ANNALS OF CARNEGIE MUSEUM

VOL. 77, NUMBER 1, PP. 93–127 20 JULY 2008

PHYLOGENY AND CLASSIFICATION OF *HYPHERPES* AUCTORUM (COLEOPTERA: CARABIDAE: PTEROSTICHINI: *PTEROSTICHUS*)

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ABSTRACT

Based on an exemplar sample of pterostichine species (Carabidae: Pterostichini), 28S rDNA and COI and COII mtDNA sequence data are used to reconstruct a phylogenetic hypothesis for generic and subgeneric taxa putatively in or related to the subgenus *Hypherpes* Chaudoir (Coleoptera: Carabidae: *Pterostichus* Bonelli). The monophyly of *Pterostichus* is equivocal as the position of the subgenus *Bothriopterus* Chaudoir varies depending on methods of sequence alignment and gap region treatment. *Pterostichus* is found to be monophyletic in the combined data analysis if *Cyclotrachelus* Chaudoir and *Tapinopterus* Schaum are included in a larger concept of the genus. It is recommended that these be treated as subgenera of *Pterostichus*. Taxa currently included in *Hypherpes* are found to form a monophyletic group. No taxon previously suggested as a close relative of *Hypherpes* was found to be in, or closely related to *Hypherpes*. The sister-group of *Hypherpes* remains unclear, but there is some support for a clade of *Pseudoferonina* Ball + *Cryobius* Chaudoir as the adelphotaxon. Taxa included in current classifications of *Hypherpes* compose a group that is in fact a complex of *Hypherpes sensu stricto* and two other subgenera, *Leptoferonia* Casey and *Anilloferonia* Van Dyke, which have been treated as junior synonyms of *Hypherpes*. Our analyses show that these three taxa are well supported as subgenera and reciprocally monophyletic, with the only change to previous taxonomic concepts of included species being the transfer of *Pterostichus rothi* (Hatch) from *Anilloferonia* to *Leptoferonia*. It is recommended that all three of these subgenera be recognized rather than being subsumed under *Hypherpes*. In *Leptoferonia* the DNA data support all species groups that were established by Hacker using morphological characters, with the exception of the *inopinus*-group. Significant reduction of the compound eyes has occurred independently at least five and possibly seven times in the *Hypherpes* complex. As many as five separate instances of eye reduction may have occurred in *Leptoferonia* alone. Maddison's concentrated changes test was used to show that there is a significant correlation between microphthalmy and autapomorphic sequence data as represented by longer than average terminal branch lengths based on Bayesian estimates of change per site. However, taxon pair contrasts show no consistent pattern of absolute difference of evolutionary rate or directionality of differences between small-eyed taxa and their sister species or sister clade. Repeated patterns of allopatric distributions are found for species-pairs of *Leptoferonia*, which consist of divisions along a north/south axis near the Pacific Coast and in the Sierra Nevada Range, or east/west divisions between coastal species and inland or Sierran species. In addition to allopatric biogeographic patterns, instances of sympatry in closely related species are interpreted to have been the result of two reduced-eye species moving into the deep litter and soil layer, thereby ecologically differentiating from near-surface leaf-litter and log dwelling species. *Pterostichus morionides* (Chaudoir), which is restricted to the Sierra Nevada Mountains in western North America, is found to be sister to *P. adoxus* (Say) and *P. tristis* (Dejean), the only species of *Hypherpes* in eastern North America. This grouping (mta-clade) was further tested by using a subset of taxa for 18S rDNA, CAD and wg sequence data and was found in some or all most-parsimonious trees for these data. In cases where they did not form a clade, they usually formed a convex group. Although counterintuitive due to the unusual disjunct biogeographic connection of these two areas and the generally dissimilar form of the adults, the mta-clade is very well supported by the DNA sequence data.

KEY WORDS: biogeography, eye reduction, ground beetles, Pterostichina

INTRODUCTION

Of the nearly 250 North American species of pterostichine ground beetles (Coleoptera: Carabidae: Pterostichini), about 100 (40%) are presently included in the subgenus *Hypherpes* Chaudoir (Bousquet and Larochelle 1993; Bousquet 1999), and recent discovery and description of species suggest that there remains a significant number of species yet to be named (Kavanaugh and LaBonte 2006; LaBonte 2006; Will 2007). All but two of the known species of *Hypherpes* are found in the region from Alaska to Baja California and east to New Mexico. The two remaining species are found from Gerogia to southeastern Canada, west to Wisconsin and east to the Atlantic coastal states. The beetles typically recognized as *Hypherpes* are mostly large (10.0 mm and larger), conspicuous (Figs. 1A, 2), common, easily collected and potentially important predators in agricultural systems (e.g., Riddick and Mills 1994, 1995, 1996a, 1996b), and yet their fundamental taxonomy and classification remains unsettled. This group is essentially the last species-rich group of large-sized ground beetles in North America that remains largely untouched by modern (post 1960) phylogeny-based revision. Much work was done by T. L. Casey (1913, 1918, 1924). Though significant, it is based on his implicit, peculiar and arguably flawed species concept. Regionally limited treatments have dealt with the relatively small number of species in the northern latitudes (Hatch 1953, Lindroth 1966), and a significant number of species-level synonymies have been established (Bousquet and Larochelle 1993; Bousquet 1999). One subgroup, *Leptoferonia* Casey, was revised by Hacker (1968). However, all of these authors have

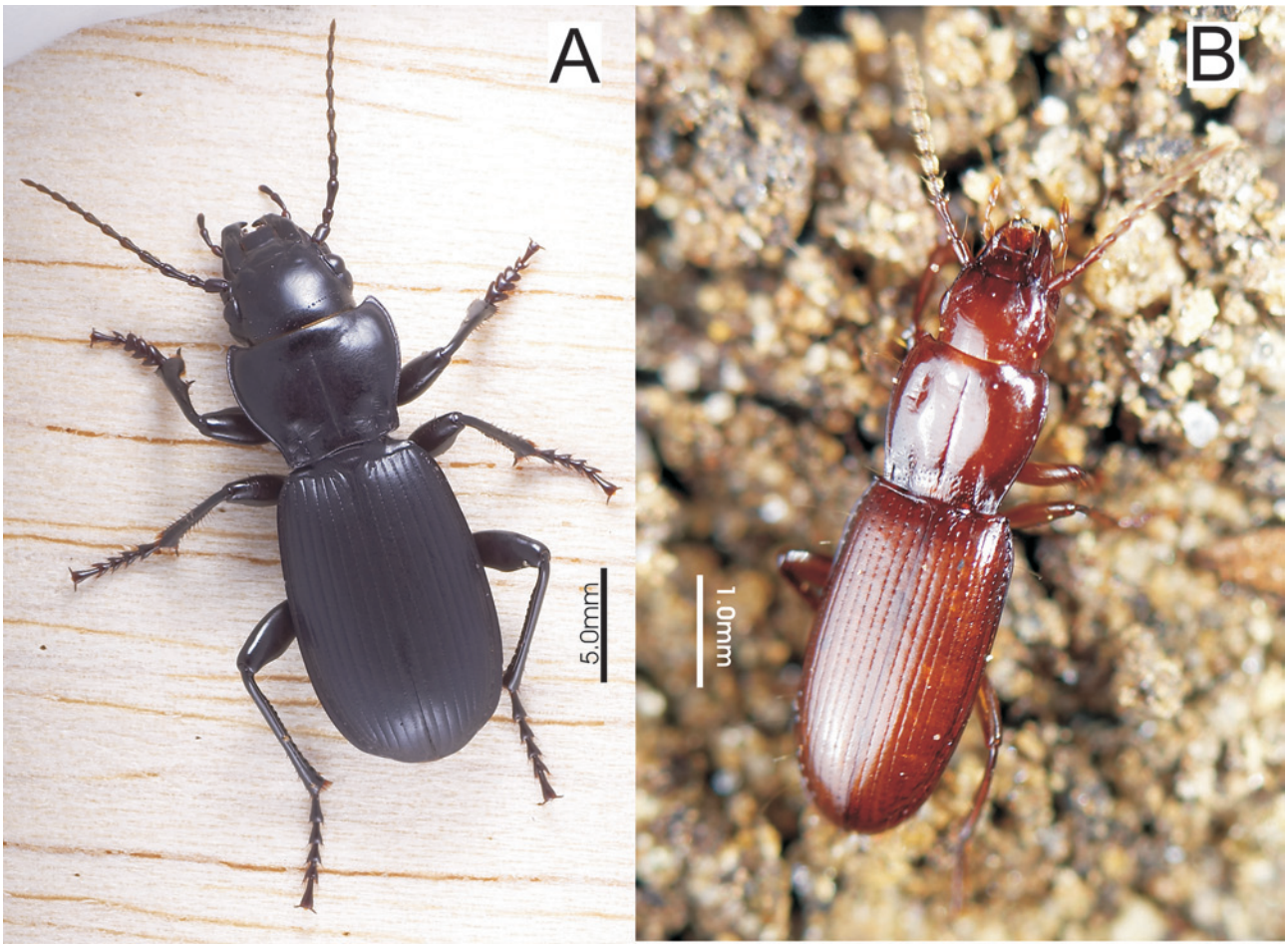

Fig. 1.—*Hypherpes* complex species (dorsal view). **A**, *Pterostichus (Hypherpes) lama*; **B**, *Pterostichus (Leptoferonia) pemphredo*.

expressed significant doubt about the monophyly of *Hypherpes* (or species groups that approximate the subgenus) and none has been able to suggest a set of synapomorphic characters for the group. Bousquet (1999:161) put it succinctly "There is no sound evidence that *Hypherpes*, as presently or previously conceived, represents a monophyletic group." However, authors have consistently identified species as belonging to *Hypherpes* or presumed allied subgenera, based on a suite of adult morphological characteristics that in combination give individuals in the group a distinctive appearance. In general, for subgeneric groups of *Pterostichus* Bonelli morphological characteristics that mark phylogenetic history are few, with most characterizable features being plesiomorphic and widely distributed in the genus or autapomorphic for species or small species groups. Characteristics such as the absence of dorsal setigerous punctures of the elytra, short mesepimeron and medial seta of the metacoxa are all shared by *Hypherpes* taxa, but are also found in various other pterostichines. Patterns of setation on the elytra and legs are quite variable across carabids and the reduced length of the mesepimeron and changes in other thoracic sclerites is probably linked to flight-wing reduction and the loss of the ability to fly (Darlington 1936). In this situation, DNA sequence data are particularly well suited and likely essential to understanding the phylogenetic relationships of the included species.

The taxonomic history of *Hypherpes* and related taxa is discussed in detail by Bousquet (1999). Major works have treated the group differently. For instance, Lindroth (1966) spread *Hypherpes* taxa over several species groups, namely the *amethystinus*, *sphodrinus* and *mancus*-groups. The *amethystinus*-group of Bousquet and Larochelle (1993) is equivalent to the subgenus *Hypherpes* as delimited by Bousquet (1999), and this is a narrower concept than the *Hypherpes* complex or *Hypherpes*-like taxa *sensu* Ball and Roughley (1982). In the set of analyses presented here we have tested the broader concept of Ball and Roughley (1982), and the narrower or subordinate concepts of other authors, except for the notable omission of the Mexican *Hypherpes*-like taxa: *Allotriopus* Bates, *Mayaferonia* Ball and Roughley, and *Percolaus* Bates. No DNA-quality specimens were available for these taxa.* Herein, what we refer to as the *Hypherpes* complex is equivalent to the

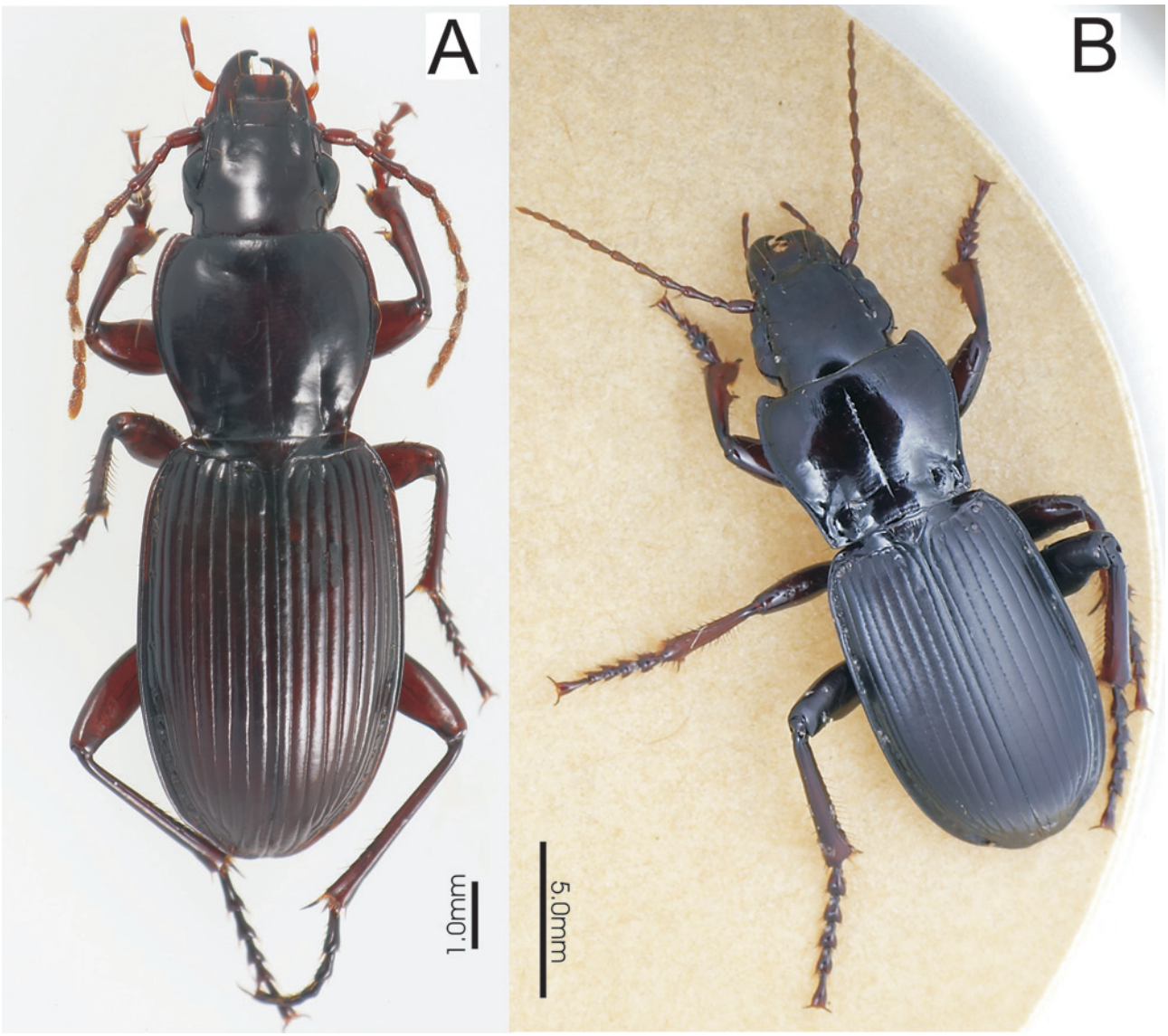

Fig. 2.—*Hypherpes* complex species (dorsal view). **A**, *Pterostichus (Hypherpes) tristis*; **B**, *Pterostichus (Hypherpes) morionides*.

subgenus *Hypherpes* of Bousquet (1999). We do so because we recognize *Anilloferonia*, *Leptoferonia* and *Hypherpes* as distinct subgenera. The primary purpose of this study is to test the monophyly of *Hypherpes,* and to establish an appropriate subgeneric classification for the taxa that are in or related to *Hypherpes* based on DNA sequence data and to discuss various implications of the patterns found.

A recent study by Sasakawa and Kubota (2007) using DNA sequence data advanced our understanding of the phylogeny of *Pterostichus* subgenera. However, even though the study herein includes an even greater sampling of genera and subgenera, neither that study nor ours is sufficient to address the phylogenetic arrangement and evolutionary trends in Pterostichini *as a whole*. Lacking from these analyses are Southern Hemisphere genera, many *Pterostichus* subgenera from Central and Western Europe and important lineages from Africa. Limited to taxa in or near *Pterostichus*, i.e., Pterostichina, results of these studies are meaningful. Data and taxon sampling in this study were not specifically designed to recover the phylogeny at the subtribal-level, but some relationships that are extremely well supported and found in all or nearly all analyses are discussed and merit further investigation.

MATERIALS AND METHODS

Taxon sampling

The overall taxon sampling strategy includes three foci (Appendix 1). First of these is a broad sample of exemplar

species representing subgenera of *Pterostichus* and putatively closely related genera. Primarily these are taxa from North America and any taxon implicated at any point in a "close" relationship to *Hypherpes*. Second is a sample of species and individuals that represent morphological, taxonomic and geographic diversity within *Hypherpes*, including multiple individuals of species that show significant morphological variation and/or have a wide geographic distribution. Third, we attempted to sample all species and geographic variants in the subgenus *Leptoferonia* (*sensu* Hacker 1968).

Multiple individuals of 14 species or putative species are included in the analyses as terminals. These are summarized as single terminals represented as a list of numbers (Appendix 1) except in figures that include support measures, branch lengths or cases where the individuals are paraphyletically arranged. Of the 14, three cases represent different parts of the species' range (*P*. (*Cryobius*) *riparius* (Dejean), *Pterostichus* (*Anilloferonia*) *lanei* (Hatch), *P*. (*H*.) *crenicollis* LeConte), but individuals sampled are not significantly different morphologically. Two cases (*P*. (*H*.) *morionides* (Chaudoir) and *P*. (*H*.) *tristis* (Dejean)) are duplicated in order to ensure accuracy of the extraction and sequencing processes due to the questionable mta-clade result (see below). Seven taxa, (*P*. (*H*.) *inermis* Fall, *P*. (*L*.) *lobatus* Hacker, *P*. (*L*.) *fenyesi* Csiki, *P*. (*L*.) *fuchsi* Schaeffer, *P*. (*L*.) *idahoae* Csiki, *P*. (*L*.) *infernalis* Hatch, and *P*. (*L*.) *inanis* Horn) were selectively sampled from available material based on obvious morphological variation, e.g., extreme differences in body length and/or variation in secondary sexual features. Sampling across multiple individuals for these taxa is intended to reveal DNA sequence variation that might correspond to apparent morphological variation. The sampling was not designed to be a significant test of species boundaries. Instead, analyses act as a test of group convexity (Estabrook 1978, 1986). Convex grouping of multiple individuals of the same taxon reinforces existing species limits, whereas a polythetic pattern would suggest a need to explain the conflict between the pattern found with DNA data and species limits as diagnosed by morphological features. Two species (*P*. (*Leptoferonia*) *angustus* (Dejean), *P*. (*H*.) *lama* (Ménétriés)) were sampled extensively relative to their known range and morphological variation. However, in the case of *P. (L.) angustus*, samples have a significant gap of more than 300 km along the south Coastal Ranges between specimens sampled in Santa Clara Co. and those from Santa Barbara Co., California. In the case of *P. (H.) lama,* sampling includes 24 sites that cover much of the species' range within California (samples lacking from the southern Sierra and Transverse Ranges and far north east Sierras), but only single individuals are included from Oregon, Washington and British Columbia. Museum specimens are known from as far to the east as Elko, Nevada, within 100 km of the Nevada-Utah border. However, we presently have no specimens for DNA extraction available from Nevada. This leaves a potentially significant portion of the range of *P. (H.) lama* unsampled.

We conducted a variety of analyses altering the set of included taxa, the alignment method and treatment of gaps (Table 1). In all cases where topology was the parameter of interest, we used parsimony as the optimality criterion for tree searching. For purposes of classification we rely on these analyses. An explicit model of sequence evolution was implemented using a mixed-model partitioned Bayesian analyses for the "standard" and 15 taxon analyses (see below) in order to examine branch lengths and to calculate clade support values. What is herein referred to as the ***extended analysis*** includes nine genera, 38 subgenera (counting *Leptoferonia* and *Anilloferonia*) and 157 terminals for 28S sequence data only. This includes all North American genera except *Abaris* Dejean and *Hybothecus* Chaudoir, which are excluded as they are only distantly related and of South American origin. *Stereocerus* Kirby, which is likely related to *Myas* Sturm but not near *Hypherpes,* and *Abax* Bonelli which is introduced from Europe, were excluded. Of the 23 North American subgenera of *Pterostichus* all are represented in the extended analysis except *Paraferonia* Casey. No specimens of this monotypic subgenus suitable for DNA extraction were available.* Two of the North American subgenera, *Lenapterus* Berlov and *Metallophilus* Chaudoir, are represented in the extended analysis by Old World species whose sequences were retrieved from GenBank.* An additional 15 subgenera whose 28S DNA sequence data were available in GenBank are included in the extended analysis. What is referred to as the ***standard analysis*** includes a subset of 140 terminals from the extended analysis. These are all terminals for which we had specimens available for DNA extraction and could complete the matrix of 28S, COI and COII. This matrix includes representatives of nine genera and 21 *Pterostichus* subgenera.

Both the extended and standard analyses include the same broad sample of *Hypherpes* species. Since *Hypherpes* is in need of taxonomic and morphological review at the species level, previous taxonomic concepts and classifications are used as a first-pass guide to diversity. This was augmented by selectively collecting species and individual variants of species representing morphological and geographical diversity. All of Casey's (1913) "groups" are represented, and if the group contains more than one species, by multiple species. All generic concepts previously based on *Hypherpes* species that are presently considered consubgeneric (Bousquet 1999) are represented. All species of *Anilloferonia* are included (*P. malkini* (Hatch) is thought to be a synonym of *P. lanei* (Hatch), (J.R. Labonte, personal communication)). Of the 26 species of *Leptoferonia*, 23 are included. Three species that were not available for sequencing, *P. falli* Van Dyke, *P. enyo* Will, and *P. deino* Will, are only known from their type series. *Leptoferonia* subspecies and geographic variants as noted by Hacker (1968) are represented by individuals from across the species' range except for *P. pumilus willamettensis* Hacker, for which we had no specimens for DNA extraction.

TABLE 1. Analyses, tree statistics and results.

Column headings are **#** - analysis number; **#Taxa** - number of individual terminals included in the analysis; **Data** - sequence type in the matrix; **Alignment method** - Clustal using default parameters, Dialign using online submission at Bibiserv (GC indicates matrices with GapCoder characters added); **#Char** - total size of aligned matrix; **#p.i. Char** - number of parsimony informative characters based on Winclada's "mop uninformative" function; **#Trees** - number of parsimony trees found; **Length**, **CI**, and **RI** are standard tree statistics based on matrix excluding uninformative and invariant sites; **Sister to *Hypherpes* complex** - adelphotaxon of *Hypherpes* complex found in each analysis; ***Hypherpes* complex**, ***Anill***(*oferonia*), ***Lepto***(*feronia*), ***Hyp***(*herpes*) and **mta** (clade of *P. morionides, P. tristis and P. adoxus*); **yes** - found to be monophyletic in consensus tree; **no** - not monophyletic in consensus tree; **na** - not tested. Marked with an *** and bold** - the 157 terminal analysis, referred to in the text as the *extended analysis*, otherwise the 140 terminal analysis, referred to as the *standard analysis*. Tree searching was done using parsimony as the optimality criterion unless otherwise noted.

#	# Taxa	Data	Alignment method	# Char	# p.i. Char	# Trees	Length	CI	RI	Sister to *Hypherpes* complex	*Hypherpes* complex	*Anill*	*Lepto*	*Hyp*	*mta*
1	157	28S	Clustal	1356	544	12150	3126	31	77	*Cryobius*	yes	yes	yes	yes	yes
2	157	28S	Dialign	1723	571	3420	2924	32	76	*Eosteropus*	yes	yes	no	no	yes
3	**157***	**28S**	**Clustal-GC**	**1912**	**885**	**72**	**3849**	**34**	**80**	**Cryobius**	**yes**	**yes**	**yes**	**yes**	**yes**
4	157	28S	Dialign-GC	2221	905	768	4171	31	76	*Eosteropus*	yes	yes	no	yes	yes
5	140	28S	Clustal	1349	499	24	2684	33	77	*Gastrosticta*	yes	yes	yes	no	yes
6	140	28S	Dialign	1656	515	216	2480	34	75	*Pseudoferonina*	yes	yes	yes	yes	yes
7	140	28S	Clustal-GC	1833	801	4800	3306	36	80	large clade	yes	yes	yes	yes	yes
8	140	28S	Dialign-GC	2079	798	1080	3487	32	75	*Pseudoferonina*	yes	yes	yes	yes	yes
9	140	COI–COII	manual	1569	568	46512	3840	23	67	large clade	yes	yes	yes	yes	yes
10	140	COI–COII pos.3 off	manual	1045	184	>100K	830	29	80	*Cyclotrachelus*	yes	yes	no	no	yes
11	140	COI–COII pos.3 off. TNT Estimated Consensus	manual	1045	184	na	na	na	na	unresolved	yes	yes	no	no	yes
12	**140***	**28S,COI–COII**	**Clustal-GC**	**3402**	**1369**	**15504**	**7283**	**28**	**73**	***Pseudoferonina* +*Cryobius***	**yes**	**yes**	**yes**	**yes**	**yes**
13	140	28S, COI–COII Bayesian Analysis	Clustal-GC	3402	1369	na	na	na	na						
14	15	18S	Clustal	1994	42	6	65	72	80	na	yes	na	na	na	no
15	15	CAD	manual	2231	235	8	523	55	53	na	yes	na	na	na	yes
16	15	wg	Clustal	463	56	26	126	60	53	na	yes	na	na	na	no
17	15	All sequences	Clustal-GC	3346	528	1	1214	55	57	na	yes	na	na	na	yes
18	15	All sequences Bayesian Analysis	Clustal-GC	3346	528	na	na	na	na	na	yes	na	na	na	yes

Selection of Sequence Data

DNA loci were selected for their proven broad phylogenetic utility (i.e. coverage of information for relationships of various ages), sampling from both nuclear and mitochondrial genomes, and ease of acquisition. Five of the six sequences analyzed here are relatively well know and have been used in previous studies of Carabidae; 28S (e.g., Kim 2000; Cryan 2001; Ober 2002), COI–COII (Sanchez-Gea 2004; Sasakawa and Kubota 2005), wg (e.g., Soto and Ishikawa 2004; Sasakawa and Kubota 2007; Ribera et al. 2005), 18S (e.g., Maddison 1999; Ribera et al. 2005). The use of the CAD gene is still in its early exploration for Carabidae, but its utility has been shown in Diptera (Moulton and Wiegmann 2004).

Sequence-Data Acquisition Methods

Genomic DNA samples were prepared from fresh beetles, beetles preserved in 95–100% EtOH, beetles frozen and then preserved dried with Drierite (anhydrous calcium sulfate, W.A. Hammond Drierite Company), or pinned museum specimens. DNA was extracted from femur or dissected pronotal muscle tissue using the DNeasy Tissue Kit (Qiagen, Valencia, CA).

About 1000 base pairs (bp) of the D1–D3 region of 28S rDNA were amplified with the forward primer **D1** (5′–GGG AGG AAA AGA AAC TAA C–3′; Ober 2002) and either the reverse primer **D3i** (5′–GCA TAG TTC ACC ATC TTT C–3′, designed for this study and used for specimens KWW 030, 035, 037–039, 050, 069, 087, 089, 092, 103, 196, 197, 206, 208) or **D3** (5′–KRC MKA GMW CAC CAT CTT T–3′; Ober 2002) under the following PCR conditions: initial denaturation at 94ºC for 2:00 minutes; 35 cycles with 94º denaturation for 0:20 , 53º annealing for 0:17, 65º extension for 0:50; and a final 72º extension for 7:00. PCR reactions were composed of 2µl template DNA, 0.3 µl HotMaster Taq DNA Polymerase (Eppendorf), 5 µl HotMaster Taq buffer, 1 µl of 10mM dNTP mix, 5 µl of 5pmol/µl solutions of each primer and enough autoclaved ddH20 to bring the total reaction volume to 50µl. Some reaction mixes included 1µl of 10µM Bovine Serum Albumin solution (Fisher).

Approximately 800bp of the COI mitochondrial region were amplified using the primers **JER** (5′–CAA CAT TTA TTT TGA TTT TTT GG–3′) and **PAT** (5′–TCC AAT GCA CTA ATC TGC CAT ATT A–3′; Simon *et al*. 1994) under the following PCR conditions: initial denaturation at 94ºC for 2:00 minutes; 35 cycles with 94º denaturation for 0:20, 53º annealing for 0:15, 65º extension for 0:50; and a final 72º extension for 7:00. PCR reactions were composed of 2µl template DNA, 0.3 µl HotMaster Taq DNA Polymerase (Eppendorf), 5 µl HotMaster Taq buffer, 1 µl of 10mM dNTP mix, 2.5µl (in some cases 3µl was used) of 5pmol/µl solutions of each primer and enough autoclaved ddH20 to bring the total reaction volume to 50µl. Some reaction mixes included 1µl of 10µM Bovine Serum Albumin solution.

Approximately 710bp of the COII mitochondrial region were amplified using the forward primer **COII-1F** (5′–CTT TTR TTA GAA AAT GGC AAC AT–3′; Cryan *et al.* 2001) and the reverse primer **COII TK-N-3782** (5′–GAG ACC ATT ACT TGC TTT CAG TCA TCT–3′; Emerson *et al.* 2000). PCR conditions and reaction mixes were the same as the COI amplifications.

The COI genes for specimens KWW431 and KWW397 and the COII gene for KWW431 were amplified and sequenced using the following reaction mixture: 30.3 µl ddH20, 5 µl buffer, 3.5 µl $MgCl_2$ 50mM solution, 4 µl dNTPs 10mM solution, 2.5 µl of each primer at 10pmol/µl concentration, 0.2 µl Apex Taq (BioResearch Products), and 2 µl template DNA. PCR conditions for these specimens were the same as above except the extension temperature was 72°C and annealing temperatures were as follows: KWW431 COI (45°C), COII (50°C); KWW397 COI (56°C). The primers for COI and COII were the same for all specimens.

Approximately 400–450bp of the wingless gene (wg) were amplified with an initial amplification with the primers **wg1** (5′–GAR TGY AAR TGY CAY GGY ATG TCT GG–3′; Ober 2003) and **B3wg2** (5′–ACT CGC ARC ACC AGT GGA ATG TRC A–3′; Maddison 2008), and a subsequent amplification of the product of the first reaction with primers **5wgB** (5′–ACB TGY TGG ATG CGN CTK CC–3′; Maddison 2008) and **B3wg2**. The reaction conditions for both runs was an initial 94°C denaturation for 2:00 minutes; 35 cycles with 94º denaturation for 0:20, 55º annealing for 0:10, 65º extension for 0:48; and a final 72º extension for 7:00. PCR reactions were composed of 2µl template DNA (or 2µl of amplified product), 0.3 µl HotMaster Taq DNA Polymerase (Eppendorf), 5µl HotMaster Taq buffer, 1 µl of 10mM dNTP mix, 2.5 µl of 5pmol/µl solutions of each primer and enough autoclaved ddH20 to bring the total reaction volume to 50µl.

A 1940–1990bp region of 18S rDNA gene was amplified with primers **5′18S** (5′–GAC AAC CTG GTT GAT CCT GCC AGT–3′) and **18L** (5′–CAC CTA CGG AAA CCT TGT TAC GAC TT–3′). The amplified region was sequenced with three pairs of primers covering overlapping sections of the gene. For some taxa, only the first two primer pairs successfully yielded sequences, resulting in a sequence of around 1450bp. The three primer pairs were **5′18S** (see sequence above) and **909R** (5′–GTC CTG TTC CAT TAT TCC AT–3′); **18Sai** (5′–CCT GAG AAA CGG CTA CCA CAT C–3′) and **18Sb**i (5′–GAG TCT CGT TCG TTA TCG GA–3′); and **760F** (5′–ATC AAG AAC GAA AGT–3′) and **18L** (see sequence above). All 18s primers are from Maddison *et al.* (1999). PCR reactions mixes were the same as for COI, with 3µl of 5pmol/µl primer solution for each reaction. The PCR protocol was 94°C initial denaturation for 2:00 minutes; 35 cycles with 94° denaturation for 0:20, 54° annealing for 0.16, and 65° extension for 0:50; with 65° final extension for 7:00.

Approximately 2200bp of the nuclear protein-coding gene CAD were amplified with 3 pairs of primers

covering overlapping sections of the gene. The primer pairs were: **338F** (5′–ATG AAR TAY GGY AAT CGT GGH CAY AA–3′; Moulton and Wiegmann 2004) and **680R2** (5′–TAR GCR TCY CTN ACW ACY TCR TAY TC–3′; Maddison 2008); **581F4** (5′–GGW GGW CAA ACT GGW YTM AAY TGY GG–3′; Maddison 2008) and **843R** (5′–GCY TTY TGR AAN GCY TCY TCR AA–3′; Moulton and Wiegmann 2004); and **CD791F2** (5′–GTN ACN GGN CAA NCA ACT GCC TG–3′; Maddison 2008) and **1098R** (5′–TTN GGN AGY TGN CCN CCC AT–3′; Moulton and Wiegmann 2004). PCR reactions were the same as for COI, with 3µl of 5pmol/µl primer solution for each reaction. The PCR reaction conditions were 94°C initial denaturation for 3:00 minutes; 4 cycles of 94° denaturation for 0:30, 55° annealing for 0:30, and 65° extension for 1:30; 4 cycles of 94° for 0:30, 52° for 0:30, and 65° for 0:30; 34 cycles of 94° for 0:30, 45° for 0:30, and 65° for 1:30; and 65° final extension for 2:00.

Amplified reactions were cleaned using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA). Sequencing of both strands of the PCR product for all genes was performed by the DNA Sequencing Facility, Department of Molecular and Cell Biology at the University of California at Berkeley, using an Applied Biosystems 48-capillary 3730 DNA Analyzer.

Computer Methods and Analyses

Sequence Processing and Alignment.—Raw sequence files were processed using Sequencher 4.5 (Genecodes Corp.) and final base-calls of ambiguous residues were made manually in that program. Matrices were assembled using Mesquite (Maddison and Maddison 2001). Alignment of variable length sequences was done with ClustalX (Thompson 1997) using default parameters or Dialign via the online submission at BiBiServ (Morgenstern 1998; Morgenstern 2004). These two alignment programs were used to give some indication of the robustness of results relative to their fundamentally different methods of aligning variable length sequences. Scoring of gap regions was done using Gapcoder (Young and Healy 2003).

Tree Searching and Topology Support Measures.—Winclada (Nixon 1999-2002) was used to submit matrices to NONA (Goloboff 1999) for parsimony tree searches using Hold=100,000; Mult*100; Hold/10. Bootstrap and Jackknife analyses were done using TNT (Goloboff 2003). "Traditional" search mode was used for 1000 replicates each. MacClade (Maddison and Maddison 2000) was used to generate the command file for the decay analysis. The decay analysis (Bremer 1994) was performed by submitting the MacClade generated command file to Paup* (Swofford 2002). Given the possibility that multiple changes at degenerate third positions in coding sequences may be uninformative or misleading (but see Wenzel and Siddall 1999) an analysis of the COI–COII matrix with third positions deactivated was done. This resulted in >100,000 most parsimonious trees (MPTs), so in addition to the parsimony analysis a consensus tree estimation was done using TNT with "precision" set to 5 and "accuracy" set to 4. Codon position was determined using the "set codon position" and "minimize stop codons" functions in Mesquite.

Branch Lengths and Clade Support Values.—MrBayes (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) was used for Bayesian inference of phylogenies. It has been demonstrated that partitioning data, rather than use of a compromise model for all data, is preferable for Bayesian analysis (Brandley 2005). The number of partitions should likely represent shared patterns of evolution. Eight partitions were established in the standard analysis matrix that represent loci and codon postions, which are typically accepted as data partitions (28S; COI positions 1,2,3; COII positions 1,2,3; and GapCoder characters). For analysis of the subset of 15 taxa the data were divided into 15 partitions (28S; COI positions 1,2,3; COII positions 1,2,3; 18S; CAD positions 1,2,3; wg positions 1,2,3 and GapCoder characters). GapCoder characters summarize gap regions in aligned variable length sequences and are treated like morphological characters. Invariant and autapomorphic characters were removed from the GapCoder data and "coding = variable" and "rates = gamma" were used for this data partition (Huelsenbeck and Ronquist 2001 program documentation). The default GTR model was used and the specific parameter estimates for each partition were independently estimated. Four simultaneous Markov chain Monte Carlo chains were run set at the default settings. The standard matrix analysis was run for 10,142,000 generations; trees were sampled every 1000 generations. The run was stopped when the average standard deviation of split frequencies had stabilized at <0.01. Parameter files were read into Tracer (Rambaut and Drummond 2004) and the combined trace graphs for each parameter visually inspected for the generation at which they appeared relatively stable. The largest value for the least stable looking parameter was noted. The MCMC file was inspected for the first generation for which the standard deviation of split frequencies had reach a value <0.01. The burn-in value (number of generation where the likelihoods are thought to be below stationarity) was set to the larger value of either that estimated by inspecting traces or 25% of the first generation for which the standard deviation of split frequencies had reach a value <0.01 (D.R. Maddison, personal communication). The burn-in value used was 2400. The 15 taxon analysis was run for 5,173,000 generations; trees were sampled every 1000 generations. When the run was stopped the average standard deviation of split frequencies had stabilized at <0.006. The burn-in value of 1300 was selected as described above.

Testing for Correlation between Microphthalmy and Terminal Branch Length.—The concentrated changes test (Maddison 1990) was used to test the apparent

non-random association of branches with relatively large values of expected change per site and reduction of eye size for taxa in the *Hypherpes* complex. Terminal branch lengths for the individual representing the species or the average of the terminal branch lengths for species represented by more than one individual as calculated by MrBayes were used. Branch lengths for 61 species were placed into three states; "Less than mean ±SE", "mean ±SE" and "greater than mean ±SE" (coded 0,?,1 respectively). Branch length was used as the dependent character. The independent character was scored as "normal eye" (0) and "small eye" (1). Small-eyed species are those that have the compound eye significantly reduced to nearly absent (*Leptoferonia caligans* Horn, *L. deino*, *L. pemphredo* Will, *L. blodgettensis* Will *L. enyo* Will, *L. rothi*, *Anillof-eronia lanei* and *A. testaceus* (Van Dyke)). As noted above three of these are not included in the phylogenetic analyses but we place them with relative confidence based on morphology (Will 2007) (Fig. 9). As these three species do not have branch length values and since species sampling is very different between *Leptoferonia* (nearly complete) and the *Hypherpes* complex (about 1/3 of estimated species sampled), six different simulations were conducted: 1. *Hypherpes* complex (61 taxa), species without branch lengths, scored as "?"; 2. *Hypherpes* complex (61 taxa), species without branch lengths scored as "0"; 3. *Hypherpes* complex (58 taxa); 4. *Leptoferonia* (26 taxa), species without branch lengths scored as "?"; 5. *Leptoferonia* (26 taxa), species without branch lengths scored as "0?"; 6. *Leptoferonia* (23 taxa).

Sister species and taxon pair contrasts for species within *Leptoferonia* were done to examine potential evolutionary rate differences between microphthalmic species and their sister species or sister clades. The absolute value of the difference of the total evolution (i.e., summed branch length) from the most recent common ancestor for each left and right descendent for sister species and for species pairs representing the maximum and minimum total evolution for species that are sister to a clade was calculated. For comparisons involving small-eyed vs. normal-eyed pairs, the normal-eyed species branch lengths were subtracted from the small-eyed species branch lengths and the sign recorded. We plotted total evolution as a function of number of nodes but found no significant correlation, so a node-density correction was not included. This method is similar to those used by Omland (1997) and Bromham (2002). A two-tailed t-test assuming unequal variance and $\alpha = 0.05$ was used to assess whether there is a significant difference between the average magnitude of branch lengths found for taxon pairs of dissimilar (small-eyed vs. normal-eyed) and the average of pairs with similar eye states.

Interspecific and Intraspecific Variation.—In order to compare the variation within species represented by multiple individuals and between species for each partition of the combined 140 taxon "standard" analysis, Paup* was used to output an uncorrected ("p") distance matrix for COI, COI and 28S (aligned using Clustal default settings). Average distances were used for species represented by multiple individuals.

Availability of Data and Specimen Vouchering

Sequences were submitted to GenBank with accession numbers as listed in Appendix 1. All matrices, tree files, output files and command files are available at http://nature.berkeley.edu/~kiplingw/Hypherpes_data.html or from the lead author. Specimen vouchers are housed in the laboratory of K.Will and Essig Museum of Entomology, University of California, Berkeley.

Source of Distributional Data and Subgeneric Classification

Species ranges are approximated for *Leptoferonia* from data in Hacker's (1968) revision and augmented by our collecting and data from museum specimens (Essig Museum of Entomology, UC Berkeley, CA). Initial generic and subgeneric classification recognized herein follows Bousquet (1999) and Lorenz (2005a, 2005b).

RESULTS

Taxonomic Results

Slightly higher RI and CI scores resulted for trees based on the 28S matrices that were aligned using ClustalX (default settings) with the matrix expanded by the use of Gapcoder. We used the 28S matrix so aligned to combine into the standard analysis, and the same alignment method was also preferred for the expanded analysis.

Analysis of the COI–COII matrix with third positions deactivated resulted in >100,000 MPTs. The consensus tree for this set of trees and the TNT estimated consensus tree (which is even less resolved) (Fig. 5) includes a monophyletic *Hypherpes* complex. Although less resolved than the consensus tree for COI–COII with third position active, the deactivated third position consensus tree is generally not in conflict with other analyses. Since greater resolution is achieved when information provided by third positions is included (Figs. 3,4), we use the entire COI–COII matrix to combine into the standard analysis.

Because results are highly consistent across all analyses (Table 1, Fig. 14) for the *Hypherpes* complex, we base most of the discussion on details of the extended and standard analyses and point out instances where various analyses conflict with these results. In most analyses there was little difference in the results for different alignments methods. There is more variation in the results for outgroup taxa as is expected given the sampling focus of the study.

Results within Pterostichina.—A monophyletic group of all *Pterostichus* subgenera, with *Tapinopterus* and *Cyclotrachelus* nested within, was found in all analyses

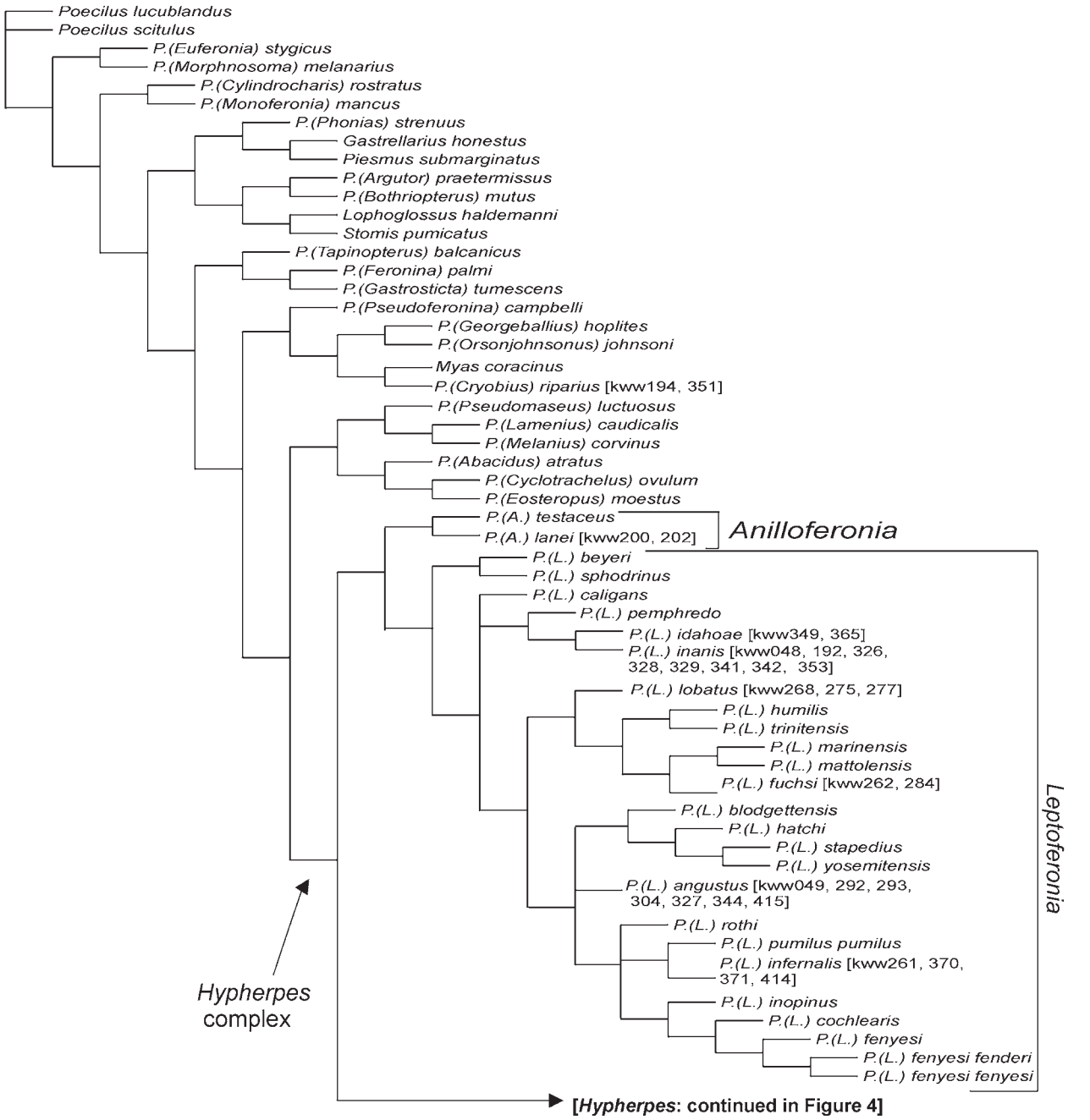

Fig. 3.—Consensus tree of 46,512 most parsimonious trees from 140 taxon, COI–COII analysis, in part. *P.* = *Pterostichus*.

that used Clustal default alignment of 28S data and an expanded matrix including GapCoder characters, whether these were the 28S data alone or combined with the mtDNA data (Figs. 6,8,10,11). However, the position of *P. (Bothriopterus) mutus* (Say) varied in other treatments, grouping with one or more of the non-*Pterostichus* genera. Analyses of COI–COII data alone did not recover a monophyletic *Pterostichus* (Fig. 3).

The arrangement of outgroups (non-*Hypherpes* complex taxa) varied significantly among the different analyses and the taxon sets. However, among the outgroup taxa some clades were present in nearly all analyses that have high bootstrap and jackknife scores (99–100), high decay support values (28–42) and high Bayesian clade support values (at or near 1.0) in the standard analysis of the combined data and extended analysis of 28S data (Figs. 8,10). These

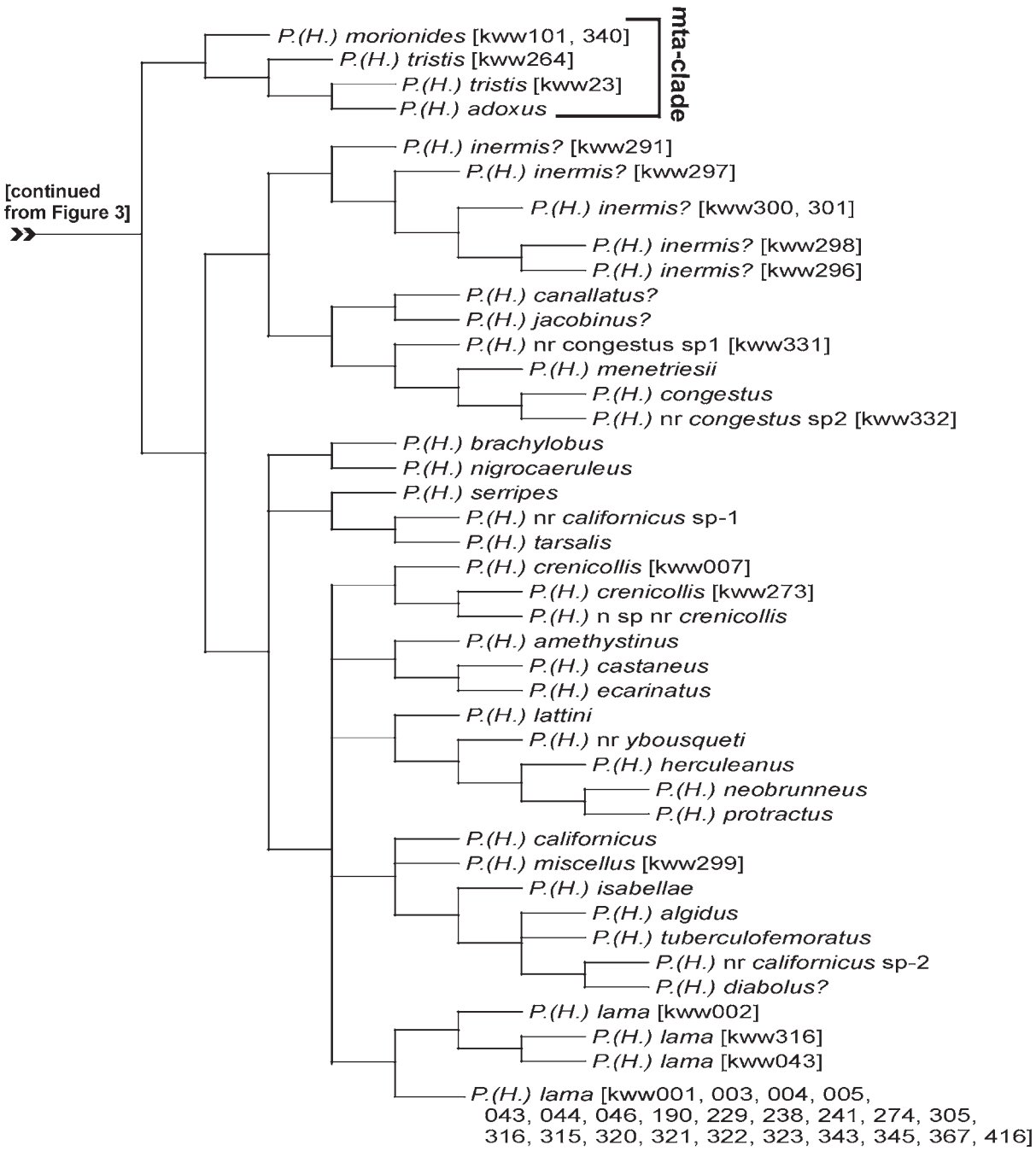

Fig. 4.—Consensus tree of 46,512 most parsimonious trees from 140 taxon, COI–COII analysis, *Hypherpes* subtree. *P.(H.) = Pterostichus (Hypherpes).*

include (*Gastrellarius* Casey + *Piesmus* LeConte); (*Cylindrocharis* Casey + *Monoferonia* Casey); (*Euferonia* Casey, *Morphnosoma* Lutshnik, *Petrophilus* Chaudoir); (*Pseudomaseus* Chaudoir (*Lyrothorax* Chaudoir (*Lamenius* Bousquet + *Melanius* Bonelli))); and (*Georgeballius* Habu (*Abea* Morita+ *Micronialoe* Park, Kwon & Lafer)). Each of these is significant in understanding the relationships among *Pterostichus* subgenera and they are discussed below.

Under all taxon sets, alignment methods, gap codings and sequence data types, the monophyly of the *Hypherpes* complex (*Hypherpes* + *Leptoferonia* + *Anilloferonia*) is supported (Figs. 8,14). In the standard analysis bootstrap and jackknife scores were 99 and 100, respectively, and the decay analysis score was 14 (Fig. 8). These measures mark the *Hypherpes* complex node as robust to perturbation and well supported (Grant and Kluge 2003).

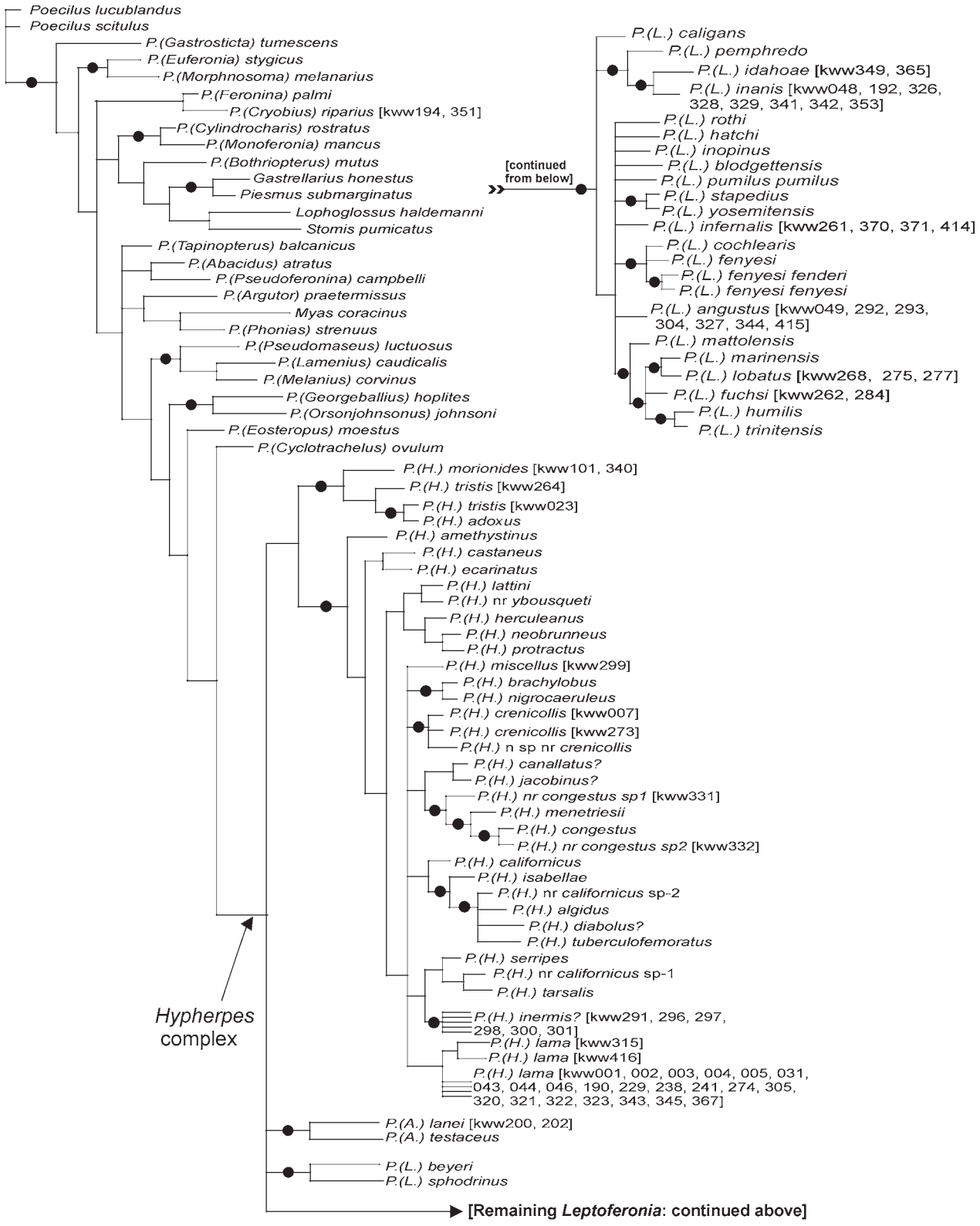

Fig. 5.—Consensus tree of 100,000 most parsimonious trees from 140 taxon, COI–COII with 3rd positions deactivated analysis and TNT consensus tree estimation. Nodes marked with black dots are those found in the TNT estimated consensus tree. *P.* = *Pterostichus.*

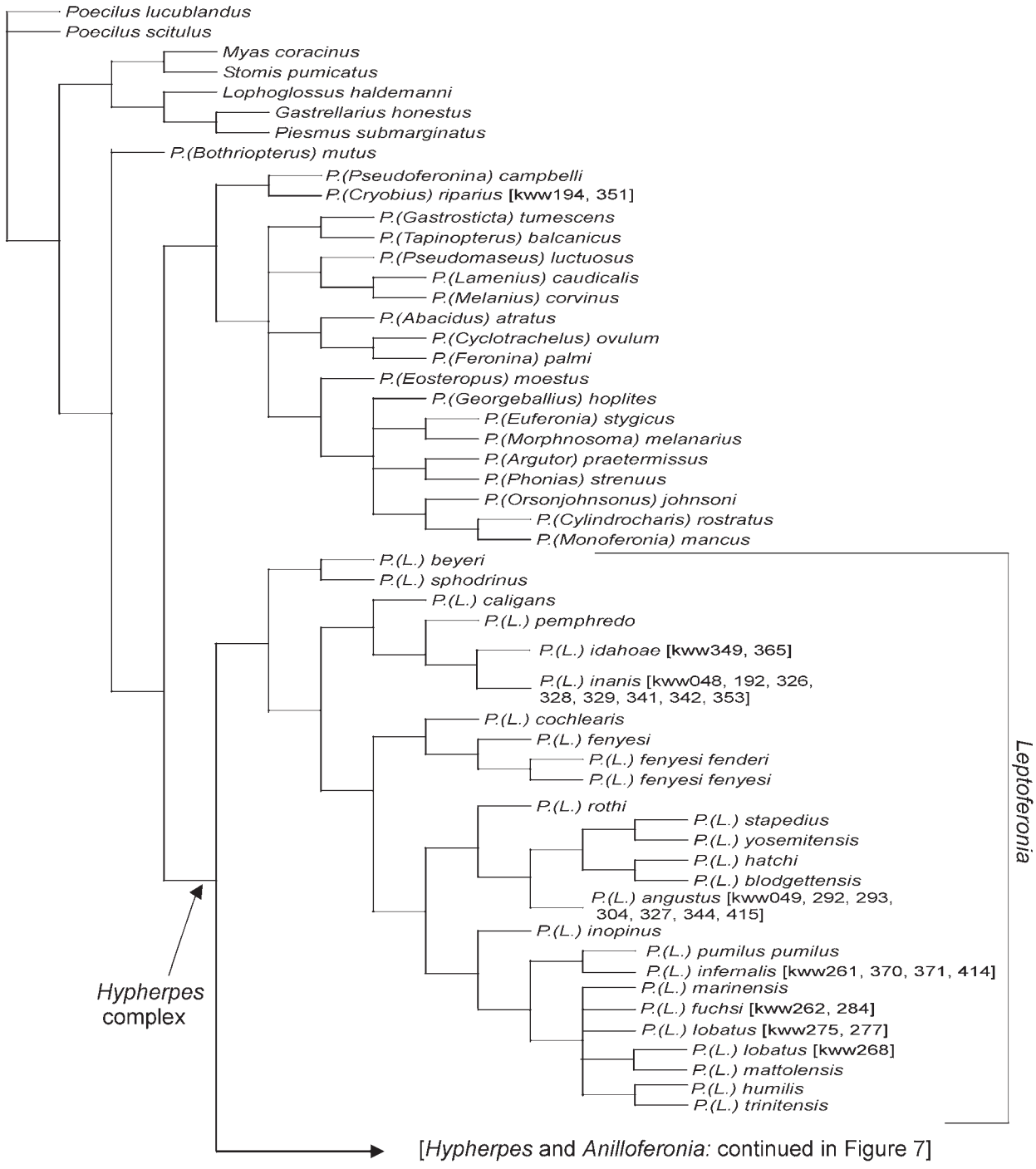

Fig. 6.—Consensus tree of 4,800 most parsimonious trees from 140 taxon, 28S analysis using Clustal default alignment and Gapcoder, in part. *P.* = *Pterostichus.*

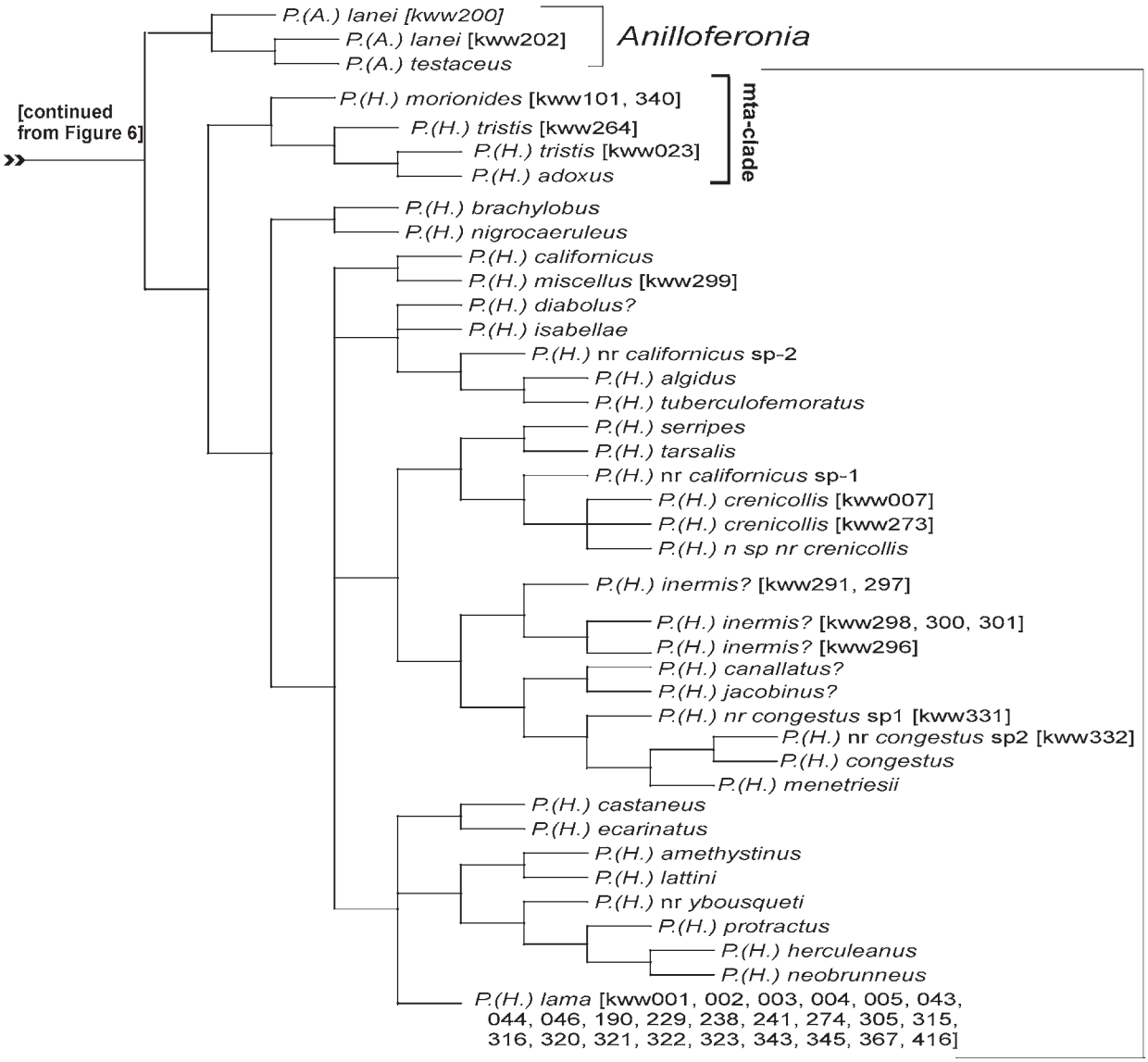

Fig. 7.—Consensus tree of 4,800 most parsimonious trees from 140 taxon 28S analysis using Clustal default alignment and Gapcoder, *Anilloferonia* + *Hypherpes* subtree. *P.* = *Pterostichus.*

No single taxon or clade was consistently found to be the sister group to the *Hypherpes* complex. *Cryobius* and/or *Pseudoferonina* were most frequently placed as sister to or near the *Hypherpes* complex, either in the grade subtending the *Hypherpes* complex or in a clade of taxa sister to the *Hypherpes* complex (Table 1). In the consensus tree from the standard analysis (Fig. 8) the clade of (*Pseudoferonina* + *Cryobius*) is the adelphotaxon to the *Hypherpes* complex. However, no taxon or group is recovered as the adelphotaxon in the bootstrap or jackknife analyses at the 50% level and the decay analysis score for the (*Pseudoferonina* + *Cryobius*) + *Hypherpes* complex node is only 3 (Fig. 8).

Results within the *Hypherpes* Complex.—Within the *Hypherpes* complex *Anilloferonia* (*P. lanei* + *P. testaceus* (Van Dyke) excluding *P. rothi* (Hatch)) is a well-supported sister-pair found in all analyses. *Leptoferonia* (including *P. rothi*) is monophyletic in all analyses except in the COI–COII analysis with third positions deactivated (Fig. 5) and the 157 terminal analysis of 28S using Dialign alignment, whether with or without gaps scored with Gapcoder. In the two analyses using 28S data the clade of *P. beyeri* Van Dyke + *P. sphodrinus* LeConte is variously positioned in resulting trees either as sister to the rest of the *Hypherpes* complex, sister to *Hypherpes sensu stricto* or sister to the remaining *Leptoferonia*. In all trees found using Dialign alignment with gaps scored with Gapcoder the *P. morionides* (Chaudoir), *P. tristis* (Dejean) and *P. adoxus* (Say) clade (referred to as the mta-clade) is sister to the

Fig. 8.—Consensus tree of 15,504 most parsimonious trees from 140 taxon, 28S,COI–COII, combined analysis using Clustal default alignment and Gapcoder (standard analysis), in part. Numbers on branches are bootstrap(jackknife)/[decay] values. Bootstrap and jackknife values of 50% or less are not shown. *P.* = *Pterostichus.*

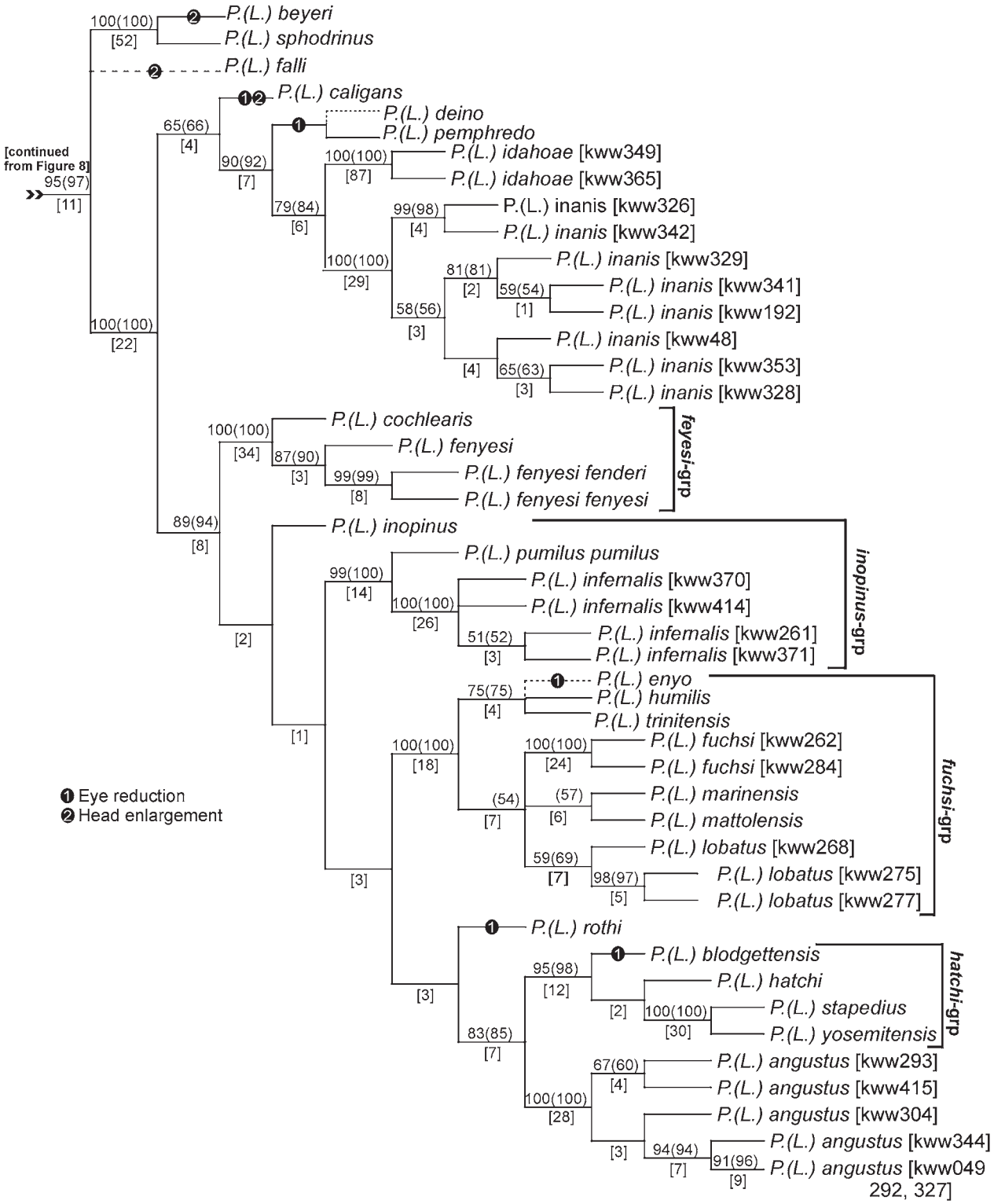

Fig. 9.—Consensus tree of 15,504 most parsimonious trees from 140 taxon, 28S, COI–COII, combined analysis using Clustal default alignment and Gapcoder (standard analysis), *Leptoferonia* subtree. Numbers on branches are bootstrap(jackknife)/[decay] values. Bootstrap and jackknife values of 50% or less are not shown. Species groups are from Hacker (1968). Morphological characters are indicated by numbers mapped on filled circles: **1**, eye reduction; **2**, head enlargement. Tentative placement of *Pterostichus falli*, *P. deino*, and *P. enyo* is indicated by dashed branch lines. These taxa were not included in the analyses of DNA and are placed based on general morphological similarity. *P.* = *Pterostichus.*

Fig. 10.—Consensus tree of 72 most parsimonious trees from 157 taxon, 28S analysis using Clustal default alignment and Gapcoder (extended analysis). Numbers on branches are Bootstrap(Jackknife)/[decay] values.Bootstrap and Jackknife values of 50% or less are not shown. Terminals included in *Hypherpes* complex taxa are condensed. *P.* = *Pterostichus.*

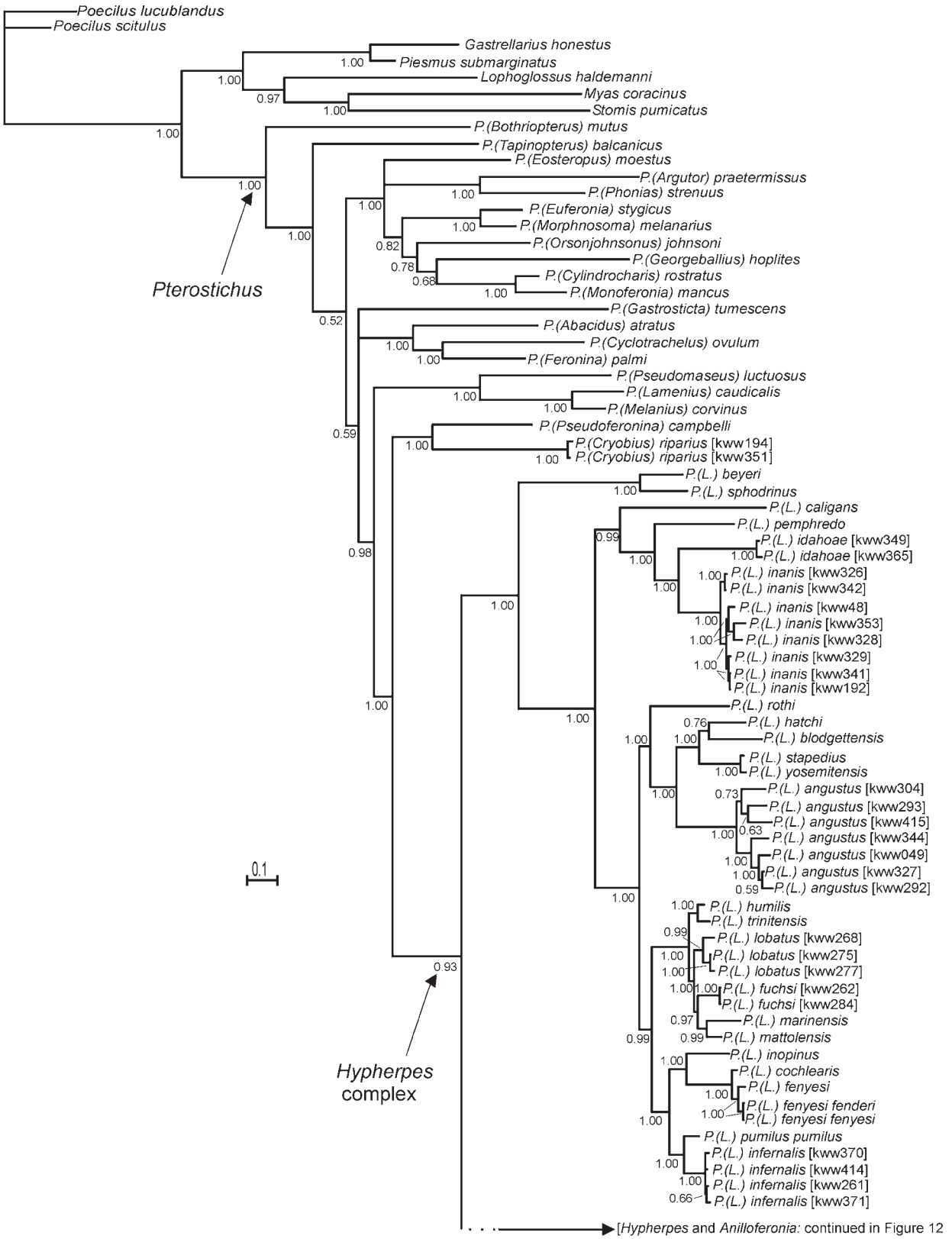

Fig 11.—Majority rule consensus tree (in part) from Bayesian analysis of the combined COI–COII and 28S data. Numbers on branches are Bayesian clade support values. *P.* = *Pterostichus*.

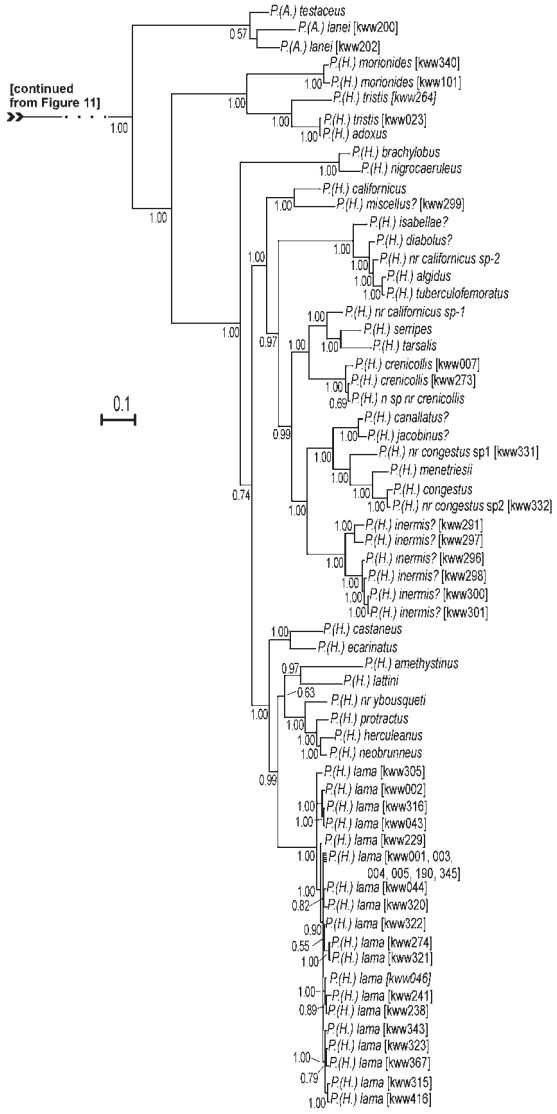

Fig 12.—Majority rule consensus tree (in part) from Bayesian analysis of the combined COI–COII and 28S data. Numbers on branches are Bayesian clade support values. *P.* = *Pterostichus*.

remaining *Leptoferonia*. When third positions are deactivated in the mtDNA matrix the node consisting of (*P. beyeri* + *P. sphodrinus*) + the remaining *Leptoferonia* species is unresolved (Fig. 5). In the standard and extended analyses the bootstrap, jackknife and decay scores all strongly support *Leptoferonia* monophyly (Figs. 8,10). The topology within *Leptoferonia* is fairly consistent in all analyses except for differences in the COI+COII analyses (Fig. 3) and in a few of the 28S data only analyses. In these cases a few nodes were not supported in the consensus and they represent little topological conflict.

All species groups designated by Hacker (1968) are recovered as monophyletic except for the *inopinus*-group (Fig. 14). *Pterostichus inopinus* (Casey) is either in a paraphyletic grade with the other members of the *inopinus*-group (*P. pumilis* Casey + *P. infernalis* Hatch) or sister to the *fenyesi*-group (COI+COII and Bayesian analyses only).

Hypherpes sensu stricto is monophyletic in all analyses except for the 157 terminal analysis using Dialign alignment without the use of Gapcoder, in the 140 terminal analysis using the ClustalX default parameter alignment and in estimated consensus using COI–COII with third positions deactivated. In the 157 terminal analysis using Dialign the mta-clade is sister to *Leptoferonia* and in the 28S 140 taxon ClustalX alignment *Anilloferonia* is sister to the mta-clade.

Subset Analyses of the mta-Clade

Six MPTs were found using the 18S data set for the subset of 15 taxa (Fig. 13A). The mta-clade is not found in their consensus. However, of the six, four include the mta-clade and two have the mta taxa as paraphyletic (Fig. 13B). All eight MPTs found for the CAD data set for the subset of 15 taxa (Fig. 13C) included the mta-clade. In the wg dataset for the subset of 15 taxa the mta-clade was not recovered in the consensus tree of the 26 MPTs (Fig. 13D). The mta-clade was recovered in six of the 26 trees. For the wg dataset, *P. morionides* was variable in its position, but was always within one node of *P.tristis* + *P. adoxus*. Most frequently (18 of 26 trees) *P. morionides* was sister to *P. menetriesii* LeConte (Fig. 13E). In some cases this pair was sister to *P. tristis* + *P. adoxus*. The combined matrix of all sequences for the subset of 15 taxa resulted in a single MPT and partitioned Bayesian analysis of this matrix also results in the same topology with uniformly high clade support values (Fig. 13F). This tree also includes the mta-clade. The general topology (e.g., in-group monophyly, monophyly of subgenera, etc.) in as much as it is tested, in all four subset analyses, was consistent with the larger standard and extended analyses.

Comparative Results

Interspecific and Intraspecific Variation.—Intra-specific variation for multiply sampled taxa was markedly lower than inter-specific variation in the *Hypherpes* complex for COI, COII and 28S except in three cases in COI (Table 2). Variation within *P. angustus*, *P. lanei* and *P. tristis* slightly overlapped with the minimum inter-specific variation for COI.

Species represented by multiple individuals in the analyses were always found to form convex groups, usually clades, but in a few cases paraphyletic grades. The two most densely sampled taxa, *P. lama* and *P. angustus,* showed some consistent structure. Within *P. lama* a clade of three individuals from near-coast sites along the Santa Lucia Range from Point Lobos south to Gamboa Point (kww002, 043, 316) form the sister group to the remaining *P. lama* samples (Fig. 4,8,12). There is a consistent north and south division between *P. angustus* samples from Sonoma,

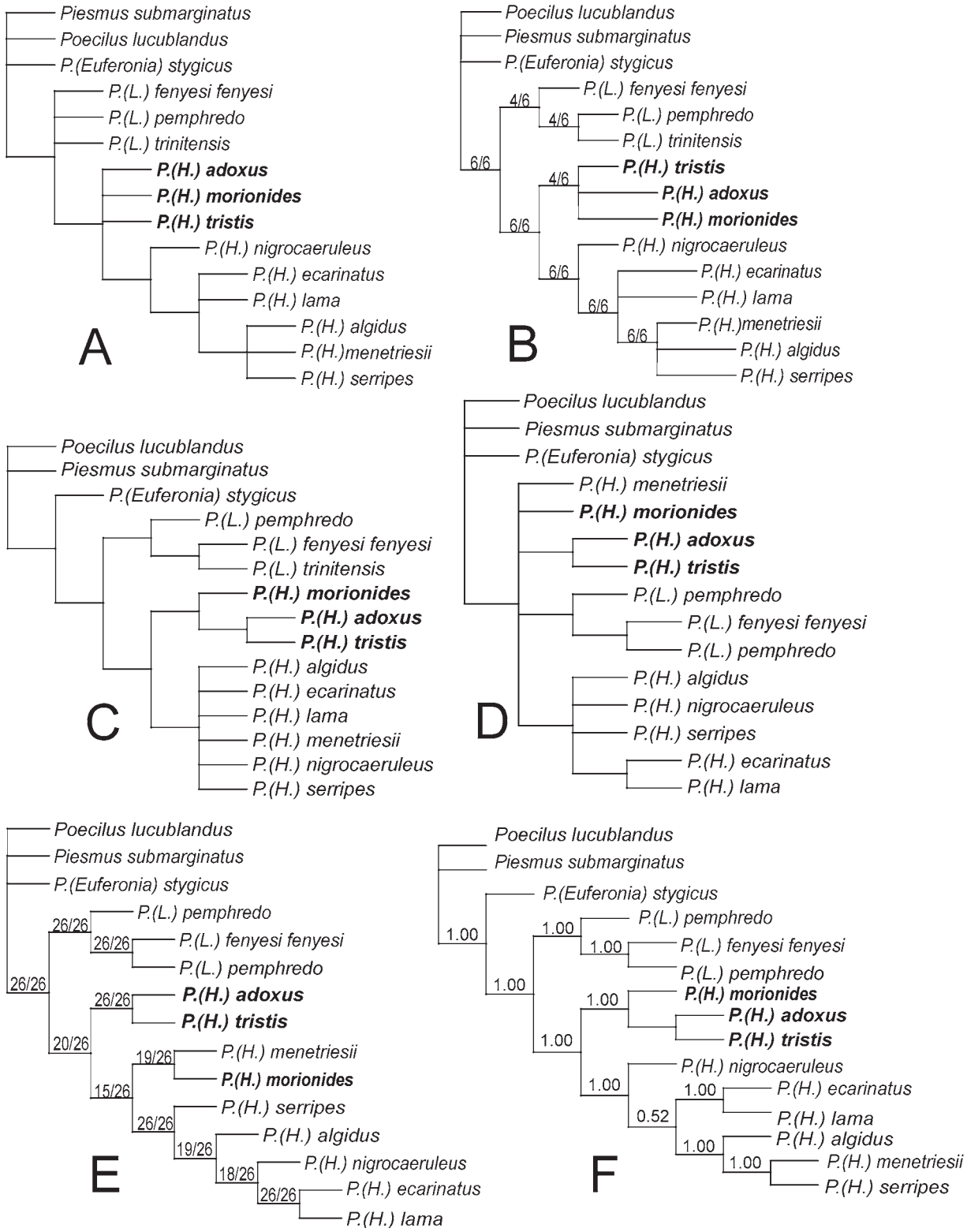

Fig 13.—Trees from 15 taxon subset analyses. **A**, 18S consensus tree of six most parsimonious trees; **B**, 18S majority rule tree; **C**, CAD consensus tree of 8 most parsimonious trees; **D**, wg consensus tree of 26 most parsimonious trees; **E**, wg majority rule tree; **F**, single most parsimonious tree found in combined analysis of all sequence data using both parsimony criteria and Bayesian analysis. For majority rule trees the numbers on branches are raw counts of the frequency the clade is found out of the total number of trees. Numbers on branches in **F** are Bayesian clade support values. The mta-clade shown in bold in all trees. *P.* = *Pterostichus*.

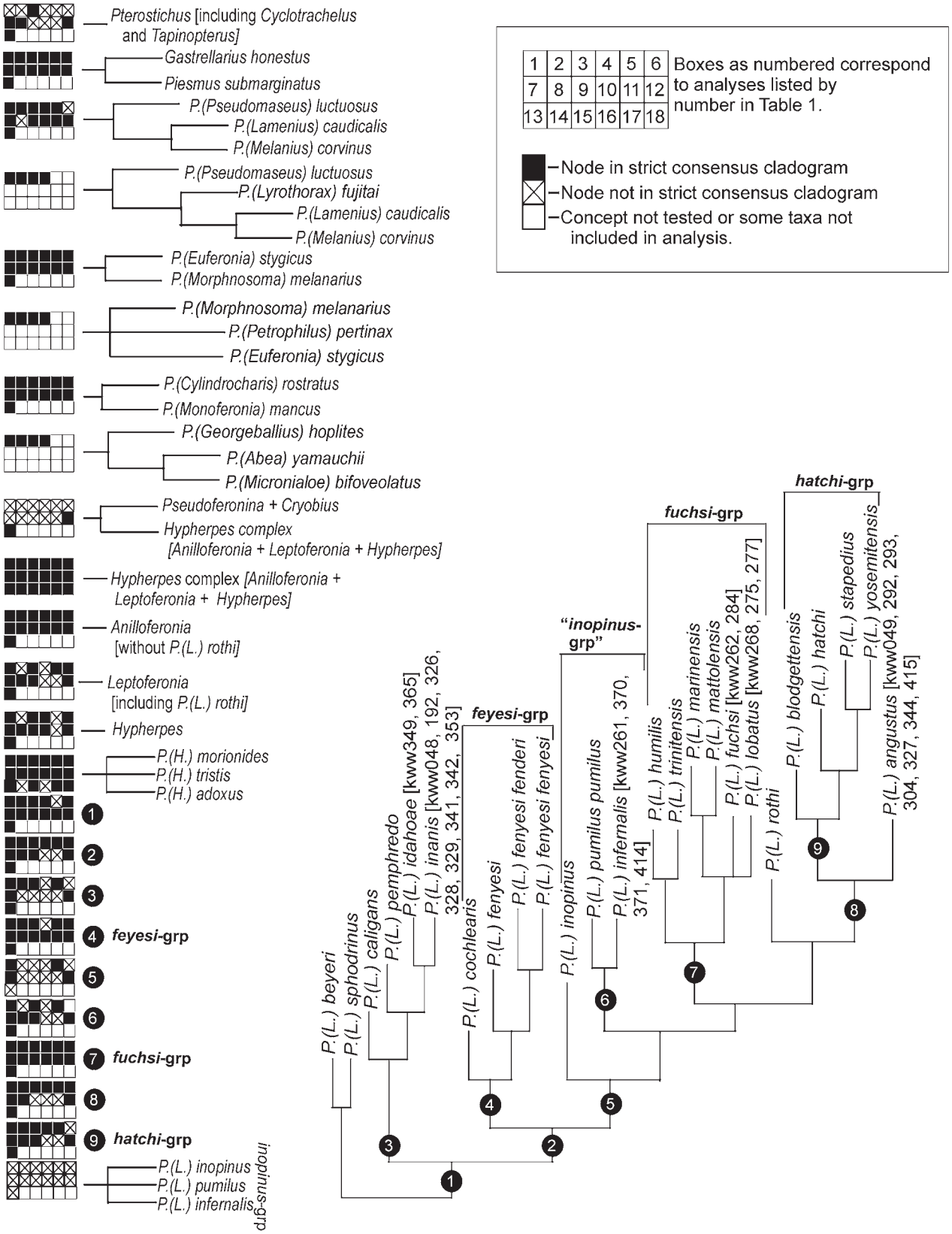

Fig. 14.—Summary figure of all analyses for various clades and groups that are discussed in the text. *P.* = *Pterostichus.*

TABLE 2. Inter- and intraspecific p-distance, as average [range], for sequence data of species represented by multiple individuals and for inter-species comparisons over all *Hypherpes* complex species.

n, number of individuals sampled. Interspecific values, and intraspecific values that exceed interspecific values, in bold.

n	spp	COI	COII	28S
2	*P. (A.) lanei*	**3.03**	2.40	2.27
2	*P. (H.) morionides*	0.71	1.02	0.00
2	*P. (H.) tristis*	**3.00**	3.38	1.32
2	*P. (H.) crenicollis*	0.87	0.71	0.09
2	*P. (L.) fuchsi*	0.12	0.14	0.00
2	*P. (L.) idahoae*	1.18	0.71	0.09
3	*P. (L.) lobatus*	1.27 [0.87–1.69]	0.75 [0.28–1.12]	0.92 [0.00–0.92]
3	*P. (L.) fenyesi*	1.28 [1.26–1.29]	1.03 [0.01–1.55]	0.18 [0.00–1.80]
4	*P. (L.) infernalis*	0.51 [0.25–0.98]	1.64 [0.14–3.81]	0.28 [0.09–0.36]
6	*P. (H.) inermis*	1.36 [0.00–2.12]	0.82 [0.00–3.11]	0.90 [0.00–1.91]
7	*P. (L.) angustus*	2.08 [0.09–**3.14**]	2.54 [0.81–3.77]	1.40 [0.27–2.18]
10	*P. (L.) inanis*	0.85 [0.00–2.18]	0.62 [0.00–1.40]	0.71 [0.00–1.29]
24	*P. (H.) lama*	0.51 [0.00–1.15]	0.73 [0.00–2.10]	0.13 [0.00–0.83]
57	**inter-spp**	**5.80 [2.50–8.11]**	**6.85 [4.03–9.29]**	**5.82 [3.08–9.50]**

Solano, Napa and Contra Costa counties and those sampled from San Mateo and Santa Barbara counties. The position of the single sample from Santa Clara County is equivocal (Fig. 9).

Microphthalmy and Long Branch Correlation.—All six variations of the concentrated changes test resulted in a concentration higher than expected by chance with p values varying, but all values are <0.002 for the character "branches with greater than mean +-SE length" falling on branches characterized by small eyes.

Contrast of Sister Species and Species Pairs.—No significant difference was found between same eye-state species pairs and different eye-state species pairs for the magnitude of change in each since their most recent common ancestor (P(T<=t) two tail =0.302 for maximum values and =0.0933 for minimum values). In cases where eye state was different, the sign of small-eye minus normal-eye branch lengths was "+" in three cases and "-" in four cases (Table 3).

DISCUSSION

Relationships Among Included Outgroup Taxa

Results for the relationships of the various subgenera of *Pterostichus* and related genera are generally consistent with those found by Sasakawa and Kubota (2007) in their analysis of 28S and wg sequence data for a smaller set of taxa. However, Sasakawa and Kubota (2007) did not demonstrate monophyly of *Pterostichus*, as *Bothriopterus* Chaudoir grouped outside the remaining subgenera in their analysis. In the present analyses, *Pterostichus* is found to be monophyletic if *Tapinopterus* and *Cyclotrachelus* are included (Figs. 8, 10), assuming combined data are used and gap regions contribute to the phylogeny. There is little doubt that *Cyclotrachelus* and *Tapinopterus* each form monophyletic groups. However, given that they clearly nest within *Pterostichus* we propose that these groups be subsumed as subgenera under the larger concept of *Pterostichus*. Bousquet (1999) proposed that *Cyclotrachelus* and *Tapinopterus*, *Abax*, *Percus* Bonelli, *Eosteropus* Tschitschérine and other genera form a group referred to as the molopite complex. These taxa are purported to share synapomorphic larval features. There is no evidence from this analysis that the molopite complex forms a monophyletic group, or even a set of closely related taxa. Likewise Sasakawa and Kubota (2007) did not find evidence for the complex. The molopite complex as discussed by Bousquet (1999) is probably polyphyletic, but an analysis including all appropriate taxa has not yet been published. One of the presumed molopite taxa, *Tapinopterus*, is superficially similar to *Hypherpes* in having a medial seta on the hind coxa and lacking the dorsal seta on the third elytral interval. No close relationship was found between *Tapinopterus* and *Hypherpes* in this study.

Across the generic and subgeneric level, results herein are partially, but not entirely, consistent with groupings presented by Bousquet (1999). This analysis does not test the proposed monophyly of a group with an apophysis on

TABLE 3. Absolute value of species pair contrast.

Sign given for species pairs involving different eye states. **n**, normally developed eye; **r**, reduced eye ; *P. = Pterostichus.*

Species Pair	Difference	Sign	Eye States
P. beyeri-sphodrinus	0.015879		n-n
P. stapedius-yosemitensis	0.016507		n-n
P. humilis-trinitensis	0.018554		n-n
P. marinensis-mattolensis	0.063324		n-n
P. cochlearis-fenyesi	0.020049		n-n
P. pumilus-infernalis	0.035911		n-n
P. blodgettensis-hatchi	0.053630	+	r-n
-max contrast			
P. caligans-idahoae	0.013183	+	r-n
P. pemphredo-idahoae	0.092002	-	r-n
P. rothi-angustus	0.141247	-	r-n
-min contrast			
P. caligans-pemephredo	0.105185		r-r
P. pemphredo-inanis	0.038423	-	r-n
P. rothi-stapedius	0.046745	-	r-n

the left paramere of the male aedeagus (Bousquet 1999) as our choice of rooting is between *Poecilus* Bonelli, which lacks the apophysis, and the remaining taxa, which have the apophysis. Bousquet (1999) pointed out that *Gastrellarius, Piesmus* versus *Stomis* Clairville share a similar form of spermatheca. All analyses herein found *Gastrellarius honestus* (Say) + *Piesmus submarginatus* (Say) as sister taxa. A sister group relationship between these two genera suggests that the apically coiled spermatheca is synapomorphic. Additionally, species of both genera are distributed exclusively in eastern North America and live under the bark of logs (*Gastrellarius blanchardi* (Horn) and *G. unicarium* (Darlington) are also known from the leaf litter). However, *Stomis pumicatus* (Panzer) was never found to form a monophyletic group with these taxa. The coiled apex of the spermatheca is most likely a feature derived independently in *Stomis* and *Gastrellarius* + *Piesmus*, and perhaps several times in pterostichines.

Two taxa from the Appalachian states and surrounding region, *Cylindrocharis* and *Monoferonia,* are superficially similar to *Hypherpes,* but were not found to be closely related to the *Hypherpes* complex. These two taxa did group as sister taxa in all analyses. The *Cylindrocharis* + *Monoferonia* relationship has not been proposed previously, but bears more investigation. *Orsonjohnsonus* Hatch has also been associated with these taxa and *Hypherpes* (Ball and Roughley 1982). *Orsonjohnsonus* was not found to be related to *Hypherpes* in any analyses, but it is sister to *Cylindrocharis* + *Monoferonia* in the standard analysis. However, this relationship is not well supported and the placement of *Orsonjohnsonus* varies in other analyses.

The clade of (*Morphnosoma* (*Euferonia* Casey + *Petrophilus*)) was recovered in all analyses. This relationship is consistent with recent discussion and analyses using male genitalic features (Sasakawa and Kubota 2005, 2006). This complex, including *Euryperis* Motschulsky, *Morphnosoma*, *Euferonia*, *Feroperis* Lafer, and *Moritapterus* Berlov is considered to constitute a single subgenus, *Petrophilus* by Sasakawa and Kubota (2005, 2006). As such, this group forms a clade with a classic East Asian + eastern North American biogeographic pattern that is well known in plants (Wen 1999) and has been found in some insect groups (Nordlander 1996).

Lindroth (1966) included the North American species of *Pseudomaseus* Chaudoir, *Lamenius* and *Melanius* in a single group, the *corvinus*-group. This implies a close relationship, but explicit synapomorphic features were not given and none found by Bousquet (1999). However, the clade (*Pseudomaseus* (*Lyrothorax* (*Lamenius* + *Melanius*))) is recovered in all analyses of DNA sequence data, evidence that these are closely related taxa. *Lyrothorax*, which is superficially similar to the other subgenera, was studied by Nemoto (1989) who suggested monophyly of the subgenus based on characteristics of the endophallus. However, he did not suggest a sister-group for *Lyrothorax*. Again this clade shows an East Asian + eastern North American biogeographic pattern.

Georgeballius hoplites (Bates), which is superficially similar to some *Hypherpes* taxa, has been associated with the subgenus. Originally it was described in the *Hypherpes*-like taxon *Allotriopus* by Bates (1882), however, Habu (1984) subsequently created the genus *Georgeballius* for this taxon. Habu also created *Carllindrothius* for "*Hypherpes*" *colonus* Bates. Both of these Japanese taxa were compared to *Hypherpes* by Habu (1984) and *C. colonus* was also examined by Lindroth (1966). Both authors concluded that these taxa were not close relatives of *Hypherpes*. Habu (1984) suggested that *Georgeballius* and *Carllindrothius* were close relatives to each other. In this analysis and in analyses by Sasakawa and Kubota (2007), the clade (*Georgeballius* (*Abea* + *Micronialoe*)) is consistently recovered and well supported. These taxa, and probably *Carllindrothius*, are likely part of an East Asian complex of taxa that is not particularly closely related to *Hypherpes,* although they are superficially similar.

Establishing the sister group of the *Hypherpes* complex is important as it would allow for unambiguous optimization of characters at the ancestral node (Watrous and Wheeler 1981; Maddison 1984). The taxon or set of taxa found to be sister to the *Hypherpes* complex varied and is dependent on the alignment method (Table 1). *Cryobius* and/or *Pseudoferonina* were most commonly found to be the adelphotaxon and the standard analysis has *Cryobius* + *Pseudoferonina* as sister to the *Hypherpes* complex. This relationship is only weakly supported, however (Fig. 8). *Cryobius* shares the additional medial seta of the hind coxa with species of the *Hypherpes* complex. *Pseudoferonina* and many, but not all, species of the *Hypherpes* complex have the median lobe of the aedeagus with a lightly sclerotized median strip. This could be a plesiomorphic character state or it may have been derived many times in pterostichines.

Assuming *Cryobius* + *Pseudoferonina* is the sister group to the *Hypherpes* complex, relatively small body

size (overall length 12.0 mm or less) can be optimized as the ancestral condition and large body size (>12.0 mm up to 30.0 mm) as the derived condition in *Hypherpes*. Body size does overlap between the small-sized species in *Cryobius*, *Pseudoferonina, Leptoferonia* and *Anilloferonia* (4–12.0 mm) and *Hypherpes* (8.0–30.0 mm). However very few *Hypherpes* species have an average length in the 9.0–12.0 mm range, though some species do frequently have individuals whose length is 9.0–10.0 mm (e.g., *P. castaneus* (Dejean)). Body-size increase has been shown to be correlated with phyletic divergence in other carabid groups (Liebherr 1988) and is thought to be particularly likely to occur in brachypterous groups. This pattern is, however, far from perfect. A notable exception in *Hypherpes* is the complex of species presently represented by *P. inermis* Fall and *P. miscelus* Casey from southern California. Their size ranges from 8.0–11.0 mm and they are brown-colored, somewhat convex and ventricose. As such, they are similar to, though still on average larger than, species of *Leptoferonia* from north coastal California. In this analysis they are found to be derived within *Hypherpes sensu stricto*, suggesting a reversal to smaller body size.

Patterns and Relationships Within the *Hypherpes* Complex

Repeated Evolution of Microphthalmy.—Within the *Hypherpes* complex *Leptoferonia* is a monophyletic group composed of those species included in the subgenus by Hacker (1968), minus *Stomis termitiformis* (Van Dyke), and adding of *P. rothi* and four species described by Will (2007). *Pterostichus rothi* was originally described in *Anilloferonia*, which at the time included all known small-sized North American pterostichines with reduced eyes. Aside from characteristics of the *Hypherpes* complex and the reduced eye size, *P. rothi* does not share any obvious synapomorphies with *P. lanei* and *P. testaceus*. *Pterostichus rothi* is significantly larger (8.0–9.0 mm vs. 5.0–6.0 mm) and is at least superficially similar to *P. angustus* (Dejean). Absolute eye size varies with the size of the individual so that size of the eye is best considered relative to the size of the head. In *P. beyeri*, *P. falli* and *P. caligans* Horn, the size of the head relative to the rest of the body is very large and the relative eye size is small. However, the eye itself only appears to be markedly reduced in size in *P. caligans*. Extreme eye reduction, i.e., reduced to very few ommatidia or to the point of having only a paler "scar", in species with more normally proportioned heads is more common than very small eyes in the large-headed taxa. In combination with normally proportioned heads, eye reduction has evolved once in *Anilloferonia* and perhaps as many as five times in *Leptoferonia.* The large headed *P. caligans* is potentially a sixth instance of eye reduction. Instances of eye reduction have occurred in separate lineages within *Leptoferonia* including *P. enyo* in the *fuchsi*-group, and *P. rothi* and *P. blodgettensis* Will in the *hatchi*-group. An additional one to three instances of eye reduction are found in *P. caligans*, *P. deino* and *P. pemphredo* Will (Fig 1B). *Pterostichus deino* was not sampled for sequence data in this analysis. However, based on morphological characters of the elytral margin and elytral form (Will 2007), it is most likely related to *P. caligans* and/or *P. pemphredo* (Fig. 9). Most likely *P. deino* is sister to *P. pemphredo* as they are similar in general body form, overall size, shape of the male genitalia and both are found in the Sierra Nevada Mountains. Given this arrangement, eye reduction can be most parsimoniously optimized as either occurring in parallel in *P. caligans* and the common ancestor of *P. deino* + *P. pemphredo*, or in the common ancestor of the entire clade including these taxa and *P. idahoae* Csiki + *P. inanis* Horn. In the latter case, the full eye would have to be regained in the common ancestor of *P. idahoae* + *P. inanis*. We prefer the delayed transformation hypothesis that invokes parallel reduction of eyes, deeming regaining ommatidia as more difficult than loss. With this assumption eye reduction has occurred six times in the *Hypherpes* complex, once in *Anilloferonia* and five times in *Leptoferonia* (Figs. 8, 9). Significant eye reduction is not known from any species of *Hypherpes sensu stricto.*

Microphthalmy and Increased Branch Length Correlation.—Of the eight species in the Hypherpes complex that have extremely small eyes, three represent the three longest terminal branches in the complex, and two are of a length significantly greater than mean +-SE. One falls within the range of the mean +-SE bin, and terminal branch lengths are not known for the final two. One falls within the range of the mean +-SE bin. At this level one can say that they never have short terminal branches. The concentrated changes tests confirm that the general pattern observed is not likely to be random. Differences in branch lengths may be due to substitution rate and/or time length differences among individual branches. Extant species descending from a common node (most recent common ancestor) are by definition of the same age. Give the substitution model employed, any differences between left and right descendents then would be a difference in rates of sequence evolution. In the case of eye reduction and autapomorphic changes in COI, COII and 28S sequences, we found that there is no significant difference in relative rate between the contrast of small-eyed species and their normal-eyed sister and pairs of same eye-state species. In fact, the direction of the difference is about equally likely to be positive or negative. This suggests that there is no relative difference in the tempo of sequence evolution. The apparent concentration of apomorphic branches in small-eyed taxa without a change in relative rates could be explained by a greater extinction rate or lower rate of cladogenesis. One possible explanation of this pattern would be the existence of undiscovered species that group with the known small-eyed taxa. However, we feel that *Leptoferonia* has been sufficiently sampled making this less likely to be the case.

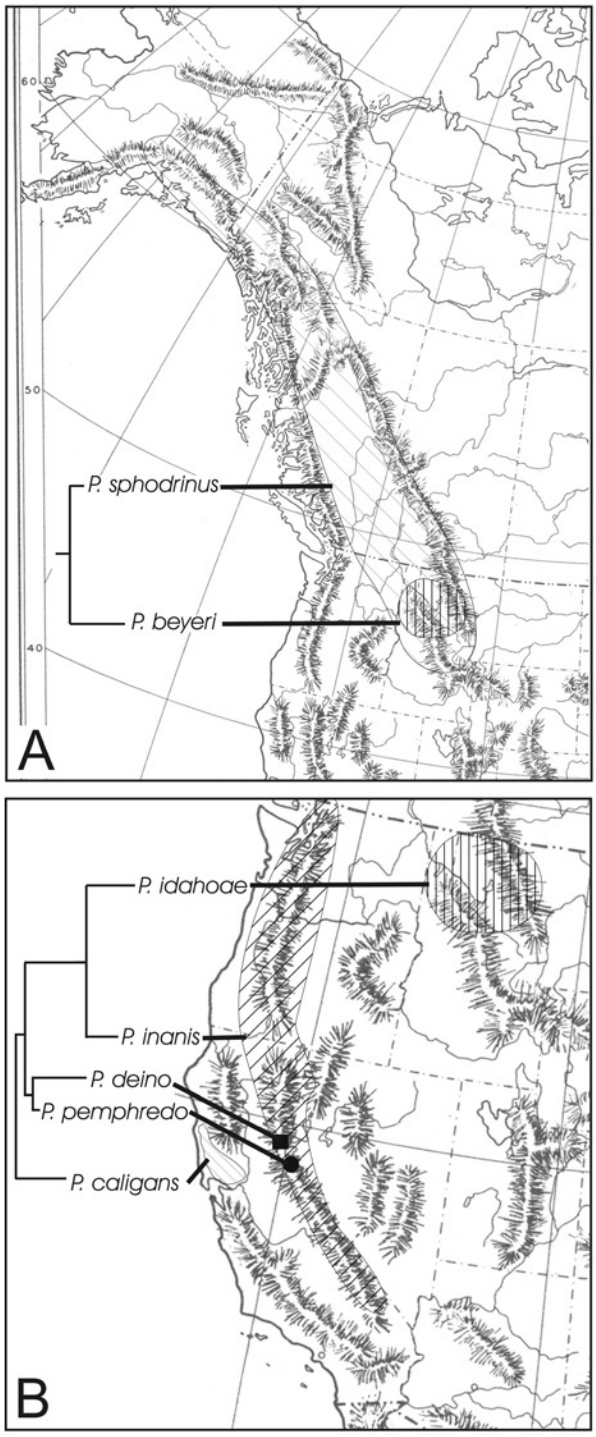

Fig. 15.—Map showing portions of western North America with approximated distributional ranges of sister species and clades of *Leptoferonia*. **A**, *Pterostichus sphodrinus* and *P. beyeri;* **B**, *Pterostichus idahoae, P. inanis, P. deino, P. pemphredo*, and *P. caligans*. The black square (*P. deino*) and dot (*P. pemphredo*) represent single locality records.

Biogeographic Patterns in *Leptoferonia*.—In *Leptoferonia* there are three main clades (Fig. 9). The clade of *P. beyeri* + *P. sphodrinus* is sister to the remaining species. *Pterostichus sphodrinus* is a widespread, common and often locally abundant species. This species' range includes the most northern (Alaska) and eastern (Montana) extreme for the subgenus (Fig 15A). In part of its range it is sympatric with its sister species, *P. beyeri,* which is restricted to northern Idaho and the western edge of Montana. The second clade of *P. caligans* to *P. inanis* consists of relatively large-sized species (except *P. pemphredo* and *P. deino*) that are ecologically diverse and geographically widespread. *Pterostichus caligans*, *P. pemphredo,* and most likely *P. deino,* are deep soil-dwelling species, whereas *P. idahoae* and *P. inanis* are forest-litter species. Biogeographically there is a division between the near coast inland range (Mendocino, Sonoma and Napa counties, CA) of *P. caligans* and the Sierra/Cascade+western Rocky Mountains of Idaho and Montana range of *P. pemphredo* + *P.deino* and *P. idahoae* + *P. inanis,* respectively (Fig. 15B). The remaining clade of *Leptoferonia* includes the majority of the species and repeated patterns of species pair biogeographic separation (Figs. 16–17). Three species pairs have north/south allopatric distributions along the coastal fog belt (Figs. 17A–B), *P. cochlearis* Hacker + *P. fenyesi* Csiki (a slight overlap of their ranges exists north of Eureka, California), *P. mattolensis* Hacker + *P. marinensis* Hacker and *P. pumilis* + *P. infernalis* Hatch. The species pair of *P. humilis* Casey + *P. trinitensis* Hacker has a coastal/inland division (Fig. 17B). A third species not included in the analysis, *P. enyo,* is mostly likely related to *P. humilis* and *P. trinitensis* based on morphological characters. This species has differentiated from its sister taxa ecologically by moving into the deep soil and litter. The final patterns are coastal+inland/Sierra, as found in the clade *P. angustus* + *hatchi*-group (Fig. 16). *Pterostichus angustus* adults are active from late autumn to early spring and are found in counties around San Francisco, north to Mendocino, east to Contra Costa and south to Santa Barbara. This species is found in oak habitat and not redwood forests. Its sister group, the *hatchi*-group, includes four spring-active species from the conifer forests of the Sierra Nevada Mountains. The *hatchi-group* species have allopatric distributions along a north/south axis (Fig. 16). Apparent east-west running barriers such as the Grand Canyon of the Tuolumne and Yosemite Valley divide the populations along the north to south axis and species are not known to range above 2800 m or below 1000 m elevation. These species are sympatric with the wide ranging *P. inanis* (Fig. 15B). *Pterostichus inanis*, however, is found down to much lower elevations, to the point that the barriers no longer prevent migration and interbreeding. The exception to the allopatric pattern among the Sierran species of the *hatchi*-group is the sympatry of *P. hatchi* and *P. blogettensis*. Like the case of *P. enyo* and its relatives, *P. humilis* and *P. trinitensis*, *P. blodgettensis* has differentiated ecologically by moving into the deep soil (Will 2007). Sister species, or presumed closely related species,

that show differentiation between leaf litter dwelling and deep-soil species are known from other beetle groups in California (Peck 1997) and other groups of carabids (Liebherr 2006).

Anilloferonia* and *Hypherpes sensu stricto.*—Anilloferonia*** consists of two distinctive species with highly reduced eyes that are restricted to the Pacific Northwest. The relationship of this subgenus to *Leptoferonia* and *Hypherpes* is not clear. In the consensus of the trees resulting from the standard analysis, *Anilloferonia* is sister to *Hypherpes*, but support for this node is weak and sensitive to various methods of alignment (Fig. 8,10). In some analyses *Anilloferonia* is sister to *Leptoferonia* (e.g., COI+COII, Fig. 3) or sister to *Leptoferonia* + *Hypherpes* (e.g., the 157 taxa analysis using Dialign and GapCoder).

The clade of *Hypherpes sensu stricto* is well supported in nearly all analyses and has high bootstrap/jackknife and decay scores (Figs. 8,10). The exemplars represents only 33 species of what will probably be a group of nearly 100 species when all are described. However, our samples represent the morphological and geographic diversity of the group. Because species-level studies of potentially important characters—e.g., male genitalia, female reproductive tract and secondary sexual characters as undertaken for *Leptoferonia*—it is premature to read much from the present phylogeny of *Hypherpes*. Support for several cases of sympatric sister taxa are indicated: (*P. brachylobus* Kavanaugh & Labonte + *P. nigrocaeruleus* van Dyke), (*P. crenicollis* LeConte + *P.* n sp nr *crenicollis*) and (*P. serripes* LeConte + *P. tarsalis* LeConte). Sympatry of sister species is generally considered unusual, as pointed out by Kavanaugh and LaBonte (2006). *Hypherpes* may be an excellent group to determine how such closely-related, ecologically similar species can coexist, given that the pattern of geographic allopatry or ecological differentiation in sympatry as seen in *Leptoferonia* is not obvious in *Hypherpes*.

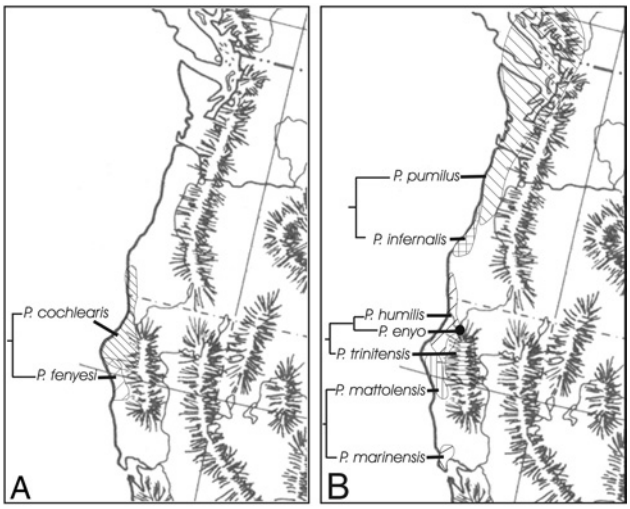

Figure 17.—Map showing portions of western North America with approximated distributional ranges of sister species and clades of *Leptoferonia*. **A**, *Pterostichus cochlearis* and *P. fenyesi;* **B**, *Pterostichus pumilus, P. infernalis, P. humilis, P. enyo, P. trinitensis, P. mattlensis*, and *P. marinensis*. The black dot (*P. enyo*) represents a single locality record.

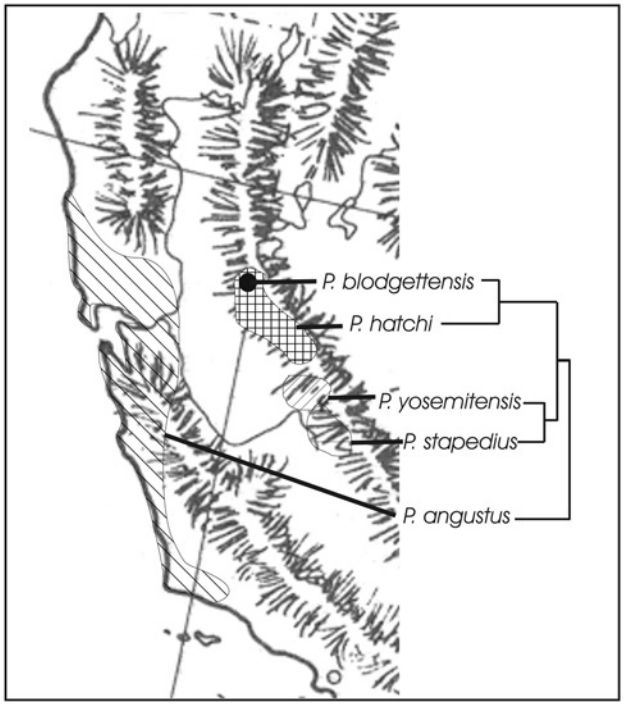

Fig. 16.—Map showing portions of western North America with approximated distributional ranges of *Leptoferonia: Pterostichus blodgettensis, P. hatchi, P. yosemitensis, P. stapedius*, and *P. angustus*. The black dot (*P. blodgettensis*) represents a single locality record.

The mta-Clade.—One unusual feature of the *Hypherpes sensu stricto* is the clade arranged as (*P. morionides* (*P. tristis* + *P. adoxus*)). As *P. tristis* and *P. adoxus* are the only two eastern North America species of the entire *Hypherpes* complex, their inclusion in, or relationship to *Hypherpes* has always been questioned. Lindroth (1966) placed *P. tristis* and *P. adoxus* in *Monoferonia* (his *mancus*-group), an exclusively eastern taxon. Casey (1913) put *P. tristis* and *P. adoxus* in a separate group (under various species names now synonymized) primarily based on the fact that they have an eastern distribution. In all analyses we found *P. tristis* and *P. adoxus* to be members of the *Hypherpes* complex and in nearly all cases as members of *Hypherpes sensu stricto.* In a few analyses the mta-clade was found to be sister to a subset of *Leptoferonia*. The position of the mta-clade as a whole is slightly sensitive to alignment and gap treatment method. The monophyly of the mta-clade, however, is not sensitive to alignment methods and is found in all analyses (Table 1, Fig 14). *Pterostichus morionides* is a deep black colored, relatively large (body length about 20.0–25.0 mm), broad and heavy bodied species with broadly expanded tarsi and a prominently enlarged

subocular region and large head (Fig. 2B). The range of *P. morionides* is the central Sierra Nevada Range in coniferous forests. Both *P. adoxus* and *P. tristis* (Fig. 2A) are typically brown colored, often somewhat iridescent, smaller (body length about 11.0–14.0 mm) and relatively long-legged. Their heads are of normal proportions. Given that we observe groups of generally similar species from the same or adjacent areas throughout our phylogeny of the *Hypherpes* complex, the mta-clade seemed like a highly unlikely result. Yet the results based on the 28S and COI+COII data show very high support for the mta-clade (Figs. 8,10). The clade was further tested using wg, CAD and 18S data for a subset of taxa and the results are fundamentally the same (Figs. 13,14, Table 1). With many different data sources all giving the same or very similar results, we have overturned our *a priori* hypothesis, but have not yet determined what evolutionary and biogeographic events could have lead to the current disjunct distribution. Such a pattern is not common, but is known in some animal groups, e.g., salamanders (Session and Kezer 1987), millipedes (Hoffman 1963) and spiders (Catley 1994, see included references). It was suggested that for these groups there are two characteristics that could explain their common geographical patterns—low vagility and a high susceptibility to desiccation—factors restricting them to humid forests (Catley 1994). This would be consistent with a broader distribution of these taxa in late Tertiary deciduous forests that extended across central North America (Axelrod 1960). At the end of the Tertiary, vicariance of eastern and western sister groups would have occurred as the central part of the continent became drier and shifted from forest to grasslands. This particular scenario, however, does not adequately fit *Hypherpes*. Whereas *Hypherpes* species are flightless and thus relatively poor dispersers (though they are remarkably good walkers), many species, including *P. morionides,* are well adapted to dry conditions and seasonal drought in the Sierran forests. Additionally it seems unlikely that *Hypherpes* species could have been distributed across the center of the continent and then have been completely extirpated from the region after the retreat of the deciduous forest. One would expect disjunct species in the moister forests of the Ouachita-Ozark Highlands or even in the grasslands. Many species of *Hypherpes* are well adapted to desert areas and seasonally dry forests of Southern California (Will, unpublished data).

Species-Level and Population-Level Diversity and Patterns.—Sequence variation for COI, COII and 28S was consistently greater between species than within (Table 2), and all species represented by two or more individual samples were found to group in clades or, in a few species, paraphyletic grades. This suggests that for this sample of taxa, the morphological diagnoses and our initial hypotheses of species boundaries generally based on pronotal form, male genitalia and setation, are usually a sufficient indication of species boundaries. Notable exceptions are species that do show ostensible intraspecific variation in pronotal form and setation (*P. angustus*, *P. inanis* and *P. inermis*?) but individuals group together in clades in all analyses. Samples of *P. tristis* and *P. crenicollis* are persistently recovered as paraphyletic and this arrangement is well supported in the standard analysis (Fig. 8). *Pterostichus tristis* and its sister species *P. adoxus* have historically been synonymized and reinstated (Perrault 1973). Given the divergence in sequence data found in the small sample presented here, a study of this complex of forms, looking at species boundaries across their ranges, should prove fruitful.

The most widely sampled species in the analyses is *P. lama*. Localities of the 24 sampled individuals span over 1800 km, covering nearly all of the species' north to south range, and from sea level to nearly 3000 m in the Sierra Nevada (records can by plotted on maps via the Essig Museum database http://bscit.berkeley.edu/eme/). Samples of this species form a clade that is highly supported (Figs. 8,10). Remarkably, though this is one of the most widespread species in the complex, and it is a very large-sized, flightless species (suggesting low vagility), the level of sequence variation between samples is quite low (Table 2). *Pterostichus angustus*, whose range is less than a third of the area of *P. lama*, has 3.5 to 10 times as much variation among the sequences used. Relative rate of change and/or ages of these species could account for this difference. It seems likely that either *P. lama* has a relatively slow rate of evolution, is a very young species or is capable of a greater level of interbreeding between apparently disparate populations (e.g., trans-Central Valley in California) than would be expected given its large size and flightless nature. Three individuals from along the Santa Lucia Range from Point Lobos south to Gamboa Point (kww002, 043, 316) do stand out as consistently forming a clade separate from other *P. lama* samples (Fig. 8). Preliminary investigation of the male genitalia suggests that this may be a distinct species, but sampling from the west side of the Santa Lucia Range and an assessment of variation in the broader range of *P. lama* is needed.

Concluding Remarks

We have established that *Leptoferonia*, *Anilloferonia* and *Hypherpes* are each well supported clades that should be recognized as subgenera of *Pterostichus*. *Leptoferonia* is now sufficiently well-studied and described such that higher-level questions regarding biogeography and character evolution can be addressed. *Hypherpes* is still at a level where the greatest effort and benefit will come from alpha-level taxonomic work within the subgeneric framework set out here.

ACKNOWLEDGMENTS

We thank all of the generous collectors that have provided material for this study (see Appendix 1) and David Maddison (University of Arizona, Tucson) and Wendy Moore (California Academy of Science,

San Francisco) for significant assistance and information that helped us in sequencing specimens for this study. Extraction and PCR for KWW397 and 431 were done by W. Moore. Extraction and PCR for various other taxa were done by A. Seago and S. Lew (University of California, Berkeley). This research was funded (in part) by the USDA-CSREES in partnership with the California State Agricultural Experiment Station.

***Note added in proof.** After submission of the manuscript we received specimens of *Pterostichus (Paraferonia) lubricus* Leconte, *P. (Lenapterus) punctatissimus* (Randall) and *P. (Allotriopus) serratipes* (Chaudoir). Preliminary results found by adding these to the 157 taxon 28S data set are not significantly different from results presented here. None of these taxa are found to be closely related to the *Hypherpes* complex.

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APPENDIX I. Specimen identification codes (*continued on subsequent pages)*.

Names followed by (?) are identifications based on the species' description that need to be confirmed against the holotype. These specimens match their description, but appear to represent polymorphic or multiple species based on characters not included in the description. Names prefaced with "nr" nearly match the described species and probably represent a form within the variation of that species. Names prefaced with "n sp nr" nearly match the described species but have a significant deviation, e.g., male genitalia or secondary sexual characteristics that separates them as highly likely to be undescribed species. The internal code is used to associate DNA and voucher specimens and is included in the tree diagrams for species represented by multiple individuals. Collector codes: MSC- Michael Caterino; ASG- Aman Gill; MH- M. Hartmann; DAH- Drew Hildebrandt; DHK- David Kavanaugh; PWK- Peter Kovarik; JRL- James LaBonte; ADL- Adam Leaché; SEL- Stephen Lew; DRM-David Maddison; CJM- Christopher Marshall; KS- K. Sasakawa; AES- Ainsley Seago; KD- K. Desender; JS- Jose Serrano; IGW- Ian Will; KWW- Kipling Will; DSY- Dou-Shwan Yang.

Species	**Origin**	**internal code**	**GenBank # 28S**	**GenBank # COI**	**GenBank #COII**	**GenBank # 18S**	**GenBank # CAD**	**GenBank # wg**
Gastrellarius honestus (Say 1823)	Tompkins Co. NY. coll. KWW	KWW021	EU142328	EU142467	EU142607	na	na	na
Lophoglossus haldemanni (LeConte 1848)	MS. coll. DH	KWW056	EU142437	EU142576	EU142716	na	na	na
Myas coracinus (Say 1823)	Vinton Co., OH. coll. KWW	KWW188	EU142438	EU142577	EU142717	na	na	na
Piesmus submarginatus (Say 1823)	FL. coll. PK	KWW019	EU142439	EU142578	EU142718	EU142290	EU142305	EU142320
Poecilus lucublandus (Say 1823)	Tompkins Co. NY. coll. KWW	KWW013	EU142440	EU142579	EU142719	EU142291	EU142306	EU142321
Poecilus scitulus LeConte 1848	Imperial Co., CA. coll. KWW	KWW183	AF398677	EU142580	EU142720	na	na	na
Pterostichus (Abacidus) atratus (Newman 1838)	Tompkins Co. NY. coll. KWW	KWW020	EU142441	EU142581	EU142721	na	na	na
P. (Abea) yamauchii Morita 1992	GenBank	na	AB243459	na	na	na	na	na
P. (Argutor) praetermissus (Chaudoir 1868)	Franklin Co., OH. coll. KWW	KWW309	EU142442	EU142582	EU142722	na	na	na
P. (Badistrinus) bandotaro Tanaka 1958	GenBank	na	AB243462	na	na	na	na	na
P. (Bothriopterus) mutus (Say 1823)	Tompkins Co. NY. coll. KWW	KWW024	EU142443	EU142583	EU142723	na	na	na
P. (Cryobius) riparius (Dejean 1828)	Kodiak Island, AK. coll. DHK	KWW194	EU142444	EU142584	EU142724	na	na	na
P. (Cryobius) riparius	Flathead Co., MT. coll. IGW	KWW351	EU142445	EU142585	EU142725	na	na	na
P. (Cyclotrachelus) ovulum (Chaudoir 1868)	Leon Co., FL. coll. DRM	KWW098	EU142327	EU142466	EU142606	na	na	na
P. (Cylindrocharis) rostratus (Newman 1838)	Tompkins Co. NY. coll. KWW	KWW016	EU142446	EU142586	EU142726	na	na	na
P. (Eosteropus) moestus (Say 1823)	Hocking Co., OH. coll. KWW	KWW310	EU142447	EU142587	EU142727	na	na	na
P. (Euferonia) stygicus (Say 1823)	Tompkins Co. NY. coll. KWW	KWW014	EU142448	EU142588	EU142728	EU142292	EU142307	EU142322
P. (Eurythoracana) kajimurai Habu & Tanaka 1957	GenBank	na	AB243498	na	na	na	na	na
P. (Feronina) palmi Shaeffer 1910	Yancey Co., NC. coll. DRM	KWW097	EU142449	EU142589	EU142729	na	na	na
P. (Gastrosticta) tumescens LeConte, 1863	Hinds Co., MS. coll. DH	KWW290	EU142450	EU142590	EU142730	na	na	na
P. (Georgeballius) hoplites (Bates 1883)	Mt. Ozuooriayama, Japan coll. KS	KWW372	EU142451	EU142591	EU142731	na	na	na
P. (Japeris) defossus Bates 1883	GenBank	na	AB243460	na	na	na	na	na

APPENDIX 1. Specimen identification codes, *continued.*

Species	Origin	internal code	GenBank # 28S	GenBank # COI	GenBank #COII	GenBank # 18S	GenBank # CAD	GenBank # wg
P. (Lamenius) caudicalis (Say 1823)	Vinton Co., OH. coll. KWW	KWW313	EU142452	EU142592	EU142732	na	na	na
P. (Lenapterus) subrugosus Straneo 1955	GenBank	na	AB243484	na	na	na	na	na
P. (Lianoe) mirificus Bates 1883	GenBank	na	AB243449	na	na	na	na	na
P. (Lyrothorax) fujitai Tanaka & Ishida 1972	GenBank	na	AB243483	na	na	na	na	na
P. (Melanius) corvinus (Dejean 1828)	Vinton Co., OH. coll. KWW	KWW312	EU142453	EU142593	EU142733	na	na	na
P. (Metallophilus) interruptus (Dejean 1828)	GenBank	na	AB243487	na	na	na	na	na
P. (Micronialoe) bifoveolatus Park, Kwon & Lafer 1996	GenBank	na	AB231285	na	na	na	na	na
P. (Monoferonia) mancus (LeConte 1852)	Rabun Co., GA. coll. DRM	KWW431	EU142454	EU142594	EU142734	na	na	na
P. (Morphnosoma) melanarius Illiger 1798	GenBank	DRM357	AF398707	EU142595	EU142735	na	na	na
P. (Nialoe) brunneipennis Straneo 1955	GenBank	na	AB243501	na	na	na	na	na
P. (Oreophilus) bicolor Dejean 1831	GenBank	na	AB243490	na	na	na	na	na
P. (Orsonjohnsonus) johnsoni Ulke 1889	Multnomah Co., OR. coll. JRL	KWW271	EU142455	EU142596	EU142736	na	na	na
P. (Petrophilus) pertinax Tschitschérine 1895	GenBank	na	AB243448	na	na	na	na	na
P. (Phonias) strenuus (Panzer 1797)	Nova Scotia. coll. DRM	KWW093	EU142456	EU142597	EU142737	na	na	na
P. (Platypterus) truncatus (Dejean 1828)	GenBank	na	AB243492	na	na	na	na	na
P. (Platysma) niger (Schaller 1783)	GenBank	na	AB243470	na	na	na	na	na
P. (Pseudethira) nepalensis Straneo 1977	Nepal, Karnaii Prov. coll. MH	KWW025	EU142461	na	na	na	na	na
P. (Pseudoferonina) campbelli Bousquet 1985	Lincoln Co., OR. coll. JRL	KWW204	EU142457	EU142598	EU142738	na	na	na
P. (Pseudomaseus) luctuosus (Dejean 1828)	Tompkins Co. NY. coll. KWW	KWW022	EU142458	EU142599	EU142739	na	na	na
P. (Pterostichus) rutilans (Dejean 1828)	GenBank	na	AB243491	na	na	na	na	na
P. (Rhagadus) microcephalus (Motschulsky 1860)	GenBank	na	AB243454	na	na	na	na	na
Stomis pumicatus (Panzer 1795)	Zonnebeke, Belgium. coll. KD	DRM1156	EU142459	EU142600	EU142740	na	na	na
P.(Tapinopterus) balcanicus Ganglbauer 1891	Spain, Pirin Mtns. coll. JS	KWW396	EU142460	EU142601	EU142741	na	na	na
Pterostichus (Anilloferonia) lanei (Hatch 1935)	Benton Co., OR. coll. JRL	KWW200	EU142323	EU142462	EU142602	na	na	na
P. (A.) lanei	Marion Co., OR. coll. JRL	KWW202	EU142324	EU142463	EU142603	na	na	na
P. (A.) testaceus (Van Dyke 1926)	Hood River Co., OR. coll. JRL	KWW354	EU142326	EU142465	EU142605	na	na	na

APPENDIX 1. Specimen identification codes, *continued*.

Species	Origin	internal code	GenBank # 28S	GenBank # COI	GenBank #COII	GenBank # 18S	GenBank # CAD	GenBank # wg
P. (Hypherpes) adoxus (Say 1823)	Hocking Co., OH. coll. KWW	KWW311	EU142329	EU142468	EU142608	EU142278	EU142293	EU142308
P. (H.) algidus LeConte 1852	Thurston Co., WA. coll. DHK	KWW010	EU142330	EU142469	EU142609	EU142279	EU142294	EU142309
P. (H.) amethystinus Mannerheim 1843	Thurston Co., WA. coll. DHK	KWW008	EU142331	EU142470	EU142610	na	na	na
P. (H.) brachylobus Kavanaugh & LaBonte 2006	Lincoln Co., OR. coll. DHK	KWW009	EU142332	EU142471	EU142611	na	na	na
P. (H.) californicus (Dejean 1828)	Contra Costa Co., CA. coll. KWW	KWW207	EU142333	EU142472	EU142612	na	na	na
P. (H.) nr californicus sp-1	Mariposa Co., CA. coll. AL	KWW193	EU142335	EU142474	EU142614	na	na	na
P. (H.) nr californicus sp-2	Sonoma Co., CA. coll. KWW	KWW186	EU142334	EU142473	EU142613	na	na	na
P. (H.) canallatus Casey 1913 (?)	Los Angeles Co., CA. coll KWW	KWW334	EU142336	EU142475	EU142615	na	na	na
P. (H.) castaneus (Dejean 1828)	Jackson Co., OR coll. SEL	KWW338	EU142337	EU142476	EU142616	na	na	na
P. (H.) congestus (Ménétriés 1843)	San Diego Co., CA. coll. KWW	KWW189	EU142338	EU142477	EU142617	na	na	na
P. (H.) nr *congestus* sp-1	San Bernardino Co., CA. coll. KWW	KWW331	EU142377	EU142516	EU142656	na	na	na
P. (H.) nr *congestus* sp-2	San Bernardino Co., CA. coll. KWW	KWW332	EU142378	EU142517	EU142657	na	na	na
P. (H.) crenicollis LeConte 1873	Humboldt Co., CA. coll. KWW	KWW273	EU142340	EU142479	EU142619	na	na	na
P. (H.) crenicollis	Benton Co., OR. coll. DHK	KWW007	EU142339	EU142478	EU142618	na	na	na
P. (H.) n sp nr *crenicollis*	Humboldt Co., CA. coll. KWW	KWW283	EU142379	EU142518	EU142658	na	na	na
P. (H.) diabolus Casey 1913 (?)	Contra Costa Co., CA. coll. KWW	KWW047	EU142341	EU142480	EU142620	na	na	na
P. (H.) ecarinatus Hatch 1936	Flathead Co., MT. coll. IGW	KWW350	EU142342	EU142481	EU142621	EU142280	EU142295	EU142310
P. (H.) herculeanus Mannerheim 1843	Tuolumne Co., CA. coll. DRM	KWW337	EU142343	EU142482	EU142622	na	na	na
P. (H.) inermis Fall 1901 (?)	Riverside Co., CA. coll. KWW	KWW291	EU142344	EU142483	EU142623	na	na	na
P. (H.) inermis (?)	Los Angeles Co.,CA. coll. KWW	KWW297	EU142383	EU142522	EU142662	na	na	na
P. (H.) inermis (?)	San Luis Obispo Co., CA. coll. KWW	KWW298	EU142384	EU142523	EU142663	na	na	na
P. (H.) inermis (?)	Santa Barbara Co., CA. coll KWW	KWW296	EU142388	EU142527	EU142667	na	na	na
P. (H.) inermis (?)	Santa Barbara Co., CA. coll. KWW	KWW300	EU142385	EU142524	EU142664	na	na	na

APPENDIX 1. Specimen identification codes, *continued*.

Species	Origin	internal code	GenBank # 28S	GenBank # COI	GenBank #COII	GenBank # 18S	GenBank # CAD	GenBank # wg
P. (H.) inermis (?)	Santa Barbara Co., CA. coll KWW	KWW301	EU142386	EU142525	EU142665	na	na	na
P. (H.) isabellae LeConte 1851 (?)	Los Angeles Co., CA. coll. ASG	KWW333	EU142345	EU142484	EU142624	na	na	na
P. (H.) jacobinus Casey 1913 (?)	Los Angeles Co., CA. coll. KWW	KWW330	EU142346	EU142485	EU142625	na	na	na
P. (H.) lama (Ménétriés 1843)	Alpine Co., CA. coll. KWW	KWW001	EU142367	EU142506	EU142646	na	na	na
P. (H.) lama	Monterey Co., CA. coll. KWW	KWW002	EU142358	EU142497	EU142637	na	na	na
P. (H.) lama	El Dorado Co., CA. coll. KWW	KWW003	EU142349	EU142488	EU142628	na	na	na
P. (H.) lama	El Dorado Co., CA. coll. KWW	KWW004	EU142350	EU142489	EU142629	na	na	na
P. (H.) lama	El Dorado Co., CA. coll. KWW	KWW005	EU142351	EU142490	EU142630	na	na	na
P. (H.) lama	Monterey Co., CA. coll. SEL	KWW043	EU142356	EU142495	EU142635	na	na	na
P. (H.) lama	Sierra Co., CA. coll. KWW	KWW044	EU142363	EU142502	EU142642	EU142281	EU142296	EU142311
P. (H.) lama	Lincoln Co., OR. coll. KWW	KWW046	EU142357	EU142496	EU142636	na	na	na
P. (H.) lama	Mariposa Co., CA. coll. DSY	KWW190	EU142370	EU142509	EU142649	na	na	na
P. (H.) lama	Marin Co., CA. coll. KWW	KWW229	EU142354	EU142493	EU142633	na	na	na
P. (H.) lama	Thurston Co., WA. coll. DHK	KWW238	EU142369	EU142508	EU142648	na	na	na
P. (H.) lama	BC, Canada. coll. ASG	KWW241	EU142347	EU142486	EU142626	na	na	na
P. (H.) lama	Humboldt Co., CA. coll. KWW	KWW274	EU142352	EU142491	EU142631	na	na	na
P. (H.) lama	Santa Clara Co., CA. coll. KWW	KWW305	EU142362	EU142501	EU142641	na	na	na
P. (H.) lama	Riverside Co., CA. coll. MC	KWW315	EU142359	EU142498	EU142638	na	na	na
P. (H.) lama	Monterey Co. CA. coll. KWW	KWW316	EU142348	EU142487	EU142627	na	na	na
P. (H.) lama	Tehama Co., CA. coll. KWW	KWW320	EU142364	EU142503	EU142643	na	na	na
P. (H.) lama	Trinity Co., CA. coll. KWW	KWW321	EU142365	EU142504	EU142644	na	na	na
P. (H.) lama	Madera Co., CA. coll. KWW	KWW322	EU142353	EU142492	EU142632	na	na	na
P. (H.) lama	Santa Barbara Co., CA. coll. MC	KWW323	EU142360	EU142499	EU142639	na	na	na
P. (H.) lama	Tulare Co. CA. coll. MC	KWW343	EU142366	EU142505	EU142645	na	na	na
P. (H.) lama	Mendocino Co., CA. coll. KWW	KWW345	EU142355	EU142494	EU142634	na	na	na
P. (H.) lama	Ventura Co., CA. coll. MC	KWW367	EU142368	EU142507	EU142647	na	na	na

APPENDIX 1. Specimen identification codes, *continued*.

Species	Origin	internal code	GenBank # 28S	GenBank # COI	GenBank #COII	GenBank # 18S	GenBank # CAD	GenBank # wg
P. (H.) lama	Santa Barbara Co., CA. coll. MC	KWW416	EU142361	EU142500	EU142640	na	na	na
P. (H.) lattini Labonte 2006	Benton Co., OR. coll. JRL	KWW369	EU142371	EU142510	EU142650	na	na	na
P. (H.) menetriesii LeConte 1873	Marin Co., CA. coll. KWW	KWW210	EU142372	EU142511	EU142651	EU142282	EU142297	EU142312
P. (H.) miscellus Casey 1913 (?)	Santa Barbara Co., CA. coll KWW	KWW299	EU142387	EU142526	EU142666	na	na	na
P. (H.) morionides (Chaudoir 1868)	Madera Co., CA. coll. KWW	KWW340	EU142373	EU142512	EU142652	na	na	na
P. (H.) morionides	Plumas Co., CA. coll. KWW	KWW101	EU142374	EU142513	EU142653	EU142283	EU142298	EU142313
P. (H.) neobrunneus Lindroth 1966	Thurston Co., WA. coll. DHK	KWW336	EU142375	EU142514	EU142654	na	na	na
P. (H.) nigrocaeruleus Van Dyke 1925	Benton Co., OR. coll. JRL	KWW272	EU142376	EU142515	EU142655	EU142284	EU142299	EU142314
P. (H.) protractus LeConte 1860	WY, Teton Co., WY. coll. JRL	KWW270	EU142381	EU142520	EU142660	na	na	na
P. (H.) serripes (LeConte 1875)	Mariposa Co., CA. coll. KWW	KWW191	EU142382	EU142521	EU142661	EU142285	EU142300	EU142315
P. (H.) tarsalis LeConte 1873	El Dorado Co., CA. coll. KWW	KWW045	EU142389	EU142528	EU142668	na	na	na
P. (H.) tristis (Dejean 1828)	Tompkins Co. NY. coll. KWW	KWW023	EU142390	EU142529	EU142669	na	na	na
P. (H.) tristis	Tompkins Co. NY. coll. KWW	KWW264	EU142391	EU142530	EU142670	EU142286	EU142301	EU142316
P. (H.) tuberculofemoratus Hatch 1936	Benton Co., OR. coll. JRL	KWW269	EU142392	EU142531	EU142671	na	na	na
P. (H.) nr *ybousqueti* Berlov 1999	Trinity Co., CA. coll. KWW	KWW335	EU142380	EU142519	EU142659	na	na	na
Pterostichus (Leptoferonia) angustus (Dejean 1828)	Sonoma Co., CA. coll. KWW	KWW049	EU142399	EU142538	EU142678	na	na	na
P. (L.) angustus	Solano Co., CA. coll. KWW	KWW292	EU142398	EU142537	EU142677	na	na	na
P. (L.) angustus	San Mateo Co., CA. coll. ASG	KWW293	EU142395	EU142534	EU142674	na	na	na
P. (L.) angustus	Santa Clara Co., CA. coll. KWW	KWW304	EU142397	EU142536	EU142676	na	na	na
P. (L.) angustus	Napa Co., CA. coll. KWW	KWW327	EU142394	EU142533	EU142673	na	na	na
P. (L.) angustus	Contra Costa Co., CA. coll. KWW	KWW344	EU142393	EU142532	EU142672	na	na	na
P. (L.) angustus	Santa Barbara Co., CA. coll. KWW	KWW415	EU142396	EU142535	EU142675	na	na	na
P. (L.) beyeri Van Dyke 1925	Idaho Co., ID. coll. KWW	KWW366	EU142400	EU142539	EU142679	na	na	na
P. (L.) blodgettensis Will 2007	El Dorado Co., CA. coll. KWW	KWW368	EU142430	EU142569	EU142709	na	na	na
P. (L.) caligans Horn 1891	Mendocino Co., CA. coll. KWW	KWW265	EU142401	EU142540	EU142680	na	na	na

APPENDIX 1. Specimen identification codes, *continued*.

Species	Origin	internal code	GenBank # 28S	GenBank # COI	GenBank #COII	GenBank # 18S	GenBank # CAD	GenBank # wg
P. (L.) cochlearis Hacker 1968	Humboldt Co., CA. coll. KWW	KWW276	EU142402	EU142541	EU142681	na	na	na
P. (L.) fenyesi Csiki 1930 (ssp unclear)	Humboldt Co., CA. coll. KWW	KWW280	EU142403	EU142542	EU142682	na	na	na
P. (L.) fenyesi fenderi Hacker 1968	Humboldt Co., CA. coll. KWW	KWW279	EU142404	EU142543	EU142683	na	na	na
P. (L.) fenyesi fenyesi Csiki 1930	Humboldt Co., CA. coll. KWW	KWW282	EU142405	EU142544	EU142684	EU142287	EU142302	EU142317
P. (L.) fuchsi Schaeffer 1910	Napa Co., CA. coll. KWW	KWW262	EU142406	EU142545	EU142685	na	na	na
P. (L.) fuchsi	Sonoma Co. CA., coll. KWW	KWW284	EU142407	EU142546	EU142686	na	na	na
P. (L.) hatchi Hacker 1968	El Dorado Co., CA. coll. KWW	KWW239	EU142408	EU142547	EU142687	na	na	na
P. (L.) humilis Casey 1913	Humboldt Co., CA. coll. KWW	KWW278	EU142409	EU142548	EU142688	na	na	na
P. (L.) idahoae Csiki 1930	Flathead Co., MT. coll. IGW	KWW349	EU142410	EU142549	EU142689	na	na	na
P. (L.) idahoae	Idaho Co., ID. coll. KWW	KWW365	EU142411	EU142550	EU142690	na	na	na
P. (L.) inanis Horn 1891	Alpine Co., CA. coll. KWW	KWW048	EU142412	EU142551	EU142691	na	na	na
P. (L.) inanis	Mariposa Co., CA. coll. AL	KWW192	EU142416	EU142555	EU142695	na	na	na
P. (L.) inanis	Madera Co., CA. coll. KWW	KWW326	EU142414	EU142553	EU142693	na	na	na
P. (L.) inanis	Tehama Co., CA. coll. KWW	KWW328	EU142418	EU142557	EU142697	na	na	na
P. (L.) inanis	Fresno Co., CA. coll. KWW	KWW329	EU142413	EU142552	EU142692	na	na	na
P. (L.) inanis	Madera Co., CA. coll. KWW	KWW341	EU142415	EU142554	EU142694	na	na	na
P. (L.) inanis	Mariposa Co., CA. coll. KWW	KWW342	EU142419	EU142558	EU142698	na	na	na
P. (L.) inanis	Hood River Co., OR. coll. JRL	KWW353	EU142417	EU142556	EU142696	na	na	na
P. (L.) infernalis Hatch 1936	Lincoln Co., OR. coll. DRM	KWW261	EU142420	EU142559	EU142699	na	na	na
P. (L.) infernalis	Coos Co., OR. coll. SEL	KWW370	EU142421	EU142560	EU142700	na	na	na
P. (L.) infernalis	Douglas Co., OR. coll. KWW	KWW371	EU142422	EU142561	EU142701	na	na	na
P. (L.) infernalis	Benton Co., OR. coll. CJM	KWW414	EU142423	EU142562	EU142702	na	na	na
P. (L.) inopinus (Casey 1918)	Benton Co., OR. coll. DHK	KWW011	EU142424	EU142563	EU142703	na	na	na
P. (L.) lobatus Hacker 1968	Mendocino Co., CA. coll. KWW	KWW268	EU142425	EU142564	EU142704	na	na	na
P. (L.) lobatus	Humboldt Co., CA. coll. KWW	KWW275	EU142426	EU142565	EU142705	na	na	na
P. (L.) lobatus	Humboldt Co., CA. coll. KWW	KWW277	EU142427	EU142566	EU142706	na	na	na

APPENDIX 1. Specimen identification, *continued*.

Species	Origin	internal code	GenBank # 28S	GenBank # COI	GenBank #COII	GenBank # 18S	GenBank # CAD	GenBank # wg
P. (L.) marinensis Hacker 1968	Marin Co., CA. coll. KWW	KWW266	EU142428	EU142567	EU142707	na	na	na
P. (L.) mattolensis Hacker 1968	Mendocino Co., CA. coll. KWW	KWW267	EU142429	EU142568	EU142708	na	na	na
P. (L.) pemphredo Will 2007	El Dorado Co., CA. coll. KWW	KWW240	EU142431	EU142570	EU142710	EU142288	EU142303	EU142318
P. (L.) pumilus pumilus Casey 1913	Pierce Co., WA. coll. AES	KWW263	EU142432	EU142571	EU142711	na	na	na
P. (L.) rothi (Hatch 1951)	Benton Co., OR. coll. JRL	KWW201	EU142325	EU142464	EU142604	na	na	na
P. (L.) sphodrinus LeConte 1863	Flathead Co., MT. coll. IGW	KWW348	EU142433	EU142572	EU142712	na	na	na
P. (L.) stapedius Hacker 1968	Madera Co., CA. coll. KWW	KWW339	EU142434	EU142573	EU142713	na	na	na
P. (L.) trinitensis Hacker 1968	Humboldt Co., CA. coll. KWW	KWW281	EU142435	EU142574	EU142714	EU142289	EU142304	EU142319
P. (L.) yosemitensis Hacker 1968	Mariposa Co., CA. coll. KWW	KWW352	EU142436	EU142575	EU142715	na	na	na