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Molecular phylogenetic evidence for multiple dispersal events in gomphocerine grasshoppers

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Abstract

The gomphocerine grasshoppers, comprising over 1000 species, occur on all continents excepting Australia and Madagascar. This work provides an independent examination of previous accounts of their taxonomic relationships and intercontinental connections, focusing on selected North American and Eurasian taxa. Our study is based on portions of four mitochondrial genes (coding for cytochrome b, cytochrome oxidase subunits I and II, and NADH dehydrogenase subunit V) which were sequenced and phylogenetically analyzed using weighted and unweighted maximum parsimony, maximum likelihood and Bayesian methods. Maximum resolution was achieved using weighted parsimony (counting transversions at third codon positions only) and Bayesian methods, and treating all four sequences, totalling 1892 bp, as a unit. The subfamily is provisionally accepted as monophyletic. The tribe Chrysochraontini is monophyletic, whereas the monophyletic status of Aulocarini and Dociostaurini is unclear. Tribes, Arcypterini, Chorthippini and Gomphocerini, are not monophyletic and require further scrutiny. Regarding biogeographic origins of the subfamily, our molecular data generally support Vickery's assertion that there were multiple periods of dispersal, most likely from Eurasia to North America. Assigning the range 50 to 70 Mya to the time of gomphocerine divergence, we provide estimates of the times of these biogeographic events.

Keywords

Orthoptera, Acrididae, Gomphocerinae, Acridinae, phylogeny, biogeography, mitochondrial DNA, molecular clock

Introduction

Among the approximately 30 subfamilies of Acrididae, the Gomphocerinae are, next to the Catantopinae, the most speciose. In terms of distribution they are second to the Oedipodinae, occurring on all continents except Australasia and Madagascar (Vickery & Kevan 1985). First established as a subfamily in 1904 (Dirsh 1975), the Gomphocerinae include species of both agricultural and general interest. For instance, some members, such as the Moroccan locust, *Dociostaurus maroccanus* Thunberg, in Eurasia and Africa; the bigheaded grasshopper, *Aulocara elliotti* (Thomas), in North America; and the South American plague grasshopper, *Rhammatocerus schistocercoides* Rehn 1906, are pests of economic concern. Understandably, much research has been devoted to these organisms. However, other less harmful species have been studied as well, some serving as model organisms for addressing fundamental issues in, for example, speciation and population genetics (*e.g.*, Hewitt 1993, Lunt *et al.* 1998), color pattern inheritance (reviewed in Dearn 1990) and insect communication (*e.g.*, Ragge 1986). It is, of course, the latter feature that many nonspecialists associate with the term "grasshopper" and, in that respect, gomphocerines are often considered the quintessential "grig" (Vickery & Kevan 1985). Most species do possess a highly developed bio-acoustic system, typically consisting of species-specific songs in males, for the most part (Jago 1971). A few genera such as *Aulocara* Scudder also communicate visually (Otte 1981).

Over the past century, there have been varied opinions on the assignment of specific genera to Gomphocerinae or Acridinae (or to some extent, Oedipodinae or Truxalinae). Much, but not all, of the controversy has centered on the acoustic apparatus. Fundamentally, the two subfamilies are distinguished by the presence (Gomphocerinae) or absence (Acridinae) of stridulatory pegs on the inner surface of the hind femur (Otte 1981). Evolutionary loss of pegs has apparently occurred fairly frequently, and not unexpectedly has contributed to classification problems. For example, the genus *Stethophyma* Fischer has been placed, at various times, in: a) Oedipodinae, owing to a similarity of its tegminal stridulatory apparatus (Vickery & Kevan 1985); b) Gomphocerinae, on the basis of behavior and overall morphology wherein femoral stridulatory pegs are believed to have been lost (Otte 1981); and, more recently, c) Acridinae, because, upon closer examination (Storozhenko & Otte 1994), the position of the tegminal stridulatory pegs proved to be unlike that in (a).

There have also been different views on the organization of taxa (see Jago (1971) and Guliaeva *et al.* (2005) for summaries), including the recent elevation of several subgenera to genus status. These changes are now reflected in the current online version of the Orthoptera Species File OSF2 (Otte *et al.* 2006). OSF2 organizes species into 19 tribes of which 15 are represented on more than one continent, which of course has biogeographic implications. In his study of the biogeographic history of the Orthoptera, Vickery (1989) speculated that the antecedents of North American gomphocerines had mixed origins, some migrating from Eurasia and some from South America via ancient or recent land bridges (see also Rehn 1958). With respect to the North American-Eurasian connection, Vickery (1986, 1989, 1997) proposed that incursions into North America took place at different times, from a very recent entry prior to the last glaciation, to a more ancient time when Laurasia was intact.

To properly assess statements on taxonomic or indeed intercontinental affiliations, an accurate phylogeny would be useful. In his comprehensive study of world-wide gomphocerines, Jago (1971), employing 31 morphological characters, presented two sets of dendrograms of 123 taxa, one set based on character correlations and the other on D. Eades' distance method (Eades 1970). While no measures of statistical confidence were given, the dendrograms

Table 1. Species used, sources/location and GenBank accession numbers of mtDNA sequences.

Subfamily /Tribe	Species	Source/Location	Accession Nos. (CO1, ND5, C02, CB)
Gomphocerinae /Arcypterini	*Pararcyptera brevipennis* (Brunner-von Wattenwyl 1861)	Montpellier, France	DQ230713, DQ230747, DQ230783, DQ230813
	Ptygonotus gansuensis Zheng & Chang 1994	Gansu Prov., China	DQ230736 DQ230772, DQ230805, --
	Ramburiella turcomana (Fischer-Waldheim 1846)	Malatya, Turkey	DQ230710, DQ230744, DQ230780, DQ230810
/Aulocarini	*Aulocara elliotti* (Thomas C.1870)	Torrington, Wyoming	DQ230719, DQ230754,--, --
	Aulocara femoratum Scudder 1899	Torrington, Wyoming	DQ230722, DQ230757, DQ230792, --
	Ageneotettix deorum (Scudder 1876)	Torrington, Wyoming	DQ230718, DQ230753, DQ230789, --
/Chorthippini	*Chorthippus curtipennis* (Harris 1835)	Pinawa, Manitoba	DQ230709, DQ230743, DQ230779, DQ230809
	Chorthippus parallelus (Zetterstedt 1821)	Budapest, Hungary	DQ230723, DQ230758, DQ230793, --
	Euchorthippus pulvinatus (Mařan 1957)	Sierra Nevada, Spain	DQ230711, DQ230745, DQ230781, DQ230811
	Glyptobothrus biguttulus (L., 1758)	Massif Central, France	DQ230731, DQ230767, DQ230801, DQ230823
	Glyptobothrus binotatus (Charpentier 1825)	Sierra Nevada, Spain	DQ230724, DQ230759, DQ230794, --
	Glyptobothrus jacobsi (Harz 1975)	Sierra Nevada, Spain	DQ230725, DQ230760, DQ230795, --
	Glyptobothrus vagans (Eversmann 1848)	Sierra Nevada, Spain	DQ230727, DQ230762, DQ230797, DQ230818
/Chrysochraontini	*Chloealtis abdominalis* (Thomas C. 1873)	Jameson, Saskatchewan	DQ230751, DQ230787, DQ230817, --
	Chrysochraon dispar (Germar 1831)	Massif Central, France	DQ230730, DQ230766, DQ230800, DQ230822
	Euthystira brachypterous (Okskay 1826)	Budapest, Hungary	DQ230726, DQ230761, DQ230796, --
/Dociostaurini	*Xerohippus anatolicus* Ramme1951	Malatya, Turkey	DQ230715, DQ230749, DQ230785, DQ230815
	Dociostaurus jagoi Soltani 1978	Sierra Nevada, Spain	DQ230734, DQ230770, --, DQ230825
	Dociostaurus maroccanus (Thunberg 1815)	Montpellier, France	DQ230714, DQ230748, DQ230784, DQ230814
/Gomphocerini	*Aeropedellus clavatus* (Thomas C. 1873)	Dilke, SK	DQ230708, DQ230741, DQ230777, --
	Aeropedellus variegatus (Fischer-Waldheim 1846)	Montpellier, France	DQ230712, DQ230746, DQ230782, DQ230812
	Bruneria brunnea (Thomas C. 1873)	Dilke, SK	DQ230707, DQ230740, DQ230776, --
	Gomphocerus rufus (L. 1758)	Massif Vercors, France	DQ230733, DQ230769, DQ230803, DQ230824
	Omocestus burri Uvarov B.P. 1936	Sierra Nevada, Spain	DQ230732, DQ230768, DQ230802, --
	Omocestus panteli (Bolivar I. 1887)	Sierra Nevada, Spain	DQ230728, DQ230763, DQ230798, DQ230819
	Phlibostroma quadrimaculatum (Thomas C. 1871)	Dilke, SK	--, DQ230742, DQ230778, DQ230808
	Stenobothrus lineatus (Panzer 1796)	Budapest, Hungary	DQ230729, DQ230764, --, DQ230820
	Stenobothrus nigromaculatus (Herrich Schaffer 1840)	Massif Central, France	--, DQ230765, DQ230799. DQ230821
	Stenobothrus zubowskyi Bolivar I. 1899	Malatya, Turkey	DQ230716, DQ230750, DQ230786, DQ230816
/Mermiriini	*Achurum carinatum* (Walker F. 1870)	Unknown location, Florida	DQ230717, DQ230752, DQ230788, --
/Paropomalini	*Cordillacris crenulata* (Bruner 1889)	Torrington, Wyoming	DQ230721, DQ230756, DQ230791, --
	Cordillacris occipitalis (Thomas C. 1873)	Torrington, Wyoming	DQ230720, DQ230755, DQ230790, --
Acridinae	*Duroniella fracta* (Krauss H.A. 1890)	Malatya, Turkey	DQ230738, DQ230774, DQ230807, DQ230827
	Covasacris albitarsis Liebermann J. 1970	Benito Juarez, Argentina	DQ230739, DQ230775, --, DQ230828
	Stethophyma gracile (Scudder 1863)	Last Mountain, Saskatchewan	DQ230737, DQ230773, DQ230806, DQ230826
	Stethophyma grossum (L. 1758)	Massif Central, France	DQ230735, DQ230771, DQ230804.--
Oedipodinae	*Locusta migratoria* (L. 1758)	GenBank	X80245

have provided working hypotheses for further study [Jago did discuss "affinities" between the gomphocerines of different continents; however, he made no statements regarding biogeographic origins]. To date, three phylogenetic studies, mostly involving mitochondrial DNA (mtDNA), have been published. Two are limited by involving either too few species (Flook & Rowell 1997, Flook *et al.* 1999) or too few genes (Guliaeva *et al.* 2005). More recently, Bugrov *et al.* (2006) published a molecular phylogeny of a number of gomphocerines, all Eurasian.

In contrast, the present research focuses on both Eurasian and North American gomphocerines, some with supposed intercontinental connections. Four mitochondrial genes are targeted. Our objectives are 1) to test the earlier biogeographic hypotheses concerning the subfamily's northern continental origins; and 2) to add to our understanding of taxonomic relationships and organization within the Gomphocerinae.

Materials and Methods

Species, along with sources, are listed in Table 1. Included are 21 Eurasian and 11 North American gomphocerines. Collectively, these represent eight of the tribes listed in the OSF2. Two North

Table 2. Primers used for PCR and DNA sequencing and position of primer binding.

Gene	Primer Pair	Primer sequence	Primer binding[a]
Cytb	CB9	5′GCCGAGACGTGAATAATAATGGAT3′	10607
	CB10	5′CTGCGAATCCTCCTCAAACTC3′	10906
	CBG1	5′GGACGAGGAATTTATTACGG3′	10693
	CBGR1	5′ATTGAACTAAATCTGTTCC3′	10885
COII	co2a	5′GGTCAAACAATTGAGTCTATTTGAAC3′	3212
	co2e	5′CCACAAATTTCTGAACATTGACCA3′	3642
	co2G29	5′TATTGCATTACCATCACTACG3′	3265
	co2G323	5′GATTTAGTCGTCCAGGTGTAGC3′	3581
COI	mtd6	5′GGAGGATTTGGAAATTGATTAGTTCC3′	1652
	mtd11	5′ACTGTAAATATATGATGAGCTCA3′	2310
	CO1G1	5′GCACCAGATATAGCATTTCC3′	1661
	CO1GR3	5′CAATAAATCCTATTAATCC3′	2285
ND5	ND5J	5′ACTCACCTCAACCAGAATCAA3′	6452
	ND5N	5′ACTCATGCTTTATTTAAGGCTTTA3′	7140

[a]Position of 5′nucleotide relative to *L. migratoria* (Flook *et al.* 1995).

American species have congenerics on the Eurasian continent. Two genera of the subfamily Acridinae were included and explored as possible outgroups for further studies of Gomphocerinae. In addition, one Eurasian and one North American species of the enigmatic genus *Stethophyma* were included to determine whether the issue of its subfamily association could be resolved. The oedipodine *Locusta migratoria* (L.) served as the outgroup.

DNA was extracted from specimens using either the DTAB/CTAB method outlined in Philips and Simon (1995) or using a QIAGEN DNeasy tissue kit (Mississauga, Canada). Portions of the mitochondrial genes encoding NADH dehydrogenase subunit V (ND5), cytochrome oxidase subunit I (CO1) and II (CO2), and cytochrome b (cytb) were amplified and sequenced. Primer sequences are described in Table 2. PCR gene amplification conditions, as well as DNA sequencing methods, are described elsewhere (Litzenberger & Chapco 2001a, 2001b).

Sequences were easily aligned by visual inspection, imported into MacClade (Maddison & Maddison 2004) and analyzed using the software packages PAUP* (version 4.0b8 – Swofford 2003) and MrBayes (Version 3.0b4, Huelsenbeck & Ronquist 2001). Both standard maximum parsimony (MP) and weighted maximum parsimony (wMP), following Farris' (1969) iterative reweighting scheme, were used. Searches were repeated using all substitutions at the first two codon positions and only transversional substitutions at the third position (methods referred to as MP123TV and wMP123TV), previous studies (Chapco et al. 2001) having shown a tendency for transitional saturation to occur at this position. In addition to parsimony, maximum likelihood (ML) and Bayesian methods were applied. Prior to implementing these analyses, the program Modeltest (Version 3.6, Posada & Crandall 1998) was employed to identify the minimum number of parameters required to explain the data. Levels of support for parsimony-derived relationships were estimated through 1000 bootstrap replicates. Owing to computer run-time restrictions, only 100 bootstrap replicates were performed using ML. Bayesian analyses provided measures of nodal support in the form of posterior probabilities (PP). For all analyses, the four sequences were treated as a combined unit, a procedure that, as in all our previous studies (Chapco *et al.* 2001, Litzenberger & Chapco 2001a, b), always yielded trees with greater resolution and support when compared to those based on single genes.

A subsidiary objective was to estimate the times of divergence for nodes of interest. First, we applied the maximum likelihood ratio test (Page & Holmes 1998) to determine whether sequences evolved in a clock-like manner. Because sequences did not in fact conform to a model of rate constancy, we estimated divergence times by employing a semiparametric penalized likelihood (PL) method which can accommodate rates that vary over lineages (Sanderson 2002). To this end, the program r8s, version 1.70 (Sanderson 2004) was used. As recommended by Sanderson, the TN algorithm was applied in conjunction with PL. A cross-validation analysis was first performed to determine the most likely smoothing parameter (a measure of the relative contributions of parametric and nonparametric models that underlie PL), necessary for estimating optimal divergence times. Zero length branches were collapsed. More extensive descriptions of the method and rationale are provided by Sanderson (2002). The program yields estimates of absolute times of divergence if at least one divergence time is known and provided as input. Often, these times are based on the fossil record. In the present case, we have relied on estimates obtained by Rowell and Flook (2004). They, citing Gaunt and Miles (2002), set the split between Oedipodinae and two other subfamilies (Melanoplinae and Proctolabinae) at about 100 Mya. Accordingly, times of divergence for the three subfamilies fell within the range 50 to roughly 65 Mya. Although Flook and Rowell (1997) suggest that Gomphocerinae is somewhat younger than Oedipodinae, they speculate that all these subfamilies had evolved in the early Tertiary or the Cretaceous. Therefore, to estimate times of divergence for nodes of interest, we took the broad approach of assigning a range of calibration points, 50 to 70 Mya, to the root of those gomphocerines that emerged unequivocally as a monophyletic cluster (see below).

Results and Discussion

The overall A+T content among the four regions spanning 1892 bp of the mitochondrial genome was 70.8%, typical of mtDNA in many insect species (Simon *et al.* 1994). Base compositions did not differ significantly among the 37 taxa, averaging 40.8% (A), 15.8% (C), 13.4% (G) and 30.0% (T). Across all genes, 853 sites were variable, of which 599 were phylogenetically informative.

While all parsimony methods recovered the same major clades depicted in Fig. 1a, maximum resolution was achieved using wMP123TV. Modeltest identified the general time reversal model (GTR) with variable rates (G) and invariable sites (I), as the one best fitting the data. Parameter estimates that emerged from this analysis were then used in ML searches and bootstrapping. Since run times for ML bootstrapping proved excessive, search procedures were repeated by constraining, using the "backbone" option, the highly supported clades B to E, as revealed in Fig. 1a. A best tree with -LnL = 16019.84 was recovered. At deeper levels, bootstrap values were not as large as those for wMP123TV. A variety of models were analyzed using Bayesian methods, and again the one that emerged with the highest likelihood was GTR + G + I. Parameter values were very similar to estimates obtained using Modeltest. The Bayesian topology (Fig. 1b) was essentially the same as that obtained using ML, but resolution of the former was superior, with most PP values in excess of 80%. High PP values are apparently not unexpected and are viewed as somewhat liberal (Suzuki *et al.* 2002). In terms

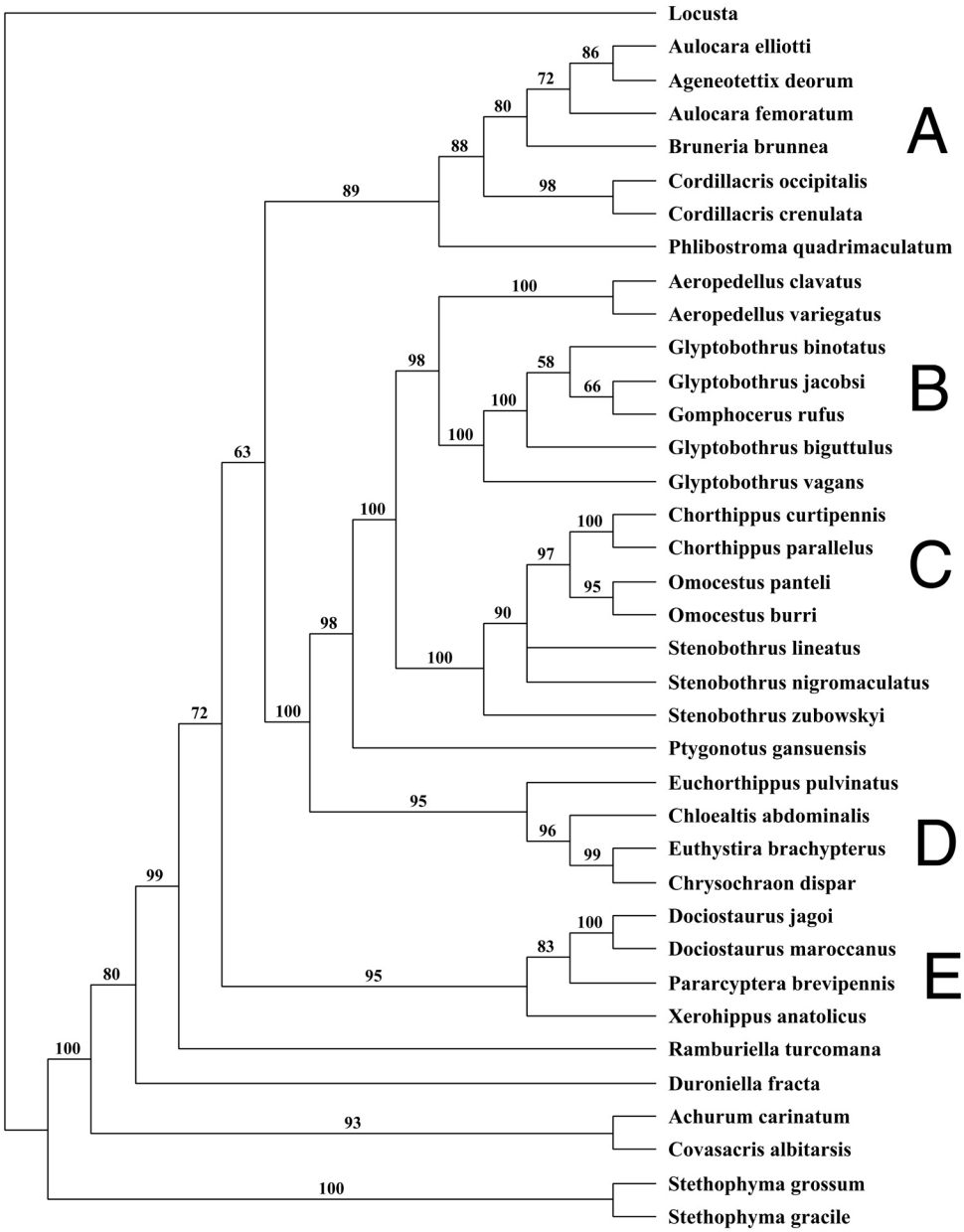

Fig. 1. Relationships recovered using two different methods. Clades A (or A') to E described in text.

Fig. 1a. Maximum parsimony tree obtained by scoring all substitutions at first two codon positions and transversions only at third-codon positions. Homoplasy was minimized by applying successive rounds of weighting using rescaled consistency indices. Numbers indicate bootstrap levels of support using 1000 replicates.

of identifying major clades, both wMP123TV and Bayesian analyses generally concur. However, there are some differences and, where these appear, outcomes from both methods will be described.

Excluding *Achurum* for the moment (see below), we conclude that the Gomphocerinae do constitute a monophyletic group. However, most tribes as defined in the OSF2 are not monophyletic. For purposes of discussion, the following groupings in the two trees (Figs 1a, b) are highlighted: A (or A′) to E.

Groups A and A′.— All members are North American. Elements of the three tribes: Aulocarini, Paropomalini and Gomphocerini are joined within these groups. Parsimony methods (Fig. 1a) link together the three species *Aulocara elliotti* (Thomas C.), *Aulocara femoratum* Scudder and *Ageneotettix deorum* (Scudder), in agreement with conventional taxonomy in which all are placed within the tribe Aulocarini (Otte *et al*. 2005). The two *Aulocara* species, however, are not directly linked: instead, *A. elliotti* and *Ageneotettix deorum* are connected. Bayesian methods also support the latter association, but place *A. femoratum* more distally. Interestingly, in Jago's (1971) study, *A. elliotti* was also directly connected to *Ageneotettix deorum* with *A. femoratum*'s position more remote.

Both A and A´(Figs 1a, b) contain the paired species of *Cordillacris* Rehn, a member of tribe Paropomalini. There is some agreement with Jago's study in which *Cordillacris* [*crenulata* (Bruner)] emerges as part of a large clade that encompasses *Aulocara* and *Ageneotettix* among other genera. Also, all analyses place *Bruneria* McNeill, a member of the tribe Gomphocerini, basally to *Aulocara elliotti* and *Ageneotettix deorum* within A (or A´) (see C below). The position of *Phlibostroma* Scudder is unclear; it emerged as either part of A using weighted parsimony (Fig. 1a) or an unresolved branch using the Bayesian procedure (Fig. 1b). [Maximum likelihood also placed the species basally within A, but bootstrap support was less than 50%.]

Group B.— This clade, recovered in all analyses with high levels of support, comprises two connected subgroups: one consisting of the

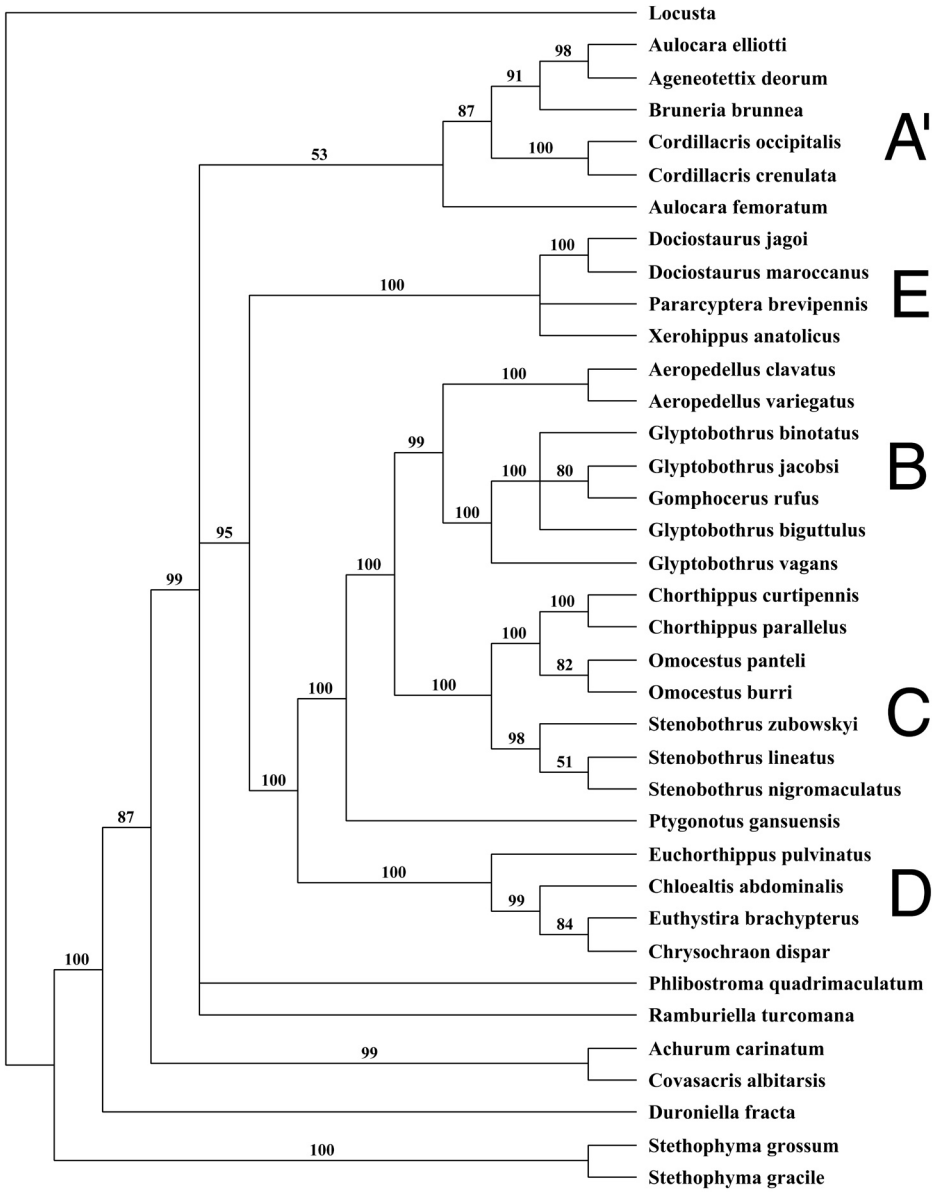

Fig. 1b. Bayesian tree based on GTR + G + I model. Eight Monte Carlo Markov chains, one cold and 7 heated, were run simultaneously for 3×10^6 generations. Trees were saved every 1000 generations, yielding 3000 saved trees; the last 1500 were used to estimate the topology, parameter values and posterior probabilities, indicated in the figure.

four species of *Glyptobothrus* Chopard with *Gomphocerus* Thunburg embedded internally and the other subgroup consisting of the two *Aeropedellus* Hebard species. Jago (1971) identified a large clade that included *Glyptobothrus*, *Gomphocerus*, *Aeropedellus* and *Chorthippus* (among other genera), claiming that *Gomphocerus* was derived from *Aeropedellus*. Connections involving these taxa are discussed in the next section.

Group C.— This clade, emerging in all analyses, consists of two species of *Chorthippus* Fieber directly linked to two species of *Omocestus* Bolivar, L., and basal to these are three species of *Stenobothrus* Fischer-Waldheim. The latter appears as a monophyletic assemblage in Fig. 1b and as a paraphyletic group in Fig. 1a. These genera belong to either tribe Chorthippini or Gomphocerini, but clearly neither tribe can be regarded as monophyletic, given that other members are positioned elsewhere. Jago (1971) also concluded that neither tribe is monophyletic. Recently, Storozhenko (2002) elevated *Glyptobothrus* Chopard from its previous status as a subgenus of *Chorthippus* to that of genus. However, *Glyptobothrus* cannot be regarded as monophyletic either, given the bisection of clade B by *Gomphocerus*, a member of the Gomphocerini. It is interesting to note that in Guliaeva *et al.*'s (2005) analysis of 16S rDNA sequences, *Gomphocerus* and *Glyptobothrus* are also directly connected.

There has been some dispute concerning the classification of *Bruneria brunnea* (Thomas C.) in the literature. At various times it was regarded as a subgenus of *Stenobothrus* (Jago 1971, Otte 1981) or as a genus in its own right (Vickery & Kevan 1985). The latter view is reflected in the current version of the OSF (Otte 2006). In Jago's (1971) dendrograms, *Bruneria* forms a close cluster with other species of *Stenobothrus*. In our molecular trees however, *Bruneria* McNeill is topologically far removed from *Stenobothrus* and instead, occupies a strongly supported connection with members of tribe Aulocarini within clade A (or A´). In contrast, *Omocestus*, also previously viewed as a subgenus of *Stenobothrus*, remains closely associated with that genus.

Apart from *Bruneria*, *Euchorthippus* Tarbinsky (see D below) and *Phlibostroma*, there is an overall intertwining of members of tribes Gomphocerini and Chorthippini. It should be pointed out that several earlier researchers [summarized and referenced in Guliaeva *et al.* (2005)] had combined these taxa into one tribe, labelled as

either Gomphocerini or Chorthippini. In their analysis of several *Eurasian gomphocerines*, Bugrov *et al*. (2006), identified two monophyletic groups within this collective, labelling one Gomphocerini (consisting of genera *Chorthippus*, *Aeropus* and *Stauroderus*) and the other Stenobothrini (consisting of *Omocestus* and *Stenobothrus*). [It should be noted that there is very little overlap between the precise species used in their study and ours.] The one glaring difference between our findings and those of Bugrov *et al.*, is that in our study, *Omocestus* links directly with *Chorthippus* rather than with *Stenobothrus*. For now, it is recommended that genera A*eropedellus*, *Chorthippus*, *Glyptobothrus*, *Omocestus* and *Stenobothrus* be placed into a single tribe, pending further clarification.

Group D.— This group, comprising three members of the tribe Chrysochraontini, consistently emerged as a monophyletic clade in all analyses. This conclusion was arrived at earlier by Jago (1971) and more recently by Bugrov *et al.* (2006). Occupying a basal position within D, in all trees, is *Euchorthippus*, a member of the Chorthippini. There is nothing in the morphological or acoustic literature to suggest such an association. It is interesting to note, however, that the same association was recently uncovered by Guliaeva *et al*. (2005), using a different genus of Chrysochraontini. This result contrasts with the findings of Jago (1971) in which *Euchorthippus* is directly associated with *Pararcyptera* Tarbinsky (= his *Arcyptera* Serville) and far removed from the Chrysochraontini.

Group E.—Members of the tribe Dociostaurini form part of this group and are closely associated with *Pararcyptera brevipennis* (Brunner-von Wattenwyl), a member of the tribe Arcypterini. Analyzing different species, Bugrov *et al*. (2006) also demonstrated a connection between these two tribes. Based on parsimony, Dociostaurini is not monophyletic; however, based on Bayesian (and ML) methods, support for monophyly is ambivalent. Both species of *Dociostaurus* Fieber are directly paired. In Jago's study, members of Dociostaurini are fairly distant from *Pararcyptera* and instead, have a close association with the North American genera, *Aulocara* and *Ageneotettix* (see Biogeography).

Other relationships.— As mentioned above, the position of *Phlibostroma* is unclear. Vickery (1989) includes it among several North American genera that have South American connections. Perhaps when Neotropical gomphocerines are investigated, the roots of *Phlibostroma* will be clarified.

The tribe Arcypterini is clearly not monophyletic, with three members scattered throughout each tree. In the parsimony tree, *Ramburiella* Bolivar L. occupies a basal position to groups A to E (with moderate bootstrap support), whereas in the Bayesian tree it is part of an unresolved polytomy involving A′, B to E and *Phlibostroma*. Another member, *Ptygonotus*, is basal to the B + C cluster.

Occupying basal positions to all of the above are the gomphocerine: *Achurum* Saussure, two acridines: *Covasacris* Liebermann, J. and *Duroniella* Bolivar, L. and two species of *Stethophyma*. A very strong association between *Achurum* and *Covasacris* emerged in all analyses. The pair is external to *Duroniella* in the parsimony tree, whereas in the Bayesian tree, the positions are reversed. The two *Stethophyma* species occupy basal positions in all trees. It is interesting to note that some populations of *Achurum carinatum* (Walker F.) are acridine-like in not having femoral pegs (Otte 1981). It is possible that *Covasacris* and *Duroniella*—and perhaps *Stethophyma* (see below)—are gomphocerines whose ancestors did possess the stridulatory elements. Another explanation, and one that might possibly account for the different basal branching orders between trees, is long-branch attraction (Felsenstein 1978). Sampling additional taxa may help resolve the discrepancy (Lyons-Weiler & Hoelzer 1997). In any case, it would appear that some members of Gomphocerinae and Acridinae are too intertwined for the latter to be considered useful as an outgroup to the former.

Further, with respect to *Stethophyma*, it should be noted that Rowell and Flook (2004) positioned the genus *Mecostethus*, a member of the same tribe as *Stethophyma* (Storozhenko & Otte 1994), basally to eight species of Oedipodinae. While it is therefore tempting to infer that *Stethophyma* is an oedipodine, it should also be noted that no members of Gomphocerinae or Acridinae were included in the Rowell & Flook study. Also, given that many tribes are proving to be polyphyletic, the inference may be premature, pending confirmation that *Stethophyma* and *Mecostethus* are indeed directly related, as suggested by morphology. An ideal study would be one that includes these species and the three aforementioned subfamilies.

Biogeography.—Having rejected the uniform molecular hypothesis (Ln non-clock = -16019.84; Ln clock = -16083.72; 2 × difference = 127.8 ***), we assigned a range of divergence values, 50 to 70 Mya, to the root node linking all species between, and including, *Aulocara elliotti* to *Ramburiella turcomana* Fischer-Waldheim [*Achurum* was excluded given its ambiguous phylogenetic position]. Divergence dates cited in the following discussion of intercontinental taxonomic connections emerged using the program r8s.

With respect to relationships between Eurasian and Nearctic taxa, our phylogenetic analyses corroborate some of Vickery's (1986, 1989, 1997) claims, but not all. Five sets of intercontinental associations, suggested by Vickery are discussed below.

Ageneotettix, *Aulocara* and *Cordillacris* are considered relatives of the Old World *Dociostaurus* (Vickery 1989). Jago's (1971) morphometric-based dendrograms support that association, with the four genera comprising part of a large clade. Vickery offers a number of biogeographic accounts, of which one involves the entry of ancestral forms into the Nearctic via the Bering land bridge during the Pleistocene. The possibility that the North American incursion may have been earlier, before the sundering of the Laurasian continent, is also proposed. Our parsimony tree does support a connection, but a fairly remote one, with the *Dociostaurus* group (E) basal (with a low bootstrap value of 63%) to the *Aulocara* group (A). In the Bayesian and ML trees, their branching relationship is unclear, with A′, E (along with B, C, and D), *Phlibostroma* and *Ramburiella* forming a polytomy. Knowing the branching order would help settle whether these gomphocerines originated in Eurasia or North America, but for now, the matter has to be considered unresolved. Nevertheless, application of r8s to the molecular data does shed light on the timing of events and an earlier incursion is favored. The estimated time of divergence between groups A and E is 48.6 to 68.0 Mya, very soon after the gomphocerine divergence and well before the Pleistocene. This range coincides roughly with the time when the two northern continents were still connected by eastern and western land bridges and climatic conditions were favorable for insect movement (Noonan 1986, Novacek 1999).

In contrast, connections between New and Old World congeners of *Aeropedellus* and *Chorthippus* occurred much more recently. Estimates place respective times of divergence at 5.3 to 7.1 and 3.4 to 4.4 Mya. These values agree roughly with Vickery's (1989) claim that migrations took place across Beringia during warm interglacial periods of the Pliocene-Pleistocene. Vickery favours an Old World origin, given that *Aeropedellus* and *Chorthippus* are much better rep-

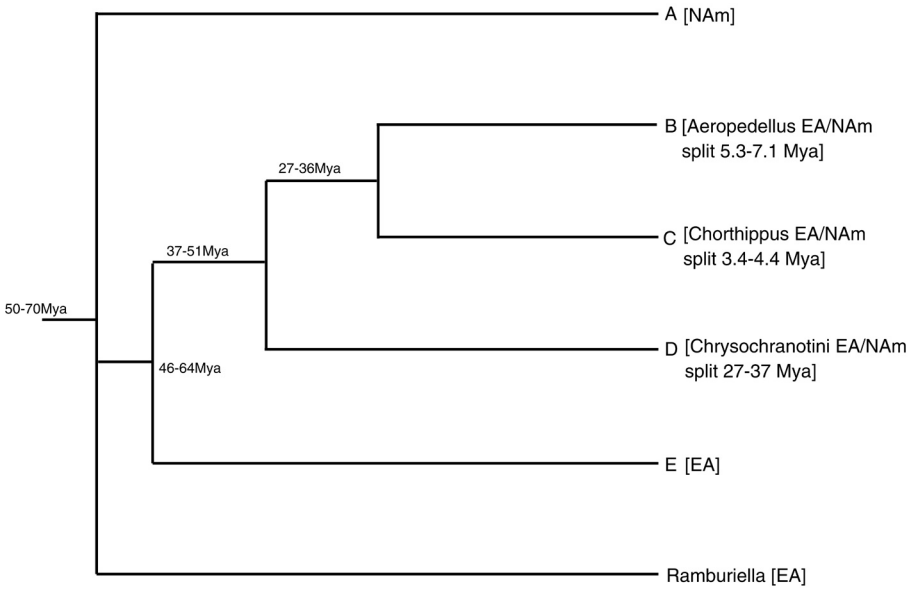

Fig. 2. Major phylogeographic events as interpreted from the collective analyses.

resented in the Palearctic. Despite Futuyma's (1998) cautioning against using this "relative-abundance" approach to deduce incursion polarities, the conjecture has merit, because within each of the B and C clades, Eurasian taxa are basal and paraphyletic to the Nearctic taxa, requiring only a single migration step in each case. If clade B (or C) had a North American common ancestor, then at least two migration events would be required to explain the Eurasian distributions.

Vickery (1986, 1989) also considered the Bering land bridge a means by which the North American Chrysochraontini, represented here by *Chloealtis*, were established from Eurasian antecedents, but at a time predating the *Aeropedellus* and *Chorthippus* migrations. Our age estimates do indeed support an earlier divergence. Calculations place the time of the *Chloealtis* – *Chrysochraon*/*Euthystira* split at about 27.3 to 37.3 Mya, the first half of the Oligocene. Climate, however, was cooler during this interval in comparison with the late Cretaceous/early Tertiary period (Noonan 1986, Askevold 1991). Still, movement could have taken place as stated, given that some species of *Chloealtis* are cold-adapted (Vickery & Kevan 1985). Vickery suggests that, because three of five species of *Chloealtis* have western distributions (Capinera *et al.* 2004), entry was by way of Beringia. However, until a molecular phylogenetic analysis is applied to all *Chloealtis* species, the possibility that one of the Atlantic bridges, still intact at the time (Askevold 1991), served as a conveyance cannot be discounted.

Vickery (1986, 1989) regards *Stenobothrus*, a close Eurasian relative of *Bruneria*, as the more ancient of the pair and the progenitor of the North American genus. He speculates that movement may have occurred during the Pleistocene or that, alternatively, *Bruneria* may have evolved previously from isolated descendants of a Holarctically distributed common ancestor. If there is a connection between *Bruneria* and *Stenobothrus*, it is a distant one (Fig. 1). Either their similar morphologies have changed very little over time or have evolved convergently.

While the subfamily affiliation of *Stethophyma* remains problematical, it is clear that the two species occupying the two northern continents are strongly connected. Vickery (1989, 1997) proposed various times of incursion into North America, from a very recent entry prior to the last glaciation, to a more ancient time when Laurasia was intact. The genetic distance separating *S. grossum* and *S. gracile* is much greater than that separating the pair of *Aeropedellus* or *Chorthippus* species. The estimated divergence time separating the two species makes them remarkably old, approximately 41.5 to 61.1 Mya, slightly after the split of clades A and E.

Figure 2 summarizes the major events as interpreted from the collective results. Owing to the uncertainty surrounding the order of branching of *Ramburiella*, clade A and clades B to E, we have represented their base as an unresolved polytomy, setting its root at 50 to 70 Mya. Based on parsimony in terms of minimal number of migrations, we favor a pattern in which the largely Eurasian clusters B, C, and D diverged from E (also Eurasian), rather than from the North American cluster A [this latter relationship was recovered using parsimony, but support was weak]. Groups B, C, D and E shared a common ancestor soon after the gomphocerine divergence, 46 to 64 Mya. About 37 to 51 Mya, the ancestor of B, C and D appeared, followed by a divergence event involving pre–B and pre-C about 10 Mya later. Also 10 Mya later, within D, a split between Nearctic and Palearctic Chrysochraontini occurred, with the former having evolved from Eurasian progenitors. More recently, during the Pliocene, North American species of *Aeropedellus* and *Chorthippus* evolved from their Eurasian ancestors. In conclusion, our phylogenetic analyses support Vickery's (1989, 1997) views that (a) there were multiple dispersals from Eurasia to North America; (b) some events were recent and some more ancient; and (c) land bridges on either side of the North American continent probably served as conveyances.

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