Phylogenetic review of desert firefly taxonomic characters (Coleoptera: Lampyridae: *Microphotus*)

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Species are often difficult to discern when morphological attributes do not reflect taxonomic boundaries. Cladistic analysis using individuals as OTUs allows for morphological attributes to be determined as either taxonomic characters or traits. This approach is used to review 16 taxonomic attributes that define *Microphotus* species. In addition, mitochondrial cyctochrome oxidase I and luciferase DNA data are analyzed for phylogenetic signal and for the potential use for species diagnosis. Analysis of morphological attributes for 317 *Microphotus* individuals yielded 5000 trees with poor resolution. Individuals of each species where not monophyletic thus failing to reject the hypothesis that the 16 taxonomic attributes are traits, hence they are not taxonomic characters. Separate cladistic analyses of mitochondrial COI and luciferase gene data for ten and seven OTUs, respectively, each produced one parsimonious tree. Topologies of these trees were incongruent by the placement of *M. chiricahuae*, *M. pecosensis*, and *M. fragilis*. Cladistic analysis of all data for 11 OTUs produced two equally parsimonious trees which were unresolved for two nodes. All data sets supported the trees, however, the luciferase data showed the most conflict with the other data sets. The data support the synonymy of *M. decarthrus* Fall 1912 and *M. fragilis* Oliver 1912 based on lack of morphological variation and geographic and temporal proximity of collection localities. Additional data is needed to make a more robust statement of *Microphotus* phylogeny and taxonomy.

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Introduction

Wheeler and Platnick (2000) define a species based on a unique combination of characters shared among all individuals. In this definition, phylogenetic characters are attributes found in all individuals of a terminal lineage, whereas traits are attributes that are not universally distributed among comparable individuals in a terminal lineage (Nixon & Wheeler 1990). One cannot know prior to phylogenetic analysis whether supposed characters are in fact either phylogenetic characters or traits. In some studies, variation among individuals is either ignored or concatenated for an OTU which may or may not represent a species. This action results in an artificial suite of characters not necessarily observed in any real organism (Vrana &Wheeler 1992). The use of individuals as OTUs tests the phylogenetic utility of suspected characters especially those that are polymorphic (Vrana & Wheeler 1992). Via a cladistic analysis, morphological attributes that support monophyly of individuals are indeed characters and provide evidence of species limits (Wheeler & Platnick 2000). Poor resolution of individual relationships indicates that suspected characters are likely traits.

This philosophical approach is most applicable in situations where taxonomic limits of species overlap as with *Microphotus*. These species are nocturnal glowworm fireflies that occur throughout the southwestern United States and adjacent parts of Mexico. Males are recognized by the following characteristics (Green 1959, LeConte 1866): 1) large, prominent eyes touch or nearly so under the head; 2) antennae are shorter than the pronotum and composed of eight to ten antennomeres with a small, glassy, bead-like process on

the tip of the terminal segment; 3) elytra shorter than the abdomen and usually pale colored with darker tips; and 4) mouthparts reduced and barely visible between the eyes. All males possess a medial triangular or lobate process on the penultimate abdominal ventrite. Females are apterous and larva-like in appearance. Although originally described otherwise, both males and females possess paired photic organs on abdominal segment VIII. Females emit intermittent glows to attract males. Males, while capable of emitting weak glows or flickers, apparently do not use bioluminescence in sexual communication.

Few taxonomic treatments of the genus have been conducted. LeConte (1866) first described *Microphotus* and prior to 1959, eight species and one subspecies were described by LeConte (1866, 1874), Fall (1912), and Oliver (1911, 1912). Green (1959) revised the genus, adding a new name (*M. chiricahuae* Green 1959), synonymizing three names (*M. robustus* Oliver 1911, *M. rinconsis* Fall 1912 and *M. abbreviatus* Oliver 1912), and elevating the only subspecies (*M. octarthrus pecosensis* Fall 1912) to species status. Currently, seven species are recognized.

Few diagnostic morphological characters separate the species. In his revision, Green (1959) relied primarily on male genitalia and secondarily on elytral length, color and number of anntenomeres to define species limits. Relatively few specimens representing few disjunct populations were dissected or examined. Thus, illustrations of male terminalia for six of the seven species represent a bias of morphological variation (Green 1959). These drawings do not represent type specimens or type localities. Cursory observation of additional specimens suggested that taxonomic characters would not diagnosis the species.

This study examines the characters used by Green to define species limits using individuals as OTUs to assess the taxonomic utility of these characters. In addition, DNA sequences from the mitochondrial cytochrome oxidase I gene and the nuclear luciferase gene are examined for their utility in defining species limits within *Microphotus*. These genes were chosen because of their potential to reveal phylogenetic information at different taxonomic levels (Graybeal 1994). Cytochrome oxidase I (mtDNA COI) has been used extensively for beetles, especially when examining closely related species and populations (Caterino et al. 2000). The luciferase gene may be influenced by sexual selection because of its intimate association with sexual communication. Luciferase is the only enzyme that interacts with the substrate luciferin to create the bioluminescent sexual signal utilized by many firefly species. Variation in mating behavior in part is likely to arise in response to molecular changes to luciferase (Kim et al. 2004). Therefore, nucleotide variation in the luciferase gene may reflect variation in species specific mating behavior. Phylogenetic analysis of these characters should reveal distinct species boundaries represented as monophyletic groups.

Materials and Methods

Adult *Microphotus* specimens were obtained on loan from the following institutions and individuals: Department of Entomology, California Academy of Sciences, San Francisco, CA (CAS, David Kavanaugh, Roberta Brett); California State Collection of Arthropods, Plant Pest Diagnostics Branch, California Department of Food and Agriculture, Sacramento, CA (CDFA, Chuck Bellamy); Cornell University Insect Collections, Department of Entomology, Cornell University, Ithaca NY (CU, James Liebherr); Essig Museum of Entomology, University of California-Berkeley, CA (ESSIG, Cheryl Barr); J. M. Cicero Collection, Tucson, AZ (JMC); The University of Kansas Natural History Museum, Snow Entomological Museum, Lawrence, KS (KSEM, Robert Brooks); Kathrin-Stanger Hall Collection, Austin, TX (KSH); Department of Entomology, Museum of Biological Diversity, Ohio State University, Columbus OH (OSU, Peter Kovarik); Santa Barbara Museum of Natural History, Santa Barbara, CA (SBMNH, Michael Caterino). Specimens were labeled with voucher numbers and returned to the loaning institution.

Additional specimens were collected at localities throughout the southwestern United States during the summer months in 2001-2003. Adult males were collected with ultraviolet light traps and pitfall traps "baited" with light emitting diodes designed to mimic the females' advertising signal (Branham 2003). Specimens were killed in 95% ethanol and stored at -80°C.

Genitalic dissection and morphological character examination.

Green's (1959) dissections were preserved mounted on points. We observed that drying caused the

Table 1. Morphological characters used for phylogenetic analysis

1. Number of antennomeres, including scape, pedicel and individual flagellomeres (0=less than 8, 1=8, 2=9, 3=10)
2. Eyes contiguous behind mouthparts, measured ventrally from behind the mouthparts to the back of the head (0=greater than half distance, 1=less than half distance)
3. Elytral length, measured from base to apex (0=greater than 3 times the length of pronotum, 1= less than 3 times the length of pronotum)
4. Elytral color (0=pale with dark tips, 1=uniform color)
5. Elytral shape (0=explanate, 1=parallel sided)
6. Pronotal size/ shape, measured laterally at widest point and from apex to base at widest point (0=wider than long, 1=as wide as long)
7. Pronotal base (0=truncate, 1=emarginate)
8. Medial longitudinal line of pronotum (0=impressed, 1=not impressed) (Fig. 1, A)
9. Transparent spots on pronotum over eyes (0=present, 1=absent) (Fig. 1, B)
10. Circular convex area of pronotum, measured from apex of pronotum to the base of circular convex area (0= from apex to greater than 1/2 way to base, 1= from apex to less than 1/2 way to base) (Fig. 1, C)
11. Inner margins of lateral lobes of aedeagus, in dorsal view (0=converging toward apex, 1=diverging toward apex, 2= straight) (Figs 2F, 2G, 2D)
12. Median lobe of aedeagus, in dorsal view (0=shorter than lateral lobe, 1=equal to length of lateral lobe, 2=longer than lateral lobe) (Figs 2E, 2F, 2G)
13. Median lobe of aedeagus, in lateral view (0=visible above lateral lobes, 1=visible below lateral lobes, 2=visible between lateral lobes) (Figs 2E, 2B, 2D)
14. Distal dorsal curvature of lateral lobe, in lateral view (0=no curvature, 1=concave, 2= convex) (Figs 2G, 2C, 2B)
15. Lateral projections on medial lobe of aedeagus, in dorsal view (0=present, 1=absent) (Figs 2 A, 2B)
16. Width of medial lobe, in dorsal view (0=uniform, 1=wider apically) (Figs 2D, 2A)

terminalia to alter shape, particularly the tips of the lateral lobes which had a tendency to bend outward. We preserved terminalia in glycerin to prevent drying, and although there is still some bending of the lateral lobe tips, it is less extreme in most cases. When available, 10 male specimens per locality were examined. In cases where fewer than 10 individuals were available, at least half of the available male specimens were examined. Terminal abdominal segments were removed with forceps and soaked in warm 10% potassium hydroxide until genitalia could be easily exerted. Male genitalia were rinsed in 95% ethanol and stored in glycerin for examination under a stereo dissecting scope at 50X power**.** A drawing tube aided all illustrations. Illustrations of male terminalia represent the more "typical" variation for the species observed by the authors. Three hundred and seventeen individual male specimens (66 *M. octarthrus* Fall 1912, 99 *M. angustus* LeConte 1874, 6 *M. chiricahuae* Green 1959, 10 *M. decarthrus* Fall 1912, 5 *M. fragilis* Oliver 1912, 48 *M. pecosensis* Fall 1912, and 83 *M. dilatatus* LeConte 1866) were examined. Sixteen characters traditionally used in species delimitation (Green 1959, Table 1, Figs 1 & 2) were examined and scored for all individuals. A matrix of character states recorded for these specimens is available at http://hisl.tamu.edu.

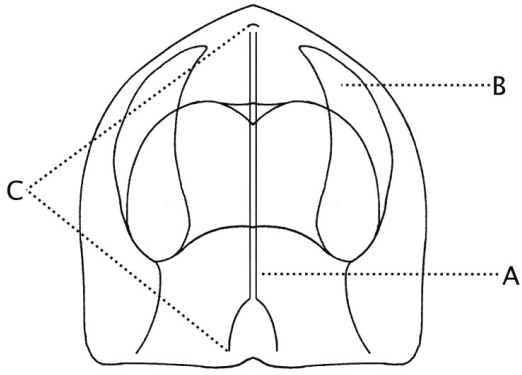

Fig. 1. Pronotum of *M. fragilis*. A = median longitudinal line, B = windows (transparent spots), C = circular convex area.

DNA extraction, amplification and sequencing

DNA was obtained from at least one thoracic leg and/ or thoracic muscle tissue from freshly collected specimens. DNA from dried specimens was obtained from the entire thorax, which was ground with a conical stainless steel rod in a 1.5 ml microfuge tube. In both cases, DNA was extracted using a DNeasy kit (Qiagen, Inc. Valencia, CA, USA) following the manufacturer's protocols. Partial mtDNA COI and luciferase sequences were separately replicated via polymerase chain reaction (PCR) with 35 μL of Nanopure water, 5 μL of 5X Promega buffer, 4 μL of 25 mM Promega $MgCl_2$, 1 μL of 40 mM dNTPs, 0.2 μL of 100 U Promega *Taq* polymerase, 2 μL of extracted DNA and 2 μL of 5 mM solution of each PCR primer. A

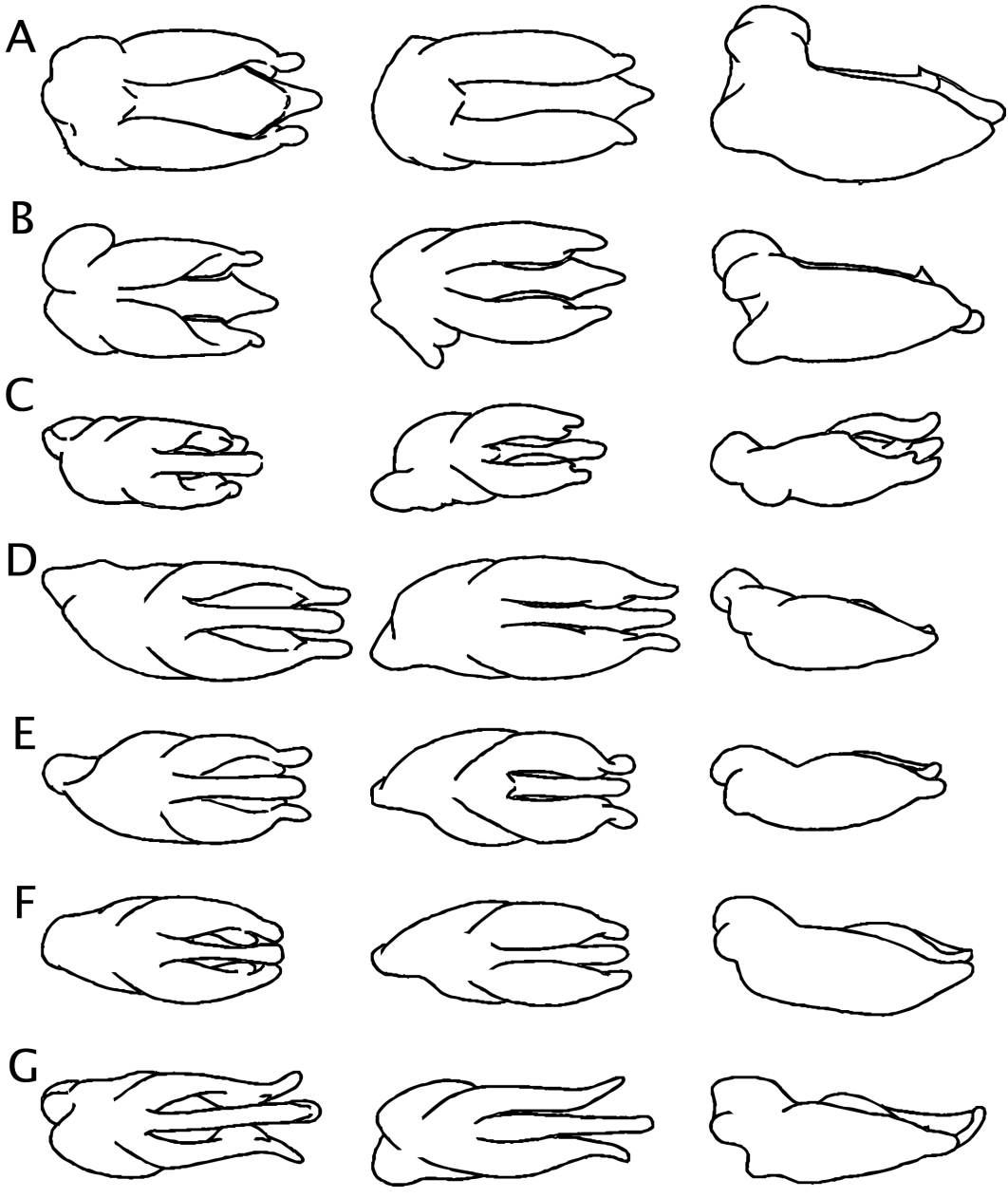

Fig. 2. Genitalia of *Microphotus*. Dorsal, ventral, and lateral view from left to right. A = *M. chiricahuae*, B = *M. pecosensis*, C = *M. octarthrus*, D = *M. dilatatus*, E = *M. decarthrus*, F = *M. fragilis*, G = *M. angustus* .

combination of PCR primers (Simon et al. 1994) was used to amplify approximately 700 base pairs: C1-2183 (alias Jerry, CAACATTTATTTGATTTTTTGG) and TL2-N-3014 (alias Pat, TCCATTGCACTAATCTGCCATATTA); C1-J-2441 (alias Dick, CCAACAGGAATTAAATTTTAGAGATTAGC) and TL2-N-3014; a new *Microphotus* specific primer, C1-J-2807 5'-

ATTCTGACTACCCAGATGTCTACTC (Mike) and TL2-N-3014 (Pat) . New PCR primers, 5'-AAGAGGTATGCACAGGTTCCAGG (Luc 1) and 5'-TAAGTGCTGTTGCTGTTTCGCG (Luc 2), were designed based on *Pyrocoelia rufa* cDNA luciferase sequence (GenBank accession number AF328553, Lee et al., 2001) and used to amplify approximately 750 base pairs of the luciferase gene. This region includes 2 introns of approximately 90 and 52 bp beginning 13 bp and 312 bp from the 3' of the sense primer (Luc 1), respectively. Mitochondrial COI DNA sequences were amplified via PCR in a Peltier thermal cycler (PTC-200) using the following conditions: an initial denaturation at 95°C for 150 s, annealing at 45°C for 30 s, and extension at 72°C for 60 s for a total of 36 cycles, followed by 72°C extension for 5 min. Luciferase sequences were amplified via PCR in a Peltier thermal cycler (PTC-200) under the following conditions: 95°C for 150 s, 55°C for 30 s, and 72°C for 60 s for a total of 36 cycles, followed by 72°C for 5 min.

All PCR products were subjected to electrophoresis in a 1X Tris borate-EDTA buffer at 100 V for 30 min in 1.5% agarose gel stained with ethidium bromide (10 mg/ ml solution) and visualized with UV light. Unincorporated dNTPs and primers were removed with either a Qiagen PCR cleanup kit or an of EXO-SAP solution (USB Corp., Cleveland, Ohio, U.S.A.). Five microliters of PCR product was added to 1 μL of EXO and 1 μL of SAP. The EXO-SAP cocktail was heated in a Peltier thermal cycler (PTC-200) at 37°C for 15 min followed by 80°C for 15 min. Cycle sequencing was performed with flourescently dyed terminator nucleotides (Big Dye kit, Applied Biosystems, Foster City CA) in a cocktail of 8 μL of PCR grade water, 2 μL of Big Dye, 3 μL of primers, and 2 μL of cleaned PCR product. Both strands of PCR product were sequenced. Cycle sequencing products were cleaned with 10% Sephadex solution and then visualized on an ABI 377 automated sequencer (Applied Biosystems).

DNA sequence analysis

The chromatographs of complementary DNA were edited into consensus sequences with Sequence Navigator™ (Gene Codes Corporation, Ann Arbor, Michigan). These sequences were submitted to Genbank (AY971782-AY971800). Alignment of individual sequences was straightforward as amino acids were conserved thus no nucleotide deletions or insertions were needed to maintain positional homology of nucleotides. The first luciferase intron exhibited some length variable among *Microphotus* species. Three, one-nucleotide; one three-nucleotide, and one nine-nucleotide indel positions were present. At the position of mis-alignment, gaps were inserted until sequences were aligned. Alternative placement of gaps was not parsimonious. Only one indel was detected in the second intron.

Phylogenetic reconstruction

A data matrix for the morphological characters was created using MacClade 4.0 (Maddison & Maddison 2000). The data matrix was analyzed in PAUP (Swofford 2002) under a maximum parsimony framework. A heuristic search of potential trees was performed with 35 replicates of random stepwise addition and branch swapping via subtree-pruning-regrafting. All other settings were default (all characters are of type 'unord', all characters have equal weight, multistate taxa interpreted as uncertainty, starting tree(s) obtained via stepwise addition, steepest descent option not in effect, branches collapsed (creating polytomies) if maximum branch length is zero, MulTrees' option in effect, topological constraints not enforced, trees are unrooted). Bootstrap values were obtained with 1000 replicates and default PAUP settings. The data matrix was also analyzed using the parsimony ratchet in NONA (Goloboff 1993). One tree was held and one character sampled for 200 iterations. All other settings were default.

An additional optimal tree search for separate and combined COI and luciferase data sets for a reduced number of OTUs was carried out in PAUP (Swofford 2002) under a maximum parsimony framework. A heuristic search of potential trees was performed with 1000 replicates of random stepwise addition, branch swapping via tree-bisection-reconnection, and default settings as described above. Molecular data were missing for *M. decarthrus*. Only 192 bp of COI were included for *M. dilatatus*. Luciferase sequences were missing for *M. dilatatus*, *M. octarthrus* 7 DR, and *M. octarthrus* NC. Bootstrap values were determined with 10000 replicates via fast heuristic search and default settings as described above. Partitioned Bremer support (PBS) was determined for the combined data sets with TreeRot v.2 (Sorenson 1999).

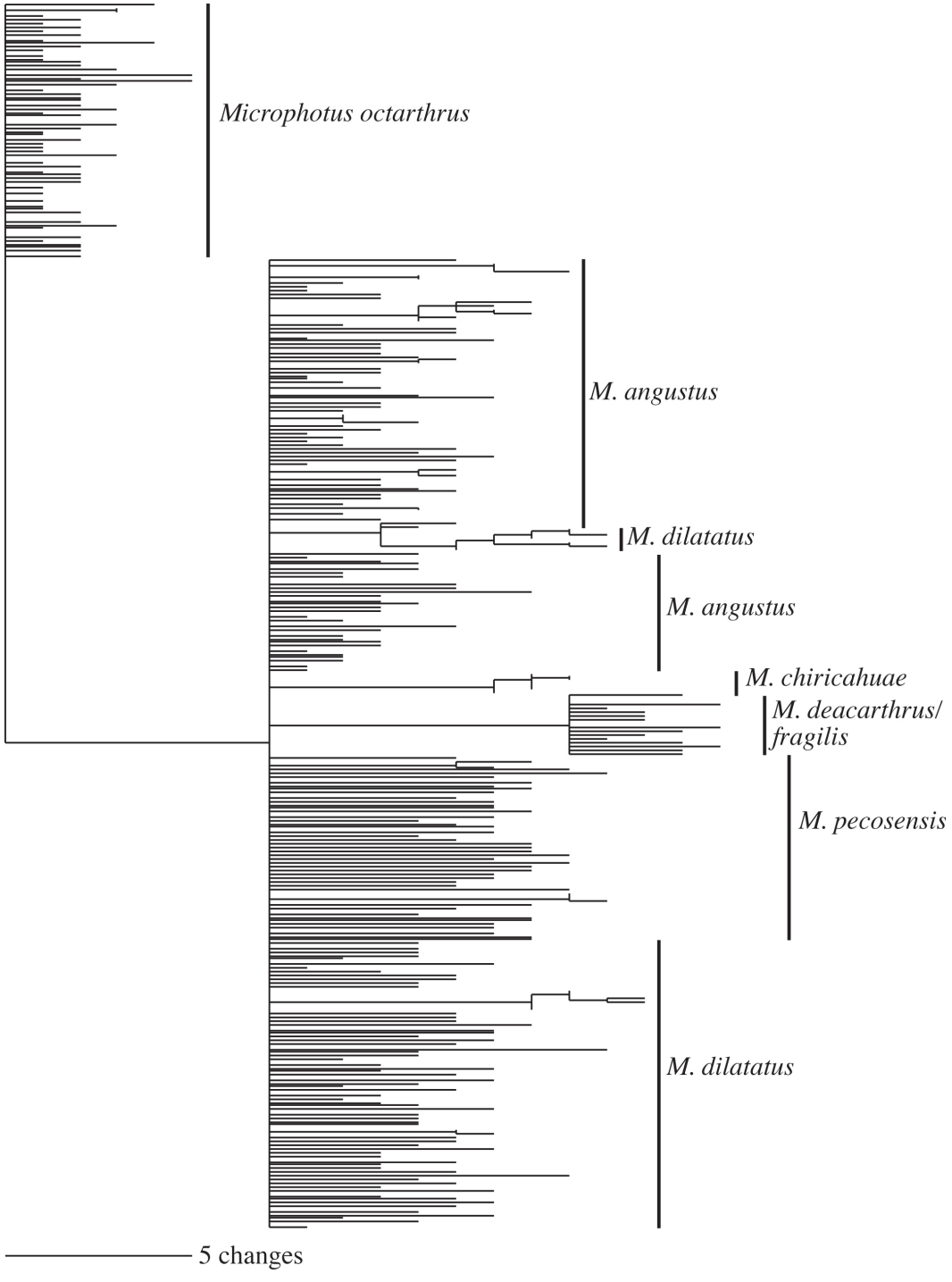

Fig. 3. An unrooted phylogram of *Microphotus* individuals based on morphological data. The tree shown is strict consensus of 5000 trees (CI = 0.445, RI = 0.6626). Branches represent individuals.

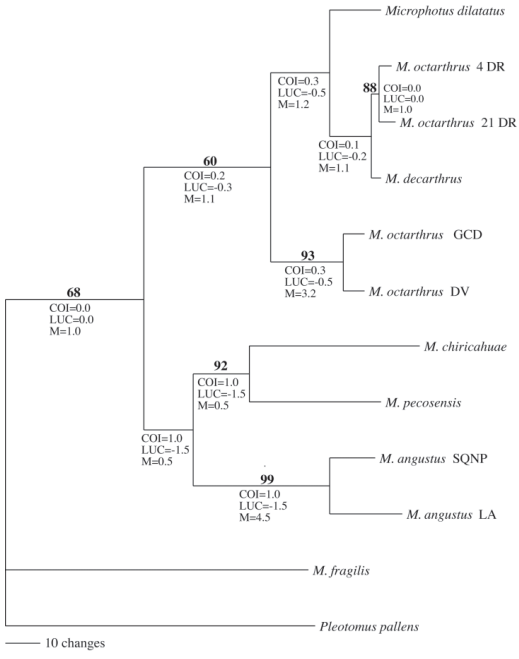

Fig. 4. Phylogram of *Microphotus* species based on combined morphological and molecular data. The tree shown is 1 of 2 equally parsimonious trees (CI = 0.7861, RI = 0.6556). Bold numbers above branches indicate bootstrap values. Bootstrap values less than 50 indicate clades unresolved in strict consensus of the equally parsimonious trees. Numbers below branches indicate partitioned Bremer support (COI = mt cytochrome oxidase I, LUC = luciferase, M = morphology). Total Bremer support for the tree was 12. Numbers following taxa indicate individuals. Letters following taxa indicate populations (GCD = New Mexico, Catron Co., Gila Nat'l. For.; NC = Arizona: Graham Co., Coronado Nat'l. For., Noon Creek; DR = Texas, Val Verde Co., Devils River State Natural Area; SQNP = California, Tulare Co., Sequoia Nat'l. Park, Potwisha; LA = California, Los Angeles Co., Los Angeles).

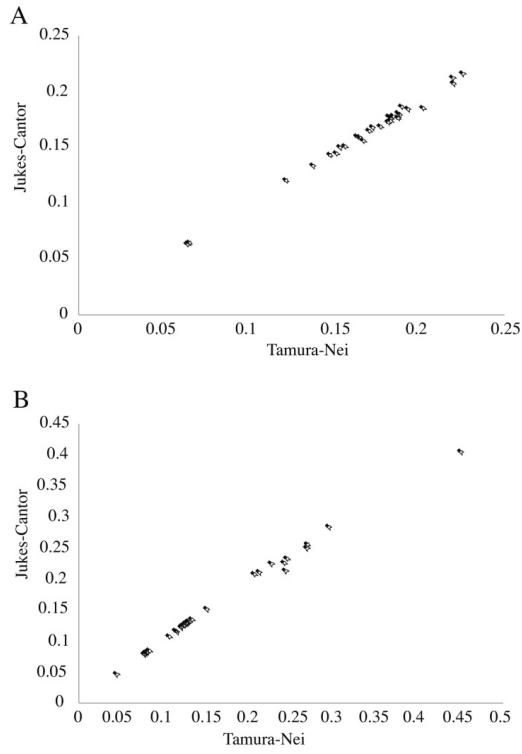

Fig. 5. Tamura-Nei vs. Jukes-Cantor pairwise genetic distances. The near correlation of distances suggests that saturation of the data is not a concern. A = mtCOI, B = luciferase

Results

Species determination remained problematic because morphological characters were polymorphic. Within *M. dilatatus*, *M. angustus*, and *M. octarthrus*, the number of antennomeres varied between individuals. *M. dilatatus, M. angustus,* and *M. octarthrus* had eight to nine, nine to ten, and seven to eight antennomeres, respectively. In addition, some specimens within these species had asymmetrical antennae, in which one antenna is one flagellomere longer than the other. Elytral characters varied among individuals. Both dark elytral tips and uniform elytral color occurred among populations of nearly all *Microphotus* species. In addition, parallel-sided and explanate elytral shapes occurred within all *Microphotus* species. Continuity of the eyes behind the mouthparts, pronotal shape and size, the shape of the pronotal base, and impression of the medial longitudinal line of the pronotum all varied among individuals of nearly all species. Furthermore, a large range of variation of the genitalic characters was found.

Cladistic analysis of 16 morphological characters for 317 individuals yielded 5000 trees of length 279. A strict consensus of these trees yielded little resolution among the named species except for *M. octarthrus*. The characters exhibited much homoplasy; total consistency index (CI) was 0.445. Monophyly of *M. octarthrus* was supported by a unique set of homoplastic characters. Absence of monophyly for the remaining species failed to reject the hypothesis that the characters do not reflect taxanomic limits (Fig. 3).

Phylogenetic reconstruction using molecular data improved resolution among the species, which likely was a consequence of an increase of parsimony informative characters. Simultaneous analysis of 470 bp of mtCOI gene, 755-bp of luciferase gene, and 16 morphological characters for 11 OTUs yielded 2 trees of length 533 (CI= 0.7861, RI=0.6556) (Fig. 4). Nucleotides were mostly comprised of AT (mean = 0.682) and the overall transition/transversion ratios for COI and luciferase were 2.16 and 1.0, respectively. Despite the observed nucleotide substitution biases, these data were not saturated as indicated by the linear relationship between JC and TN values (Fig. 5). In total 155 characters were phylogenetically informative; COI, luciferase, and morphology exhibited 87, 52 and 16 informative characters, respectively. Although bootstrap values > 60% were found for the majority of clades, support differed for each data set. For example, COI and morphological data exhibited relatively higher amounts of PBS as compared to luciferase. It is surprising that luciferase conflicted with the other data sets as observed by the negative branch support. This might have been a result of missing data from several of the OTUs however the effect of missing data on PBS is unexplored (Damgaard & Cognato 2003). Thus we analyzed the COI and luciferase data sets separately and together using the same tree search criteria as above. Cladistic analysis of mtCOI yielded one parsimonious tree of length 258 (CI=0.6611, RI=0.6164). Bootstrap values > 60% were recovered for less than half the clades; *M. chiricahuae, M. fragilis*, and *M. pecosensis* were poorly resolved (Fig. 6). Cladistic analysis of luciferase yielded one parsimonious tree of length 204 (CI=0.9020, RI=0.7436). Bootstrap values > 50 % were recovered for a majority of clades, and *M. pecosenis* and *M. chiricahuae* were better resolved (Fig. 7). The simultaneous analysis of genes yielded two equally parsimonious trees of length 470 (CI= 0.8170, RI=0.6627). Bootstrap values greater than 50% were observed for a majority of clades. Negative PBS values for basal clades indicate conflict in the data sets. Conflict is not observed in the peripheral clades which are mostly supported by the luciferase data (Fig. 8). Differences in PBS for each gene observed among simultaneous analysis trees is likely due to differences in taxon sample and tree topology.

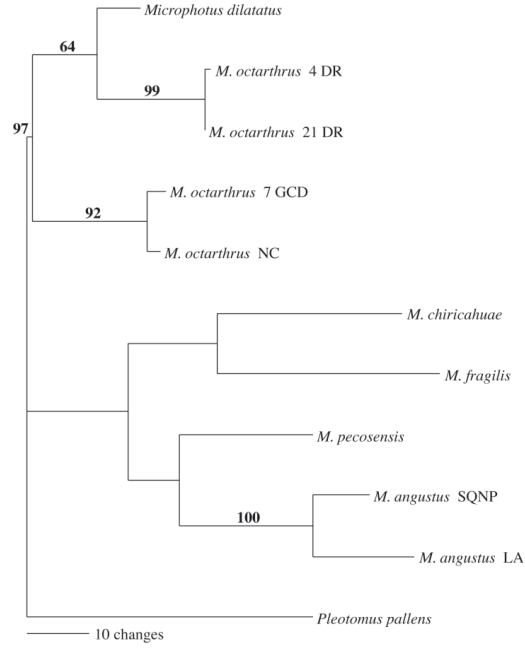

Fig. 6. Phylogram of *Microphotus* species based on mtCOI data. The tree shown is the most parsimonious tree (CI = 0.6611, RI = 0.6164). Bold numbers above branches indicate bootstrap values. Numbers following taxa indicate individuals. Letters following taxa indicate populations (GCD = New Mexico, Catron Co., Gila Nat'l. For.; NC = Arizona: Graham Co., Coronado Nat'l. For., Noon Creek; DR = Texas, Val Verde Co., Devils River State Natural Area; SQNP = California, Tulare Co., Sequoia Nat'l. Park, Potwisha).

Discussion

The low CI associated with the morphological trees indicates a high amount of homoplasy among morphological characters. This indicates that the characters used by Green to define species limits for *Microphotus* are traits rather than characters, and combinations of homplasies rather than synapomorphies diagnose species. Based on shared morphological characters and sympatric range, there is little evidence that supports *M. decarthrus* and *M. fragilis* as separate species. In addition, previous taxonomic observations suggest the synonymy of these species (Green 1959). Molecular data may diagnose these species however the lack of fresh specimens precluded further analysis. Nevertheless, given the taxonomic confusion and problematic diagnosis of these two species, *M. fragilis* is synonymized with *M. decarthrus* (syn. nov.).

Until a more thorough study including behavior, more molecular data, and individuals sampled across the range of *Microphotus* is undertaken, the remaining species should be recognized. Although morphological characters appear homoplastic, unique combinations of these characters allow for the diagnosis of currently defined species. Synonymizing or describing additional species would only further confound species limits.

Reproductive barriers are expected to exist among *Microphotus* species given that many species occur in sympatry. It has been suggested that mating behavior including female advertising posture, male approach, and coupling time are potential reproductive barriers and may be useful in species delimitation (Cicero 1981). However, sex ratios among *Microphotus* species appear to be highly disproportionate; females are encountered less frequently than males (Cicero, personal communication). This has limited the study of mating behavior only to one population in each of three species (Cicero, 1981). Other species, such as *M. angustus* and *M. chiricahuae,* are relatively restricted in distribution. Although it is expected that these species would be less isolated by distance, the patchwork of suitable habitat within California may effectively isolate populations of *M. angustus*. Morphological differences in genitalia are observed among *M. angustus* individuals of different populations. These differences may indicate cryptic species but integration of these characters is observed among *M. angustus* and *M. dilatatus* individuals.

Molecular data has resolved species limits for many insect species resulting in taxonomic revi-

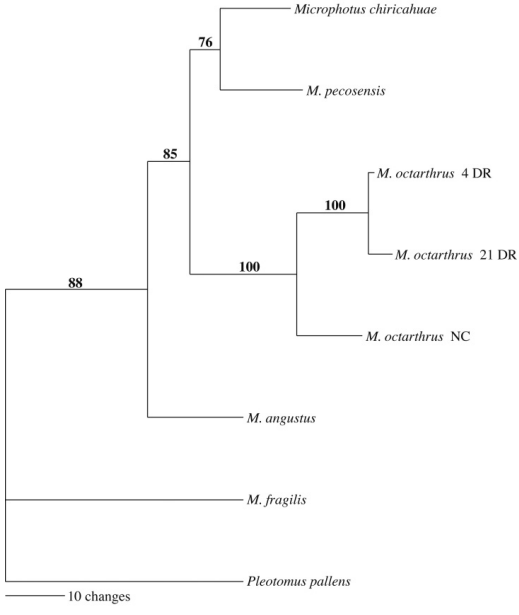

Fig. 7. Phylogram of *Microphotus* species based on luciferase data. The single most parsimonious tree obtained is shown above (CI = 0.9020, RI = 0.7436). Bold numbers above branches indicate bootstrap values. Numbers following taxa indicate individuals. Letters following taxa indicate populations NC = Arizona: Graham Co., Coronado Nat'l. For., Noon Creek).

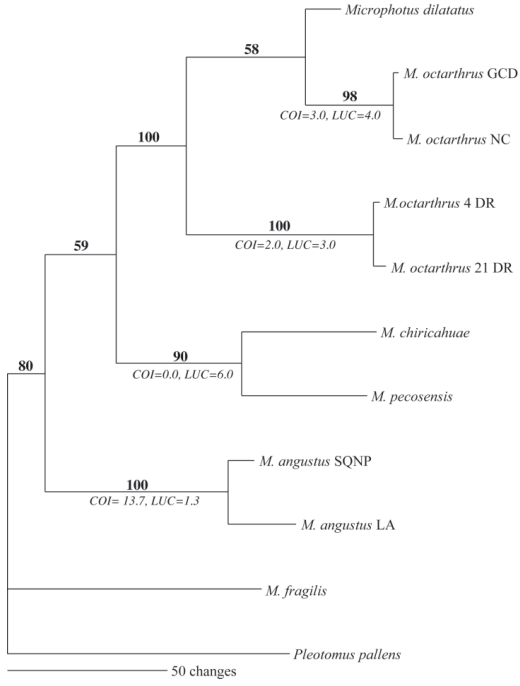

Fig. 8. Phylogram of *Microphotus* species based on combined molecular data. The tree shown is 1 of 2 most parsimonious trees (CI = 0.8170, RI = 0.6627). Bold numbers above branches indicate bootstrap values. Bootstrap values less than 50 indicate clades unresolved in strict consensus of the equally parsimonious trees. Numbers below branches indicate partitioned Bremer support; no number equals 0 PBS for both datasets (COI = mt cytochrome oxidase I, LUC = luciferase). Total Bremer support for the tree was 33. Numbers following taxa indicate individuals. Letters following taxa indicate populations (GCD = New Mexico, Catron Co., Gila Nat'l. For.; NC = Arizona: Graham Co., Coronado Nat'l. For., Noon Creek; DR = Texas, Val Verde Co., Devils River State Natural Area; SQNP = California, Tulare Co., Sequoia Nat'l. Park, Potwisha; LA = California, Los Angeles Co., Los Angeles).

sion (e.g., Morgan et al. 2000). Our data suggest that both COI and luciferase provide many characters that will help resolve a phylogeny of *Microphotus* species (Fig. 5). However, our data is limited and we do not speculate whether or not nucleotide and/or amino acid changes of the luciferase gene associate with species boundaries. As advocated for morphological taxonomic characters (Vrana & Wheeler 1992) and as demonstrated in this study, species limits and taxonomy of *Microphotus* may best be determined through the phylogenetic analysis of individuals. To implement a well-founded revision, molecular data should be generated for scores of individuals sampled through species distributions. However, this endeavor is limited by the infrequency of finding live specimens.

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References

Branham, M. A. (2003) A new technique for collecting glowworm fireflies (Coleoptera: Lampyridae). *The Coleopterists Bulletin* 57: 114-115.

Caterino, M. S., Cho, S., & Sperling, F. A. H. (2000) The current state of insect molecular systematics: a thriving Tower of Babel. *Annual Review of Entomology* 45: 1-54.

Cicero, J. M. (1981) Evolution of the glow-signal in *Microphotus* (Coleoptera: Lampyridae). MS Thesis, Department of Entomology, The University of Arizona. 27 pp.

Damgaard, J. & Cognato, A. I. (2003) Sources of character conflict in a clade of water striders (Heteroptera: Gerridae). *Cladistics* 19(6): 512-526.

Fall, H. C. (1912) New Coleoptera chiefly from the southwest. *Canadian Entomologist* 44: 40-48.

Goloboff, P. (1993) NONA, Program and Documentation. Version 2.0.

Graybeal (1994) Evaluating the phylogenetic utility of genes: a search for genes informative about deep divergences among vertebrates. *Systematic Biology* 43(2): 174-193.

Green, J. W. (1959) Revision of the species of *Microphotus,* with an emendation of the Lampyrini (Lampyridae). *The Coleopterists' Bulletin* 13: 81-96.

Kim, J. G., Kim, I., Bae, J.S., Jin, B. R., Kim, K. Y., Kim, S. E., Choi, J. Y., Choi, Y. C., Lee, K. Y., Sohn, H. D., & Noh, S. K. (2004) Genetic subdivision of the firefly, *Luciola lateralis* (Coleoptera:Lampyridae), in Korea determined by mitochondrial COI gene sequences. *Korean Journal of Genetics* 23 (3): 203-219.

LeConte, J. L. (1866) New species of North American Coleoptera, Part I. *Smithsonian Miscellaneous Collections, 2nd edition* 6 (167): 1-177.

LeConte, J. L. (1874) Descriptions of new Coleoptera chiefly from the Pacific slope of North America. *Transactions of the American Entomological Society* 5. 43-72.

Lee, K.S., Park, H. Y., Bae, J. K., Goo, T.W., Kim, I., Sohn, H. D., & Jin, R. J. (2001) Molecular cloning and expression of a cDNA encoding the luciferase from the firefly, *Pyrocoelia rufa*. *Journal of Biotechnology* 92: 9-19.

Maddison, D.R. & Maddison, W.P. (2000). MacClade 4.0. Sinauer Associates Inc. Sunderland, MA.

Morgan, M. Knisley, C. B., & Vogler, A. P. (2000) New taxonomic status of the endangered tiger beetle Cincindela limbata albissima (Coleoptera: Cincindelidae): evidence from mtDNA. *Annals of the Entomological Society of America* 93(5): 1108-1115.

Nixon, K.C. & Wheeler, Q. D. (1990) An amplification of the phylogenetic species concept. *Cladistics* 6: 211-223.

Oliver, E. (1911) Revision des Lampyrides. *Revue Scientifique du Bourbonnaise et du Centre de la France* 24. 63-85.

Oliver, E. (1912) Lampyrides faisant partie des collections du Musee de Washington. *Annals de la Society Entomologique de Belgique* 56: 24-27.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Lui, H., & Flook, P. (1994): Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87: 651-701.

Sorenson, M. D. (1999) *TreeRot, version 2*. Boston University, Boston, MA.

Swofford, D. L. (2002) *PAUP*: Phylogenetic Analysis Using Parsimony. Version 4.0b10* Sinauer Associates Inc. Sunderland, MA..

Vrana, P. & Wheeler, Q. D. (1992) Individual organisms as terminal entities laying the species problem to rest. *Cladistics* 8: 67-72.

Wheeler, Q. D. & Platnick, N. I. (2000) The phylogenetic species concept (*sensu* Wheeler and Platnick). [pp. 55-69] In Q. D. Wheeler & R. Meier [Eds.] *Species Concepts and Phylogenetic Theory. A Debate*. Columbia University Press, New York. v+230 pp.

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