyphanes*) *spinolai* from Chile and Argentina, Fig. 4C, to the Holarctic *B.* (*Ocydromus*) *scopulinum*, Fig. 2H), and thus at first glance their placement in the *Ocydromus* Series seems reasonable.

Toledano (2008b) notes that the so-called South American "*Peryphus*" are unlikely to be related to true, Northern-Hemisphere

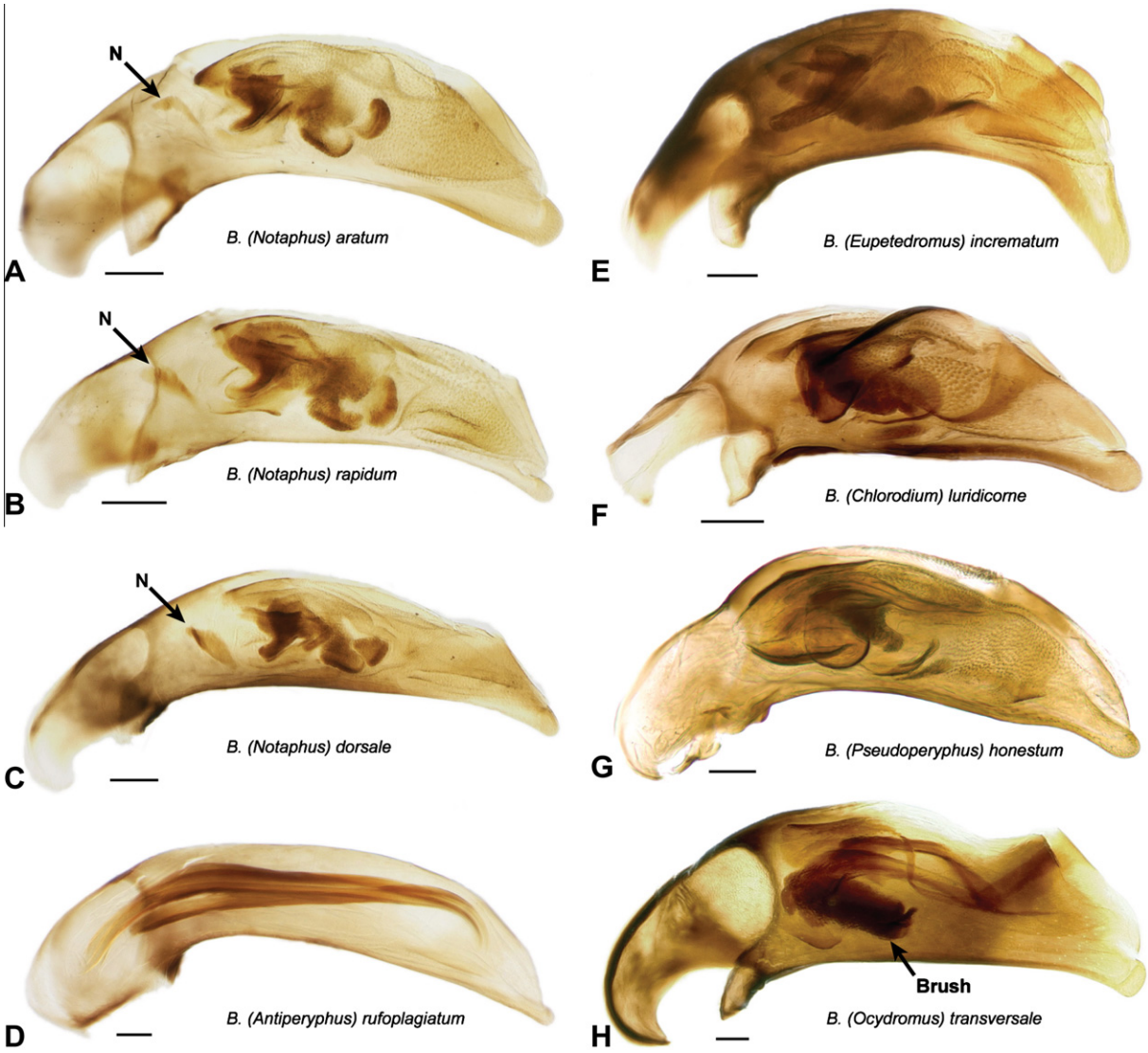

Fig. 14. Variation of the aedeagus of male genitalia of *Bembidion*. Note the greater similarity of *B. aratum* (A) to *B. rapidum* (B) than to *Eupetedromus* (E), especially in the context of the extent of variation throughout the genus. "N" shows the "N-sclerite" characteristic of some members of the *Bembidion* Series. "Brush" shows the brush sclerite; it is present in all pictured species except *B. rufoplagiatum* (D). Scale bar 0.1 mm. (A) *B.* (*Notaphus*) *aratum*, USA: New Mexico: Grant Co., Gila River near Gila; voucher DNA2284. (B) *B.* (*Notaphus*) *rapidum*, Canada: Ontario: Burlington; voucher DNA1754. (C) *B.* (*Notaphus*) *dorsale*, Canada: Alberta: Taber, Oldman River; voucher DNA1895. (D) *B.* (*Antiperyphus*) *rufoplagiatum*, Chile: Reg. X: Rio Gol Gol; voucher DNA1454. (E) *B.* (*Eupetedromus*) *incrematum*, Canada: Nova Scotia: Upper Kennetcook, Hanna Brook; voucher DNA1411. (F) *B.* (*Chlorodium*) *luridicorne*, Turkmenia: Kapet-Dag, Geo-depe; voucher DNA1298. (G) *B.* (*Pseudoperyphus*) *honestum*, USA: North Carolina: Huntdale; voucher DRM V100165. (H) *B.* (*Ocydromus*) *transversale*, USA: Wyoming: Laramie; voucher DNA2097.

members of the *Ocydromus* Series, instead considering them possibly related to *Notaphus*. My results indicate that all South American subgenera except for *Notaphus* and *Nothocys*, including, among others, *Notholopha*, *Pachmophena*, and the more recently described subgenus *Ecuadion*, form a clade, the *Antiperyphanes* Complex. Further, the *Antiperyphanes* Complex is, like *Notaphus*, a member of the *Bembidion* Series (Fig. 9B), and thus is not closely related to the Holarctic *Ocydromus* and *Peryphus*.

The *Antiperyphanes* Complex is supported by three nuclear protein genes when analyzed independently (Table 5.28), as well the ribosomal + mitochondrial genes when analyzed together (although with no bootstrap support, Table 4.28). Support for this clade in the seven-gene analysis is MLB = 98 and PB = 88.

Thus, it appears that a single clade in South America has diversified into many different body forms and habitats, paralleling lineages in the Northern Hemisphere. For example, *Nothonepha* (Fig. 4A), lives in similar habitats to the tachyine genus *Elaphropus*, which they resemble in size and form. *Notholopha* (Fig. 4B) and *Pachmophena*, which are of a similar size and shape to *Bembidion* (*s. str.*), include species that run rapidly and live in habitats distant from water, as do many *Bembidion* (*s. str.*). *Antiperyphanes* (Fig. 4C) lives on gravel river shores, as do many of their similarly formed northern counterparts in the *Ocydromus* Complex. Others, such as *Plocamoperyphus* (Fig. 4D), living on the beaches of the Pacific Ocean, have similar patterning to *Bembidion* (*Leuchydrium*) *tigrinum* (Fig. 2J), a member of the *Ocydromus* Complex, which lives on beaches of the Pacific Ocean in western North America.

The internal phylogenetic structure of the *Antiperyphanes* Complex (Fig. 9B) suggests that some changes need to be made to the classification of South American *Bembidion*; for example, *Plocamoperyphus* appears to be derived from within *Antiperyhanes*. However, I refrain from making changes until a more detailed study of the South American fauna, including a denser sampling of species, is completed (Maddison, in prep.).

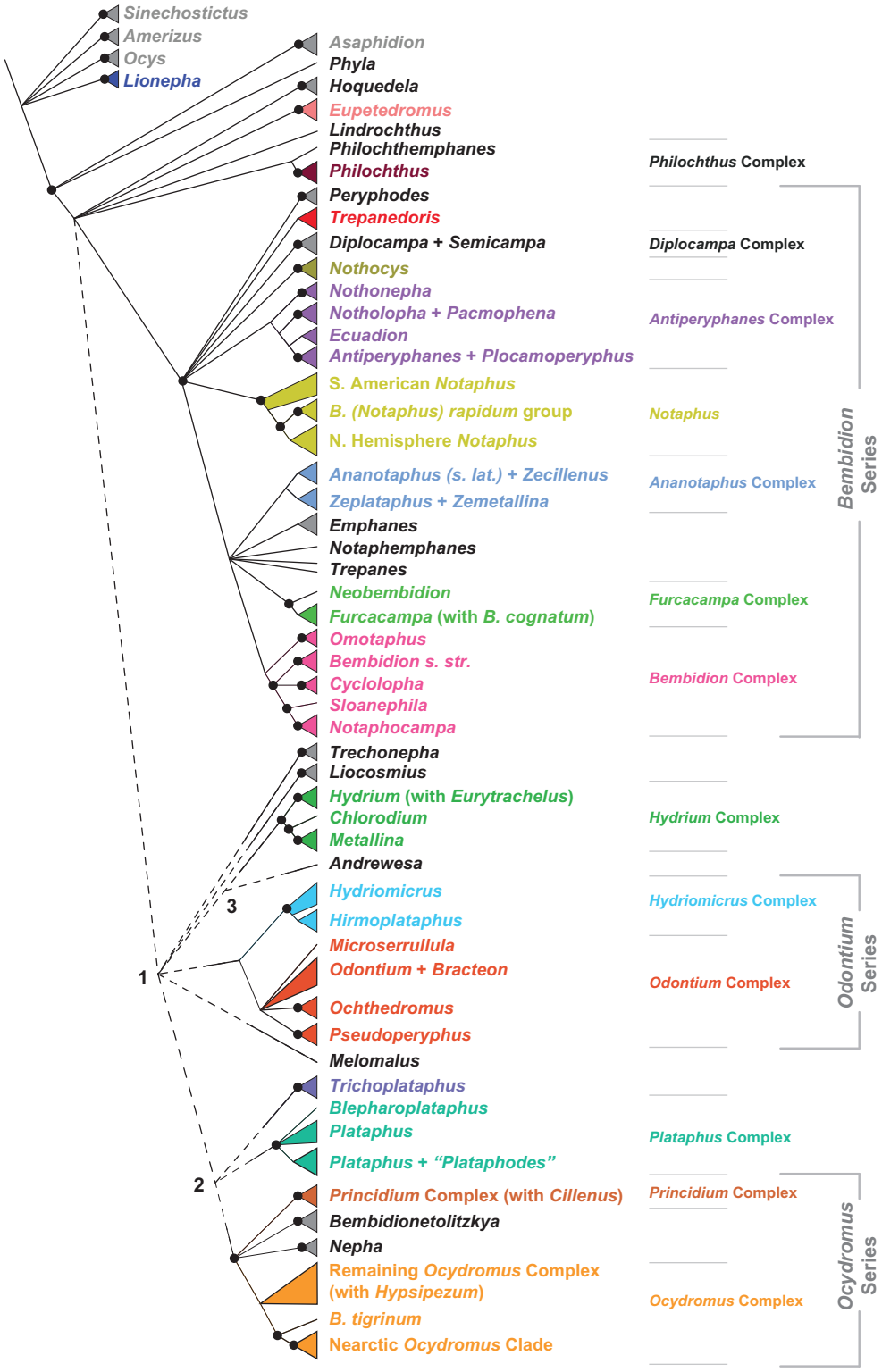

Fig. 15. Summary tree of relationships in Bembidiina. All clades in this tree, except for the one marked "2", are supported by maximum likelihood bootstrap values ⩾90 for the seven-gene matrix and I + Γ model. All clades have MLB ⩾50 for all seven analyses in which a single gene was removed from the matrix, thus indicating that their support comes from at least two genes. All solid (not dotted) lines and triangles indicate clades that are also supported by two independent genes (one by at least MLB ⩾50 and the other at least with the clade in the ML tree or MPTs). Those clades that are also supported by MLB ⩾90 under both an I + Γ and Γ models as well as PB ⩾90, and with bootstrap support by at least two separate genes (and not just two ribosomal genes) are marked with a black spot. The dotted lines for numbered clades have (1) MLB ⩾90 for the combined analysis but no individual gene support, although there is support from CAD and *wingless* if the only non-*Bembidion* in the analysis is *Asaphidion*; (2) MLB = 82 with weak individual support from three genes; (3) MLB ⩾90 for the combined analysis but contradictory individual gene support. A quadrilateral indicates a group is paraphyletic. All taxa below *Asaphidion* belong to the genus *Bembidion*.

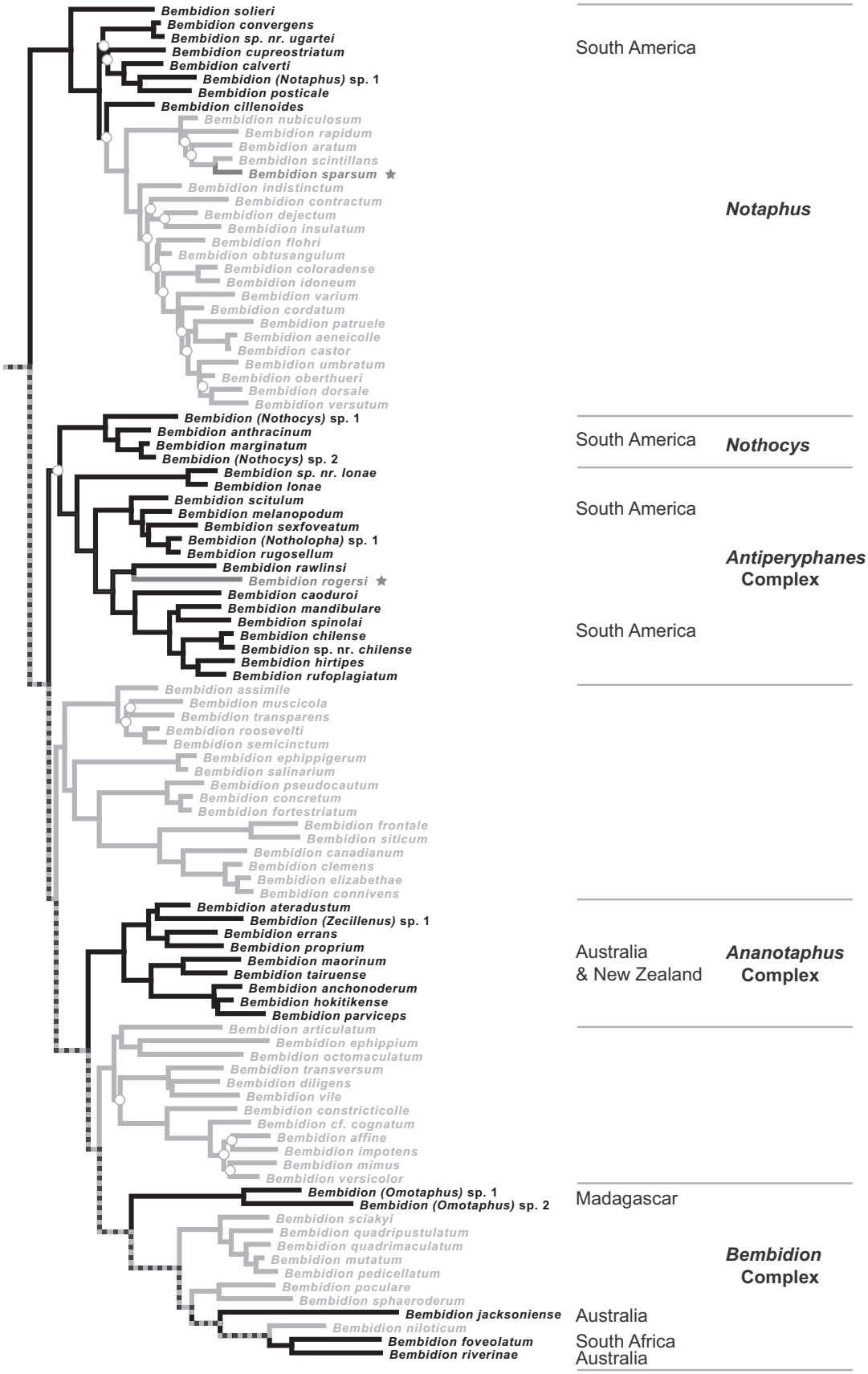

Fig. 16. Geographic distribution of species of the *Bembidion* Series shown on the tree of Fig. 8B. Species living in the Southern Hemisphere (including all of temperate South America) are shown in black, and their regions of origin are shown on the right. Those from the Northern Hemisphere (exclusive of northern South America) are shown in gray. Branches are colored according to a simple parsimony reconstruction, with checkered lines indicating ambiguous reconstruction. The two species marked by a star (*B. sparsum*, *B. rogersi*) are from Costa Rica; all other North American species are from the United States or Canada.

4.12. Bembidion Series: Diplocampa Complex

Diplocampa and *Semicampa* (Fig. 4E) are two subgenera of small *Bembidion* (2.5–3.5 mm) that are characterized by doubled frontal furrows, either just on the clypeus (*Semicampa*) or throughout their lengths (*Diplocampa*). Together they form a clade that is very well supported by CAD, ArgK, topoisomerase, and to a lesser extent by *wingless*, 18S, and COI (Table 4.29). A possible merger of the subgenera awaits study of additional species.

4.13. Odontium Series

The *Odontium* Series includes a pair of Complexes, *Hydriomicrus* and *Odontium*, that are in some ways dissimilar in appearance and habit.

The *Hydriomicrus* Complex (Fig. 2A) includes two subgenera, *Hirmoplataphus* (nine species in North America, and two in the Palaearctic) and *Hydriomicrus* (five species in North America). They are relatively small, most being between 3.5 and 4.5 mm in length; many live in gravel or under cobble on the shores of rivers, although some are found on sand shores (e.g., *B. brevistriatum*), sphagnum bogs (*B. quadratulum*), or *Darlingtonia* bogs (*B. innocuum*). The monophyly of this group is well-established by results from CAD, topoisomerase, and 28S (Table 5.30), as well as the combined analyses (Table 4.30).

The *Odontium* Complex (Fig. 2B–E) is a more speciose, Holarctic group that includes larger-bodied beetles (most adults are 5–7 mm in length). All but the subgenus *Pseudoperyphus* tend to live on open, mostly barren sand and clay beaches; most are difficult to catch on warm days, as they fly readily when approached. The monophyly of this group is indicated by five of the seven genes (Table 4.31), if *Microserrullula* is included, and is firmly supported by the combined analyses of seven genes (MLB = 99, PB = 94).

Maddison (1993), in delimiting the *Odontium* Complex (in that work called the *Odontium* Subgeneric Group), was uncertain about whether *Pseudoperyphus* (Fig. 2B) and *Microserrullula* (Fig. 2E) were members, as the morphological states that suggested relationship had not yet been interpreted cladistically. *Pseudoperyphus* lacks a derived state of larvae present in *Odontium*, *Bracteon*, and *Ochthedromus*, but has very similar male genitalia (although the polarity of the latter's states are unclear). In contrast, *Microserrullula* adults have several external features, possibly derived, that link it to *Odontium* and *Bracteon*, but they have male genitalia that are modified enough to make it difficult to homologize endophallic structures with those of *Odontium*. My current results indicate that *Pseudoperyphus* and *Microserrullula* are indeed members of the *Odontium* Complex.

There is conflicting evidence about the exact placement of *Microserrullula* within the complex. In the ML tree of all data (Fig. 8C), *B.* (*Microserrullula*) *xanthacrum* appears as the sister group of the remainder. However, this is only clearly supported by CAD (Table 5.32), and there are contradictory indications (Table 4.32). The single *Microserrullula* sampled is on a long branch in CAD, ArgK, topoisomerase, and COI, and it is possible that the signal about its placement is being hidden by changes along the long branch; addition of more species of *Microserrullula* may help determine its relationships.

The *Odontium* Series includes adults with angulate shoulder margins (Fig. 7F), in particular members of *Odontium*, *Bracteon*, *Ochthedromus*, and *Microserrullula*, as well as adults without angulate shoulder margins (Fig. 7E), among members of *Pseudoperyphus*, *Hirmoplataphus*, and *Hydriomicrus*. Previous classifications have placed *Bembidion* with angled shoulder margins, including members of the *Hydrium* Complex, *Phyla*, *Hoquedela*, *Andrewesa*, and *Pekinium* Csiki, together with *Odontium* (Jeannel, 1941; Toledano, 2008a). It is apparent that this trait is scattered around the phylogeny (Fig. 13B).

4.14. Ocydromus Series: Cillenus and the Princidium Complex

B. (*Cillenus*) *laterale* (Fig. 2G) is one of the 30 or so *Bembidion* species worldwide that live in the intertidal zone, and the only one included in this study. As with many of the intertidal species, *B. laterale* has a shape that is unusual for *Bembidion*, with a wide head and long mandibles. This form has been proposed to be an adaptation for feeding on intertidal amphipods (Green, 1956; Lindroth, 1980). These and other derived traits have led many authors to treat *Cillenus* as a separate genus (Lorenz, 2005; Marggi et al., 2003; Ortuño and Toribio, 2005; Perrault, 1981). In contrast, Lindroth (1980) and Toledano (2000) maintain *B. laterale* within *Bembidion*. Sequences from 28S, 18S, and *wingless* suggested that *B. laterale* is related to *B. mexicanum* (Maddison and Ober, 2011), the only member of the *Ocydromus* Complex sampled in that paper.

The evidence from seven genes overwhelming indicates that *Cillenus* is related to the subgenera *Princidium*, *Testedium* (Fig. 2F), and *Paraprincidium*; this *Princidium* Complex, including *Cillenus*, is supported by six of the seven genes, with CAD, *wingless*, and ArgK showing high bootstrap values (Table 5.33). The AllData matrix shows MLB = 100 and PB = 100 (Table 4.33).

As mentioned above, the *Princidium* Complex is part of the *Ocydromus* Series, and thus *Cillenus* is simply a member of *Bembidion* that has some derived features. That intertidal *Cillenus* might arise from this group is not too surprising: at least some other members of the *Princidium* Complex (e.g., *B.* (*Testedium*) *bipunctatum* (Linneaus)) are salt tolerant, and occur along sea shores (Lindroth, 1985).

4.15. Ocydromus Series: the Ocydromus Complex

The *Ocydromus* Complex (Fig. 2H–J) is the most speciose complex in *Bembidion*, and contains many brown, black, or bluish *Bembidion* living on river shores, in open fields, in alpine areas, and other habitats. The group is Holarctic, with its center of diversity in the Palaearctic.

The existence of this clade is not surprising, although two of its members might be: *Hypsipezum* and *Leuchydrium*. The subgenus *Hypsipezum* contains small, brown beetles found at high elevation in the mountains of eastern Africa; this group has frequently been classified as a separate genus (e.g., Lorenz, 2005). *Leuchydrium* contains only *Bembidion tigrinum* (Fig. 2J), a large species living on the shore of the Pacific Ocean from California to British Columbia. *Bembidion tigrinum* superficially resembles some of the intertidal members of subgenus *Chinocillenus*, but as Lindroth (1976) notes, that is likely a result of convergence. Its mottled color pattern is very unusual among *Ocydromus* Complex species, but it is a condition not uncommon in beetles that live on open beaches, e.g., the unrelated *B.* (*Plocamoperyphus*) *mandibulare* (Fig. 4D).

That the *Ocydromus* Complex, including *Hypsipezum* and *Leuchydrium*, is monophyletic appears most likely. It is supported in the seven-gene analysis by an MLB = 100 and PB = 99 (Table 4.34), with evidence provided by three of the nuclear protein-coding genes (Table 5.34).

The phylogeny within the *Ocydromus* Complex hints that this group needs much more thorough study, and a revision of its classification. One of the unexpected clades is a group of 13 species, shown as the bottom species in Fig. 9C, from *B. actuosum* through *B. transversale*. This group contains three of the six sampled members of the subgenus *Peryphus*, one of four *Peryphanes*, both *Testediolum*, all *B. transversale* group members, and one *Ocydromus incertae sedis*; the clade is thus scattered across the current classification. I can see no obvious synapomorphy of this group, but they

do have one thing in common: they are all Nearctic. This Nearctic *Ocydromus* Clade is well supported (Tables 4.35, 5.35) and is sister to *Bembidion tigrinum* (Tables 4.36, 5.36). There are three additional clades in the *Ocydromus* Complex, all supported by high bootstrap values for the combined matrix, and by at least two individual genes, that also speak of the need to reclassify the *Ocydromus* Complex: (1) *B. maroccanum* + *B. stephensi* + *B. dudichi*, containing two of the four sampled *Peryphanes* and the single *Ocyturanes*; (2) *B. platynoides* + *B. modestum* + *B. scopulinum* + *B. grapii*, containing one of the four sampled *Peryphanes*, the two *Ocydromus* (*s. str.*), and one *Ocydromus incertae sedis*; and (3) *B. obscurellum* + *B. rupicola* + *B. tetracolum* + *B. ripicola*, containing two of the six sampled *Peryphus* and the one sampled *Euperyphus*. I refrain from making nomenclatorial changes now, as I have sampled relatively few *Ocydromus* Complex species. Type species of genus-group names will need to be sampled before major changes are made, especially as some of the North American species I have sampled may not belong to the same lineage as the type species of the subgenera in which they are currently placed; for example, *B. commotum* and *B. nebraskense*, currently classified as *Testediolum*, may not belong to that subgenus (Paolo Bonavita, pers. comm., 2011).

4.16. Plataphus Complex

Plataphus (Fig. 5A) and *Plataphodes* (Fig. 5B) have been considered similar but distinct subgenera of north-temperate *Bembidion*, which differ slightly in the structure of the shoulder margin of the elytron. Many are relatively flat, black or brown, unspotted beetles, some with a metallic sheen. They are similar to other subgenera (e.g., *Trichoplataphus* and *Blepharoplataphus*) that have been considered related, but those differ in characters such as the presence of extra setae on the abdominal sterna. The largest species of *Bembidion* in North America, *B. planatum* (Fig. 5C), has traditionally been placed in the subgenus *Plataphus* (e.g., Lindroth, 1963), but Toledano (2008c) has recently removed it to the subgenus *Melomalus*.

My data shows very clearly that *Plataphus* + *Plataphodes* + *Blepharoplataphus*, without *B. planatum*, form a clade among the species I have sampled. This *Plataphus* Complex is supported by individual analyses of six genes, although only well-supported by 18S (Table 5.37), but with very high MLB and PB values for both the NucProt and RiboMito combined matrices (Table 4.37). Inclusion of *B.* (*Melomalus*) *planatum* in this group yields a non-monophyletic group, as suggested weakly by each individual gene (Table 5.38) and more strongly by the combined analyses (Table 4.38).

Within the *Plataphus* Complex, *B.* (*Blepharoplataphus*) *hastii* is the sister species of the rest, as indicated by the weak support in four genes for a *Plataphus* + *Plataphodes* clade (Table 5.39), and strong support from the combined analyses (Table 4.39).

Within the *Plataphus* + *Plataphodes* clade, there are relatively few well-supported clades. There is very strong evidence against monophyly of *Plataphus* exclusive of *Plataphodes* (Tables 4.40 and 5.40), in good part because there is a clade that consists of all sampled *Plataphodes* plus four of the eight sampled *Plataphus* (*B. stillaguamish*, *B. gordoni*, *B. rufinum*, and *B. simplex*) (Tables 4.41 and 5.41). There is also some evidence against the monophyly of *Plataphodes* (Tables 4.42 and 5.42). For this reason, I merge *Plataphodes* into *Plataphus*, to form a larger, monophyletic *Plataphus*.

The placement of *Trichoplataphus* (Fig. 5D) relative to the *Plataphus* Complex is unclear. There is some slight evidence, mainly from the *wingless* gene, that these two groups might be sisters, but the bulk of evidence speaks against a relationship (Tables 5.43 and 4.43).

4.17. Hydrium Complex

There are two groups of species of large, relatively convex, mostly greenish *Bembidion* whose placement has varied from author to author. The subgenus *Hydrium* as traditionally defined includes one described species, the brilliant, green, shiny, convex *B. levigatum* (Fig. 5H), which is common on the upper, steep banks of sandy river shores throughout much of the USA east of the Rockies. *Bembidion levigatum* is characterized in part by having many extra setae on the elytra, a trait that only a few other *Bembidion* have (e.g., *B.* (*Antiperyphus*) *hirtipes*, *B.* (*Hydriomicrus*) *semistriatum*). It was perhaps because of this unusual characteristic that Lorenz (2005) removed *Hydrium* from *Bembidion*. A group of nine species that some authors call the subgenus *Eurytrachelus* contains adults which are somewhat similar in form to *B. levigatum*, but lack the extra setae. Among the species I have sampled, *B. interventor*, *B. nitidum*, and *B. obliquuulum* are considered members of *Eurytrachelus* in the modern literature (e.g., Lorenz, 2005). Jeannel (1941) considered the two groups to be closely related, and merged them under *Hydrium*. Most other authors (e.g., Lindroth, 1963) have kept them separate.

My data indicate that *B. levigatum* is simply a derived member of "*Eurytrachelus*", as its status as a sister to part of *Eurytrachelus* is supported by four genes (Table 5.45), and it is well supported by analyses of combined matrices (Table 4.45). As *Hydrium* is the older name for this group, all members of what was called *Eurytrachelus* are transferred here back into *Hydrium*.

Two subgenera that share the angulate shoulder margin (Fig. 7F) found in *Hydrium* are evidently related: *Metallina* (Fig. 5G) and *Chlorodium*. This trio of subgenera form the *Hydrium* Complex, which is very strongly supported by combined analyses (Table 4.44), and five of the seven genes (Table 5.44).

There are some hints that the sister group of the *Hydrium* Complex is another subgenus with angulate shoulder margins, *Andrewesa* (Fig. 5L). Although individual gene analyses generally speak against this, with only *wingless* in support (Table 5.46), the combined results are moderately strong (Table 4.46). Further sampling in both the *Hydrium* Complex and *Andrewesa* may clarify a possible relationship.

4.18. Trechonepha + Liocosmius?

The relationships of two small, western North American subgenera to the remainder of *Bembidion* are not clear. *Trechonepha* (Fig. 5E) is a subgenus of two recognized species, both brown with pale legs, which live under leaf litter on moist, dark soil or sand, often in shaded forest habitats. *Liocosmius* (Fig. 5F) is a subgenus of three described and at least three undescribed species, all spotted, with delicate, thin appendages; they are most frequently found on sandy shores of creeks and rivers. Neither subgenus belongs to any of the series in *Bembidion*.

Although there is no definitive evidence for their placement, three genes (CAD, 28S, and COI) suggest that these two subgenera might be sister groups (Table 5.47).

4.19. The Philochthus Complex and B. wickhami

Philochthus (Fig. 5I) is a Palearctic subgenus of about 30 species, characterized in part by a notched and sinuate hind prothoracic margin (Fig. 7B).

Philochthus does not belong where typically placed. Although Jeannel (1941) considered them to be related to *Bembidion*, and Toledano (2005) to *Sloanephila* and *Notaphocampa*, they are not members of the *Bembidion* Series (Fig. 9A), or any other series (Fig. 9A). The combined matrices (Table 4.48) indicate a relationship with the Asian subgenus *Philochthemphanes*, although the evi-

Table 7
A revised classification of the genus *Bembidion*. Those taxa not sampled in my study are contained in [], indicating that their placement is speculative. Other genera in Bembidiina are *Sinechostictus*, *Amerizus*, *Ocys*, *Lionepha*, and *Asaphidion*. Placement of *Caecidium*, *Orzolina*, and *Sakagutia* outside of *Bembidion* has been based upon their autapomorphies, and thus they could easily be derived from within *Bembidion*.

Bembidion
- *Odontium* Series
  - ***Hydriomicrus* Complex**
    - *Hirmoplataphus*, *Hydriomicrus*
  - ***Odontium* Complex**
    - *Odontium*, *Bracteon*, *Ochthedromus*, *Pseudoperyphus*, *Microserrullula*
- *Ocydromus* Series
  - ***Princidium* Complex**
    - *Princidium*, *Testedium*, *Paraprincidium*, *Cillenus* [*Actedium* Motschulsky]
  - ***Ocydromus* Complex**
    - *Ocydromus*, *Peryphus*, *Terminophanes*, *Ocyturanes*, *Asioperyphus*, *Peryphanes*, *Testediolum*, *Euperyphus*, *B. transversale* group, *Hypsipezum*, *Leuchydrium*
  - **Other Subgenera**
    - *Nepha*, *Bembidionetolitzkya*
    - [*Omoperyphus* Netolitzky, *Pamirium* Netolitzky, *Peryphiolus* Netolitzky, *Politophanes* Müller-Motzfeld, *Thaumatoperyphus* Netolitzky]
- *Bembidion* Series
  - ***Bembidion* Complex**
    - *Bembidion*, *Cyclolopha*, *Notaphocampa*, *Omotaphus*, *Sloanephila*
  - ***Ananotaphus* Complex**
    - *Ananotaphus*, *Australoemphanes*, *Gondwanabembidion*, *Zemetallina*, *Zeplataphus*, *Zecillenus* [*Nesocidium* Sharp, *Zeactedium* Netolitzky, *Zeperyphodes* Lindroth, *Zeperyphus* Lindroth]
  - ***Antiperyphanes* Complex**
    - *Antiperyphanes*, *Antiperyphus*, *Nothonepha*, *Pacmophena*, *Notholopha*, *Ecuadion*, *Plocamoperyphus* [*Chilioperyphus* Jeannel, *Notoperyphus* Bonniard de Saludo, *Pseudotrepanes* Jeannel]
  - ***Furcacampa* Complex**
    - *Furcacampa*, *Neobembidion*
  - ***Diplocampa* Complex**
    - *Diplocampa*, *Semicampa*
  - ***Other* Subgenera**
    - *Notaphus*, *Nothocys*, *Trepanedoris*, *Notaphemphanes*, *Peryphodes*, *Trepanes*, *Emphanes*
    - [*Apteromimus* Wollaston, *Endosomatium* Wollaston, *Pseudophilochthus* Wollaston, *Gnatholymnaeum* Sharp]
- Unplaced to Series
  - ***Plataphus* Complex**
    - *Blepharoplataphus*, *Plataphus* (*including Plataphodes*)
  - ***Hydrium* Complex**
    - *Hydrium*, *Metallina*, *Chlorodium* [*Neja* Motschulsky]
  - ***Philochthus* Complex**
    - *Philochtemphanes*, *Philochthus*
- **Other subgenera**
  - *Hoquedela*, *Lindrochthus*, *Eupetedromus*, *Trechonepha*, *Liocosmius*, *Melomalus*, *Trichoplataphus*, *Andrewesa*, *Phyla*
- *Incertae sedis*
  - [*Armatocillenus* Dupuis, *Aureoplataphus* Netolitzky, *Bembidromus* Toledano, *Chinocillenus* Netolitzky, *Corallicillenus* Uéno, *Desarmatocillenus* Netolitzky, *Jedlickion* Toledano, *Josefia* Toledano, *Lymnaeoperyphus* Nakane, *Lymnaeum* Stephens, *Microsinocys* Toledano, *Necpericompsus* Netolitzky, *Nipponobembidion* Habu and Baba, *Novicillenus* Uéno and Habu, *Pekinium* Csiki, *Peryphophila* Netolitzky, *Pseudometallina* Netolitzky, *Pseudosinocys* Toledano, *Taiwanobembidion* Habu, *Talanes* Motschulsky]

dence from individual analyses of four individual genes that support this is slight (Table 5.48).

These two subgenera combined, the *Philochthus* Complex, may be sister to the enigmatic *B. wickhami* (Fig. 5J) from western North America. Although exact placement of *B. wickhami* is not indicated by any bootstrap analyses, it is placed as the sister group of the *Philochthus* Complex in the maximum likelihood tree of the AllData matrix (Fig. 8A). This result is supported independently by the NucProt and RiboMito ML trees; the only single-gene analysis that shows this relationship is maximum likelihood analysis of topoisomerase. A possible relationship between *Philochthus* and *B. wickhami* (as *B. carlhi*) was suggested by Erwin and Kavanaugh (1981), based upon several morphological similarities, although some of the character states noted have evolved multiple times independently or are plesiomorphic within *Bembidion*. However, there is one notable derived character that links *B. wickhami* and *Philochthus*, the sinuate and notched hind margin of the pronotum, which is similar in the two groups. Corroboration from multiple sources suggests that *B. wickhami* may be a relative of the *Philochthus* Complex. Although there are no known species whose sampling could split the *B. wickhami* branch, it is possible that sampling more species of *Philochthemphanes* will yield a more confident placement of *B. wickhami*.

Because of the distinctiveness of *B. wickhami*, well-separated from other *Bembidion*, I here create a new subgenus, *Lindrochthus*, to house it. The name, to be treated as masculine, is formed from a combination of Carl Lindroth's last name and the ending "chthus", to suggest its similarity to subgenus *Philochthus*. With *B. wickhami* as the type species, *Lindrochthus* is distinguished from other *Bembidion* by having the hind margin of the prothorax sinuate and notched, crista clavicularis present, lateral bead at shoulder not angulate, elytral setae ed3 and ed5 in elytral interval, although near adjacent striae. From *Philochthus* it is distinguished by the less abruptly sinuate hind margin of the prothorax, and the reduced number of elytral striae (with at most two to four evident striae), in addition to the molecular characteristics that place *Lindrochthus* outside of the *Philochthus* + *Philochthemphanes* clade. For a more complete description of *B. wickhami*, see Erwin and Kavanaugh (1981).

4.20. Summary of phylogenetic relationships

A summary of the phylogenetic relationships among the major lineages of Bembidiina is shown in Fig. 15; there is robust evidence for all clades shown in this tree (except for those marked with

dotted lines, clades 1, 2, and 3), as they are strongly supported by the combined analyses as well by at least two independent genes.

A summary of a new classification of *Bembidion* resulting from this work is given in Table 7, including speculation as to where some unsampled subgenera might fall. Additional changes will be needed once the *Ocydromus* and *Ananotaphus* complexes are better sampled. The inclusion of more species will likely also require merging of subgenera.

4.21. A Gondwanan origin of Northern Hemisphere Bembidion?

In discussing *Bembidarenas setiventre*, which he took to be a member of *Bembidion* subgenus *Plataphus*, Jeannel (1962: 538) states [my translation of his French], "The presence of this *Plataphus* raises again the issue of bipolarity in the faunas of particular groups of cold climate. In most cases, the problem was solved by taxonomic studies showing that in reality it is convergence between species of different lineages." Thus, although he notes that apparently amphitropical groups are often found to be separate, independent radiations in the two hemispheres, he claims that the *Plataphus* Complex, *Ocydromus* Series (his "*Peryphus* Series"), and *Notaphus* are truly amphitropical, with members in both the Holarctic region and southern South America.

My results indicate that although Jeannel was correct about *Notaphus*, the *Plataphus*-like and *Ocydromus*-like forms in the Southern Hemisphere are convergent with the true, Northern-Hemisphere *Plataphus* and *Ocydromus*. Jeannel's (1962) suggestion that Holarctic *Notaphus* arose from a South American lineage is corroborated by the seven genes studied here, as the South American species form a grade from within which the Holarctic clade arose (Figs. 8B, 9B, and 15).

Toledano (2005, 2008b) extends the observation of a potential southern origin of *Notaphus*. He proposed a "Copernican revolution" in phylogenetic studies of Bembidiina, in that we perhaps should view several lineages in the Northern Hemisphere as having arisen from Gondwanan stocks, rather than the reverse. He suggests this for lineages around *Notaphus*, i.e., more or less the *Bembidion* Series as defined here (Toledano, 2008b), as well as for *Australoemphanes* in the south and *Emphanes* in the north (Toledano, 2005). He speculates that the entire subtribe of Bembidiina might have had an origin in Gondwana (Toledano, 2005).

Among the more striking results from the molecular data are the biogeographic patterns and consistency of groups. Most of the species from Australia and all of the species from New Zealand form an endemic clade (the *Ananotaphus* Complex), including *Australoemphanes*, and this clade is not phylogenetically intermingled with Northern Hemisphere forms. All species in South America other than *Notaphus* and *Nothocys* form an endemic clade (the *Antiperyphanes* Complex), and this clade is not intermingled with *Notaphus*, nor is it (or any part of it, such as *Ecuadion*) derived from within *Notaphus* (against Toledano, 2008b).

Toledano's (2008b) proposal that a group more or less equivalent to the *Bembidion* Series may have arisen in the Southern Hemisphere can neither be confirmed or refuted with my data, although it is an open possibility. A simple parsimony-based reconstruction of hemisphere (Northern or Southern) on the phylogeny of the *Bembidion* Series shows that it is equally parsimonious to presume a Northern or Southern Hemisphere origin (Fig. 16).

However, it appears unlikely that Bembidiina as a whole arose in the Southern Hemisphere. Although many lineages within the *Bembidion* Series are southern (Fig. 16), there are very few members of Bembidiina outside of this series in the Southern Hemisphere. The *Ocydromus* Complex members in the mountains of eastern and southern Africa are likely southern extensions of otherwise Northern Hemisphere groups (Paolo Bonavita, pers. comm.), and the same is likely true of the few Indonesian members of the *Ocydromus* complex. *Sinechostictus* in Indonesia (Luca Toledano, pers. comm.) presumably follow the same pattern, as almost all species except for a high-elevation Javanese species are north of the Equator. A third group, *Microserrullula*, now straddles the equator, but as it is but a subgenus in the otherwise-Holarctic *Odontium* Series, its presence in the Southern Hemisphere is likely not indicative of an ancient home there for all Bembidiina. The placement of the two Southern Hemisphere groups that I have not sampled, the endemic radiation on St. Helena (Basilewsky, 1972) and the intertidal *Desarmatocillenus* Netolitzky (Lindroth, 1980), may alter the pattern slightly, but there are no indications from morphological data that these taxa will alter the reconstruction of Bembidiina as originating on what are now Northern Hemisphere landmasses.

4.22. Implications for morphological evolution

The exclusion of *Bembidarenas* and *Phrypeus* from Bembidiina means that all Bembidiina, with three exceptions, have the derived state of a brush sclerite (Fig. 14H) in the endophallus of male genitalia. The exceptions are two groups in the *Bembidion* Series (some members of the *Antiperyphanes* Complex lack the brush, Fig. 14D, as do members of the subgenus *Zecillenus* (Lindroth, 1976)), as well as subgenus *Microserrullula* within the *Odontium* Complex (Paolo Bonavita, pers. comm.). Given the deeply nested position of these taxa within *Bembidion*, their lack of brush sclerites surely results from secondary losses (Lindroth, 1976). Thus, the presence of a brush sclerite is a synapomorphy for Bembidiina, although it has been independently derived within the trechite subtribe Xystosomina (Erwin, 1994; Maddison and Ober, 2011).

The inferred tree implies that many standard morphological characters used in *Bembidion* systematics have undergone extensive evolution that would cloud their use in phylogenetics. The rampant homoplasy in the position of seta ed3 of the elytron, evident in Fig. 13A, indicates that Toledano (2005, 2008b) was correct in suggesting this character's states were not appropriate as the primary means to infer major divisions of Bembidiina. The same caution, but to a lesser extent, could be applied to the shape of the elytral bead at the shoulder (Fig. 13B). The complexity of variation in the hind margin of the pronotum and crista clavicularis, and subsequent difficulty in using them as phylogenetic markers, has been discussed above.

I hope that these results, along with the discovery of well-supported clades within Bembidiina, will inspire the search for additional, and less homoplastic, morphological characters. A notable goal will be to ascertain synapomorphies of the major clades discovered here, including the *Bembidion* Series, *Odontium* Series, and *Ocydromus* Series, as well as *Bembidion* as a whole (with or without the subgenus *Phyla*).

These characters may include traits of the endophallus of the male genitalia. The value of the structure of the endophallus for systematic inference has been emphasized by Lindroth (1963). I have shown one example here in the similarities of *B. aratum* to other *Notaphus* (Fig. 14), but a more thorough study needs to be conducted based upon more than patterns in a two-dimensional photograph. The endophallus is a complex, multi-layered structure, with lobes, sclerites, and microsculpture evident if the endophallus is everted from the aedeagus (Coulon, 2002; Maddison, 1993). Detailed studies of endophalluses of worldwide Bembidiina are needed similar to those Coulon (2002) has done for the French fauna. In addition to providing key clues to the homology of sclerites and other aspects of the endophallus, such an analysis could allow us to determine which states are derived.

One of the more intriguing implications of the inferred phylogeny is that the nature of change varies between the major lineages. I have already noted that there is much more crossing of the

equator in the *Bembidion* Series (Fig. 16) than elsewhere in Bembidiina. In addition, there are differences in the pace of morphological evolution in some features. For example, members of the *Bembidion* Series vary widely in the structure of the head, much more so than remaining *Bembidion*. Many members of *Bembidion* have grooves on the dorsal surface of the head, called frontal furrows. In all species outside of the *Bembidion* Series, these furrows are simple, and relatively shallow. If they extend upon the clypeus, the clypeal seta is contained within the furrow. Within the *Bembidion* Series, however, the furrows vary widely. There have been at least three separate derivations (in *Trepanedoris*, *Trepanes*, and *Furcacampa*) of deep, convergent frontal furrows (Figs. 3H and 4F) that extend onto the clypeus separate from a furrow containing the clypeal setae. Other species in the series have shallow, but convergent frontal furrows; others have doubled frontal furrows; others have simple frontal furrows, like *Bembidion* outside of this Series. It is not apparent why there has been extensive evolution of this trait within the *Bembidion* Series, but not outside of it.

4.23. Effect of site-to-site rate variation models

To date, there has been no formal theoretical or empirical study investigating the concern that has been raised (Yang, 2006; A. Stamatakis in RAxML 7.0.4 manual) about potential problems that might arise in estimating parameters of an $I + \Gamma$ model of site-to-site rate variation; in particular, it is not apparent that the problems would extend to inference of the phylogeny. My results suggest that the effect of using an $I + \Gamma$ model (or not) on the inferred phylogeny is not always dramatic. It affected the bootstrap value of clades by up to 18 percentage points (in most instances much less than this) in single-gene analyses and 7.2 percentage points in the AllData analyses (Table 6). Examination of many more empirical data sets, as well as theoretical work, is needed to assess this issue fully.

4.24. Nuclear copies of COI

Nuclear copies of mitochondrial DNA, or numts (Lopez et al., 1994), can confuse phylogenetic analysis and confound the use of mitochondrial DNA as a tool for species delimitation and identification (Bensasson et al., 2001; Thalmann et al., 2004; Walther et al., 2011). Within *Bembidion*, numts of COI have been found to be widespread in several species of subgenus *Pseudoperyphus* (Maddison, 2008), as well as being present in the *B.* (*Ocydromus*) *transversale* group (Maddison and Swanson, 2010). Within some species of *Pseudoperyphus*, the numts were diverse and commonly enough amplified to make sequencing of COI impossible with standard methods (Maddison, 2008).

Fortunately, outside of *Pseudoperyphus*, I found no evidence that the amplified sequences were solely numts. I have found evidence for numts in the outgroups (Anillina and Zolini), other genera of Bembidiina (*Sinechostictus*, *Amerizus*), and within *Bembidion* in the *Bembidion*, *Ocydromus*, and *Odontium* series, as well as the subgenus *Hoquedela*, but in none did the sequences generated have obvious signs of being pseudogenes (such as having stop codons, or frameshift mutations). However, because of the widespread presence of numts in trechites, it is still possible that some sequences correspond purely to numts, even if they show no double peaks in the chromatograms or no clear evidence of being pseudogenes. In the future, studies of bembidiine COI should ideally use methods that seek to avoid sequencing of numts (Calvignac et al., 2011; Moulton et al., 2010).

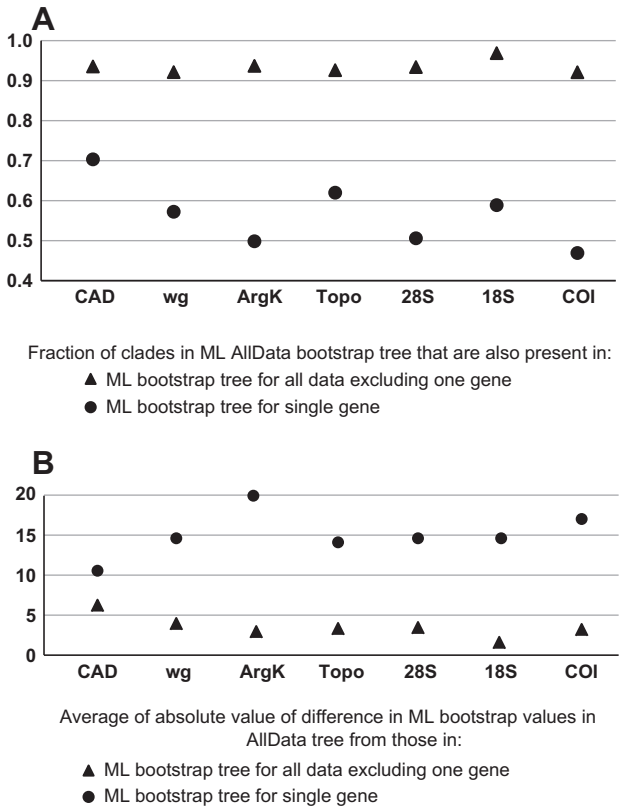

Fig. 17. Comparison of tree structure and bootstrap values of maximum likelihood bootstrap trees in single-gene analyses and multigene analyses in which a single gene is omitted to the tree structure and bootstrap values from the AllData analysis. $I + \Gamma$ model used. For topoisomerase, 18S, and COI, comparisons were made on pruned trees containing only the species sampled for those genes. (A) Fraction of the clades that are present in the maximum likelihood AllData bootstrap tree that are also present in ML bootstrap trees from other matrices. The higher the value, the more the other matrix yields a tree similar to that of the AllData tree. Note that dropping out any single gene at most causes a loss of 8% of the clades (i.e., the fraction of clades retained is >0.92). (B) Average of the absolute values of the difference in ML bootstrap values between the tree for AllData and trees from other matrices, over clades that the two trees share.

4.25. The value of each gene for phylogenetic analyses

Removing any single gene from the AllData matrix results in an ML bootstrap tree with 92–97% of the clades present in the full seven-gene matrix (Fig. 17A, triangles), in comparison to the 98.2–98.9% values for separate, identical AllData bootstrap analyses. Thus, the loss of a single gene does not have a major effect on the tree. In particular, the clades shown in Fig. 15 are all present in all of the bootstrap trees from seven analyses each missing a different gene. This is notable, as it indicates that no single gene contains the entirety of the signal for any of the clades in Fig. 15; that is, all of the clades shown in Fig. 15, and most of the clades in the AllData ML bootstrap tree, are supported by at least two genes.

CAD is the gene whose results most closely match the results from the AllData analyses, with 70% of the clades in the AllData ML bootstrap tree recovered by CAD (Fig. 17, circles). For those clades that they have in common, CAD is also the gene whose bootstrap values most closely match those of the AllData tree (note that the circle for CAD in Fig. 17B has the lowest value, thus indicating more similar bootstrap values). This could indicate that CAD is providing most of the signal for the AllData analyses, but the fact that the six-gene matrix that excludes CAD yields a tree very similar to

the AllData tree indicates that the close match CAD's tree has with the AllData tree does corroborate the value of CAD for inferring phylogeny of Bembidiina.

In contrast, COI yields the poorest results, with the ML bootstrap tree from COI sharing only 47% of the AllData tree's clades (Fig. 17A, circles), and having the second-highest average discrepancy in bootstrap values from the AllData tree (Fig. 17B, circles). However, some caution should be applied to interpretation of results from COI, topoisomerase, and 18S, as these genes were not sampled as extensively as the other four genes, with only 216 of 270 species sampled for COI, 207 for topoisomerase, and 159 for 18S.

5. Future research

I have not sampled some taxa that may yield a clearer picture of some aspects of the phylogeny of the group. For the deeper splits, sampling the remaining genera of Bembidiina (*Caecidium*, *Orzolina*, and *Sakagutia*) may help, but some key subgenera within *Bembidion* are also important to sample or sample more densely. The subgenus *Phyla* should be targeted for denser sampling, because of its apparent distance from other *Bembidion* (Fig. 15). Some unsampled subgenera in the mountains of Asia (including *Microsinocys*, *Pseudosinocys*, and *Bembidromus*) may prove critical in understanding deep relationships. For improvements to knowledge about the phylogeny within a major group, and the resulting classification, one of the more important groups to sample more densely is the *Ocydromus* Complex.

It is possible that increased knowledge about the phylogeny of *Bembidion* will lead to an eventual disassociation of the genus into multiple genera. I do not recommend this now; major changes should await further study on the unsampled subgenera, either by DNA sequencing or by examination of novel morphological characters. However, once that is done, knowledge sufficient for a functional classification will be in hand, and the decision can be made about the fate of the genus.

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Appendix A. PCR protocols and PCR and sequencing primers

A.1. Recommended protocols for each gene

The following protocols yield good sequences for over 95% of *Bembidion* species if the genomic DNA is of sufficient quality. Sequencing primers are the same as amplification primers unless otherwise noted. Cycling conditions are listed in Table A1, and primers in Table A2. Additional protocols used to obtain some sequences are given in Table A3.

- 28S: Use primer pair LS58F–LS998R or primer pair NLF184/21–LS1041R, with thermal cycling setup C1 (Table A1).
- 18S: Use primer pair SS27F–SS1893R, with cycling setup C2, and sequence with all six primers listed listed under 18S in Table A2.
- COI: Use cycling setup C1 with primer pair B1490 – Bcoi2R as first choice, or primer pair LCO1490–HCO2198 as second choice.

Table A1
PCR thermal cycling conditions for recommended protocols. Second column indicates general pattern for thermal cycling, with "S" being standard, with a start phase of 2–3 min at 94 °C, followed by the cycling phase, with each cycle consisting of 20–30 s of denaturing at 94 °C, 20–30 s of annealing at the annealing temperature, and an extension phase at 65 °C or 72 °C (as specified by the manufacturer of the Taq). "T" is for a touchdown reaction, which is like the standard protocol but with two or three rounds of cycling reactions rather than just one. Cyc: Number of cycles; for touchdown reactions, the number of cycles in each of the two or three cycling rounds is given. Ta: annealing temperature in °C; for touchdown reactions, the annealing temperatures in each of the two or three cycling rounds is given. Ext: time, in seconds, of the extension phase.

#		Cyc	Ta	Ext
C1	S	35	52	70
C2	S	35	50	150
C3	S	37	52	120
C4	S	35	54	150
C5	S	37	55	90
C6	S	31	57	90
C7	T	9, 30	60, 55	120
C8	T	9, 27	60, 57	180
C9	T	6, 6, 36	57, 52, 45	120

Table A2
Primers used for DNA amplification and sequencing. Dir: direction of primer, either forward (F) or reverse (R). Syn: primer synonym. Kind: primer used for original PCR amplification and sequencing (A) or primer used only for sequencing (S). Ref: reference for original description of primer, if known. (1) This study, (2) Ober (2002), (3) Van der Auwera et al. (1994), (4) Wray et al. (1993), (5) Maddison et al. (1999), (6) Wild and Maddison (2008), (7) Ward and Downie (2005), (8) Moulton and Wiegmann (2004), (9) Simon et al. (1994), (10) Maddison (2008), (11) Hebert et al. (2003). Primers marked with † were used for sequences obtained in the earlier part of this study; they are not recommended unless the recommended combinations fail. Primers marked with * were used to obtain the long CAD sequences submitted to GenBank for some of the taxa; they are not relevant for the portion of CAD analyzed in this study.

Gene	Primer	Syn	Dir	Kind	Sequence	Ref.
28S	LS58F	D1	F	A	GGGAGGAAAAGAAACTAAC	2
	NLF184/21		F	A	ACCCGCTGAAYTTAAGCATAT	3
	LS998R	D3	R	A	GCATAGTTCACCATCTTTC	2
	LS1041R	D3aR	R	A	TACGGACRTCCATCAGGGTTTCCCCTGACTTC	10
18S	SS27F		F	A	TATGCTTGTCTCAAAGATTAA	
	S1893R	18L	R	A	CACCYACGGAAACCTTGTTACGACTT	
	SS398F	18Sai	F	S	CCTGAGAAACGGCTACCACATC	4
	SS1054F	760F	F	S	ATCAAGAACGAAAGT	4
	SS1090R	18Sbi	R	S	GAGTCTCGTTCGTTATCGGA	4
	SS1554R	909R	R	S	GTCCTGTTCCATTATTCCAT	5
COI	B1490		F	A	TTTCAACAAACCATAAGGATATTGG	10
	Bcoi2R		R	A	GCTAATATDGCRTARATTATTCC	10
	LCO1490		F	A	GGTCAACAAATCATAAAGATATTGG	11
	HCO2198		R	A	TAAACTTCAGGGTGACCAAAAAATCA	11
wg	wg550F		F	A	ATGCGTCAGGARTGYAARTGYCAYGGYATGTC	6
	wg578F		F	A	TGCACNGTGAARACYTGCTGGATG	7
	wgAbRZ		R	A	CACTTNACYTCRCARCACCARTG	6
	wgAbR		R	A	YTCGCAGCACCARTGGAA	7
	B3wg2†		R	A	ACTCGCARCACCAGTGGAATGTRCA	10
	B5wg1†		F	A	GARTGYAAGTGTCAYGGYATGTCTGG	10
	5wgB†		F	A	ACBTGYTGGATGCGNCTKCC	10
CAD	CD338F*		F	A	ATGAARTAYGGYAATCGTGGHCAYAA	8
	CD680R2*		R	A	TARGCRTCYCTNACWACYTCRTAYTC	10
	CD581F4*		F	A	GGWGGWCAAACTGCWYTMAAYTGYGG	10
	CD843R*		R	A	TTYGARGARGCNTTYCARAARGC	8
	CD791F2		F	A	GTNACNGGNCAANCAACTGCCTG	10
	CD806F		F	A	GTNGTNAARATGCCNMGNTGGGA	8
	CD806F3*		F	A	TTAYTGYGTTGTNAARATWCCNMGNTGGGA	1
	CD821F		F	A	AGCACGAAAATHGGNAGYTCNATGAARAG	6
	CD1098R		R	A	TTNGGNAGYTGNCCNCCCAT	8
	CD1129R		R	A	ATTCTRGCTTGYTGYCTRTGYAAATCCAT	1
	CD1098R2		R	A	GCTATGTTGTTNGGNAGYTGDCCNCCCAT	6
	CD1231R		R	A	TCCACGTGTTCNGANACNGCCATRCA	6
ArgK	AK168F		F	A	CAGGTTTGGARAAYCACGAYTCYGG	6
	AK183F		F	A	GATTCTGGAGTCGGNATYTAYGCNCCYGAYGC	6
	AK270F†		F	A	GGYTTCAAGAAGACYGACAA	10
	AK933R		R	A	CCCTCAGCYTCRGTGTGYTCNCCRCG	1
	AK939R		R	A	GCCNCCYTCRGCYTCRGTGTGYTC	6
	AK950R†		R	A	TTGTTRGARATGTCRTAGATGCC	6
Topo	TP643F		F	A	GACGATTGGAARTCNAARGARATG	6
	TP675F		F	A	GAGGACCAAGCNGAYACNGTDGGTTGTTG	6
	TP932R		R	A	GGWCCDGCATCDATDGCCCA	6

Table A3
PCR thermal cycling conditions used for protocols that are not recommended for the fragments of genes analyzed, but which were used to obtain some sequences early in this study. See legend of Table A1 for more details.

Gene	Primer pair		Cyc	Ta	Ext
wg	5wgB–B3wg2	S	37	56	60
	B5wg1–B3wg2	S	35	51	50
CAD	CD338F–CD680R2	T	5, 5, 35	57, 52, 45	90
	CD581F4–CD843R	T	5, 5, 35	57, 52, 45	90
	CD806F–CD1098R	T	5, 5, 35	57, 52, 45	90
	CD791F2–CD1098R	T	5, 5, 35	57, 52, 45	90
	CD821F–CD1231R	T	5, 5, 35	57, 52, 45	90
	CD806F3–CD1129R	T	5, 5, 35	57, 52, 45	90
ArgK	AK270F–AK950R	S	35	53	60

- *Wg*: Use primer pair wg550F–wgAbRZ with cycling setup C3. If that does not yield a sufficient band, then do a nested reaction with the product of the wg550F – wgAbRZ reaction as the template, and using primer pair wg578F – wgAbR and cycling setup C4.
- CAD3: A hemi-nested reaction will almost always succeed. The first reaction should use CD806F–CD1231R and cycling setup C7, then use the product of this as a template for a reaction using CD806F–CD1098R2 and cycling setup C5.
- ArgK: Use primer pair AK168F–AK939R with cycling setup C8. If that does not yield a sufficient band, then do a nested reaction with the product of the AK168F–AK939R reaction as the template, and using primer pair AK183F–AK933R and cycling setup C6.
- Topo: Use primer pair TP643F–TP932R with cycling setup C9. If that does not yield a sufficient band, then do a hemi-nested reaction with the product of the TP643F–TP932R reaction as the template, and using primer pair TP675F–TP932R and cycling setup C9.

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.01.015.

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