SYSTEMATICS

Phylogeny of Leafhopper Subgenus *Errhomus* (*Erronus*) (Hemiptera: Cicadellidae) Based on Mitochondrial DNA Sequences

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ABSTRACT The Nearctic leafhopper genus *Errhomus* Oman (Hemiptera: Cicadellidae) exhibits high host-plant selectivity and female brachyptery, which may limit gene flow. Two subgenera, *Errhomus* (*Errhomus*) and *Errhomus* (*Erronus*), are recognized based on morphological and behavioral differences. We used mitochondrial DNA sequencing to elucidate the phylogenetic relationships among members of the subgenus *Errhomus* (*Erronus*). Portions of the NADH dehydrogenase subunit 1 (ND1) and the leucine (CUN) transfer RNA (tRNA-Leu) genes were sequenced and were analyzed with parsimony and maximum likelihood. Our results generally verified the findings of Hamilton and Zack (1999); however, both optimality criteria revealed a paraphyletic *Errhomus* (*Erronus*). *Errhomus* (*Erronus*) *calvus* does not seem to be a member of the subgenus *Errhomus* (*Erronus*) but seems to be more closely aligned with the subgenus *Errhomus* (*Errhomus*). We tested two a priori null hypotheses: 1) that the phylogeny estimated from the molecular data matched a previous phylogeny based on morphology and biogeography from Hamilton and Zack (1999) and 2) that diagnostic characters of the male genitalia (serrated aedeagal processes and triangular aedeagal shaft tip) were synapomorphies that defined major clades. We rejected these hypotheses with both Bayesian tests and parametric bootstraps.

KEY WORDS *Errhomus*, *Erronus*, leafhopper, Cicadellidae, molecular systematics

THE LEAFHOPPER GENUS *Errhomus* is an unusual and interesting group in that the females are brachypterous and are thus unable to fly. The males are macropterous, but they tend toward sedentary habits. Oman and Musgrave (1975) noted that female brachyptery could confer a selective advantage in the windy environments that many *Errhomus* species inhabit because fully winged females would be more likely to be blown away from suitable habitats. Due to female brachyptery, individuals do not readily disperse. Populations are thus characteristically disjunct, and gene flow is probably restricted. Many of the species have extremely limited distributions and may be subject to extinction events. Current distributional patterns reveal past geological, climatic, and catastrophic isolating events. These influences include river course changes, canyon formation, climatic changes, glaciation, and catastrophic flooding. For a thorough discussion of the physical events thought to have resulted in current distributional patterns, see Oman (1987) and Hamilton and Zack (1999).

Hamilton and Zack (1999) and Oman (1987) provide detailed descriptions of the morphology and biology of this taxon. Hamilton and Zack (1999) recognize 23 species and 20 subspecies in two subgenera, *Errhomus* (*Errhomus*) (hereafter referred to as the typical subgenus) and *Errhomus* (*Erronus*). Members of the genus *Errhomus* are relatively large, robust leafhoppers (Fig. 1). All known species are univoltine, and populations mature early in the growing season concurrent with their primary host plant balsamroot, *Balsamorhiza* (*Artorhiza*) spp. (Asteraceae). These leafhoppers are primarily endemic to the Pacific Northwest (Washington, Oregon, Idaho, and Montana). Other areas of endemism include the Rocky Mountains (western Wyoming and central Colorado), the Wasatch Plateau (Utah), and southern British Columbia.

The usual host plants for *Errhomus* are balsamroot and rabbitbrush, *Chrysothamnus* spp. (Asteraceae). Other host plants include lupine, *Lupinus* spp. (Fabaceae); graceful cinquefoil, *Potentilla gracilis* Dougl. (Rosaceae); western puccoon, *Lithospermum ruderale* Dougl. (Boraginaceae); and sagebrush, *Artemisia tridentata* Nutt. and *A. cana* Pursh (Asteraceae). Most members of the typical subgenus feed on balsamroot, whereas most members of the subgenus *Erronus* feed on various herbaceous plants, including balsamroot (Table 1).

There are also other characteristics that distinguish the subgenera. Females of the typical subgenus seldom migrate in search of suitable oviposition sites, and the species are usually distinguishable by genitalic characters (male and female). In the subgenus *Erronus,* females migrate annually in search of ovipo-

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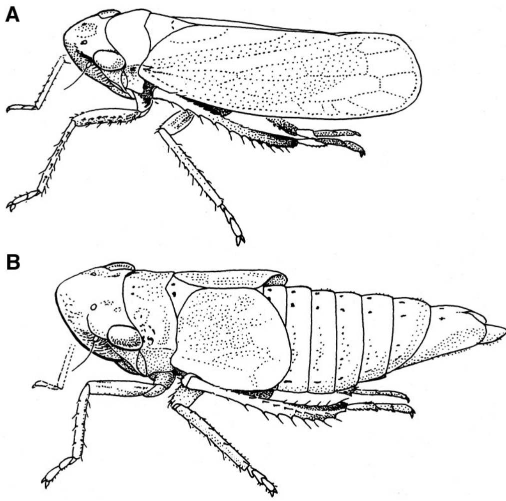

Fig. 1. Habitus of *Errhomus* (*Erronus*) *variabilis* Oman. (A) Male; (B) Female (Hamilton and Zack 1999).

sition sites (this behavior inspired the name *Erronus*, which means "the wanderer"). The species within *Erronus* show few structural differences. In fact, the females are indistinguishable and identification must rely on associations with males. Hamilton and Zack (1999) used features of the male genitalia (specifically, the structure of the aedeagal processes) as a primary means to delineate the subgenera. Their resulting phylogenetic tree (based on morphology and biogeography) is redrawn in Fig. 2A.

In the current study, we tested whether a phylogeny reconstructed using molecular sequence data was consistent with the phylogeny developed by Hamilton and Zack (1999). As null hypotheses, we used both the species-level phylogeny presented in Hamilton and Zack (1999) and the assertion that the selected characters of the male aedeagus defined major clades within *Erronus.* Because differences in tree topology may result from data indecisiveness, we used various statistical tests to determine whether trees obtained from analysis of sequence data were in significant conflict with previous morphology-based hypotheses (Sullivan et al. 2000).

Mitochondrial DNA (mtDNA) sequence data were used in this study to investigate relationships within the subgenus *Erronus.* Mitochondrial genes are a popular choice in molecular systematic studies because they are relatively easy to isolate and analyze, clonally inherited, single copy, nonrecombining, and abundant (Simon et al. 1994, Hillis et al. 1996). Because animal mtDNA evolves rapidly (Hillis et al. 1996, Avise 2000), it is useful for comparisons of closely related taxa (Simon et al. 1994). For recent divergences, mitochondrial genes are more efficient at tracking short internodes than nuclear genes (Caterino et al. 2000) because they typically have faster mutation rates.

In the current study, we examined sequence variation of a portion of the mitochondrial gene NADH dehydrogenase subunit 1 (ND1) and leucine (CUN) transfer RNA (tRNA-Leu) genes to explore phylogenetic relationships primarily within the subgenus *Erronus.* Mitochondrial tRNAs evolve at a higher rate than nuclear tRNAs (Simon et al. 1994). Furthermore, among the mitochondrial protein coding genes, the NADH dehydrogenase subunit genes are rapidly evolving at the amino acid level (Simon et al. 1994). So, both genes used in this study have high rates of evolution, which is desirable considering that the taxa under scrutiny are of relatively recent origin (Oman 1987, Hamilton and Zack 1999). Mitochondrial DNA regions are among the most commonly sequenced ones in insects (Caterino et al. 2000). Despite this fact, ND1 has not been widely used as a marker (Caterino et al. 2000). It was, however, found to be informative for reconstructing species-level relationships among leafhoppers (Dietrich et al. 1997, 1998) within the genera *Flexamia* and *Dalbulus.*

Table 1. Collection information for samples used in genetic analysis

Genus	Species	Location	GPS information	Date	Host plant
Errhomus	*montanus** (Baker)	Teton Co., WY	43° 29.831′ N, 110° 57.292′ W 2,577 m	7/14/01	Mixed forbs (not balsamroot)
	*camensis** Hamilton & Zack	Missoula Co., MT	46° 53.087′ N, 113° 33.426′ W 1,153 m	5/21/01	Balsamroot
	*rivalis** Hamilton & Zack	Missoula Co., MT	46° 49.597′ N, 114° 05.270′ W 997 m	5/21/01	Lupine
	*bracatus** Hamilton & Zack	Ravalli Co., MT	45° 55.782′ N, 114° 07.051′ W 1,239 m	5/22/01	Balsamroot
	*naomi** Hamilton & Zack	Sevier Co., UT	38° 36.084′ N, 111° 37.244′ W 2,531 m	6/24/01	Sagebrush
	*solus** Oman	Missoula Co, MT	46° 55.411′ N, 113° 26.424′ W 1, 182 m	5/21/01	Sagebrush, lupine, and cinquefoil
	*affinis** Oman	Wallowa Co., OR	45° 56.150′ N, 117° 26.909′ W 657 m	5/16/01	Balsamroot, lupine, and cinquefoil
	*calvus** Oman	Ferry Co., WA	48° 10.433′ N, 118° 43.320′ W 580 m	5/14/01	Balsamroot and mixed annuals
	*pallidus** Oman	Wallowa Co., OR	45° 25′ N, 117° 17′ W	6:7:01	Cinquefoil
	*serratus** Oman	Baker Co., OR	44° 52′ N, 117° 06′ W	5/19/01	Balsamroot
	*variabilis** Oman	Idaho Co., ID	45° 54.188′ N, 116° 10.410′ W 1,059 m	5/19/01	Balsamroot
	ochoco Oman	Wheeler Co., OR	44° 32.965′ N, 120° 19.948′ W 1,030 m	4/29/01	Balsamroot
	similis Oman	Wheeler Co., OR	44° 48.578′ N, 120° 01.248′ W 559 m	4/29/01	Balsamroot
Carsonus	sp.	Latah Co., ID	46° 45.56′ N, 116° 29.29′ W	7:6:03	Ninebark

From left to right the genus, species (with members of the subgenus *Erronus* marked with an asterisk [*]), location, global positioning system (GPS) coordinates and elevation, date collected, and host plant form which the sample was collected.

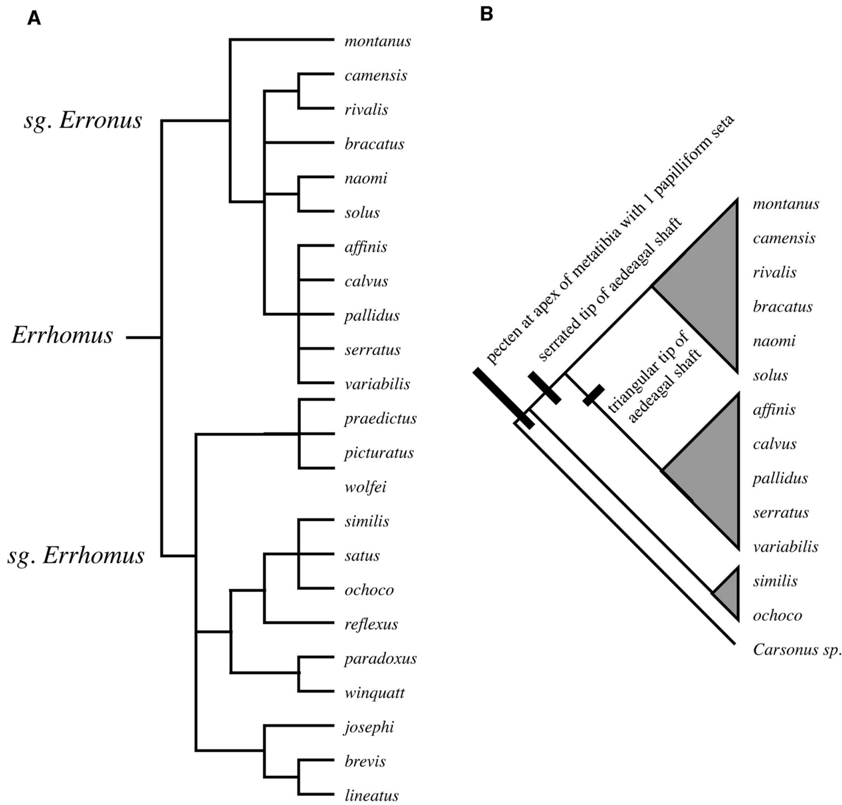

Fig. 2. (A) Phylogeny of the leafhopper genus *Errhomus* based on morphology and biogeography (Hamilton and Zack 1999); (B) Constraint tree for male genitalia test, with diagnostic synapomorphies shown on branches. Relationships of species within clades represented by the shaded triangles were treated as soft polytomies.

Materials and Methods

The subgenus *Erronus* was the focus of the current study (Fig. 2A). The ingroup was composed of the 11 recognized species in this subgenus [*E. montanus* (Baker), *E. camensis* Hamilton & Zack, *E. rivalis* Hamilton & Zack, *E. bracatus* Hamilton & Zack, *E. naomi* Hamilton & Zack, *E. solus* Oman, *E. affinis* Oman, *E. calvus* Oman, *E. pallidus* Oman, *E. serratus* Oman, and *E. variabilis* Oman] plus two species (*E. similis* Oman and *E. ochoco* Oman) from the typical subgenus. *Carsonus* sp. was included as the outgroup. Both genera, *Errhomus* Oman and *Carsonus* Oman, were placed in the subtribe Bathysmatophorina by Oman (1987). Hamilton and Zack (1999) raised Bathysmatophorina to tribal status. This newly established tribe, Bathysmatophorini, still includes both *Errhomus* and *Carsonus*.

Sample Collection and Preservation. *Errhomus* is univoltine, and its life cycle is in synchronization with that of its host (primarily balsamroot; Table 1). The males emerge early during the host plant bloom with the females emerging slightly later. After mating, the males soon die, and only the females remain. The ratio of males to females that were collected depended on when we arrived at a site. We primarily collected males if we arrived when balsamroot was just beginning to bloom, and females if we arrived after blooming. Although balsamroot typically blooms in April, this can be affected by elevation and seasonal fluctuations in climatic conditions. Samples of each species in the genus *Errhomus* were collected by hand or sweep net. Samples were taken from sites recorded by Oman (1987) and Hamilton and Zack (1999) (and field notes) in Washington, Oregon, Idaho, Montana, Wyoming, and Utah. Species identifications were based on collecting location (Table 1) and verified using the key presented in Hamilton and Zack (1999).

Errhomus was typically found in association with its primary host plant balsamroot. Some sites were teeming with *Errhomus,* and large numbers of specimens (in some cases, hundreds) could be taken by just sweeping the net through the balsamroot and adjacent vegetation. Other sites, however, required a more exhaustive search due to extremely localized populations of *Errhomus,* even within a vast stand of balsamroot. Sometimes, collecting was difficult not because of unequal leafhopper distribution but because of low overall densities. Often, finding specimens required sifting through the duff at the base of the plants and/or aspirating leafhoppers off of the leaves. After collection, the specimens were kept alive on ice. Upon return to the laboratory, specimens were stored at

−80°C. Both pinned and frozen voucher material from each collecting locality was deposited in the M.T. James Entomological Collection (JEC) at Washington State University.

Nucleic Acid Extraction and Polymerase Chain Reaction (PCR) Amplification. Abdomens were removed from all specimens before extraction and were retained as vouchers in the JEC. Total nucleic acids were isolated from the head and thorax of each leafhopper following the protocol described in Sheppard et al. (1992). PCR was used to amplify the mtDNA fragment. Preliminary sequences were generated using the primer pair ND1 + 3 and ND1-1, as published by Dietrich et al. (1998). Although amplification using this primer pair was successful, the resulting reverse sequences were not readable. To obtain "clean" reverse sequences against which the forward sequences could be confirmed, we designed an *Errhomus*-specific reverse primer [5′-cga aag gac caa taa gc(ac) cta at-3′], from a conserved region identified in the initial forward sequences of eight species. PCR using primers ND1 + 3 and ND1-1a amplified a fragment of ≈700 bases, encompassing the 3′ end of the 16S rDNA gene (not included in the aligned sequences), the entire tRNA-Leu gene, and the 5′ end of the ND1 gene. The amplifications were carried out in a total volume of 25 μl.

Sequencing and Sequence Alignment. The PCR product was purified with either Ultrafree-MC polyethersulfone filter units or Microcon-PCR filter units (Millipore Corporation, Billerica, MA) before use in the sequencing reaction. Sequencing reactions were performed with the BigDye Terminator version 3.0 cycle sequencing ready reaction mix from Applied Biosystems Inc. (Foster City, CA) and 50–100 ng of purified PCR product. The sequencing reaction products were purified using Performa DTR gel filtration cartridges from Edge Biosystems (Gaithersburg, MD). The purified reactions were sequenced on an Applied Biosystems 3100 genetic analyzer. Sequences were deposited in GenBank under accession numbers AY372369–AY372388.

The nucleotide sequences (containing 600 bases) were aligned using CLUSTAL X (Thompson et al. 1997) and then manually edited. To check for reading frame errors and termination codons, the nucleotide sequences were translated to functional peptide sequences in Se-Al (Rambaut 1996). Finally, the sequences were aligned manually in Se-Al. *E. serratus* presented three sites (position numbers 314, 378, and 379) for which base calling was problematic. To avoid potential errors, these uncertain base positions were treated as missing data. In addition, *E. bracatus, E. calvus, E. ochoco,* and *E. similis* each possessed a gap at position number 45, which was treated as missing.

Phylogenetic Analyses. Whenever possible or informative, two exemplars were included for each species (from the same collecting locality). *E. serratus, E. similis, E. montanus, E. bracatus, E. naomi, E. solus, E. calvus,* and *Carsonus* sp. were represented by one exemplar.

Phlylogenetic analyses were conducted under both parsimony and likelihood optimality criteria by using PAUP* version 4.0b (Swofford 2002). Parsimony searches (branch-and-bound with furthest addition sequence) were conducted using equal weights. The outgroup (*Carsonus* sp.) was used to root the tree. Nodal support was estimated by bootstrap analysis (Felsenstein 1985) with 1000 replicates.

For the maximum likelihood analysis, we selected an appropriate model of sequence evolution following Sullivan et al. (1997). A maximum parsimony tree was used as an estimate of the phylogeny, and log-likelihood score for each of 16 models of sequence evolution were computed. Once model parameters were optimized, we used a χ^2 approximation of the null distribution (Yang et al. 1995) to select the simplest model of sequence evolution that was not significantly different from the full model using likelihood ratio tests. To generate the ML tree, a heuristic search of the data were performed in Paup* by using TBR branch swapping and random addition sequences. The ML tree also was rooted with *Carsonus* sp., and 200 bootstrap replicates were used to estimate nodal support.

Hypothesis Testing. Two statistical approaches were used to test the a priori hypotheses. The first approach used parametric bootstrapping (Goldman 1993, Huelsenbeck et al. 1996, Sullivan et al. 2000), a method that allowed us to assess whether the optimal phylogeny is significantly better than the phylogeny predicted by either of the null hypotheses. We implemented the parametric bootstrap using maximum parsimony and maximum likelihood as optimality criteria, but we describe the process below using terminology appropriate to maximum parsimony. First, we searched the data for the most parsimonious phylogeny and noted the tree length (TL). We then designed a topological constraint that forced the operational taxonomic units to match the predictions of each null hypothesis (Fig. 2) and searched for the most parsimonious phylogeny consistent with that constraint. Using the model of sequence evolution selected above, we optimized the branch lengths on this constrained tree, and used Seq-Gen (Rambault and Grassly 1997) to simulate 1000 data sets on the constrained topology. Paup* was then used to search each simulated data set for the optimal tree and the optimal tree constrained to meet the predictions of the hypothesis. The null distribution was formed by the difference in tree length ($TL_{constrained} - TL_{unconstrained}$) for all replicates. Significance was assessed by comparing the same difference in the actual data ($TL_{constrained} - TL_{unconstrained}$) to the null distribution. The procedure was identical under maximum likelihood, except that we simulated and analyzed 100 replicates.

We also used a Bayesian approach to hypothesis testing. We used MrBayes version 3.0 (Huelsenbeck and Ronquist 2001), which implements a Markov Chain Monte Carlo approach to approximate the true posterior distribution of the topology given the data. We ran the chain for 5.1 million generations with the model of sequence evolution selected above, and, af-

ter plotting the log-likelihood scores, discarded the first 100,000 generations as the burn-in time of the chain. We sampled trees every 1000 generations, giving us a set of 5,000 trees with high log-likelihoods that we used to evaluate the posterior probability of each a priori hypothesis. We filtered this set of trees by using the phylogeny predicted by each null hypothesis (Fig. 2) and computed the proportion of trees consistent with each null hypothesis.

Results

Sequence Alignment and Variability. The final aligned sequences yielded 600 characters, including nucleotides and gaps from 20 individuals. This represents 60 bp of the tRNA-Leu gene, a 10-bp noncoding region, and 530 bp of the ND1 gene. This region corresponds to positions 12,129–12,750 of the *Drosophila yakuba* Burla sequence (Clary and Wolstenholme 1985; GenBank accession number NC_001322).

Model Selection and Genetic Distance. We selected a submodel of the GTR + I + Γ model of sequence evolution that had three rate classes ($rAC = rAT = rCG = rGT$, rAG, rCT), the proportion of invariable sites (pinv = 0.45), base frequencies (127 A = 0.245, 127 C = 0.09, 127 G = 0.157, 127 T = 0.508), and rate heterogeneity according to the gamma distribution with a shape ($\alpha = 1.34$).

Within *Erronus*, uncorrected sequence divergence ranged up to 9.0% and within the typical subgenus uncorrected divergence ranged up to 1.5%. The distance between *Erronus* and the typical subgenus was 17.4–20.2%. Distance from *Carsonus* to *Erronus* was 23.0–25.0%, and the distance between *Carsonus* and the typical subgenus was 23.8–24.9%. However, *E. calvus* did not conform to this pattern. Between *E. calvus* and the subgenus *Erronus,* to which it was previously assigned (Hamilton and Zack 1999), there was 17.9–19.9% sequence divergence. Between *E. calvus* and the typical subgenus, there was 1.5–2.1% divergence. Based on sequence divergence, *E. calvus* seems more closely related to the typical subgenus.

Phylogenetic Analyses. Of 600 bases, a total of 242 sites were variable and 158 sites were parsimony-informative. Unweighted parsimony analysis generated eight most-parsimonious trees with a TL = 386. The topologies differed only in the relationships among the exemplars of *E. pallidus* and *E. variabilis.* Maximum likelihood analysis yielded the tree ($-\ln L = 2445.23777$) presented in Fig. 3. The maximum likelihood tree is similar to the parsimony trees (not shown) in that two clearly defined clades were formed, each supported by a 100% bootstrap value. These two groups correspond to the subgenera *Erronus* and *Errhomus* as defined by Hamilton and Zack (1999), except for the placement of *E. calvus.* Thus, the major discrepancy between the phylogeny estimate from the mitochondrial DNA and the traditional classification (based on morphological data) involves *E. calvus.*

Hypothesis Testing. The results from the parametric bootstrap and the Bayesian hypothesis testing were consistent in that each rejected both of the a priori hypotheses. For the parametric bootstrap, the phylogeny constrained to match the phylogeny in Hamilton and Zack (1999) has a tree length of 493 steps, an increase of 107 steps over the most parsimonious tree (Fig. 4). The phylogeny constrained to match the male genitalia had a tree length of 486 steps, an increase of 100 steps over the most parsimonious tree. Both of these values were significantly greater than could be attributed to chance ($p_{HZ} < 0.001$; $p_{MG} < 0.001$). Results under maximum likelihood were similar; the phylogeny constrained to match the prediction of the male genitalia had a $-\ln L = 2658.22548$ ($\delta = 212.98777$), the phylogeny constrained to match the prediction of Hamilton and Zack (1999) had a $-\ln L = 2674.87912$ ($\delta = 229.6414$), and both were significantly greater than could be expected by chance ($p_{HZ} < 0.01$; $p_{MG} < 0.01$). The result of the Bayesian test was also similar, in that none of the trees in the posterior distribution were consistent with the predicted topology of either Hamilton and Zack (1999) or the male genitalia ($p_{HZ} < 0.0002$; $p_{MG} < 0.0002$).

Discussion

The level of variation observed in the mtDNA provided a high level of phylogenetic resolution. Our analysis yielded two distinct clades, agreeing with the morphological and biogeographic phylogeny of Hamilton and Zack (1999). Furthermore, within the subgenera, the relationships based on molecular data were generally consistent with Hamilton and Zack (1999).

However, in our phylogeny estimate, *E. calvus* is clearly a member of the typical subgenus, which represents the major source of conflict with the morphological data. There was a low percentage of divergence between *E. calvus* and members of the typical subgenus, and the placement of *E. calvus* was responsible for the rejection of our null hypotheses. These results strongly support the placement of *E. calvus* with members of the typical subgenus, represented here by *E. similis* and *E. ochoco.* Our phylogenetic analyses assume that selective forces are not acting in any region of the mitochondrial genome. It is possible that the phylogenetic placement of *E. calvus* in the subgenus *Errhomus* results from selective pressures common to these taxa. Because of the significant incongruity between this placement and that of Hamilton and Zack (1999), we resequenced the sample and made every effort to ensure that this was not a mistake in labeling or specimen handling.

Because of the strength of biogeographic relationships and morphological characters used by Hamilton and Zack (1999), it is difficult to explain why our study places *E. calvus* outside of *Erronus.* Perhaps the genitalic characters used by Hamilton and Zack (1999) may not accurately reflect the species relationships within the genus *Errhomus* due to convergent evolution or some other phenomenon. If *E. calvus* belongs

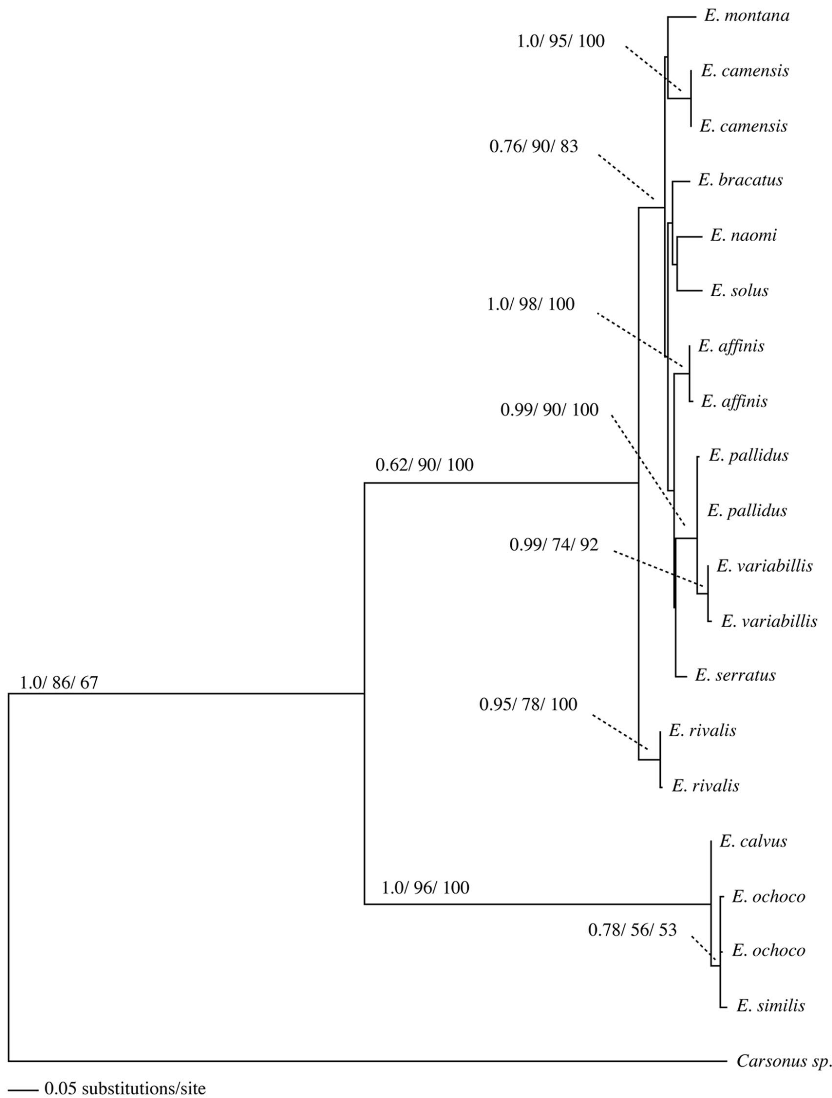

Fig. 3. Maximum likelihood phylogeny (substitution model GTR + I + Γ, −lnL score = 2445.23777). Bayesian posterior probability/ML bootstrap/parsimony bootstrap above branches. Tree rooted by *Carsonus* sp.

to the typical subgenus, then the characters defining the subgenera (Hamilton and Zack 1999) require reevaluation. For example, if the characters selected were the products of convergent evolution, then true relationships may have been clouded.

Estimates of an arthropod mtDNA clock, based on pairwise sequence divergence per million years, range from 2.0% (DeSalle et al. 1987) to 2.3% (Brower 1994). Hamilton and Zack (1999) estimated that the genus *Errhomus* is of early Miocene or pre-Miocene age (Miocene Epoch 24–5 mya). They further stated that the subgenus *Erronus* "appears to represent the sister-group of the typical subgenus, so its species are probably at least as ancient as typical *Errhomus*." According to our data, the subgenera are 17.4–20.2% divergent, indicating a mid-to-late Miocene split (7.6–10.1 mya), assuming rates similar to those proposed by Brower (1994) and DeSalle et al. (1987), which is generally consistent with Hamilton and Zack (1999).

Because the current study represents the first attempt at a molecular analysis of this genus, more molecular data from different populations and subspecies need to be gathered before a revision of the existing taxonomic classification (Hamilton and Zack 1999) may be required. First and foremost, the remaining members of the typical subgenus should be sequenced to have a complete molecular phylogeny of the genus *Errhomus.* Additional genes, including nuclear markers, would add strength to this phylogenetic analysis. A complete set of morphological data should be included together with molecular data in a total evidence analysis. Last, the clade containing

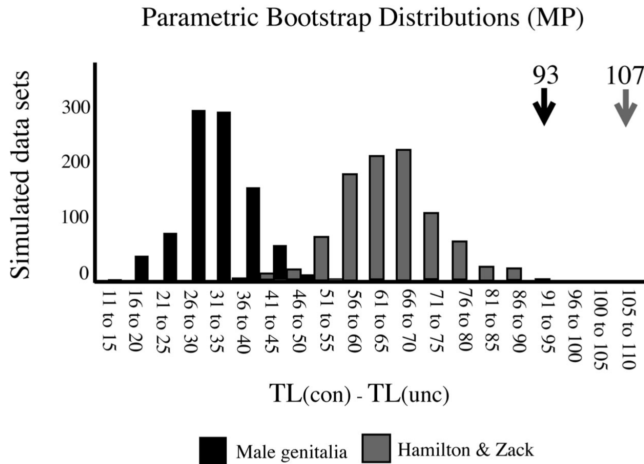

Fig. 4. Distribution of differences between unconstrained TL and TL constrained to match the morphological and biogeographic phylogeny (gray) of Hamilton and Zack (1999) and male genitalic characters (black) used by Hamilton and Zack (1999). Test statistics for parametric bootstrap are shown with arrows. Each null hypothesis was easily rejected with both parsimony (above) and ML (not shown) parametric bootstraps.

E. calvus, *E. ochoco*, and *E. similis* merits phylogenetic investigation because *E. calvus* is genetically closest to *E. similis* and morphologically similar to other *Erronus*.

Further investigation of the genus *Errhomus* is recommended not just because of its applicability to many different types of study, but because many populations are at risk of extinction. Some species, such as *E. ochoco* and *E. rivalis*, are known from only single localities. A better understanding of species relationships would allow for a more accurate risk assessment. Additionally, our study treated a limited number of taxa collected from a very limited number of geographic locations. Significantly more taxa and collections from a much greater number of locations would be required to test the biogeographic explanations for taxon formation that were proposed by Oman (1987) and Hamilton and Zack (1999).

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