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Research Article

Phylogenetic Analysis of the North American Beetle Genus *Trichiotinus* (Coleoptera: Scarabaeidae: Trichiinae)

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A hypothesized evolutionary history of the North American endemic trichiine scarab genus *Trichiotinus* is presented including all eight species and three outgroup taxa. Data from nineteen morphological traits and CO1 and 28S gene sequences were used to construct phylogenies using both parsimony and Bayesian algorithms. All results show that *Trichiotinus* is monophyletic. The best supported topology shows that the basal species *T. lunulatus* is sister to the remaining taxa that form two clades, with four and three species each. The distribution of one lineage is relatively northern while the other is generally more southern. The ancestral *Trichiotinus* lineage arose from 23.8–14.9 mya, and east-west geographic partitioning of ancestral populations likely resulted in cladogenesis and new species creation, beginning as early as 10.6–6.2 mya and as recently as 1.2–0.7 mya. Morphological character evolution is also briefly discussed. The limited distribution of *T. rufobrunneus* in Florida and *T. viridans* in the Midwest mainly due to urban development and widespread agriculture makes these two species of conservation concern.

1. Introduction

The genus *Trichiotinus* Casey, 1915, comprises a group of eight species that are commonly known as the hairy flower beetles due to their namesake trait of being covered in setae. The genus is placed in the tribe Trichiini Fleming, 1821, and the subfamily Cetoniinae Leach, 1815 [1]. The Trichiini currently includes 43 genera that are found nearly worldwide except for Australia and Madagascar [2, 3] with thirteen of these taxa located in the New World. In the Nearctic realm including Central America, nine genera exist including *Apeltastes* Howden, 1968, *Archedinus* Morón and Krikken, 1990, *Dialithus* Parry, 1842, *Giesbertiolus* Howden, 1988, *Gnorimella* Casey, 1915, *Iridisoma* Delgado-Castillo and Morón, 1991, *Paragnorimus* Becker, 1910, *Trigonopeltastes* Burmeister, 1840, and *Trichiotinus* Casey, 1915 [4, 5].

Trichiotinus is widespread in North America (Figure 1) with species distributed from Florida to Texas to southern Canada including Nova Scotia in the east to and as far north as the Northwest Territories in the west [3, 6]. While several species are widespread, others are relatively restricted including one species found mainly in Texas, another endemic to part of central Florida, and a third found in a small portion of the Midwest through to southern Ontario [7]. Adults are beautifully patterned and colored yet are thought to be harmful to flowers because they eat pollen and petals [8]. But due to their hirsute bodies, they certainly may act as successful pollinators [6]. Larvae are known to feed upon various types of dead hardwood.

The most obvious morphological characters uniting the species include the body dorsally and ventrally setose, the elytral margin bowed downward below and behind the humeral angle, the elytra with two raised intervals, and the presence of two transverse cretaceous (chalky white) bands in most but not all species [3]. Among taxa, there appear to be few morphological variations that can help hypothesize evolutionary history within the genus. A phylogenetic analysis was done using as many morphological characters that could be discovered, as well as molecular data from two genes. Furthermore, we briefly explore character evolution

Table 1: List of taxa used in the analysis, their collection origin, and accession numbers for DNA sequences on GenBank.

Taxon	Locality	CO1	28S
Trichiotinus affinis (Gory & Per.)	Kentucky, Warren Co.	KX132104	KX151980
Trichiotinus affinis (Gory & Per.)	Quebec, Gatineau	KX132105	KX151981
Trichiotinus assimilis (Kirby)	Ontario, Muskoka	KX132107	KX151983
Trichiotinus bibens (Fab.)	Kentucky, Warren Co.	KX132111	KX151987
Trichiotinus lunulatus (Fab.)	Texas, College Station	KX132112	KX151988
Trichiotinus piger (Fab.)	Illinois, Sumner	KX132108	KX151984
Trichiotinus rufobrunneus (Casey)	Florida, Tallahassee	KX132109	KX151985
Trichiotinus texanus (Horn)	Texas, College Station	KX132110	KX151986
Trichiotinus viridians (Kirby)	Illinois, Sumner	KX132106	KX151982
Outgroups			
Gnorimella maculosa (Knoch)	Quebec, Gatineau	KX132113	KX151989
Trigonopeltastes delta (Forster)	Kentucky, Warren Co.	KX132114	KX151990
Osmoderma eremicola (*Knoch*)	Tennessee, Portland	KX132115	KX151991

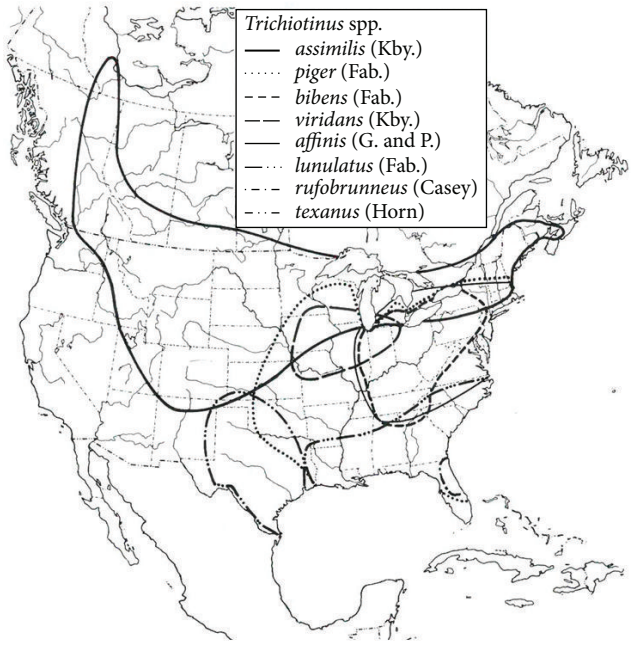

Figure 1: Distributions of the eight species of *Trichiotinus* from [3] (used with permission).

and the biogeography of the group and hypothesize the dates of cladogenesis based on CO1 divergence.

2. Materials and Methods

2.1. Sampling. Specimens were collected in various localities within the USA and Canada (Table 1). The molecular analysis used fragments of DNA sequence data from CO1 and 28S genes (ca 800 bp fragment of mtDNA cytochrome oxidase subunit 1 (COI) and ca 560 bp of D2 loop of nuclear 28S rRNA). While the CO1 gene is a standard gene used in phylogenetic studies, the nuclear 28S rRNA gene is a mosaic of highly conserved and variable regions [9] and has also proven to be useful for resolving relationships in numerous insect groups (e.g., [10, 11]), including studies of beetles (e.g., [12, 13]).

2.2. DNA Sequencing. DNA was extracted using the Omega Bio-Tek E.Z.N.A. Insect kit. Muscle tissue was removed from the pro- and mesothoracic regions and ground up to improve the extraction of DNA. Sequences were amplified by PCR using combinations of published primers [14] and 5 Prime master mix was used in the amplification process. Typical PCR cycles for COI consisted of an initial denaturation at 95°C for 2 minutes, followed by 40 cycles of 95°C for 30 seconds, 46°C for 30 seconds, and 72°C for 1 minute, followed by a final extension at 72°C for 5 minutes. The PCR reaction for 28S was similar except for an annealing temperature of 55°C. Amplification of the 16S gene was attempted but the results were very poor in early tests and work on this gene was discontinued.

PCR products were purified prior to sequence reactions and sequenced using ABI dye-terminator v3.1, following the standard protocol on an ABI3130 sequencing machine. DNA sequences were edited in Geneious version 7.1.4. Sequences were deposited in GenBank with accession numbers listed in Table 1.

2.3. Morphological Data. Morphological data was acquired by soaking specimens in lactic acid to macerate tissues before dissection. Various individual body parts were examined on slides in glycerine or dry to discover characters and states. Mouthparts were examined and found to be so similar that no useful character states from them were discovered. Some characters used to define the genus such as two protibial teeth were found to be uninformative and were excluded. Two characters were uninformative but each has three states (char. 10 and 14) and are included in the analysis. Characters and states for each taxon are listed in Table 2 and were found from the head, pronotum, elytra, pygidium, and ventrites with the descriptions as follows:

(1) Elytral third and fifth intervals: strongly convex (0); feebly convex to flattened (1).

Table 2: List of taxa and their morphological character states used in this study. Note that *Trigonopeltastes* in character seven is coded as having both states as indicated within the parentheses.

Osmoderma eremicola	-141-11	2113712??110
Gnorimella maculosa	-121-11	0302611??111
Trigonopeltastes delta	112112(0/1)	0222500??001
Trichiotinus_affinis_KY	1120000	110110010000
Trichiotinus_affinis_QC	1120000	110110010000
Trichiotinus_assimilis	1120000	110110011000
Trichiotinus_bibens	1000101	110041011000
Trichiotinus_lunulatus	1000101	100031011000
Trichiotinus_piger	0110100	100100001000
Trichiotinus_rufobrunneus	0110100	110100001000
Trichiotinus_texanus	0130100	100100001000
Trichiotinus_viridans	1120000	100120010000

(2) Head and pronotum color: bright metallic green (0) or not (1).

(3) Elytra color: (0) bright metallic green; (1) light brown; (2) a combination of black and brown (may be some greenish reflections); (3) black; (4) dark brown.

(Note that although *T. bibens* appears brownish underneath a bright metallic green, the species is classified as having state 0.)

(4) Elytral lateral margin below humeral angle: abruptly deflexed down and outward (0) or smoothly rounded (1).

(5) Elytral fourth interval: sparsely punctate (0); distinctly puntate (1).

(Note that *Osmoderma* and *Gnorimella* have elytra lacking distinct intervals and are coded as inapplicable.)

(6) Pygidium shape: approximately round with the width ~equal to the length (0); transverse, wider than long (1); narrow, longer than wide (2).

(7) Elytra: obliquely transverse narrow white bands present (0); bands absent (1).

(Note that *Trigonopeltastes delta* does not have bands present but at least one species does (*T. sallaei*). Therefore this taxon is coded as having both states present.)

(8) Pygidium cretaceous patch: covering surface (0); on lateral edges only (1); absent (2). (This character in *Gnorimella* is dimorphic where females have the patch covering the surface and males have both lateral and a central longitudinal oriented patch. Further, some males appear to have no cretaceous patch at all. This character was coded for females. Some species of *Trigonopeltastes* have a patch similar to that seen in *Trichiotinus* but were coded as seen in *T. delta* typically with the entire surface covered.)

(9) Ventrite cretaceous band: on 5th laterally (0); absent (1); covering entire surface on 1st–5th (2); covering lateral surface on 1st–5th (3).

(10) Pronotal setae: present, erect, and dense (0); absent (1); setae recumbent and on outer edge in a line in triangular pattern (2).

(11) Elytra: dull or opaque area on lateral declivous portion: absent (0); present on posterior 2/3 (1); present on entire surface (2).

(12) Male paramere teeth: 2 teeth at apex (0); 1 tooth at apex (1); no teeth and apex tapered (2); no teeth and apex abruptly expanded and rounded (3); no teeth and apex gradually expanded and pointed (4); 1 tooth ~medially (5); no teeth and broadly curved at apex (6); no teeth and narrowly curved at apex (7).

(13) Short cretaceous (i.e., chalky white) longitudinal band just posterior of scutellum: present (0); absent (1).

(14) Scutellum shape: elongate triangular and sides slightly rounded (0); broadly triangular and sides rounded (1); elongate triangular and sides straight (2).

(15) Female genitalia, dorsal view: stylus elongate and transverse (0) or broader, blunt, and/or obliquely positioned (1).

(16) Female genitalia, dorsal view: apical tooth short and small (0) or not (1).

(17) Metatarsal length: distinctly longer than metatibia (0) or shorter than metatibia (1).

(18) Elytral striae: present (0); absent (1).

(19) Elytral surface: in part smooth and shiny (0); velvet-like throughout surface (1).

2.4. Analyses. All sequences were assembled and edited by eye using the program Geneious, version 7.1.4 (Genecodes Corp., Ann Arbor, MI). Alignment of the CO1 and 28S data sets was done using Muscle, version 3.8.425 [15], with the default parameters including 8 iterations, max. number of trees to build = 1, and optimization = anchor.

The data matrix was first constructed using WinClada [16]. Parsimony analysis was done using Nona and TNT [17, 18]. All characters were coded as unordered, and the matrix was analyzed with equal weights. The search was implemented using the following parameters, for example, in TNT: hold 10 000, hold/50, Mult $*$ 1000 (random addition sequence, 1000 replicates and TBR branch swapping). Character evolution and node support were done using WinClada.

JModelTest version 2.1.7 [19] was used to determine the best model to use in the Bayesian analyses. For the 28S sequence data the best model was determined to be HKY + I while for the CO1 the best model was TIM2 + G. As the latter is not a model available in Mr. Bayes, the next best available, a GTR + G model of nucleotide substitution was used.

Bayesian analyses were executed in the programs MrBayes, version 3.1 [20]. For Bayesian analyses, a relative burn-in of 25.0% and 1,000,000 generations were used as well as the other default values. For the 28S analysis,

the commands lset nst = 6 and rates = propinv were used while the CO1 analysis used lset nst = 6 and rates = gamma. The morphological analysis used the standard morphological model with lset rates = gamma, coding = variable, prset symdirihyperpr = fixed (infinity), and ratepr = variable. These same commands were used in the total evidence partitioned data analysis. The standard deviation of split frequencies between the two simultaneous analyses decreased below 0.01 within 330,000, 265,000, 505,000, and 10,000 generations for the CO1, 28S, morphology, and concatenated total data, respectively, and visual analysis of trace plots of the likelihoods of sampled trees was also examined to determine when the MCMC chains had reached stationarity.

Clade support based on the majority rule values was discovered in Mr. Bayes while node support was evaluated in WinClada. Bootstrap and jackknife values were calculated using 1000 replications and 10 search replications with one starting tree per replication and without tree bisection-reconnection (TBR). Each data set was explored individually and in combination as total evidence with both parsimony and Bayesian analyses. Trees were rooted between the ingroup and the three outgroups.

2.5. Molecular Dating. The application of a global molecular clock has been shown to be difficult both methodologically and philosophically [22, 23]. In addition, its applicability is difficult to assess. Nevertheless, the COI region is extensively used due to its relatively consistent rate of change among lineages [24] and the idea that it is better to have a possibly poor estimate rather than no estimate at all. Hence this partial CO1 sequence was used herein for dating branch divergences.

The most used calibration in insects assumes a rate of 2.3% sequence divergence per one million years [25, 26]. Based on the published rates for other groups of beetles [27–31] the clock with the rates of 0.0075 and 0.012 (Beast 1.4.8 [32]) was used to calculate the time to the most recent common ancestor (MRCA) for each clade. To account for rate heterogeneity among sites, a gamma distribution was used. The Yule speciation model generated a tree using a lognormal relaxed clock. Two independent runs of the MCMC for five million generations (sampling every 5,000 generations) were performed for each clock rate. Burn-in was set to 10%. Tracer 1.3 [33] as used to evaluate the convergence of the chains in both runs.

3. Results

We successfully obtained DNA sequences for the two target gene regions for all 12 of the ingroup and outgroup taxa included in this study. CO1 data ranged from 799 to 801 bases in length with 147 informative characters. 28S sequence data was generally about 560 bases with 36 informative characters. Two taxa had significantly shorter 28S sequences due to poor quality sequencing and included *Gnorimella* (346 bases) and *T. assimilis* (368 bases).

Trichiotinus is strongly supported as a monophyletic genus using either parsimony or Bayesian analyses (Figures 2–4). Based on the included outgroups, the most likely sister genus seen in all but two analyses is *Trigonopeltastes*; in the Bayesian CO1 analysis *Gnorimella* is the sister clade (Figure 3(a)) while in the parsimony 28S analysis one of two trees shows *Gnorimella* + *Trigonopeltastes* as the sister clade (strict consensus in Figure 3(d)). Within the ingroup, *Trichiotinus lunulatus* is the sister to the other seven species in both parsimony and Bayesian total evidence analyses as well as the topologies using only the CO1 data. Clades supported in most analyses include ((*T. piger* + *T. rufobrunnea*) + *T. texanus*) and usually + *T. bibens* (Figure 2, clade 1) as well as (*T. affinis* + *T. viridans*) and usually + *T. assimilis* (Figure 2, clade 2). All clades within *Trichiotinus* have strong support in the total evidence analyses (Figure 2) giving one confidence in this hypothesis of evolution. All support levels are greater than 89% in the Bayesian topology while in the parsimony analysis the support is above 92/95 for the bootstrap and jackknife values, respectively, with the exception of clade 1 (Figure 2) with values of 52/50 supporting this node. The parsimony total data analysis produced a single tree of 609 steps and CI = 71 and RI = 57.

Parsimony analysis of the morphological data alone produces a single 40-step tree (CI = 90, RI = 87) that is similar to the total evidence topology except that *T. bibens* + *T. lunulatus* together form a clade that is sister to the other *Trichiotinus* species instead of *T. lunulatus* as sister to all remaining species (Figure 4). Additionally, *T. piger* is sister to *T. texanus* instead of *T. rufobrunneus*. In contrast to parsimony, the Bayesian morphological topology is relatively unresolved with a hexatomy; the only clades within *Trichiotinus* that appear are a trichotomy consisting of *T. texanus, T. rufobrunneus*, and *T. piger* and a second clade composed of *T. lunulatus* + *T. bibens*.

The CO1 data analyzed using parsimony (Figure 3(b)) is identical to that found with total evidence analyses. In contrast, the Bayesian topology is similar but less resolved with a trichotomy created via the unresolved position of *T. bibens* (Figure 3(a)). Topologies from the 28S data in either Bayesian (Figure 3(c)) or parsimony analyses (two trees discovered, strict consensus in Figure 3(d)) strongly support clade 1, but clade 2 is disrupted in part by the unusual placement of *T. assimilis*. This may be due to the reduced sequence length for this species due to poor quality and the necessity of greater editing; while other species generally had about 540 bases, *T. assimilis* had only 368 bases included in the analysis. One should also note that only 36 bases were informative for the full length of this sequence. One other unusual aspect to this single gene analysis is the shift to a basal position of both *T. affinis* and *T. viridans* and is in stark contrast to their placement in all other analyses.

4. Discussion

The genus *Trichiotinus*, based on the total evidence topology, is composed of three main lineages; a single species, *T. lunulatus*, is sister to all other species and these in turn form two sister clades (labeled 1 and 2) as seen in Figure 2. Morphologically, the eight species within the genus *Trichiotinus* are quite similar, with only what might be considered minor differences. But at least in North America, the genus is unique

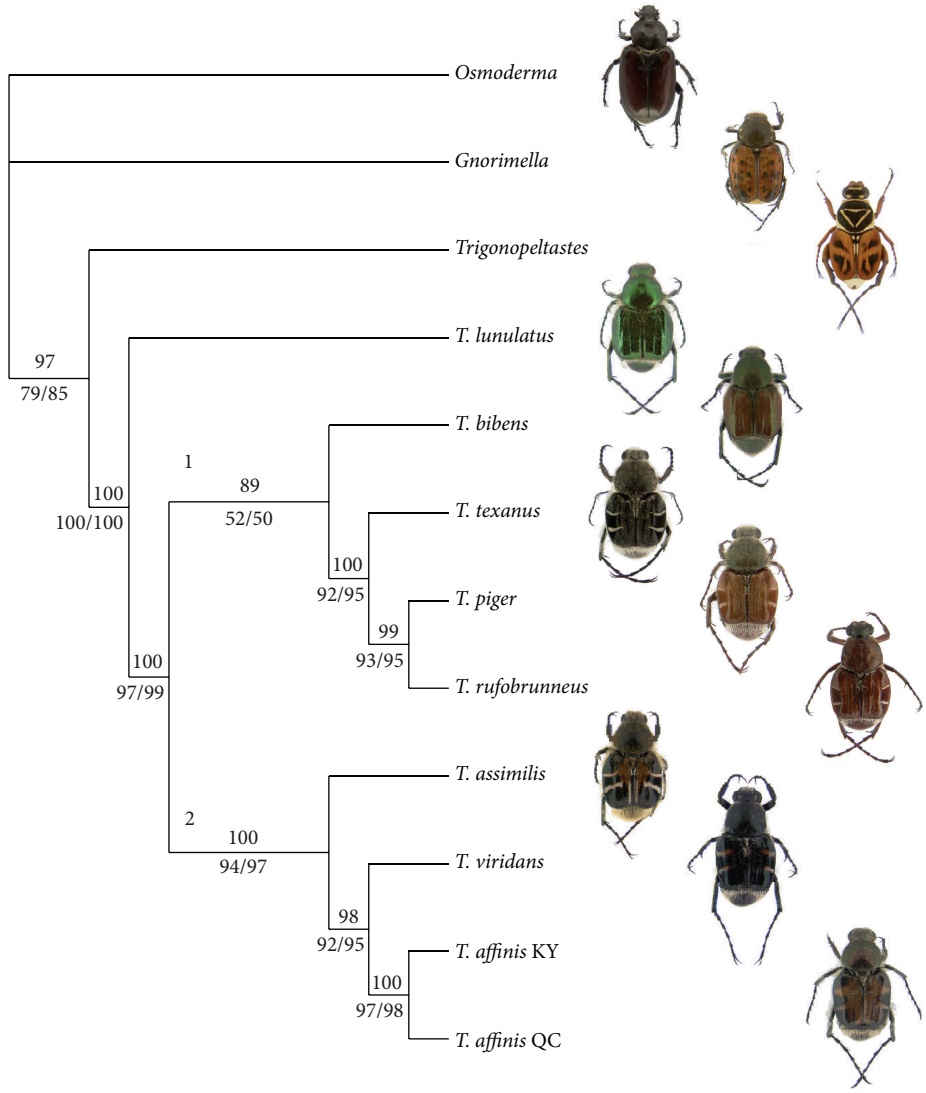

Figure 2: Topology of *Trichiotinus* found using both parsimony and Bayesian analyses and with all three data sets combined (CO1, 28S, and morphology) as well as a parsimony analysis using just the CO1 data. This is considered the best supported topology for the genus. Clade support values from the Bayesian analysis (above) and bootstrap/jackknife values (below) from the parsimony analysis are shown adjacent to each node, respectively. The two major clades discussed are labeled as 1 and 2 and the two included *T. affinis* sampled from Kentucky and Quebec are indicated.

and distinct from other genera by the presence of an elytral lateral margin below humeral angle that is deflexed down and distinctly projected outward (character 4, state 0). Other characteristics used to define the genus from closely related taxa are not as useful, including the pygidium cretaceous patch on lateral edges only (character 8, state 1). While this is different from the included *Trigonopeltastes delta*, other species in this genus as well as other genera also have a similar shaped pygidial patch (see [3]).

The sister relationship of *T. bibens* + *T. lunulatus* as seen in the tree based upon morphological tree (Figure 4) is based on states that use the bright metallic green color of the head + pronotum and the elytra. Hence this relationship should be considered weakly supported and further is not seen in the molecular or total evidence topologies. This clade is sister to the lineage that is supported by a single character, the presence of obliquely transverse white bands on the elytra (character 7, state 0). Based on the total evidence tree, this state either evolved twice within both clade 1 and clade 2 (Figure 2) with the selection pressure perhaps due to becoming morphological similarity to bees or (and less likely) was lost in *T. bibens*.

4.1. Distribution. All species of *Trichiotinus* are found primarily in the mid and eastern parts of North America although one species (*T. assimilis*) extends into the western states within the mountain time zone and north into the territories of northwestern Canada (Figure 1). Species appear to fall into two main geographic patterns that may reflect some degree of temperature range preference or tolerance, as

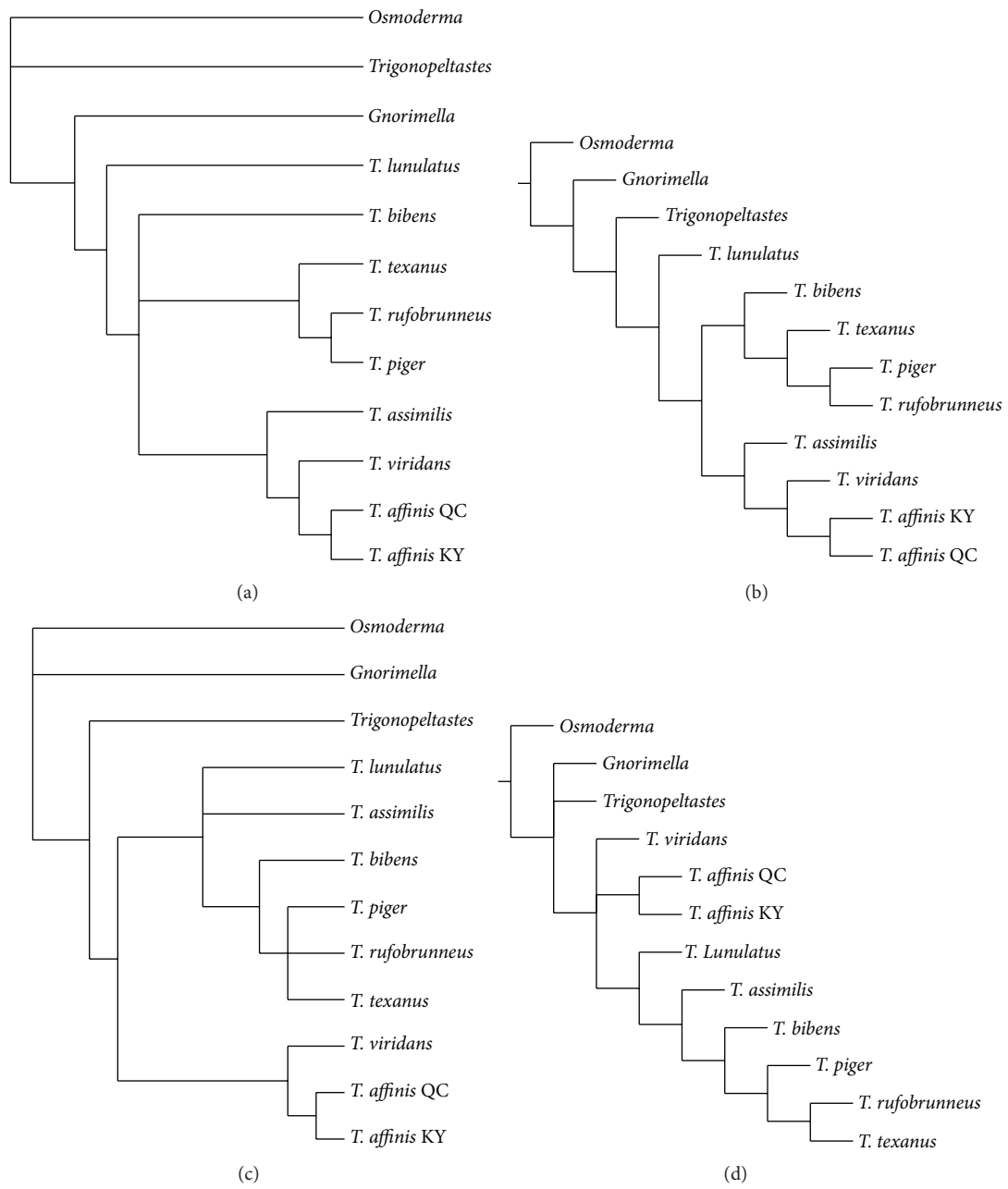

Figure 3: The Bayesian and parsimony analyses using molecular data. (a) Bayesian analysis using the CO1 data. The tree is similar to that found with the total evidence except for an unresolved trichotomy near the base of the topology; (b) parsimony analysis using CO1 data. The tree is identical to that found with the total evidence; (c) Bayesian analysis using 28S data; (d) Parsimony analysis using 28S data, strict consensus of two topologies.

no obvious geographical barriers exist. Clade 2 (*Trichiotinus assimilis*, *T. viridans*, and *T. affinis*) has a distribution largely in the northern and central part of the USA and appears to have a colder temperature tolerance. All other species (*T. lunulatus* and clade 1) are restricted to the middle and southern parts of the Midwest and eastern USA reflecting a higher temperature tolerance.

Adults are good fliers and forage on a variety of flowers while larvae are known to feed on various species of decaying hardwoods. Hence the restricted distributions of some species are somewhat puzzling. In particular, *T. viridans* has an odd distribution in the Midwest but that may reflect an association with the northern half of the mid-western oak-savanna habitats (see [34] for oak-savanna distribution figure). *Trichiotinus rufobrunneus* also has a very small distribution within Florida and again is likely restricted to oak scrub habitats.

4.2. Evolution in the Genus. Although there are issues with time estimations using CO1 data, our conservative approach

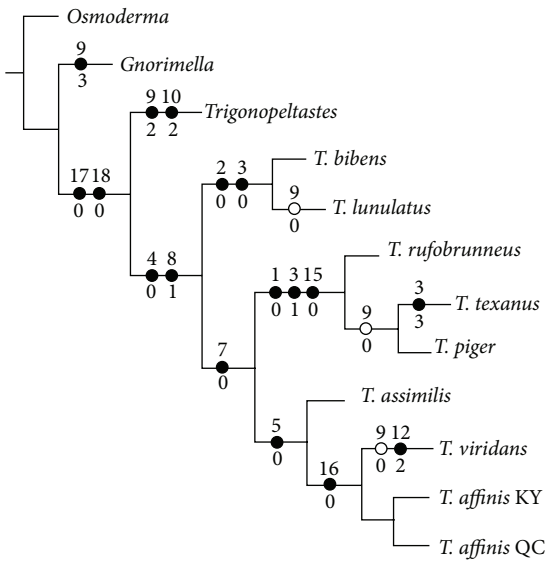

Figure 4: Topology based on morphological data from the parsimony analysis showing clade support (characters above and character states shown below). Solid black dots indicate character states without homoplasy. The Bayesian analysis of morphology was similar but much less resolved.

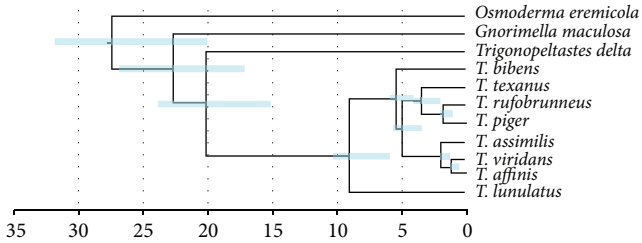

Figure 5: Tree showing divergence via the molecular clock. Dates are millions of years earlier and include the probable minimum and maximum age of cladogenesis.

supports the origin of this genus in the early Miocene (14.9–23.8 mya) (Figure 5). The sister clade most often appearing in this study, *Trigonopeltastes*, is a mainly a Mexican and Central American Neotropical genus. However, as no Old World representatives were included as outgroups, it is possible that the sister clade is from that region. Howden [3] speculates that *Trichius* is a possible sister lineage, but that perhaps a different Asian clade, of which he only knew from descriptions, might also be likely. If the genus does have evolutionary ties with an Asian lineage, there were strong links between Asia and North America from the late Paleocene to the middle-late Oligocene, when disjunction arose between North America and Asia [35]. Hence, the split of the common ancestor into two lineages, with one being the ancestral *Trichiotinus* species, may be related to the Middle Miocene Climatic Transition (MMCT) when temperatures on the planet began a rapid decrease [36]. More complete phylogenetic study will be needed to better hypothesize the sister clade but was beyond the scope of this study.

The earliest split in the *Trichiotinus* clade occurred in the late Miocene 10.6–6.2 mya (Figure 5). One lineage resulting from this event is represented by *T. lunulatus* while the second includes the seven remaining species. Based on Figure 2 (clade 1 and clade 2), the next cladogenic event resulted in two major lineages. Although only clade 2 appears as monophyletic in the molecular clock topology (Figure 5), for clade 1 the origin is estimated at 6.3–3.6 mya, reflecting the maximum age when *T. bibens* of clade 1 split from the remaining six taxa and the minimum age when the remaining three taxa of clade 1 separated from clade 2.

Clade 1 includes all species with a more southern distribution compared to clade 2, although *T. piger* confounds this somewhat with a distribution extending north into southern Canada. Nonetheless, possibly due to a cooling climate, this lineage may have been isolated in the southwestern part of the current range. In contrast, clade 2 may have been isolated in the southeast. Without the ability to shift further south due to a possible distribution on the Florida peninsula (even with the continental shelf exposure during maximum glaciation events), this lineage may have become by necessity more tolerant of colder temperatures that may be reflected in a generally more northernly present day distributions for all of the species in clade 1. All three species (*T. affinis*, *T. assimilis*, and *T. viridans*) are currently found no further south than approximately 34° north latitude in northern Alabama. *T. assimilis* in particular appears to be more cold tolerant than any other species as evidenced by a distribution as far north as the territories of Canada (Figure 1).

4.3. Further Cladogenesis. All remaining divergence and speciation within the genus occurred no earlier than four million years to as recently as 700,000 years earlier (Figure 5). During the late Pliocene about 3.6 mya the climate deteriorated and became more variable through and into the Pleistocene glacial/interglacial cycles [37]. About 3 mya, large ice sheets appeared in the high latitudes of the Northern Hemisphere and continued to grow rapidly for another million years. Additionally, the climate became more variable as seen in the 41,000 yr obliquity cycles due to axial tilt shift in the Earth. Hence the cause of additional cladogenesis in *Trichiotinus* is most easily attributed once again to successive glaciation events dividing ancestral populations into western and eastern blocks long enough for speciation and reproductive isolation to occur.

At least some evidence suggests that there was a broad band of warm mixed (temperate) forest/woodland across central North America during the middle Miocene [38]. Glaciation would have destroyed habitat and shifted these forests much further south than they currently exist (Figure 6). And with ice extending furthest in the Midwest compared to the eastern and western parts of the USA, the hardwood tree species needed by *Trichiotinus* may have been divided into western and eastern populations.

Trichiotinus is dependent upon decaying hardwoods as larval food including oak [6]. Jackson et al. [39] present evidence for a split in distribution of oaks (*Quercus* spp.) during the most recent glacial maximum on either side of the Mississippi drainage and may be indicative of the effects

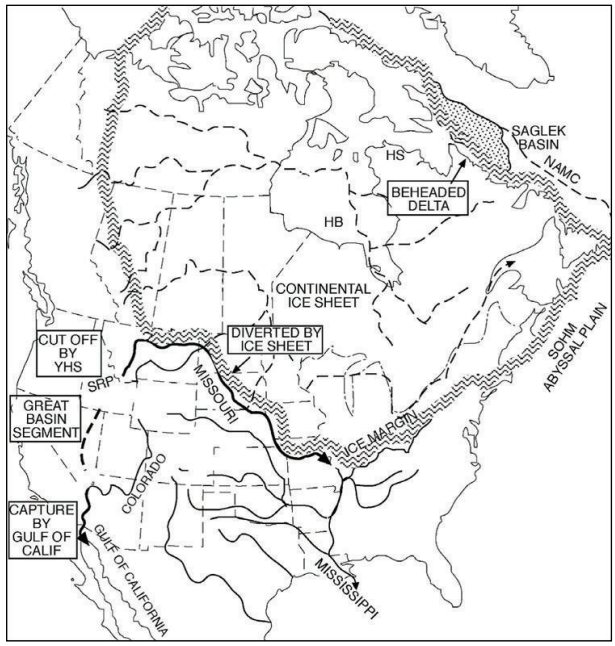

Figure 6: Maximum extent of the last Pleistocene glaciation in North America. Modified from [21].

of earlier glacial maxima as well. P. A. Delcourt and H. R. Delcourt [40, 41] also postulated the presence of spruce (*Picea glauca*) forests in the Lower Mississippi Valley. This extension south of these more cool adapted forests all the way to the gulf coast that divided the hardwood forests into eastern and western blocks was thought to be due to glacial meltwater flow creating a cooler climate locally [42].

During the Pre-Illinoian Stage, an interglacial event with higher sea levels likely isolated a population of *T. piger* in Florida that speciated into *T. rufobrunneus* sometime between 1.2 and 2.0 mya. Perhaps similar to the Florida Scrub Jay, both species appear to be largely restricted to xeric oak scrub and scrubby pine flatwoods habitats in Florida. Lastly, the Nebraskan Glaciation event that occurred from 780,000 to 900,000 years earlier [43] correlates closely with a dated cladogenesis event in *Trichiotinus* of 700,000 to 1.2 mya. The western population evolved into *T. viridans* while the eastern population is now recognized as *T. affinis*.

4.4. Conservation. Future studies or perhaps some degree of population monitoring should be considered to address potential conservation concerns for *T. rufobrunneus* due to their limited distribution in Florida as well as *T. viridans* that is found only in the "corn and soybean deserts" of the upper Midwest, where unfortunately little natural habitat remains. Distribution records are largely based on data from 1935 [6] and it is likely that a reduction in distributions of these and other species has occurred since this time. Future studies could map out current distributions as well as abundances; at least in Kentucky, beetles can be difficult to find in what appears to be good forest habitat for both larvae and flowers used for adult feeding for unclear reasons (Philips, unpublished).

Disclosure

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Competing Interests

The authors declare that they have no competing interests.

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