SYSTEMATICS OF THE NORTH AMERICAN BEETLE SUBGENUS PSEUDOPERYPHUS (COLEOPTERA: CARABIDAE: BEMBIDION) BASED UPON MORPHOLOGICAL, CHROMOSOMAL, AND MOLECULAR DATA

DAVID R. MADISON
Department of Entomology, University of Arizona, Tucson, Arizona 85721
beetle@ag.arizona.edu

ABSTRACT

The subgenus Pseudoperyphus Hatch of Bembidion Latreille is revised. External structure, male genitalia, chromosome number, and DNA sequences from seven genes (28S rDNA, 18S rDNA, cytochrome oxidase I, wingless, CAD, arginine kinase, and RNA polymerase II) reveal the presence of nine species. Five of the species belong to the B. chalceum subgroup: B. chalceum Dejean, B. rothfeldsi, new species (type locality: Bridgewater, Vermont), B. bellorum, new species (type locality: Tygart Valley River near Valley Head, West Virginia), B. antiquum Dejean, and B. lousiella, new species (type locality: North Aspy River near Cape North, Nova Scotia). The remaining species belong to the B. honestum subgroup: B. honestum Say, B. arenobilis, new species (type locality: Danville, Virginia), B. integrum Casey, and B. rafotinctum Chaudoir. The group is most diverse in Vermont, where seven species live. The geographic ranges of many species overlap: of the 36 possible species pairings, 28 are found in sympatry. The morphological character system most closely correlated with species boundaries, as indicated by DNA sequences, is shape of the flagellum of the male genitalia, and it is likely involved in reproductive isolation. The shape of the pronotum is also implicated as important for species recognition. Two species, B. chalceum and B. rothfeldsi, show derived haploid chromosome numbers distinct from the typical n=12 found in other Pseudoperyphus and most other Bembidion, with n=13 and n=17 respectively. Each of the nine species is inferred to be monophyletic in the Bayesian gene tree of at least one gene, but five of the species are paraplethnic for at least one gene, with two species (B. antiquum and B. arenobilis) being paraplethnic for as many genes as they are monophyletic. Evidence for nuclear copies of cytochrome oxidase I (COI) in several species makes inference of the COI gene tree difficult, and is one reason that COI is not the gene of choice to identify Pseudoperyphus specimens, contrary to standard DNA barcoding protocols. The best single gene for species identification is 28S rDNA; the worst are COI and wingless. A key to species and geographic distribution maps are provided.

KEY WORDS: barcode, Bembidini, Carabidae, chromosomes, cryptic species, gene tree, morphology, numts, Pseudoperyphus

INTRODUCTION

Members of the carabid genus Bembidion Latreille, subgenus Pseudoperyphus Hatch, are abundant beetles on gravel or cobble river shores throughout North America east of the Rocky Mountains, with a few localities west of the Rockies to the Pacific coast. They are especially frequent on flat, gravel islands (Figs. 1A, 1B), where they are often the commonest small (5-7 mm) predator.

The most recent revision divides the subgenus into four species, the two most widespread and common of which (Bembidion chalceum Dejean and Bembidion honestum Say) are considered difficult to separate and are “generally confused in collections” (Lindroth 1963). In fact, Lindroth’s concepts of the two species are themselves equivocal; for example, he variously identified one form (herein recognized as Bembidion antiquum Dejean) in collections as B. honestum or B. chalceum. The difficulty of separating Lindroth’s concepts of B. chalceum and B. honestum led T.L. Erwin to note, on a label attached to a specimen in the Museum of Comparative Zoology, “chalceum and honestum cannot be separated in large series. Lith [Lindroth] covers extremes p. 247-248 – I think it’s one species.”

In 1980, while studying chromosomes of Bembidion in the late Klaus Rothfels’s laboratory, I collected a sample of what I took to be B. chalceum from the Oxtongue River in Ontario, Canada. Much to my surprise, the sample contained some specimens with 13 pairs of chromosomes, and others with 17 pairs. Subtle morphological differences between the n=13 and n=17 specimens made it clear that two distinct species were involved, and that what has typically been called “Bembidion chalceum” is a mixture of at least two species. Further exploration of adult structure, chromosomes, and DNA sequences led to the discovery that the suite of specimens within Lindroth’s (1963) concepts of B. chalceum and B. honestum is in reality a complex of at least seven species, four of which are new. As the new species in some aspects form a complex chain of morphological intermediates between typical B. chalceum and B. honestum, previous confusion of subtle distinctions between species was to be expected. This paper examines morphological, cytogenetic, and molecular variation within the group, revising the nine species of Pseudoperyphus now known.

This paper is dedicated to my mentor, George E. Ball. George, both by example and effort, makes people around him better, and this paper exists because of the inspiration he has provided to me. His never-failing integrity and honesty, his consideration for others, and his devotion to advancing knowledge about the organisms we love has served as my touchstone for the past three decades.

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Fig. 1.—Habitats of *Pseudoperyphus*. A, type locality of *Bembidion louisella*, North Aspy River, Cape Breton Island, Nova Scotia. 151 *B. louisella* were collected on this gravel island, in the company of *Bembidion rusticum*. B, type locality of *Bembidion bellorum*, Upper Tygart Valley River, near Valley Head, West Virginia. About 65 *B. bellorum* were found on the gravel island in the center of the photograph, along with two other common species of *Bembidion*, *B. planum* and *B. rusticum*, as well as a specimen each of *B. carolinense* Casey and *B. nigrum* Say. C, type locality of *Bembidion arenobilis*, Dan River, Danville, Virginia. Specimens were most abundant at the edge of sand ledges, among dead leaves accumulated in the sand. D, Quechee Gorge, Vermont, showing the habitat of *Bembidion rufotinctum*. Specimens are found by splashing cracks in the bedrock.

**MATERIALS AND METHODS**

Approximately 7300 specimens of *Pseudoperyphus* were examined from the collections listed below. Each collection's listing begins with the coden used in the text, and is followed by the curator in parentheses, or the full address if a private collection:

BMNH – The Natural History Museum, London  
(Max Barclay)

CAS – California Academy of Sciences  
(David H. Kavanaugh)

CMNH – Carnegie Museum of Natural History  
(Robert L. Davidson)

CSCA – California State Collection of Arthropods  
(Chuck Bellamy)

CUIC – Cornell University Insect Collection  
(James K. Liebherr)

DAHC – Drew Hildebrandt, 710 Laney Road, Clinton,  
MS 39056 U.S.A.

DRM – David R. Maddison, Department of Entomology,  
University of Arizona, Tucson, AZ 85721 U.S.A.

FMNH – Field Museum of Natural History  
(Alfred Newton, Margaret Thayer)

JCC – Janet C. Ciegler, 2636 Pine Lake Drive, West Co  
lumbia, SC 29169 U.S.A.

KWW – Kipling W. Will, Wellman Hall, University of  
California, Berkeley, CA 94720 U.S.A.

MCPM – Milwaukee Public Museum (Susan Borkin)

MCZ – Museum of Comparative Zoology  
(Philip D. Perkins)
Collecting Methods

In addition to specimens borrowed from collections, numerous beetles were collected by hand. During daylight, specimens were found by moving gravel or cobbles to expose beetles underneath, or by splashing the shore with water. At night, most specimens were found on the surface, unhidden.

Specimens tend to be most common on flat gravel or sand islands in rivers, and less common on the main shore; for this reason, when islands were accessible, they were preferentially collected. In general, specimens are most abundant on the downstream or upstream tips of islands or bars.

Specimen Fixation and Preservation

Specimens for morphological examination were killed and preserved in *Acer* sawdust to which ethyl acetate was added. Specimens to be cytogenetically characterized were fixed in 3:1 absolute ethanol:glacial acetic acid, with the abdomen opened to expose tissues to fixative. Most specimens for DNA sequencing were collected into 100% ethanol (with the abdomen partly removed to increase penetration) or silica gel.

Some specimens, including holotypes of the four species described herein, were prepared to yield molecular and chromosomal data as well as high-quality morphological specimens. This was done by grasping the chilled, live male in one hand, and carefully extracting the genitalia through the rear using forceps held in the other hand. The attached accessory glands and testes were slowly pulled through the rear using forceps held in the other hand. The remainder of the beetle was placed in sawdust containing a few drops of ethyl acetate, and re-associated with the genitalia after mounting.

Morphological Methods

Methods for studying adult structures, and terms used, are given in Maddison (1993). Study of the flagellum of the male genitalia typically requires examination of the right side of the aedeagus, as the flagellum is obscured from the left side by darker membranous layers, especially of the flagellar sheath (Maddison 1993). For this reason, the male genitalia are figured from the right side as well as the more standardly-illustrated left side.

Measurements for Apparent Body Length (ABL) were from apex of the labrum to apex of the longer elytron. Habitus photographs were taken with a Nikon Coolpix 990 attached to an Olympus SZH dissection scope. Other photographs of external body parts were taken with a Leica Z6 and JVC KY-F75U camera using Microvision’s Cartograph software for extended depth of field (EDF) processing; the images thus potentially have some artifacts caused by the EDF algorithm. The genitalic images were taken with a Diagnostic Instruments’s Spot Flex camera attached to a Leica DM4000 microscope, with EDF processing by Image Pro version 6.0’s 3D Surface Inspector module. These images thus do not match exactly what one sees under a microscope, as the software chooses only one plane of focus for each point in an image, whereas multiple focal levels contribute to the image that people see as they look at a genitalic preparation with transmitted light under a microscope. However, the images shown are judged to be representative enough to be used for identification.

Cytogenetic Methods

A total of 227 *Pseudoperopythus* specimens was examined for chromosome number (Appendices 1 and 3). Chromosome squashes were made from testes stained with Feulgen stain, as outlined by Maddison (1985). At least five cells undergoing meiotic divisions were examined in each specimen to determine the number of chromosomes. In most specimens both first and second metaphase were studied to confirm the sex chromosome system.

Chromosome counts for outgroups (Appendix 2) are derived from Maddison (1985), with the adjustment that specimens listed therein for “*B. bifossulatum*” consisted of specimens from both *Bembidion bifossulatum* LeConte and *Bembidion cheyennense* Casey.

Molecular Methods

**Taxon Sampling.**—Outgroups (Appendix 2) were chosen based upon morphological considerations (Maddison 1993) and a more extensive molecular phylogenetic study of *Bembidion* (Maddison, unpublished), which indicates that *Pseudoperopythus* belongs to the *Odontium* LeConte subgeneric group (Maddison 1993). Three members of *Bracteon* Bedel, three members of *Odontium*, and three members of *Ochthedromus* LeConte, all members of the
Table 1. Primers used for DNA amplification and sequencing.

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<th>Primer</th>
<th>Syn</th>
<th>Dir</th>
<th>Kind</th>
<th>Sequence</th>
<th>Ref</th>
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<td>2</td>
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<tr>
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<td>NLF184/21</td>
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<td>A</td>
<td>ACCGCCTGAAYTATACCATAT</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LS998R</td>
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<td>A</td>
<td>GCATAGTCACCACATCTTT</td>
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<td>LS1041R</td>
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<td></td>
</tr>
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<td>18S</td>
<td>SS27F</td>
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<td>A</td>
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</tr>
<tr>
<td></td>
<td>S1893R</td>
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<td>SS398F</td>
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<td>A</td>
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<td>Jerry</td>
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<td></td>
<td>CD791F2</td>
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**Odontium** subgeneric group, were sequenced for the seven studied genes. In the literature, *B. bifossulatum* and *B. cheyennense* are generally considered conspecific, but unpublished molecular analyses reveal that they are distinct (Maddison and Arnold, in prep.) and are so considered herein. In addition, two species were examined belonging to the *Hirmoplatus* Netolitzky subgeneric group (one member of subgenus *Hydriomicrus* Casey and one of subgenus *Hirmoplatus*), which is the sister group of the *Odontium* subgeneric group (Maddison, unpublished).
Table 2. PCR protocols used for each primer pair.

<table>
<thead>
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<th>Gene</th>
<th>Primer Pair</th>
<th>Prot</th>
<th>Cyc</th>
<th>Ta</th>
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<td>5wgB - B3wg2</td>
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A total of 249 males and 17 females of *Pseudoperyphus* was sequenced from as diverse geographic areas as possible, including 86 specimens also studied cytogenetically. Details of the specimens sampled are included in Appendix 1. Detailed locality information for each of the sequenced specimens, including latitude and longitude data, links to habitat pictures, etc., is available at [http://bembidion.org/pseudoperyphus/DNAlocalities.kml](http://bembidion.org/pseudoperyphus/DNAlocalities.kml).

**DNA Extraction.**—DNA was extracted from metathoracic muscle or male accessory glands using the Qiagen DNeasy Tissue Kit and the “DNA Extraction for Animal Tissues” protocol.

**DNA 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; Pol2: RNA polymerase II.

Fragments for these genes were amplified using the Polymerase Chain Reaction on either an MJ Research PTC-150 Miniicycler 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 Tables 1 and 2. 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 the multiple chromatograms for each sequence and initial base calls were made with Phred (Green 2002) and Phrap (Green 1999) as orchestrated by Mesquite’s Chromaseq package (D.R. Maddison and W.P. Maddison 2006; W.P. Maddison and D.R. Maddison 2006), with subsequent modifications by Chromaseq and manual inspection. Multiple peaks at a single position in both reads were coded using IUPAC ambiguity codes.

Sequences have been deposited in GenBank with accession numbers EF648610 through EF649676.

**Alignment.**—The protein-coding genes showed no evidence of insertions and deletions in the history of the sampled sequences, and so these were aligned without difficulty. 18S rDNA showed only a single-base insertion or deletion within the outgroups, but none within *Pseudoperyphus*. 28S rDNA had no insertions or deletions within *Pseudoperyphus* except for one region of at most 14 nucleotides with multiple base insertions or deletions, and three other regions of at most seven, two, and one nucleotide; these were aligned by eye. Aligned matrices are available at [http://bembidion.org/pseudoperyphus](http://bembidion.org/pseudoperyphus).

**Phylogenetic inference.**—For each gene, some specimens had identical sequences to other specimens of the same
species. For analyses of each individual gene, these redundant sequences were merged into one, to reduce the size of the matrix and speed analyses. However, if all specimens of a species have the same sequence, then monophyly of the species cannot be tested if that species is represented in the matrix by only one sequence. While it might seem as if specimens with identical sequences must appear monophyletic on a tree, that is not necessarily so (for example, if the sequences contain no apomorphies relative to closely related sequences). There are three examples of species all of whose sequences are identical in a gene: all *Bembidion bellorum*, new species, have identical 28S rDNA sequences, and identical 18S rDNA sequences, and all *B. honestum* have identical arginine kinase sequences. To test the monophyly of these species with these genes, additional matrices were subject to the full suite of analyses, with all redundant specimens represented for the species in question. Thus, additional analyses were conducted for 28S rDNA and 18S rDNA with all *B. bellorum* specimens included, and arginine kinase with all *B. honestum* included.

Analyses were also conducted on a matrix consisting of concatenated data from all genes. The matrix consisted of only those 53 specimens (eleven outgroups and 42 *Pseudoperyphus*) that were sequenced for all seven genes. Each sequence in this concatenated matrix was approximately 8100 bases in length.

For each matrix, both parsimony and Bayesian analyses were conducted.

Most-parsimonious trees were sought using PAUP* (Swofford 2002) or TNT (Goloboff et al. 2007). PAUP* was used for all matrices. For each PAUP* search, 3700 to 8000 replicates were conducted, each beginning with a starting tree formed with the random addition sequence option. For 28S, COI, wingless, CAD, ArgK, and Pho2, the large number of most parsimonious trees led to the necessity of limiting the search by allowing each replicate to save no more than 25 trees. The number of most parsimonious trees found for each matrix ranged between 454 and 216,000; shortest trees were found in at least 400 of the replicates for all but one of the matrices. Shortest trees for the wingless matrix, in contrast, were found in only three of the replicates; as a result, a more thorough TNT search was conducted, holding 10,000 trees, starting with the MULT command, followed by the XMULT command with hold=50, hits=85, and ratchet=5. TNT found shortest trees 85 times.

Bayesian analyses were conducted using MrBayes (Huelsenbeck and Ronquist 2005), with models of evolution chosen with the aid of ModelTest (Posada 2005). As MrBayes cannot implement all models examined by ModelTest, only those models available in MrBayes were considered, and the best among those was chosen. 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 invariant and the remainder following a gamma distribution (the GTR + I + Γ model). GTR + I + Γ was also the model chosen by the likelihood ratio test for COI. With the exception of COI, however, the likelihood ratio test chose a simpler model, a 2-parameter rate matrix (HKY85). Because of ambiguity regarding the model to be used by genes other than COI, two separate analyses were conducted for each of these genes, one with a GTR + I + Γ model and one with an HKY85 + I + Γ model.

A single MrBayes MCCMCMC search was conducted for each analysis, each with two runs of four chains each, for between 10 and 85 million generations, with trees sampled every 1000 generations. All searches converged, as judged by an average standard deviation of split frequencies of less than 0.01 (Huelsenbeck and Ronquist 2005), except for both wingless analyses, whose average standard deviation of split frequencies reached 0.015 after 30 million generations.

For each analysis, the trees in a burn-in period were excluded, and the majority-rules consensus tree of the remaining trees was calculated by PAUP. The burn-in period was at least 25% of the total length of the run (as only the remaining 75% were used to calculate the average standard deviation of split frequencies used as a convergence diagnostic), and extended until the likelihood scores and all parameter values reached a stable plateau, as judged by visualization tools in Tracer (Rambaut and Drummond 2004). The burn-in period ranged from 5 million generations (i.e., 5000 trees for each of two runs per analysis) to 65 million generations. The number of trees sampled for each analysis varied from 8000 to 70,000.

To confirm that branches with high Bayesian Posterior Probabilities (BPP) are not an artifact (Lewis et al. 2005), neighbor-joining bootstrap analyses were conducted of each matrix, each with 1000 replicates. Maximum likelihood distances were used, with an analysis using a GTR + I + Γ model and another using an HKY85 + I + Γ model for each matrix. Any clades present in the Bayesian analysis that were also present in at least 50% of the neighbor-joining bootstrap replicates were judged to have valid BPPs. For those clades in the Bayesian tree that failed this test, branch lengths of the majority-rules consensus tree of the sampled Bayesian trees were calculated using likelihood by PAUP*, using the same model as used in the Bayesian analyses. Any branch in this tree whose length was longer than at least one of the branches that passed the neighbor-joining-bootstrap test was also judged to have a valid BPP. All clades (except for a few within-species clades that are not discussed further) with BPP greater than 70 were confirmed using this technique.

Images of the full phylogenetic tree from each analysis are provided at http://bembidion.org/pseudoperyphus.

**Morphological Variation**

*Pseudoperyphus* adults vary in many of the morphological traits that typically vary between carabid species (e.g., microsculpture, pronotal shape, form of elytral striae and
intervals, form of male aedeagus), as documented in the taxonomic account below. There is one character system among these that is noteworthy because of the tight correlation between its variants and the molecular and cytogenetic data: shape of the flagellum of the male genitalia.

The flagellum is a grooved spike that is the apical component of the Central Sclerite Complex (CSC, Fig. 2; Maddison 1993); it apparently is the conductor of sperm, and may enter the spermathecal duct during copulation (Schuler 1960). Maddison (1993) noted that the form of the flagellum was unique to most Bembidion (Bracteon) species, and that it was the definitive character to be used for identification for some difficult-to-identify species.

Constancy of the flagellum within species of Pseudoperyphus and the distinction between species, at least in local populations, is apparent after dissection of the CSC. For example, twelve specimens of Pseudoperyphus found together along the shore of the Ottauquechee River at Quechee Gorge, Vermont, were examined. These would have been classified as B. chalceum by most authors. Chromosome counts and external body form, however, suggested that there were three species among the twelve specimens. Within each of these three species, the flagella are very similar, but are notably different between species (Fig. 2).
More generally, the basic shape of the flagellum, including its length and sinuosity of the dorsal and ventral margins, was found to be almost constant within species. The shape was also unique for each species, with two exceptions: the flagella of *B. honestum* and *Bembidion arenobilis*, new species, could not be consistently distinguished (Figs. 14G, H), and a very few specimens of *Bembidion rothfelsi*, new species, (e.g., right-most specimen in Fig. 2C) had a flagellum very similar to that of *Bembidion louisella*, new species (Fig. 14E).

**MOLECULAR RESULTS**

Levels of DNA Sequence Variation

The most variable gene, as judged by the average proportion of nucleotides by which *Pseudoperyphus* specimens differ, is COI; the least variable is 18S (Table 3). Among the five protein-coding genes examined, COI and CAD show the most number of fixed amino acid differences between species, and RNA polymerase II the least.

Patterns of variation do not always fit those expected. Even though 18S rDNA has the least overall variation, and is typically viewed as a slowly-evolving gene (Woese 1987), 18S shows a relatively high number of fixed differences between some pairs of species that are morphologically very similar (Table 4). For example, in 1446 bases of COI, *B. louisella* and *B. bellorum* show no consistent differences, and only one difference in 932 bases of 28S, but they differ consistently by 7 of 1865 bases of 18S (Table 4C).

**Multiple, Distinct Copies of Genes**

For some individual beetles, both sequencing reactions for a particular nucleotide position showed two clear peaks, as one would expect if the PCR products contained two different forms of the gene. This is not surprising for nuclear protein-coding genes (e.g., CAD and wingless), and it presumably indicates a heterozygous individual with different maternal and paternal alleles at that locus.

However, 103 of 266 specimens sequenced for the mitochondrial gene (COI) also showed double peaks in both sequencing reactions. Because of presumed uniparental inheritance of mitochondria, multiple, distinct copies within an individual are not expected (Thalmann et al. 2004). The presence of multiple copies was confirmed for several specimens by cloning (Maddison, unpublished). It is most likely that the other copies of COI are in the nuclear genome, having moved from the mitochondrion; that is, these extra copies are likely “numts”, which have been found in many animal taxa (Bensasson et al. 2001; Richly and Leister 2004; Thalmann et al. 2004). It is less likely that they represent multiple, distinct mitochondrial genomes within an individual (heteroplasmy), as

### Table 3. Variation of gene fragments studied.

<table>
<thead>
<tr>
<th></th>
<th>28S</th>
<th>18S</th>
<th>COI</th>
<th>wg</th>
<th>CAD</th>
<th>ArgK</th>
<th>Pol2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>266</td>
<td>71</td>
<td>266</td>
<td>195</td>
<td>74</td>
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<td>932</td>
<td>1865</td>
<td>1446</td>
<td>453</td>
<td>2082</td>
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<td>682</td>
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<td>average difference</td>
<td>0.0163</td>
<td>0.0048</td>
<td>0.0586</td>
<td>0.0369</td>
<td>0.02448</td>
<td>0.0466</td>
<td>0.0181</td>
</tr>
<tr>
<td>variable aa</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>4</td>
<td>13</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>fixed aa</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>1</td>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
heteroplasmic is relatively rare among animals (Thalmann et al. 2004), and as some of the copies sequenced from both *B. rothfelsi* and *B. honestum* are non-functional pseudogenes, having frameshift mutations, large deletions, or early stop codons (Maddison, unpublished). Multiple copies of COI are also amplified if the standard barcode primers LCO1490 and HCO2198 (Hebert et al. 2003) are used.

The distribution of multiple copies of COI is not random in *Pseudoperyphus*. In the *B. chalceum* subgroup, three members (*B. chalceum*, *B. bellorum*, and *B. louisella*) appear to lack multiple copies of COI, and only one specimen of *B. antiquum*, number 1173, from Burlington, Ontario, shows multiple copies. *Bembidion rothfelsi*, however, frequently has multiple copies: 29 of 38 individuals sequenced had multiple copies, from throughout the range (Missouri, North Carolina, New Hampshire, Nova Scotia, Pennsylvania, Virginia, Vermont). In the *B. honestum* subgroup, *Bembidion rufotinctum* Chaudoir and *B. arenobilis* show only a single copy of COI. In contrast, *B. honestum* (30 of 61 specimens, from Maine, North Carolina, New Hampshire, Nova Scotia, Virginia, Vermont) and *Bembidion integrum* Casey (43 of 44 specimens, including five of the six females sequenced, from Iowa, Indiana, Missouri, Mississippi, South Dakota, Texas) show multiple copies.

Because of concerted evolution in which copies of ribosomal genes are homogenized throughout a genome (Eickbush and Eickbush 2007; Hillis et al. 1991), one expects the pool of copies of ribosomal genes to be more homogeneous than other nuclear genes. In *Pseudoperyphus*, 18S rDNA follows this pattern, with most specimens showing only a single form. Only a few specimens showed a single double peak in 18S rDNA, suggesting that there is slight variation among copies in those individuals.

In contrast, 28S rDNA shows more notable patterns of heterogeneity within individuals. Three species show uniformity within individuals in 28S rDNA (*B. antiquum*, *B. bellorum*, and *B. honestum*). In each of three other species (*B. chalceum*, *B. louisella*, and *B. integrum*), a single individual was sequenced that has some internal variation in 28S rDNA. Of the ten individuals of *B. rufotinctum* sequenced, three show at least two distinct copies. In the isolated Missouri population of *B. rothfelsi*, four of five individuals show heterogeneity, whereas only one (from Vermont) of the 33 specimens from other geographic areas are heterogeneous. *Bembidion arenobilis* also shows geographic variation: whereas none of the seven specimens from Mississippi is heterogeneous, all 18 of the Virginia specimens and five of six North Carolina specimens are heterogeneous for 28S rDNA.

### Phylogeny of *Pseudoperyphus*

The phylogenies inferred from the seven genes differ one from another (Figs. 3-5), although some elements are held in common. For example, all analyses for all genes except the most-parsimonious trees for *wingless* show the *B. honestum* subgroup as a clade, with strong support. In many

<table>
<thead>
<tr>
<th></th>
<th>28S</th>
<th>18S</th>
<th>COI</th>
<th>wg</th>
<th>CAD</th>
<th>ArgK</th>
<th>Pol2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. antiquum</em>, n=24</td>
<td>24</td>
<td>9</td>
<td>24</td>
<td>21</td>
<td>13</td>
<td>13</td>
<td>6</td>
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<td>41</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><em>B. louisella</em>, n=25</td>
<td>25</td>
<td>12</td>
<td>25</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>4</td>
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<td><strong>B</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. honestum</em>, n=61</td>
<td>61</td>
<td>10</td>
<td>61</td>
<td>55</td>
<td>12</td>
<td>12</td>
<td>6</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><em>B. arenobilis</em>, n=31</td>
<td>31</td>
<td>9</td>
<td>31</td>
<td>31</td>
<td>12</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. bellorum</em>, n=8</td>
<td>8</td>
<td>5</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>5</td>
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<tr>
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<td>7</td>
<td>0</td>
<td>0</td>
<td>32</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td><em>B. louisella</em>, n=25</td>
<td>25</td>
<td>12</td>
<td>25</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>
genes, the specimens identified as belonging to a single species form a clade.

Interpretation of COI and wingless is problematic, however, because of evidence of numerous individuals containing multiple copies. The resulting DNA sequences, which are an amalgam of the two or more sequences in the genome, may not be placed phylogenetically where any of the individual sequences would be placed. For COI this is particularly problematic, as numts tend to be “fossilized”, changing less from ancestral COI sequences than do their mitochondrial counterparts (Zischler et al. 1995). Thus, there might be a tendency for individuals in which a COI numt is amplified by PCR to appear as sister to the clade in whose ancestor the numt arose. To the extent numts are amplified differentially in different individuals, variation seen within a species such as B. honestum might result more from variation in the extent of PCR amplification of different gene copies rather than true variation within the species.

The molecular data indicate that Pseudoperyphus is monophyletic (supported by 28S, COI, wingless, ArgK, and Pol2), although CAD suggests that Ochthedromus is within Pseudoperyphus, as sister to the B. honestum subgroup. Four genes (18S, COI, CAD, and Pol2) support monophyly of Pseudoperyphus+Ochthedromus, although the other three genes provide a conflicting view.

Within Pseudoperyphus, the monophyly of the B. honestum subgroup is well supported by all seven genes. Support for monophyly of the B. chalceum subgroup is somewhat less evident, with COI, wingless, CAD, and ArgK providing strong support, and 28S rDNA weak support.

Within each subgroup, however, the phylogeny of species shows no consistent pattern between trees of different genes. This might be an artifact of incorrect

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Fig. 3.—Majority-rules consensus trees from Bayesian analyses using a GTR + I + F model of 28S rDNA and 18S rDNA for Pseudoperyphus. Branch lengths as reconstructed by MrBayes; scale bar units are substitutions per site. While MrBayes reconstructs branch lengths for these data to be orders of magnitude longer than those reconstructed by PAUP* (not shown), the relative branch lengths within a tree are very similar to those reconstructed by PAUP*. Thick branches are those that are supported by BPP≥90 for both Bayesian analyses and are present in the most-parsimonious trees. Asterisks mark position of holotypes. Trees are drawn to the same horizontal scale. Outgroups not shown. As all multiple, identical sequences were condensed into a single sequence, the number of terminals for each species is not necessarily equal to the number of specimens sequenced for that species; the number of specimens sequenced for each group is indicated in parentheses after the species name. A, 28S rDNA; B, 18S rDNA.
inference of gene trees, or it might indicate true conflict between the genes, perhaps caused by deep coalescence (Maddison 1997). The phylogeny inferred from the concatenated matrix of all genes (Fig. 6) shows well-supported relationships throughout the tree, but most of these clades have strong support from some genes and strong conflict from others. Caution is also in order because of the problems inherent in combined analyses of multiple genes when gene trees might be expected to differ one from another (Kubatko and Degnan 2007). Details of the phylogeny of species of *Pseudoperyphus* will be examined more thoroughly in a separate paper (Maddison, Oliver, Arnold, and Wild, in preparation). For the purposes of this paper, of greatest importance is evidence for species boundaries provided by phylogenies for each gene (Table 5).

**GEOGRAPHIC RELATIONSHIPS OF SPECIES**

The geographic ranges of *Pseudoperyphus* species are shown in Figs. 18-24. Google Earth files that contain more detailed information about localities are available from links on http://bembidion.org/pseudoperyphus.

The geographic ranges of many *Pseudoperyphus* species broadly overlap. For example, seven of the nine species are found in Vermont (only *B. integrum* and *B. arenobilis* are missing from that state). In part because of similarity of habitat of different species of *Pseudoperyphus*, sympatric species often co-occur microsympatrically, together on the same gravel bar (Table 6). For example, *B. chalceum*, *B. rothfelsi*, *B. louisella*, *B. bellorum*, and *B. honestum* are found together on one gravel bar on the Baker River, near Rumney, New Hampshire. Four species (*B. chalceum*, *B. rothfelsi*, *B. honestum*, and either *B. louisella* or *B. anti-quum*) are found together in several localities in Vermont, New Hampshire, Maine, and North Carolina.

**SPECIES BOUNDARIES**

Four of the character systems studied herein could potentially provide strong evidence about species boundaries within *Pseudoperyphus*. Two of these are expected to be involved in reproductive isolating mechanisms (the flagellum and chromosome number), a third might be (pronotal shape), and the fourth, the gene sequences, provides evidence about recent and past gene exchange between populations.
The flagellum of the male genitalia apparently enters the female spermathecal duct during copulation (Schuler 1960), and likely conducts the sperm. The correlation observed between length of the spermathecal duct and length of the flagellum in subgenus Microserrulla Netolitzky of Bembidion (Schuler 1959) as well as Hawaiian Bembidion (Liebherr 2008) suggests that the flagellum might be involved in the processes that determine which male mates with which female, either via a classic lock-and-key mechanism or sexual selection (Eberhard 1985). Thus, differences in flagellar shape could lead to reproductive isolation.

Variation in pronotal shape also may be involved in reproductive isolation. Evidence for this comes indirectly in the extent to which the pronotum is used by taxonomists to distinguish species of carabids. More compelling is the observation that carabid males hold the females at the junction between the pronotum and mesothorax using their front tarsi, as observed for Carabus (Takami 2002) and Bembidion (Maddison, unpublished). It would not be surprising, then, if the differences noted by taxonomists are in some of the same structures used by male Bembidion to distinguish species, as has also been suggested for the mesepisternal sulcus of tiger beetles of the genus Cicindela (Freitag 1974). While this is very speculative and needs to be tested, the fact that differences in pronotal shape observed in Pseudoperyphus are similar to those between species in other groups of carabids lends support to the distinctiveness of forms of Pseudoperyphus herein recognized as species.

In general, chromosomal polymorphism within animal species is rare, as it reduces viability of offspring (White 1973). One would expect, therefore, limited gene flow between Pseudoperyphus with different chromosome numbers. In particular, the unusual chromosome numbers of B. chalceum (n=13) and B. rothfelsi (n=17) provide evidence that these forms are distinct species, not currently exchanging much genetic material with other forms.

Finally, the evidence provided by genes is compelling regarding current and past gene flow, especially if multiple, unlinked genes show no evidence of gene flow between morphological types in sympatry. In the current study, COI, a mitochondrial gene, is definitely not genetically linked to the remaining genes. 18S and 28S are expected to be linked, as they are present in arrays of tandem repeats (Eickbush and Eickbush 2007). The location in the genome of the remaining genes is unknown, but it seems highly unlikely that all are tightly linked.
Thus, if two sympatric morphological forms have multiple
genes in which there are consistent nucleotide differences,
a lack of gene flow between these forms is indicated, and
they likely represent two distinct species. For example, in
individuals with flagellar shapes as in Fig. 14D (the *B. anti-
quum* form) show consistent differences from individuals
with flagellar shapes as in Fig. 14E (the *B. louisella* form)
in six of the genes studied (Table 4A), and the two forms
are broadly sympatric. This provides strong evidence that
these two forms are different species.

For *Pseudoperyphus* species as defined herein, every
species pair (except one) differs consistently by at least
two of flagellar shape, pronotal shape, chromosome num-
ber or multiple genes.

The single exception to this is the pair of *B. hones-
tum* and *B. arenobilis*, which consistently differ only in
DNA sequences. These two forms show fixed nucleotide
differences in four of the studied genes (28S, 18S, ArgK,
and Pol2), representing perhaps three linkage groups.
As these two forms are allopatric, the evidence that they

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**Fig. 6.**—Majority-rules consensus tree from Bayesian analyses of all seven genes for those specimens for which all genes were sequenced, using a separate GTR + I + G model for each gene. Branch lengths as reconstructed by MrBayes. Thick branches are those that are supported by BPP≥90 for both Bayesian analyses and are present in the most-parsimonious trees. The Central Sclerite Complex, with flagellum, of each *Pseudoperyphus* species is shown to the right, as well as the haploid chromosome number.
represent distinct species is not as compelling. However, while allopatric, they have been found within 44 km of each other in Virginia, and in that region, the two forms are more distinct, as judged by both morphological characters and DNA sequences. Populations of *B. honestum* from the James River watershed in Virginia and *B. arenobilis* from the adjacent Roanoke River watershed are distinct for numerous morphological characters (see the Diagnoses under these species in the taxonomic section, below), and have fixed differences in five presumably unlinked genes (Fig. 7). That this distinction is present in such close populations, with no apparent intervening barriers, suggests the forms are reproductively isolated. Specimens from more distantly separated populations of these two species are more similar in appearance to one another than are the adjacent populations. For example, Nova Scotia populations of *B. honestum* are more similar to Mississippi populations of *B. arenobilis* than are specimens

<table>
<thead>
<tr>
<th>Table 5. Support for monophyly or non-monophyly of each species from DNA sequences. Abbreviations: BPP: Bayesian Posterior Probability percentage; MP: most-parsimonious.</th>
<th>28S</th>
<th>18S</th>
<th>COI</th>
<th>wg</th>
<th>CAD</th>
<th>ArgK</th>
<th>Pol2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. chalceum</em></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>B. rothfelsi</em></td>
<td></td>
<td></td>
<td></td>
<td>X, 74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. bellorum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X, 79</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. antiquum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X, 90</td>
<td></td>
</tr>
<tr>
<td><em>B. louisella</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. rufotinctum</em></td>
<td></td>
<td>X, 98</td>
<td></td>
<td>X, 95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. arenobilis</em></td>
<td></td>
<td></td>
<td>X, 98</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. integrum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X, 75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- BPP ≥ 90, clade in MP trees
- BPP ≥ 90, clade not in MP trees or BPP 50-90, clade in MP trees
- BPP 50-90, clade not in MP trees
- BPP <50, clade not in MP trees
- X BPP of paraphyly or polyphyly of clade ≥ 70 (value indicated after X)

<table>
<thead>
<tr>
<th>Table 6. Geographic relationships of the species of <em>Pseudoperyphus</em>.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
</tr>
<tr>
<td><strong>M. chalceum</strong></td>
</tr>
<tr>
<td><strong>B. rothfelsi</strong></td>
</tr>
<tr>
<td><strong>B. bellorum</strong></td>
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<tr>
<td><strong>B. antiquum</strong></td>
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<tr>
<td><strong>B. louisella</strong></td>
</tr>
<tr>
<td><strong>B. rufotinctum</strong></td>
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<tr>
<td><strong>B. honestum</strong></td>
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<tr>
<td><strong>B. arenobilis</strong></td>
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<tr>
<td><strong>B. integrum</strong></td>
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</table>
of these two species in Virginia. In addition, there are no consistent differences between COI of distant populations even though in Virginia the two species exhibit 37 fixed differences. This suggests character displacement between populations of *B. honestum* and *B. arenobilis* where they are geographically proximate.

Monophyly of Each Species

While the nine *Pseudoperyphus* species as defined in this paper are likely separate, and do not share genes extensively, they are not necessarily monophyletic in all sampled genes. In particular, one would expect that species with large population sizes, or which have diverged from their sister group relatively recently, will have some genes for which the species is paraphyletic, reflecting deep coalescence (Maddison 1997).

For one of the species of *Pseudoperyphus*, *B. rothfelsi*, the individuals sequenced are monophyletic in all seven genes (Table 7). The remaining *Pseudoperyphus* have lesser numbers of genes supporting monophyly, with two species, *B. antiquum* and *B. arenobilis*, showing as many genes that are supported as monophyletic for that species as genes that are strongly supported as non-monophyletic (Table 7).

![Fig. 7.—Distribution of known populations of *B. honestum* (circles) and *B. arenobilis* (stars) in the watersheds of the James River and Roanoke River in Virginia and northern North Carolina. The table shows the number of nucleotides in each gene showing fixed differences between these four *B. honestum* populations and six *B. arenobilis* populations. For example, all 21 specimens sequenced for 28S rDNA from the James River watershed have 2 nucleotides that are consistently different from those of all 24 specimens sampled from the Roanoke River watershed.](image)

<table>
<thead>
<tr>
<th>Number of genes</th>
<th>Monophyly</th>
<th>Non-monophyly</th>
<th>Chr</th>
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<tbody>
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</tr>
<tr>
<td><em>B. rothfelsi</em></td>
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<td>17</td>
</tr>
<tr>
<td><em>B. bellorum</em></td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>B. antiquum</em></td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>B. louisella</em></td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>B. rufotinctum</em></td>
<td>6</td>
<td>0</td>
<td></td>
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<tr>
<td><em>B. honestum</em></td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>B. arenobilis</em></td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>B. integrum</em></td>
<td>4</td>
<td>1</td>
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</tr>
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</table>
DESCRIPTONS AND IDENTIFICATION OF TAXA

Family Carabidae Latreille, 1802
Tribe Bembidini Stephens, 1827
Genus Bembidion Latreille, 1802
Subgenus Pseudoperyphus Hatch, 1950

Pseudoperyphus Hatch, 1950:100. [Type species Bembidion chalceum Dejean, by original designation.]
Bracteomimus Lindroth, 1955:49. [Type species Bembidion chalceum Dejean, by original designation.]

Diagnosis.—Adult Pseudoperyphus are relatively large Bembidion, with an ABL from 4.8 to 6.6 mm. Frontal furrows on head are relatively shallowly impressed, not convergent, and not prolonged onto the clypeus. The pronotum lacks a basolateral carina. The lateral groove of the elytron is not prolonged medially at the shoulder, and thus is not angulate as in other members of the Odontium subgeneric group. All striae are well-impressed in basal half of elytra, and at least the second is well-impressed to the apex. Discal setae (ed3 and ed5) are attached to the third stria. The metasternal process between mesocoxae is unmargined. The hind wings are full, macropterous, and the beetles occasionally fly if approached on a hot day. Abdominal sterna are not pubescent, and lack rows of small setae. The right lobe of the Central Sclerite Complex of the male genitalia has a thick and dark ventral margin. The first stylomeres of females have eight or more long setae. First instar larvae lack a pattern of spots on the head, in contrast to spotted larvae of the remainder of the Odontium subgeneric group. The sex chromosome system is XY/XX, with 11 to 16 pairs of autosomes (thus, 12 to 17 pairs of chromosomes).

Habitat.—Members of Pseudoperyphus are generally found on rocky or gravel shores of running waters. Except for B. rufotinctum and B. arenobilis, Pseudoperyphus are most abundant on flat (< 10 cm high) gravel and cobble islands bordered by riffles in rivers and streams. At the type locality of B. bellorum in West Virginia, for example, the beetles were many times more common on the gravel island shown in the center of Figure 1B than along the shorelines on either side of the river. Within such an island, they are often most abundant at the upstream and downstream ends. These islands may act in part as filters, possibly trapping both Pseudoperyphus and organisms that constitute their food.

In contrast, B. rufotinctum is found in cracks in bedrock that is exposed in fast-flowing rivers, and B. arenobilis on shores of pure sand or sand mixed with some gravel. While other Pseudoperyphus are also found on sandy river shores (e.g., B. chalceum and B. integrum near Cayuga, Indiana), most are found on shores with cobbles and gravel, with sand as a minor component.

Fig. 8.—Adult males of Pseudoperyphus. A, Bembidion chalceum (Upper Northfield, Nova Scotia, DRM voucher V100002); B, B. rothfelsi (Bridgewater, Vermont, DRM voucher V100067).
Fig. 9.—Adult males of *Pseudoperyphus*. **A**, *B. antiquum* (Howland, Maine, DRM voucher V100082); **B**, *B. louisella* (North Conway, New Hampshire, DRM voucher V100080); **C**, *Bembidion bellorum* (Valley Head, West Virginia, DRM voucher V100236); **D**, *B. rufotinctum* (Quechee Gorge, Vermont, DRM voucher V100086).
Fig. 10.—Adult males of *Pseudoperyphus*. A, *B. honestum* (Glasgow, Virginia, DRM voucher V100196); B, *B. honestum* (Sharon, Vermont, DRM voucher V100093); C, *B. arenobilis* (Danville, Virginia, DRM voucher V100189); D, *B. integrum* (Stratford, Iowa, DRM voucher V100138);
Species Included

*Bembidion chalceum* subgroup
- *B. chalceum* Dejean
- *B. rothleri*, n. sp.
- *B. bellorum*, n. sp.
- *B. antiquum* Dejean
- *B. louisella*, n. sp.

*Bembidion honestum* subgroup
- *B. rufotinctum* Chaudour
- *B. honestum* Say
- *B. arenobilis*, n. sp.
- *B. integrum* Casey

Identification of Species Using Morphological Data

Most adults of *Pseudoperyphus* are difficult to identify based on external structure of adults, with the exception of *B. rufotinctum*, as many of the distinctive characters are subtle aspects of shape, luster, or color. Comparative material will significantly aid identification of new material. For a positive identification, examination of the flagellum of the male aedeagus is often necessary, ideally by studying the aedeagus from the right side. This is certainly true of specimens of *B. louisella* and *B. antiquum*, as I have yet to discover external characters that can allow them to be reliably told apart. Two species, *B. honestum* and *B. arenobilis*, cannot yet be consistently distinguished with morphological data.

In the following key for adults, references to ed3 are to the anterior of the two discal setae in elytral stria three; ed5 is the posterior of the two discal setae.

1. Elytra with central red spot just before apex (Fig. 9D). Appendages thick and short. Pronotum aeneous, with basolateral depressions bluish. Pronotum cordate (Fig. 11F), with constricted base and strongly-rounded sides .............. *B. rufotinctum*

1'. Elytra uniform in color, without red spot. Appendages more slender. Pronotum of various colors and forms ................................................. 2

2 (1'). Microsculpture on disc of elytra isodiametric, or, if stretched into transverse meshes (Figs. 13A, 13B). Pronotum relatively narrow, with lateral margins straighter (less rounded) than other *Pseudoperyphus*, only slightly constricted in front of hind angles; basal impression smooth, with few wrinkles; basal foveae distinct, generally somewhat linear, angled anteriorly toward the midline (Fig. 11I). Male flagellum long and straight (Fig. 14I). Western, from the states bordering the Mississippi River and westward ........................................... *B. integrum*

2'. Microsculpture on disk of elytra isodiametric, or, if stretched into transverse meshes, less so than in Fig.13A (but some specimens as much as Fig. 13B). Pronotum with more rounded sides, or wider, or wrinkled, with less distinct basal foveae. Male flagellum shorter, or sinuous. Geographic range western or eastern .................................................. 3

3 (2'). Male aedeagus with dorsal margin of flagellum more or less straight, except for slight curve at tip in some specimens (Figs. 14G, 14H). Pronotum relatively flat, with basolateral depressions very shallow, and dull in luster (Figs. 11G, 11H); lateral margins not strongly rounded. Area around ed5 foveate (Figs. 12A, 12B). Elytral stria five clearly engraved to apex (Figs. 12A, 12B). Microsculpture of elytra more or less isodiametric (Fig. 13C)

.......................................................... B. chalceum subgroup, 5

3'. Male aedeagus with dorsal margin of flagellum notably sinuate (Fig. 14A-E), least so in *B. bellorum* (Fig. 14E). Area around posterior discal seta less foveate (Figs. 12D-F). Elytral stria five effaced toward apex (Figs. 12D,F), or, if clearly engraved to apex (Fig. 12E), then pronotum convex, with strongly rounded sides (Fig. 11A). Microsculpture isodiametric or transverse .................................................. 4

4 (3). Hind angles of pronotum of most specimens projecting, forming a right angle or slightly acute (Fig. 11H). In the Piedmont or coastal plains below 400 m in elevation from Virginia (south of the James River drainage) south to Mississippi; on shorelines of pure sand or sand mixed with gravel .............

.......................................................... *B. arenobilis*

4'. Hind angles of pronotum generally obtuse (Fig. 11G). In the James River drainage of Virginia and northward, or, if south of the James River watershed, in the mountains, above 500 m elevation ......

.......................................................... *B. honestum*

5 (3'). Elytral striae 2 through 7 deeply engraved at apex; seventh stria deeply engraved at level of ed5 (Fig. 12E). Flagellum of male relatively short, and gently sinuate (Fig. 14A) ....................... B. chalceum

5.' Elytral striae 2 through 7 more or less effaced near apex; seventh stria rarely deeply impressed posterior of ed5 (Figs. 12D, F). Flagellum longer, of various shapes (Figs. 14B-E) ........................................ 6

6 (5'). Pronotum convex, with relatively straight sides, more or less angulate at the anterior lateral seta (Fig 11B); lateral edge of pronotum from anterior lateral seta to front angle straight, not rounded, with front angle in general relatively prominent. Appendages darker, with palps and (usually) tibiae piceous, with bluish reflection; often with slight bluish reflection on head, sides of the pronotum, and legs. Flagellum of male with relatively straight ventral margin, slightly curved dorsally toward apex in almost all specimens (Fig. 14B); if apex is not curved dorsal, then the basodorsal edge of the CSC lacks the
thick, dark ridge shown in Fig. 14E (marked with an arrow) ............................... B. rothfelsi

6'. Pronotum less convex, wider, with lateral edge of pronotum from anterior lateral seta to front angle curved, not straight (Figs. 11C, D, E). Appendages generally paler, usually with tibia at least partly rufous and first antennomere rufous ventrally. Flagellum of male either with sinuous ventral margin (Fig. 14D) or with straight ventral margin that is not curved dorsally at apex (Fig. 14C) ..................... 7

7 (6'). Elytral intervals convex, striae well-impressed, with large punctures. Shiny, microsculpture not deeply engraved; both male and female pronota entirely shiny, even the posterior portion. Pronotum narrower, convex, with less rounded sides (Fig 11C). Dorsal edge of male flagellum abruptly angled, with blunt apex, and dorsal border near apex darkened and gently curved (arrow in Fig. 14C) ..................... B. bellorum

7' (7'). Elytral intervals flat, striae less deeply impressed, with small punctures. Duller; at least posterior portion of female pronotum dull. Pronotum broader, flatter, with well-rounded sides (Figs. 11D, 11E). Dorsal edge of male flagellum not abruptly angled, with more tapered apex .......................... 8

8 (7'). Pronotum more cordate (Fig. 11E), with the constricted region in front of hind angles more prominent, with basolateral margins parallel (or subparallel) for a short distance in front of hind angle, and thus the hind angles more acute, often protruding; widest point of pronotum often in front of anterior lateral seta; transverse basal impression more prominently wrinkled, with basal foveae less well-defined. Dorsal surface slightly shinier. Appendages slightly darker, with palps often fully infuscated. Many specimens cannot be reliably distinguished from B. antiquum without examination of male genitalia. Male flagellum with straight ventral margin (Fig. 14E). From eastern Vermont, New Hampshire, Maine, Quebec, and the maritime provinces of Canada, including Cape Breton Island and Newfoundland .......................................... B. louisella

8'. Pronotum less cordate (Fig. 11D, which shows a relatively cordate pronotum for B. antiquum), often with a less extensive posterior constricted region, hind angles more often obtuse; widest point of pronotum often behind anterior lateral seta; transverse basal impression less wrinkled in most specimens; basal foveae thinner, more clearly defined. Dorsal surface in most specimens slightly duller. Appendages slightly paler, with palps often rufous in part. Male flagellum with sinuate ventral margin (Fig. 14D). From Nova Scotia (excluding Cape Breton Island), west and south to at least Missouri, and North Carolina ..................... B. antiquum

**Bembidion chalceum** Subgroup

Members of this subgroup are characterized by the flagellum of the male genitalia, the dorsal margin of which is notably sinuate. In addition, membranes anterior to the os- tial microtrichial patch (Maddison 1993) have very large scales, and the apex of the flagellar sheath, just dorsal of the flagellum, has dark, linear scales. The apex of the ae- deagus is narrow.

**Bembidion chalceum** Dejean

(Figs. 2A, 8A, 11A, 12E, 14A, 15A, 18)


**Notes about Types.**—According to G.E. Ball, in the box labeled “Chalceum Dejean Etats-Unis, C. Dejean,” there are “11 specimens, of which only two need be seriously considered as authentic Dejean material. The first one bears the row and locality labels; the third, a square [of green paper with female symbol].” The first is designated as the lectotype. Genitalia of lectotype examined.

**Diagnosis.**—The combination of convex, somewhat small and cordate prothorax with small and deep basal foveae (Fig. 11A), elytral striae deeply engraved to apex (the se- venth, for example, is deeply engraved posteriad of ed5, Fig. 12E), convex elytral intervals, and lack of a red spot on the elytra is normally sufficient to identify specimens. The male flagellum is sinuate (Figs. 2A, 14A, 15A), but shorter than in the similarly sinuate *B. antiquum* (Figs. 2B, 14D, 16A). Unique within *Pseudoperyphus*, specimens have 13 pairs of chromosomes.

From *B. rothfelsi*, which is also relatively convex, *B. chalceum* differs in its smaller size, more convex elytral intervals, more deeply engraved striae, especially toward the elytral apex, more rounded prothorax, and in generally paler appendages.

From *B. bellorum*, *B. chalceum* differs in its more convex elytral intervals, more deeply engraved striae, stronger microsculpture, and (in many, but not all, specimens) its generally brassy or green metallic reflection.

Members of this species are relatively small, with an ABL of 4.8-5.7 mm (n=11).

**Geographic Distribution.**—This is the most widespread member of the subgenus, extending from the Pacific coast in the west to Newfoundland in the east, and from the North West Territories south to Arkansas and South Carolina (Fig. 18). Approximately 1970 specimens were examined.
Morphological Variation.—Specimens from some populations (e.g., Illinois, Alberta, Newfoundland) are larger and darker than some eastern (e.g., Vermont, southern Ontario) populations. Some specimens from Colorado have very reduced microsculpture, and thus are quite shiny; they also have pale reddish legs.

Molecular Variation.—The six specimens examined from near Hope, British Columbia, have distinct 28S rDNA, differing from all but one specimen east of the Rocky Mountains in having two unusual bases around site 670 in the sequenced fragment. British Columbia specimens have GT-GTTTTTAAAT; in contrast, most specimens from Alberta, Wyoming, and eastward have the sequence ATGTTTTA AAA. One of the five specimens sequenced from Alberta, voucher number 1671 from Burbank, is polymorphic for 28S, showing clear double peaks in the chromatograms at these two differing sites. There are also very short secondary peaks at both of these sites in a few other eastern specimens, suggesting that a small fraction of the copies of 28S in these beetles is of the western form.

Habitat.—On gravel and cobble shores of rivers. Members of this species can also be found around gravel-shored ponds, including in quarries (e.g., Cambridge, Ontario, Canada).

Bembidion rothfelsi, new species
(Figs. 2C, 8B, 11B, 12F, 13F, 14B, 15B, 19)

Fig. 12.—Elytral apices of *Pseudoperyphus*. Elytral stria numbers indicated in figure A. A, *B. arenobilis* (Virginia: Brookneal, DRM voucher V100488); B, *B. honestum* (Virginia: Bent Creek, DRM voucher V100492); C, *B. integrum* (Mississippi: Lexie, DRM voucher V100497); D, *B. antiquum* (Virginia: Glasgow, DRM voucher V100493); E, *B. chalcium* (New Hampshire: Quincy, DRM voucher V100494); F, *B. rothfelsi* (Vermont: Bridgewater, DRM voucher V100498).
Maddison V100068 Voucher Specimen” / “Chromosomes 2n=26+XY David R. Maddison” [pale purple paper] / “HOLOTYPE Bembidion rothfelsi David R. Maddison” [red paper] / “ADP 110513”. Genitalia in glycerine vial with specimen, and chromosome slide nearby; extracted DNA stored separately. GenBank accession numbers for DNA sequences of the holotype are: EF648685 (18S), EF648960 (28S), EF649383 (COI), EF649465 (CAD), EF649668 (wg), EF648771 (ArgK), EF648827 (Pol2).

Paratypes.—400 specimens, deposited in USNM, MCZ, CAS, CNC, BMNH, MNHN, CMNH, CUIC, UASM, UAIC, DRM, MSMC, MZLU, and UVCC, including 370 from the type locality, as well as all B. rothfelsi specimens listed in Appendix 1.

Type Locality.—USA: Vermont: Windsor Co., Ottauquechee River, Bridgewater 250m, 43.5878°N 72.6377°W. At the type locality, B. rothfelsi were abundant on 11 July 2003, with 372 specimens found in a small area of cobble shore. Only four other Pseudoperyphus specimens were found: two of B. chalceum, one of B. honestum, and one of B. antiquum. Bembidion planum Haldeman was also common.

Notes about Synonymy.—This is the taxon called “Bembidion sp. 17” in Maddison (1985, 1993).

Derivation of Specific Epithet.—This species is named in honor of the late Klaus Rothfels, cytogeneticist, in whose lab in 1980 I discovered the distinctness of this species through examination of chromosomes.

Diagnosis.—Externally identifiable most readily by shape of the prothorax, and, in many specimens, color. Adults are dark, in most specimens with piceous tibiae and palps; the legs, prothorax, and head often have a bluish reflection in areas. The pronotum is narrow, convex, with the lateral margin consisting of a series of more or less straight lines, rather than being rounded (Fig. 11B). Thus, the margin is
This species has been confused with *B. chalceum* in the past (see that species for a description of differences), and might be confused with *B. bellorum*, and, to a lesser extent, *B. louisella* and *B. antiquum*.

From *B. bellorum* distinguishable by the darker appendages, with pinceau first antennal article and infuscated palps, with a bluish reflection. *B. rothfelsi* specimens also have a duller luster, especially in females, with slightly more transverse microsculpture meshes on the elytral disc. The prothorax in *B. rothfelsi* is narrower and with straighter edges, with narrower reflexed margins. The punctures on the striae are also smaller in *B. rothfelsi*, most easily visible by comparing seventh striae.

From both *B. louisella* and *B. antiquum*, *B. rothfelsi* is easily distinguished by the narrower, much-less rounded prothorax, with narrower reflexed margin. The flagellum of *B. rothfelsi* is very similar to that of *B. louisella*, but the apex of *B. louisella* is straighter; in *B. rothfelsi* the apex is slightly curved dorsal in almost all specimens. In rare specimens that have an uncurved apex (e.g., Fig 2C, right-most specimen), then the basodorsal edge of the CSC lacks the thick, dark ridge characteristic of *B. louisella* males (Fig. 14E, arrow).

Members of this species are generally larger than *B. chalceum*, with an ABL of 5.7-6.2 mm (n=6).

**Geographic Distribution.**—About 950 specimens of this common species were examined, ranging from Nova Scotia south to Mississippi, and west to Missouri (Fig. 19).

**Morphological Variation.**—Some specimens from New Hampshire, identified using DNA or genitalia, have prothoraces less like a standard *B. rothfelsi* and more like *B. bellorum*. Some males have a flagellum very similar to that of *B. louisella*.

**Molecular Variation.**—None noted.

**Habitat.**—This species is generally found on smaller rivers and creeks.

*Bembidion bellorum*, new species

(Figs. 1B, 9C, 11C, 13D, 14C, 15C, 20A)


Paratypes.—73 specimens, deposited in USNM, MCZ, CAS, CNC, BMNH, MNHN, CMNH, CUIC, UASM, UAIC, DRN, MZLU, and UVCC, from the following localities. **UNITED STATES. NEW HAMPSHIRE.**
Fig 15.—Aedeagi of *Pseudoperyphus* males. Left (top) and right (bottom) views. A, *B. chalceum* (North Carolina: Huntdale, DRM voucher DNA1539); B, *B. rothfelsi* (Ontario: Marsh’s Falls, DRM voucher V000100); C, *B. bellorum* (West Virginia: Valley Head, DRM voucher DNA1904).
Fig. 16.—Aedeagi of *Pseudoperyphus* males. Left (top) and right (bottom) views. **A**, *B. antiquum* (Ontario: Ottawa, DRM voucher V000102); **B**, *B. louisella* (Nova Scotia: Cape North, DRM voucher DNA1247); **C**, *B. rufotinctum* (New Hampshire: Merrimack, DRM voucher DNA1536).
Fig. 17.—Aedeagi of *Pseudoperyphus* males. Left (top) and right (bottom) views. A, *B. honestum* (North Carolina: Huntdale, DRM voucher V100165); B, *B. arenobilis* (Virginia: Danville, DRM voucher V100163); C, *B. integrum* (Nebraska: Brule, DRM voucher V000113).
Type Locality.—USA: WV: Randolph Co., Tygart Valley River near Valley Head 690m, 38.5804°N 80.0306°W (Fig. 1B). No other Pseudoperyphus were discovered along with the 66 B. bellorum found at the type locality.

Derivation of Specific Epithet.—Named after Ross and Joyce Bell, who have contributed so much to our knowledge of carabids, and who have lived in the heart of the region containing abundant Pseudoperyphus. In addition to encouraging my work on Bembidion, Ross collected specimens of B. honestum and B. rufotinctum and fixed them for cytogenetic study at my request during the first phases of this study; these proved vital in my early understanding of the group. Ross also discovered the first substantial series of B. bellorum, from near Granby, Vermont.

Diagnosis.—Adults of this species are shiny, with dark body and relatively pale appendages (for example, the antennal scape is entirely pale rufous or at most slightly infuscated dorsally), and with larger, distinct punctures in the elytral striae. They are similar in appearance to a large, narrow Bembidion (Hirmoplataphus) nigrum Say. The apex of the flagellum is distinctive, with the dorsal margin thickened and gently curved (arrow in Fig. 14C), and the apex relatively thick.

For features distinguishing B. bellorum from B. chalceum and B. rothfelsi, see the descriptions of those two species.

Members of this species are relatively small, with an ABL of 5.1-5.8 mm (n=8).

Geographic Distribution.—133 specimens were examined, from Maine, Vermont, and New Hampshire south to Pennsylvania and West Virginia, west to Ohio and Kentucky (Fig. 20A). As this species has been found in northern Vermont only 28 km from the border with Canada, I expect that it will be discovered in southern Québec.

Morphological Variation.—None noted.

Molecular Variation.—None noted.

Habitat.—As is standard for Pseudoperyphus, found on cobble and gravel shores of rivers. On the Tygart Valley River in West Virginia, found in the company of
B. planum and B. rusticum Casey which were also common. Along the Black Fork near Hambleton found with many B. honestum and B. planum.

Bembidion antiquum Dejean
(Figs. 2B, 9A, 11D, 12D, 14D, 16A, 21)


Notes about Types and Synonomy.—According to G.E. Ball, in the box in the MNHN labeled “antiquum Dejean Etats-Unis, C. Dejean,” there are “6 specimens, of which only the first two are probably authentic Dejean material. Both are males. The first one has the Dejean name and locality. The second one is simply labeled [male symbol on square green paper].” As Dejean (1831) mentioned that he had only a single specimen, the first of these two males is considered to be the holotype. Thus, my labels designating that specimen as the lectotype are in error, as pointed out by Yves Bousquet (pers. comm. 2008). Genitalia of holotype examined.

The type specimen of Ochthedromus dilatatus LeConte is, by external form, certainly either a B. antiquum or B. louisella. Its color, luster, and pronotal shape is that of a typical B. antiquum. As the type is a female, it cannot be identified with certainty. However, as all specimens examined from Pennsylvania of similar form are B. antiquum,
and the nearest known locality of *B. louisella* is central New Hampshire (over 500 km distant), *O. dilatatus* is considered a synonym of *B. antiquum*.

**Diagnosis.**—This and the following species are the largest members of the *B. chalceum* subgroup, and are most readily identified by their size, and by having large, flat elytra, with flat intervals, and relatively shallowly-impressed striae, and by the well-rounded pronotum (Fig. 11D). Males of *B. antiquum* have a large, sinuate flagellum (Fig. 14D, 16A), resembling that of *B. chalceum*, but larger.

*Bembidion antiquum* members can be extremely difficult to tell apart from *B. louisella* using external morphological characters, although they can easily be distinguished if male genitalia or DNA sequences are available. While morphologically extreme individuals can be identified using external characters with fair certainty, many specimens cannot. *Bembidion antiquum* adults have a prothorax that is less constricted basally (Fig. 11D), with maximum width generally behind the anterior lateral seta, and with slightly more obtuse hind angles; the basal impressions are less wrinkled in *B. antiquum*, and the basal foveae are more linear and more sharply delimited. The appendages in many specimens are paler than in *B. louisella*, and many *B. antiquum* specimens have a more pronounced aeneous metallic reflection. Despite the similarities externally, the male flagellum of *B. antiquum* (Fig. 14D) is quite different from that of *B. louisella* (Fig. 14E).

This species has in the past been confused with both *B. chalceum* and *B. honestum*. It is quite different from *B. chalceum: B. antiquum* is much broader, with a large prothorax,
and with flat elytral intervals, and less-impressed striae. From *B. honestum* it is most easily distinguished by the broader, more-rounded pronotum, with deeper basolateral impressions, and by the more apically-effaced elytral striae: *B. antiquum* also has slightly more transverse elytral microsculpture meshes than does *B. honestum*.

Members of this species are relatively large, with an ABL of 5.6-6.6 mm (n=7).

**Geographic Distribution.**—From Nova Scotia and Québec south to Alabama, and west to Wisconsin and Arkansas (Fig. 21). There is in addition a single specimen in the CNC from Alberta labeled “CANADA: Lethbridge, Alb 126. 23.VII.1956 Lindroth”. In 2004, Anne E. Arnold and I collected at the exact locality (locality details provided by G.E. Ball, in litt.) in an effort to find *B. antiquum*, but only found *B. chalcem* in abundance (well over 100 specimens were found). As *B. antiquum* is otherwise not known from west of Wisconsin and Arkansas, this specimen may have been labeled in error. There is as well a single specimen in the CMNH labeled “CA”, which, if it refers to California rather than Canada, is likely incorrectly labeled.

Curiously, among the 633 specimens of *Pseudoperyphus* I have examined from New Hampshire, none is *B. antiquum*, even though *B. antiquum* lives in all surrounding states and provinces. The 90 specimens of *B. antiquum*/*B. louisella*-like *Pseudoperyphus* from New Hampshire, from six localities, are all *B. louisella*.

For comments on the geographic overlap between *B. antiquum* and the similar *B. louisella*, see the section “Geographic distribution” under the latter species, below.

**Morphological Variation.**—Specimens from the Ozark Plateau have relatively effaced microsculpture, and thus are notably shiny.

**Molecular Variation.**—None noted.

**Habitat.**—Living on the cobble and gravel shores of a wide variety of flowing waters, from large rivers to small creeks.

*Bembidion louisella*, new species  
(Figs. 1A, 9B, 11E, 13E, 14E, 16B, 22)


**Paratypes.**—174 specimens, deposited in USNM, MCZ, CAS, CNC, BMNH, MNHN, CMNH, CUIC, UASM, UAIC, DRM, MSMC, MZLU, and UVIC, including 150 from the type locality, plus all *B. louisella* specimens listed in Appendix 1, and the remainder from the following localities: CANADA: Nova Scotia: Pleasant Bay (6).

**Type Locality.**—North Aspy River near the town of Cape North, Cape Breton Island, Nova Scotia, Canada, 46.8863°N 60.5224°W (Fig. 1A). No other *Pseudoperyphus* were found along with the 151 specimens of *B. louisella* found at the type locality.

**Derivation of Specific Epithet.**—Named after my mother, Louise Juana (Devillez) Maddison, who grew up on the shores of Cape Breton Island, about 90 km from the type locality, and whose curiosity and inquisitiveness was my first inspiration as a biologist. She has been extraordinarily supportive of my work since I first became interested in beetles as a teenager, and has accompanied me on many field trips, including the 2002 trip during which the holotype was collected. The epithet “louisella” is a noun in apposition meaning “little Louise”.

**Diagnosis.**—Adults of this northeastern species resemble *B. antiquum* externally, but *B. rothfelsi* in shape of the flagellum. The prothorax is broad and cordate, with strongly rounded sides, and with relatively wide lateral margins; the widest point of the pronotum is often in front of the...
anterior lateral seta (Fig. 11E). The basolateral margins of the pronotum are parallel (or subparallel) for a short distance in front of the hind angle in most specimens. The posterior portion of the prothorax is more constricted in *B. louisella*, with more acute hind angles. The transverse basal impression of the pronotum is generally wrinkled in *B. louisella*, and the basal foveae are usually shallower, and less clearly defined. As with *B. antiquum*, elytral intervals are shallow, with relatively shallowly-impressed elytral striae, which are effaced apically. The dorsal surface is generally darker, rarely with a notable aeneous reflection. Male genitalia have the ventral margin of flagellum straight, and with a curved dorsal margin (Figs. 14E, 16B).

To distinguish *B. louisella* from *B. antiquum* and *B. rothfelsi*, see the descriptions under the latter two species. *B. louisella* cannot be reliably distinguished from *B. antiquum* except with male genitalia or DNA sequences. Members of this species are relatively large, with an ABL of 5.6-6.6 mm (n=8).

**Geographic Distribution.**—Restricted to the northeast, from Newfoundland and Quebec (common on the Gaspé) south and west to Maine and New Hampshire, as well as northeastern Vermont (Fig. 22). Within Nova Scotia, *B. louisella* and *B. antiquum* show complementary ranges, with *B. louisella* known from Cape Breton Island and the rivers flowing into the Bay of Fundy

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Fig. 22.—Geographic distribution of *B. louisella* (stars), with nearby localities for *Bembidion antiquum* (circles). Black stars and dark gray circles indicate specimens identified using one of the two definitive traits for this pair of species (male flagellum or DNA sequences). Gray stars and light gray circles indicate specimens identified only using external structure; as these two species are very difficult to tell apart by external structure, the grey records should be viewed with greater caution.
from the Cobequid Mountains, as well as the Halfway River near Hantsport, and \textit{B. antiquum} along the other rivers. Casual observation suggests that \textit{B. louisella} occurs along rivers with colder waters, but this needs to be confirmed with numerical data.

\textit{Bembidion louisella} and the similar \textit{B. antiquum} have both been found at Becancour, Québec (CSCA), and in the vicinity of Allagash, Maine (MCZ). They are also expected to coexist on the southern slopes of the Appalachian mountains in eastern New Hampshire and western Maine (e.g., possibly on the Saco River between North Conway and Cornish).

\textbf{Morphological Variation.}—Specimens from Newfoundland are somewhat larger than specimens from the mainland.

\textbf{Molecular Variation.}—No significant variation noted.

\textbf{Habitat.}—On the gravel and cobble shores of cold, clear rivers.

\textit{Bembidion honestum} Subgroup

Members of this subgroup are characterized by a relatively straight flagellum of the male genitalia; the aedeagus has a relatively broad apex (Figs. 16C, 17), and lacks the large scales present near the ostial microtrichial patch in the \textit{B. chalcium} subgroup; the aedeagus also lacks dark, linear scales on the flagellar sheath.

\textit{Bembidion rufotinctum} Chaudoir (Figs. 1D, 9D, 11F, 14F, 16C, 20B)

\textit{Bembidium rufotinctum} Chaudoir, 1868:241. \textit{LECTOTYPE} female designated by Lindroth (1963), in MNHN, not examined. Type locality “l’Amérique septentrionale.” Type locality restricted herein to Quechee Gorge, Vermont.


\textbf{Diagnosis.}—The large, central, reddish spot on the elytra (Fig. 9D), after which this species is presumably named, is the most distinctive trait of specimens. The red spot contrasts notably against the pronotum, which is often green with the basolateral depression and margins bluish. In fresh specimens, the femora and first antennal article are a bright rufous or rufotestaceous. In addition, the pronotum is strongly cordinate, and the legs are short and thick. The aedeagus is small, with a broad apex, and a small flagellum (Figs. 14F, 16C).

This species contains the smallest \textit{Pseudoperyphus}, with an ABL of 4.6-5.3 mm (n=8).

\textbf{Geographic Distribution.}—From Québec, Vermont, and New Hampshire south to South Carolina and Georgia (Fig. 20B); see Cooper (1976), Choate (1977), Davidson (1981), and Choate and Choate (1995).

\textbf{Morphological Variation.}—None noted.

\textbf{Molecular Variation.}—Specimens from North Carolina differ from those sampled from Vermont and New Hampshire at eight nucleotides in 1446 bases of COI (0.55%), but do not differ consistently in any of the other six genes.

\textbf{Habitat.}—\textit{Bembidion rufotinctum} is unusual within \textit{Pseudoperyphus} in living in cracks of emergent bedrock in river channels (Cooper 1976; Davidson 1981), as shown in Fig. 1D.

\textit{Bembidion honestum} Say (Figs. 7, 10A-B, 11G, 12B, 14G, 17A, 23)


\textbf{Notes about Types and Synonymy.}—The \textit{B. honestum} neotype is teneral, and the genitalia appear to be lacking. However, external characteristics are sufficient to allow placement of the type to either this species or \textit{B. arenobilis}, and the type locality indicates that it belongs to this species. Types of \textit{Ochtedromus basalis} LeConte and \textit{Bembidium platyderum} Chaudoir, appear, by morphological traits, to belong to this species or, less likely, to the Mississippi populations of \textit{B. arenobilis}. The type localities of both names thus argue for placement as synonyms of \textit{B. honestum}. The type of \textit{Ochtedromus basalis} has the pterocephalous and abdomen damaged, apparently by dermestids.

\textbf{Diagnosis.}—\textit{Bembidion honestum} is extremely difficult to distinguish from \textit{B. arenobilis}. Where they are geographically proximate, specimens of the two species are relatively easy to distinguish morphologically. In these regions (North Carolina and Virginia), \textit{B. honestum} specimens are darker, with darker appendages; \textit{B. arenobilis} adults have clearly rufous tibiae, and paler femora. The microsculpture of \textit{B. honestum} is more weakly impressed, and thus specimens are shinier; this is most evident on the very center of the dorsal surface of the head of males between the eyes, which in most specimens has effaced...
microsculpture in *B. honestum*, but obvious microsculpture in *B. arenobilis*. The microsculpture of the eighth elytral interval is also in general less distinct in *B. honestum* than in *B. arenobilis*, in which the microsculpture can be granulate (e.g., between eo6 and eo7). The hind angles of the pronotum are generally obtuse in *B. honestum*, in contrast to the acute and laterally protruded angles of most *B. arenobilis* specimens. Most specimens of *B. arenobilis* have the area around ed5 slightly more foveate than specimens of *B. honestum*.

However, specimens of *B. arenobilis* from the southern and western end of the range (Mississippi) are more
similar to B. honestum in morphological traits, especially when compared to B. honestum from further north, e.g., New Hampshire. While the difference in pronotal shape tends to be consistent (but not universally), the microsculpture differences are not. As there are no consistent differences in genitalia that I have found, I cannot consistently distinguish specimens based upon morphological traits. The only certain way to distinguish these two species is with molecular data.

To distinguish this species from B. integrum, see under that species.

Members of this species are relatively variable in size, with an ABL of 5.3-6.2 mm (n=10).

**Geographic Distribution.**—Approximately 1340 specimens were examined from Nova Scotia and Québec south to North Carolina (in the mountains), and west to eastern Illinois (Fig. 23). There are, in addition, state records from Kansas, Iowa, and Wisconsin. In Virginia, B. honestum occurs in the Piedmont in the James River drainage and north (Fig. 7).

The report of B. honestum from Churchill, Manitoba (Elias 1984), is based on several specimens of the subgenus Plataphus (examined).

**Morphological Variation.**—Specimens from Virginia, near the range of B. arenobilis, are darker and have broader, more well-rounded prothoraces than do specimens from further north (e.g., Vermont).

**Molecular Variation.**—There are two rather different forms of COI within this species, which differ by about 40 nucleotides. One form is found in New England (Maine, New Hampshire, Vermont) and the mountains of North Carolina; the other is found in Nova Scotia, New England (Maine, New Hampshire), Pennsylvania, and lowland Virginia. However, if one of these forms is simply a nuclear copy of COI, it may be that this geographic pattern reflects in part only variation in primer sites and thus the relative ease of PCR amplification of the nuclear and mitochondrial copies rather than variation in the COI sequence as a whole. Many of the North Carolina specimens appear to have both of these different forms within them, based upon the pattern of double peaks in the chromatograms. Cloning of COI will be necessary to resolve the true patterns of variation of mitochondrial COI as opposed to nuclear COI.

The *wingless* gene shows a similarly complex pattern with many individuals being heterozygous between two very different *wingless* forms. One form, very much like that present in B. integrum, is found in Nova Scotia, New England (Maine, Vermont), montane North Carolina, and lowland Virginia. The other form, more like that found in B. arenobilis, is found in New England (Maine, New Hampshire, Vermont), montane North Carolina, and lowland Virginia. As with COI, cloning of *wingless* would allow greater understanding of the patterns.

**Habitat.**—This species tends to live on larger, more slowly flowing rivers.

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**Bembidion arenobilis, new species** (Figs. 1C, 7, 10C, 11H, 12A, 13C, 14H, 17B, 23, 25A)


**Paratypes.**—194 specimens, deposited in USNM, MCZ, CAS, CNC, BMNH, MNHN, CMNH, CUC, UASM, UAIC, and DRM, from the following localities. **UNITED STATES. NORTH CAROLINA. Chatham Co.:** Haw River at Bynum, 90m, 35.7721°N 79.1442°W (16). **Durham Co.:** Flat River near Rougemont, 120m, 36.2276°N 78.8884°W (2). **Rockingham Co.:** Dan River, Madison, 175m, 36.375°N 79.993°W (77). **Stokes Co.:** Town Fork Creek at Walnut Cove, 180m, 36.2917°N 80.1403°W (54). **VIRGINIA. Bedford Co.:** Big Otter River at route 24, 175m, 37.246°N 79.3495°W (8). **Campbell Co.:** Altavista, Roanoke River, 155m, 37.1057°N 79.2863°W (1). **Falling River, Brookneal, 115m, 37.054°N 78.9355°W (1). **Danville City Co.:** Danville, Dan River, 36.5288°N 79.4246°W (35).

**Type Locality.**—USA: Virginia: Dan River in Danville, at Robertson Bridge, 36.5788°N 79.4328°W (Fig. 1C). No other *Pseudoperyphus* were found at the type locality; other *Odontium* subgeneric group members include *Bembidion americanum* Dejean, *Bembidion confusum* Hayward, and an undescribed species related to *Bembidion arenulum* Hayward.

**Derivation of Specific Epithet.**—Informally derived by combining two Latin words: *arenus*, meaning sand, and *nobilis*, or noble. “Noble” is in reference to the similarity to members of *B. honestum*, a species whose epithet means distinguished or noble, and “arenas” to the habitat of these beetles on well-drained, sandy shores of rivers.

**Diagnosis.**—*Bembidion arenobilis* is so similar to *B. honestum* that I originally hesitated to describe it as a distinct species. However, the populations of *B. honestum* (James River, Virginia) that are closest to those of *B. arenobilis* (Roanoke River watershed, Virginia) show no sharing of genes (see above, and Fig. 7), and these populations are distinct morphologically from one another. Virginia *B. arenobilis* are duller because of the more deeply-impressed microsculpture, with lateral margins of prothoraces more sinuate and with hind angles more acute, and with paler legs, usually having rufous femora; for further details see the diagnosis of *B. honestum*.

Members of this species have an ABL of 5.3-5.7 mm (n=6).

**Geographic Distribution.**—From the Roanoke River drainage in Virginia south in the Piedmont through North Carolina, South Carolina, Alabama, and Mississippi (Fig.

**Diagnosis.**—Specimens of this species can be difficult to distinguish from both *B. honestum* and *B. arenobilis*, as these are the other two species with flatter, less rounded prothoraces, and a straight male flagellum. The microsculpture of *B. integrum* is stretched into more transverse meshes (Figs. 13A, B) than any *B. honestum* and *B. arenobilis* (Fig. 13C). In addition, the prothorax of *B. integrum* is smoother, less wrinkled, with more distinct and angled basal foveae (Fig. 11I). Most *B. honestum* and *B. arenobilis* specimens have the area around ed3 and ed5 more distinctly foveate (Figs. 12A, B) than any *B. integrum* (Fig. 12C). The flagellum of *B. integrum* (Fig. 17C) is much longer than that of either *B. honestum* or *B. arenobilis* (Figs. 17A, B).

One unexpected difference between *B. integrum* and both *B. honestum* and *B. arenobilis* is color of veins of the hind wings, which are much paler in *B. integrum* than most specimens of the other two species (Fig. 25).

Members of this species have an ABL of 5.2-6.3 mm (n=9).

**Geographic Distribution.**—The 500 specimens studied range from western Indiana south to Mississippi, west to Texas and adjacent Mexico, and north to Montana (Fig. 24).

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**Morphological Variation.**—Specimens from Mississippi are more similar in external form to *B. honestum* than to specimens of *B. arenobilis* from North Carolina and Virginia; see the diagnosis under *B. honestum*.

**Molecular Variation.**—Three genes show fixed differences between the Mississippi populations and the North Carolina/Virginia populations: 18S rDNA, differing by one nucleotide; CAD, also differing by one nucleotide; COI, with the populations differing at five nucleotide sites. The population from Mississippi is distinct enough from the North Carolina and Virginia populations in DNA sequences that it may eventually warrant a separate name. However, geographically intermediate localities should be sampled before this is done.

**Habitat.**—Less gravel- or cobble-dependent than most *Pseudoperyphus*. The most notable feature in common to known sites is the presence of pure, well-drained sand. At some of the known localities this sand has fine gravel mixed in, but at others, including the type locality, the beetles are found on pure sand or sand containing layers of leaf litter, in which the beetles hide. Near Wiggins, Mississippi, and at six of the nine localities in Virginia and North Carolina, specimens were found in the company of an undescribed species related to *B. aenulum* (Maddison and Arnold, in prep.) which itself prefers coarse, well-drained sand shores.
Morphological Variation.—None noted.

Molecular Variation.—None noted.

Habitat.—On gravel and sand shores of rivers.

IMPLICATIONS FOR “BARCODING”

Molecular sequence data have been used to help determine the boundaries of species and uncover new species for several decades (e.g., Smith et al. 1991), and subsequent works suggest that DNA sequences can be powerful tools to aid in our discovery of boundaries to gene flow (e.g., Conn et al. 1997; Shaffer and McKnight 1996; Taylor et al. 2000).

A variant of this research effort, called “barcoding,” has emerged, which proposed that use of only a single fragment of COI will in general be sufficient to distinguish and diagnose species of animals (Hebert et al. 2003). Numerous discussions of the pros and cons of barcoding have appeared (e.g., Moritz and Cicero 2004; Rubinoff et al. 2006; Savolainen et al. 2005; Sperling 2003; Will et al. 2005), with one focus of criticism being the use of only a single short fragment of COI.

Among the seven genes studied for *Pseudoperyphus*, COI and *wingless* are least successful at delimiting species, in part because of the multiple copies in each individual. In addition, two of the species that exhibit only single copies of COI, *B. lousella* and *B. bellorum*, show no consistent differences in 1450 bases of COI.

As one would expect given the complexities of evolution, there is variation across organisms as to which gene fragments are most beneficial for species delimitation. Within *Pseudoperyphus*, if only one gene fragment can be sequenced, COI is not the gene of choice. Instead, I would recommend 28S ribosomal DNA.

FUTURE DIRECTIONS

Whatever contribution this paper might make to our knowledge of *Pseudoperyphus*, much work remains to be done. A more detailed study of adult external structure, including morphometric analyses of pronota, should prove valuable not only to understand patterns of variation, but also to acquire potential characters for identification of specimens. The female reproductive system warrants detailed study, and diagnose species of animals (ebert et al. 2003). Nu
derous molecular sequence data have been used to help determine the boundaries of species and uncover new species for several decades (e.g., Smith et al. 1991), and subsequent works suggest that DNA sequences can be powerful tools to aid in our discovery of boundaries to gene flow (e.g., Conn et al. 1997; Shaffer and McKnight 1996; Taylor et al. 2000).

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ACKNOWLEDGMENTS

This paper, long in gestation, has benefited over the years from the generous help of many people. The late Klaus Rothfels provided inspiration and the intellectual and material environment in which the key initial discoveries were made when I was an undergraduate at the University of Toronto. Over 20 years ago, Ross Bell and Brian Farrell collected specimens for chromosomal analyses that allowed a preliminary understanding of the *B. honestum* subgroup. Tana Ellis acquired 11 of the first DNA sequences, and Wendy Moore patiently trained me in molecular methods, which allowed me to gather the rest. Wendy also helped design some needed COI primers. John K. Moulton shared with me his CAD primers before publication, and gave advice that helped me acquire data from this vital gene. Phil Ward provided information about his *wingless* primers. Alex Wild designed valuable primers for *wingless*, arginine kinase, and RNA polymerase II. Julia Maddison looked at beetle cultures during my absences. François Lutzoni and Kathleen Pryer provided laboratory space during visits to North Carolina. James Bridgland (Cape Breton Highlands National Park) aided my effort in choosing a type locality for *B. lousella*. Locality information that guided field work was kindly provided by Paul Choate, Robert Nelson, Kipling Will, and Robert Davidson. David Hillis, Greg Pauley, John Acorn, and Hugh Robertson kindly assisted me with field work. Ford Doolittle provided much-needed supplies for a collecting trip to Nova Scotia. Eugene Hall and Blanche Matheson helped with speci
dimen preparation. Without the fine work of the DNA Sequencing Facility at the University of Arizona, the molecular data would not be available. Thanks, too, to Wendy Moore, Jim Liebherr, and Betsy Arnold for their thoughtful reviews of the manuscript. Yves Bouquet kindly pointed out that the type specimen of *B. antiquum* Dejean is a holotype rather than a lectotype.

My thanks as well to curators of the collections from which material was borrowed for their effort in making specimens available. The efforts of some are worthy of special note. Drew Hildebrandt provided critical live specimens from Mississippi, going on numerous trips to aid this work; my understanding of *B. integrum* and *B. arenobilis* would be much less rich without his generous help. My companions in collecting, Louise Maddison, Julia Maddison, Helen Amerongen, and Wayne Maddison, provided much help and patience.

Without the encouragement, understanding, patience, insight, and extraordinary efforts of my friend, Anne Elizabeth Arnold, who shared with me the discovery of many *Pseudoperyphus* on river shores from Nova Scotia to Alberta, and from Iowa to North Carolina to Maine, this project would have been only a small fraction of what it is.

This work was funded in part by NSF grants DEB-9981935 and EF-0531754 (for primer development), and by funds generously provided by the Department of Entomology and College of Agriculture and Life Sciences, University of Arizona.

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Appendix 1. *Pseudoperiphyus* specimens examined for DNA and chromosome number.

Specimens were identified to species using the totality of evidence: external structure, male genitalia, chromosome number, and DNA sequences. #: Maddison lab DNA voucher number; holotypes indicated by “*”. Sex: male unless indicated as a female by “f”. St: State or Province. Chr: haploid chromosome number. Other genes: genes sequenced in addition to 28S rDNA and COI, which were sequenced for all specimens. See text for gene abbreviations.

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**APPENDIX 1. Pseudoperyphus specimens examined for DNA and chromosome number, continued.**

Specimens were identified to species using the totality of evidence: external structure, male genitalia, chromosome number, and DNA sequences. #: Maddison lab DNA voucher number; holotypes indicated by “*”. Sex: male unless indicated as a female by “f”. St: State or Province. Chr: haploid chromosome number. Other genes: genes sequenced in addition to 28S rDNA and COI, which were sequenced for all specimens. See text for gene abbreviations.

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| *B. bellorum*  | 1901 |     | WV  | Valley Head    |     |                           |
| *B. bellorum*  | 1902*|     | WV  | Valley Head    | 12  | 18S, wg, CAD, ArgK, Pol2  |
| *B. bellorum*  | 1904 |     | WV  | Valley Head    | 12  | 18S, wg, CAD, ArgK, Pol2  |
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| *B. antiquum*  | 1508 |     | ME  | Howland        | 12  | 18S, wg, CAD, ArgK, Pol2  |
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| *B. antiquum*  | 1558 |     | ME  | Howland        |     | 18S, wg, CAD, ArgK        |
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Specimens were identified to species using the totality of evidence: external structure, male genitalia, chromosome number, and DNA sequences. #: Maddison lab DNA voucher number; holotypes indicated by “*”. Sex: male unless indicated as a female by “f”. St: State or Province. Chr: haploid chromosome number. Other genes: genes sequenced in addition to 28S rDNA and COI, which were sequenced for all specimens. See text for gene abbreviations.

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**APPENDIX 1. Pseudoperyphus specimens examined for DNA and chromosome number, continued.**
**APPENDIX 1.** *Pseudoperyphus* specimens examined for DNA and chromosome number, *continued.*

Specimens were identified to species using the totality of evidence: external structure, male genitalia, chromosome number, and DNA sequences. #: Maddison lab DNA voucher number; holotypes indicated by “*”. Sex: male unless indicated as a female by “f”. St: State or Province. Chr: haploid chromosome number. Other genes: genes sequenced in addition to 28S rDNA and COI, which were sequenced for all specimens. See text for gene abbreviations.

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**APPENDIX 1.** *Pseudoperyphus* specimens examined for DNA and chromosome number, *continued.*
Specimens were identified to species using the totality of evidence: external structure, male genitalia, chromosome number, and DNA sequences. #: Maddison lab DNA voucher number; holotypes indicated by “*”. Sex: male unless indicated as a female by “f”. St: State or Province. Chr: haploid chromosome number. Other genes: genes sequenced in addition to 28S rDNA and COI, which were sequenced for all specimens. See text for gene abbreviations.

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APPENDIX 2. Outgroup species and specimens sequenced for each gene.

#: Maddison lab DNA voucher number. St: State or Province. Chr: haploid chromosome number (based upon Maddison, 1985; see text). See text for gene abbreviations.

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n: number of specimens examined. St: State or Province. Chr: haploid chromosome number.

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