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A molecular phylogenetic analysis of the grasshopper genus *Melanoplus* Stål (Orthoptera: Acrididae) – an update

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Abstract

This research expands upon our previous molecular phylogenetic analysis of the genus *Melanoplus* by incorporating additional mitochondrial genes, taxa and specimens. Included are two monotypic genera suspected of close affiliation with *Melanoplus: Phoetaliotes* and *Bohemanella*. Portions of four mitochondrial genes, coding for cytochrome b, cytochrome oxidase subunits I and II, and NADH dehydrogenase subunit II, were sequenced and phylogenetically analyzed using (weighted and unweighted) parsimony and neighbor-joining methods. Maximum resolution of relationships was achieved using weighted parsimony and by treating all sequences, totaling 1716 base pairs, as a unit.

The following large clades emerged in parsimony analyses, supported by moderate to poor bootstrap values: A — *sanguinipes*, *femurrubrum*, *devastator*, *gaspesiensis*, *fasciatus*, *borealis*, *madeleineae*, *dawsoni*; B — *packardii*, *foedus*, *angustipennis*, *gladstoni*, *aspasmus*; C — *bivittatus*, *franciscanus*, *keeleri*, *calidus*, *littoralis*, *differentialis*; D — *infantilis*, *alpinus*, *aridus*, *Phoetaliotes*, *scudderi*; and E — *confusus*, *Bohemanella*, *marginatus*, *microtatus*. *M. lakinus* was basal to all species. Deviations from the conventional literature in which species are organized into species groups or series are discussed. It is concluded that many such groups are phylogenetically questionable; their validity warrants serious reconsideration.

Two phenomena – a rapid burst (or bursts) of speciation occurring early in the genus' evolution and an absence of complete lineage sorting for certain closely related species – are nicely illustrated by *Melanoplus*. We provide evidence that the massive radiation that took place within the past 4 My, inferred previously by Knowles and Otte, extends to a wider base of taxa, beyond the particular species studied by these authors.

Keywords

Melanoplus, mitochondrial DNA, phylogeny

(with respect to New World spine-throat grasshoppers): ". . .those relationships are unlikely to be resolved through the use of gross morphological characters such as those employed to date. Almost certainly it will be necessary to use molecular traits to group them properly."
— Perez-Gelabert & Otte (2000)

Introduction

The North American component of the grasshopper subfamily Melanoplinae is numerically dominated by the genus *Melanoplus* Stål. Included among the over 230 species (Vickery & Kevan 1983, Otte 1995) are species such as the economically important migratory grasshopper *M. sanguinipes* (F.) and the now-extinct Rocky Mountain grasshopper, *M. spretus* (Walsh). Species are usually identified by distinctive male genitalic characters which, according to Knowles and Otte (2000), may have evolved rapidly by sexual selection, possibly contributing to an explosive diversification within the genus during the past 4 million years (My). Various schemes, not totally concordant, have been proposed for assigning taxa to species 'series' or 'species groups' (Table 1). Hebard defined 45 species-groups (Gurney 1960), but unfortunately these listings were never published in their entirety (Hebard 1917, 1919).

This paper, as part of our ongoing efforts to understand relationships within *Melanoplus*, expands on a previous molecular analysis (Chapco *et al.* 1999) by including additional taxa and genes. This study also assesses the extent of polymorphism within species and examines its possible confounding effects with respect to achieving phylogenetic resolution at lower taxonomic levels.

Materials and methods

Sequences were obtained from 64 individuals distributed among 32 *Melanoplus* species and members of two monotypic genera with suspected close connections to *Melanoplus*: *Phoetaliotes nebrascensis* (Thomas) and *Bohemanella frigida* (Boheman). *Schistocerca gregaria* (Forskål) and *Locusta migratoria* (L.) served as outgroup taxa.

Total DNA was extracted according to Chapco *et al.* (1992) and Philips & Simon (1995). Regions of four mitochondrial genes (cytb, COII, ND2 and COI) were amplified and sequenced, following procedures described in Litzenberger & Chapco (2001a,b).

The resulting sequences were easily aligned by visual inspection and imported into MacClade (Maddison & Maddison 1992). Phylogenetic relationships were inferred using maximum parsimony (MP), weighted parsimony (wMP) [according to Farris' (1969) iterative reweighting scheme], and the neighbor-joining (NJ) method (Saitou & Nei 1987), all available in the software package, PAUP, version 4.0b8 (Swofford 2001). For the NJ analysis, distances were estimated using the K2 transformation (Kimura 1980). [Application of Posada and Crandall's (1998) MODEL-TEST program revealed that the model "TIM + I + G" best fitted the data; however, the latter returned the same bootstrap topology as that based on K2.] For all analyses, the four sequences were treated as a combined unit, a maneuver that, as in all our previous studies (Chapco *et al.* 2001; Litzenberger & Chapco 2001a, b), resulted in improved resolution and bootstrap support when compared to the outcomes of single gene studies.

Levels of support for derived relationships were estimated through 1000 bootstrap replicates. Within species, nucleotide diversities were estimated using MEGA version 2.1 (Kumar *et al.* 2001).

Table 1. List of *Melanoplus* species

Species	Species group or series
alpinus	*Infantilis*[1,3], *Collinus*[2]
angustipennis	*Angustipennis*[1,2]
aridus	*Aridus*[1-3]
aspasmus	*Marginatus*[6]
bivittatus	*Bivittatus*[1,2]
borealis	*Mexicanus*[1], *Fasciatus*[2]
bowditchi	*Flavidus*[1], *Bowditchi*[2]
bruneri	*Mexicanus*[1], *Utahensis*[2]
calidus	*Femur-nigrum*[4], *Immunis*[5]
confusus	*Confusus*[1], *Collinus*[2]
dawsoni	*Dawsoni*[1,2]
devastator	*Mexicanus*[1], *Devastator*[2]
differentialis	*Ponderosus*[1], *Robustus*[2]
fasciatus	*Fasciatus*[1,2]
femurrubrum	*Femurrubrum*[1,2]
foedus	*Packardii*[1,2]
franciscanus	-
frigidus	-
gaspesiensis	-
gladstoni	*Dawsoni*[1,2]
infantilis	*Infantilis*[1,2]
keeleri	*Keeleri*[1], *Collinus*[2]
kennicotti	*Kennicotti*[1], *Glaucipes*[2]
lakinus	*Lakinus*[2], *Occidentalis*[3]
littoralis	-
madeleineae	-
marginatus	*Marginatus*[1,6], *Plebejus*[2]
microtatus	*Marginatus*[6]
occidentalis	*Occidentalis*[1], *Flabellifer*[2]
packardii	*Packardii*[1,2]
sanguinipes	*Mexicanus*[1], *Spretus*[2]
scudderi	*Scudderi*[1], *Mancus*[2]
yarrowi	*Bivittatus*[1,2]

[1] Helfer (1987), [2] Scudder (1898), [3] Knowles & Otte (2000), [4] Hebard (1935), [5] Gurney (1960), [6] Rentz (1978)

Results and discussion

Approximately 1716 bases of the cytb, COII, COI and ND2 regions of mtDNA were sequenced (Appendix 1). For 10 museum specimens certain regions proved refractory, either to amplification or sequencing. Missing data were treated as unknown in parsimony analyses and ignored in pairwise comparisons for distance analyses. Across all genes, 680 sites were variable and 386 were phylogenetically informative. Maximum parsimony yielded 1435 equally parsimonious trees, each of step length 1438 and consistency index 39.2%: weighted parsimony recovered 240 equally parsimonious trees of length 316.4, with a somewhat higher consistency index value of 59.1%. There was general agreement between the MP and wMP topologies. The wMP majority-rule consensus tree is reproduced in Fig. 1, with MP and wMP bootstrap values placed on those branches that have at least 50% support using wMP, the latter method generally yielding higher values. Values (< 50%) for clades lacking bootstrap support are not shown. There is less agreement with the NJ tree for which resolution is generally poor (Fig. 2). Discussion will primarily refer to associations in Fig. 1, but where necessary, attention will be drawn to the NJ phylogram.

Five large "clades", labeled A to E for purposes of discussion, were recovered using parsimony (six clades: A, B, C′ to F′ using NJ). *M. lakinus* formed a separate clade basally to the above groups. While bootstrap support for these clusters varies from moderate to poor, the branching order among them is indeterminate. An examination of the NJ phylogram (Fig. 2) shows that the internodes separating these groups are quite short compared to many of the proximal branches. A similar phenomenon emerged in Knowles and Otte's (2000) study of a few species groups comprising mostly montane species of *Melanoplus*. Their interpretation was that evolution took place very rapidly within very short "opportunistic" periods, engendered by the numerous glaciation/deglaciation cycles during the Pleistocene. Statistically, their data suggest that diversification occurred sequentially, albeit rapidly, rather than in a single explosive radiation (Knowles 2000). We concur. The order, however, is not clearly resolved. Thus, we compared the likelihood of trees depicting other branching orders with the tree in Fig. 1 (represented as ABCDE: it has the highest likelihood) by the Kishino-Hasegawa (1989) test, and discovered that there are several trees (*e.g.* ACBDE) with likelihoods not significantly lower than that of ABCDE. However, not all permutations are statistically equally likely. Several trees had significantly lower likelihoods. For example, most trees in which clade E is displaced from its lower position to a more internal one (*e.g.*, ABECD) are significantly less likely. Discovering branching order may in fact be difficult to attain because the number of phylogenetically meaningful nucleotide substitutions is expected to be small within the periods represented by the short internode lengths (Hoelzer & Melnick 1994). Sexual selection, in this case involving male genitalic traits, is proposed as a possible mechanism underlying rapid evolutionary change (see references in Knowles & Otte 2000).

It would seem that, given our parallel results, which are based on a wider sampling of species groups and taxa with wider distributions, the speculations of Knowles and Otte (2000) on the evolutionary significance of the Pleistocene and the role of sexual selection, are not restricted to those taxa occupying the "sky islands" and western North America, but might also apply to all species of the genus. Assuming that mitochondrial DNA changes are approximately clock-like, application of the molecular clock (calibrated at a rate

of 1% per My per lineage, see references in Chapco *et al*. 2001) provides an estimate of 4.8 My (based on the most disparate pair of species, *devastator* and *kennicotti*) for the age of the genus: in rough agreement with the 3.6 My value of Knowles and Otte (2000). (If we use their rate of 1.15 %, then our estimate reduces to 4.1 My.)

While resolution at the higher levels is poor, this is generally not the case for many taxa within the major clusters. Phylogenetic relationships suggested by the molecular data are, in some cases, supportive of groupings proposed by early workers. There are, however, some notable deviations:

1) The clade (A), consisting of *sanguinipes*, *devastator*, *bruneri*, *borealis*, *femurrubrum*, *fasciatus*, *dawsoni*, *gaspesiensis* and *madeleineae,* is supported in 72% of bootstrap replicates (81% for MP). Helfer (1987) and Gurney & Brooks (1959) assigned the first four species to the species group Mexicanus. Interspersed among them are the remaining five species, the first three of which are assigned respectively to the species groups Femurrubrum, Fasciatus and Dawsoni. Since this is the largest clade, it will be dealt with in subsections.

1a) *Sanguinipes* and *femurrubrum*: The connection between this pair is extremely close (mean K2 distance, d = 1.15 ± 0.14 %) with four and three specimens of each species intermingled respectively (the implications of which are discussed later). A morphological trait that is used to define the Mexicanus group, a pronounced mesosternal hump (Gurney & Brooks 1959), may be of dubious phylogenetic value, given this result.

1b) *Devastator* and *sanguinipes*: In the previous study (Chapco *et al*. 1999) these two species, although close phylogenetically, were not as close as *sanguinipes* and *femurrubrum*, a somewhat surprising result given the ability of *devastator* and *sanguinipes* to hybridize (Orr *et al*. 1994). Our examination of additional specimens provides a more comprehensive picture. In particular, we included two specimens of each of three recognized subspecies (Gurney & Brooks 1959) of *M. sanguinipes*: *sanguinipes*, *vulturnus* and *defectus,* respectively occuring in the northern US and Canada, the US southeast and the US southwest. Subspecies *sanguinipes* had been used in our 1999 paper. In this study, subspecies *sanguinipes* clusters with subspecies *vulturnus* and *femurrubrum*, whereas subspecies *defectus* clusters with two specimens of *devastator*, a species that also occurs in the US southwest. Two other specimens of *devastator* occupy unresolved positions within clade A.

1c) *Gaspesiensis*, *madeleineae* and *borealis*: Both *gaspesiensis* and *madeleineae* are thought to have arisen in refugia during the Wisconsin glaciation period, or possibly in earlier glacial times, from ancestors of the now-widespread *M. borealis* (Vickery 1987, 1989). These interrelationships were studied and discussed fully in Chapco & Litzenberger (2002) and the addition of more taxa here leaves their relative positions unchanged.

1d) *Dawsoni*: Both Helfer (1987) and Scudder (1898) placed this species, along with *gladstoni,* in the species group/series Dawsoni, although Scudder regarded the latter as a hodgepodge of taxa. In our previous study, *dawsoni* and *gladstoni* were far removed from each other; they remain so with the inclusion of additional genes.

1e) *Fasciatus* and *borealis*: Scudder (1898) placed these species in the series Fasciatus, described by him as "not very homogeneous". Helfer assigned *fasciatus* and *borealis* to separate groups. Given the poor resolution within some parts of clade A, it is difficult to ascertain whether there is a direct linkage between them.

2) Recovered within the moderately supported (79%) clade B are *packardii*, *foedus*, *angustipennis*, *gladstoni* and *aspasmus*. Species *packardii* and *foedus* are difficult to distinguish morphologically, except for certain aedeagal features (Brooks 1958); both are assigned to the Packardii species group (Helfer 1987). Molecular support for connecting the two remains very strong (97%) with the inclusion of more sequence. As with *sanguinipes* and *femurrubrum*, there is an overlap of specimens. The remaining three species belong to the Angustipennis, Dawsoni and Marginatus species groups respectively (Table 1). The direct connection between the pair (*packardii*, *foedus*) and *angustipennis,* discovered earlier (Chapco *et al*. 1999), is sustained with the addition of more data. However, an association between *gladstoni* and *aspasmus*, which had not emerged previously, is indicated here, albeit with 68% bootstrap support (87% using NJ).

3) Clade C, consisting of *bivittatus*, *keeleri*, *differentialis*, *calidus*, *franciscanus* and *littoralis,* emerged in parsimony searches; however, bootstrap support was below 50%, a result which may be due to the absence of some sequences for the last three species (Appendix 1). Species *bivittatus*, *keeleri*, *calidus* and *differentialis* belong to four separate species groups or series (Table 1), whereas, to our knowledge, *franciscanus* and *littoralis* are unassigned. Some connections within clade C, however, are suggested in the older literature. Similarities between *franciscanus* and members of the Immunis species group, to which *calidus* belongs, were noted by Gurney (1960). Roberts (1942) pointed out common features between *littoralis* and *differentialis*, a linkage that is moderately supported (72%) by our molecular data. In the previous investigation (Chapco *et al*. 1999), *differentialis* was very strongly tied to *bivittatus*, but with the inclusion of more species the association, while still present, is more tenuous. Another pair, *bivittatus* and *yarrowi*, characterized by large body size, is united in the group Bivittatus (Table 1). Their relationship, nevertheless, has to be regarded as unknown, given the poor bootstrap support for clades C and E (the latter containing *yarrowi*). Interestingly, neighbor-joining does link *yarrowi* with *franciscanus* (80% bootstrap support) within clade C, but this may be an artifact reflecting the lack of complete sequence for *franciscanus* (Appendix 1).

4) Clade D, comprised of *infantilis*, *alpinus*, *aridus*, *Phoetaliotes nebrascensis* (Thomas) and *scudderi,* is weakly supported (55%); there are, however, some noteworthy internal connections supported by high bootstrap values. The previously recovered association (Chapco *et al*. 1999) involving *alpinus* and *infantilis* is preserved here with 100% bootstrap support, justifying their assignment to the same species group, Infantilis (Table 1). Scudder (1898) placed *alpinus* and *infantilis*, along with *confusus* and *keeleri,* in the series Collinus, a conglomerate not supported by our data. The connection between *Phoetaliotes* and *aridus* is extremely strong (99%) and yet there is nothing in the literature to suggest such a relationship or, indeed, that there should be an intimate association between *Phoetaliotes* and *Melanoplus* itself. If additional data were to sustain the internal position of *Phoetaliotes*, it would seem reasonable either to rename this species *Melanoplus nebrascensis* or to regard *Melanoplus* as a paraphyletic taxon. Since *Phoetaliotes* has only one distinctive feature, a disproportionately large head relative to the thorax (Scudder 1898, Helfer 1987), the former view is favored. The species *scudderi* is part of clade D, but its association is not strongly maintained.

5) Within weakly supported (59%) clade E, several interesting associations emerge. There is a moderately strong (83%) relationship between *confusus* and *occidentalis*, belonging respectively to their nominate species groups. Occidentalis also includes *lakinus*, but the latter is outside E (see below). There is an extremely tight affiliation (100 %) between *marginatus* and *microtatus*, consistent with their placement within the species group Marginatus (Rentz

1978). Marginatus includes *aspasmus*, but clearly the latter is far removed (Fig. 1). On the basis of relatively little differentiation of male genitalia, Marginatus is regarded as the most primitive of all Melanoplus species groups (Rentz 1978), a point that is discussed further below. Another highly supported cluster consists of *Bohemanella frigida*, *kennicotti*, and *bowditchi*. Over the past 50 y the first species has been assigned to the Holarctic tribe Podismini or, as *M. frigidus* Boheman, to the Nearctic tribe Melanoplini (Vickery 1987, 1989). A previous molecular investigation (Litzenberger & Chapco 2001a) presents and discusses evidence for the latter point of view. With the inclusion of more species of *Melanoplus* in this study, we were able to ascertain the relative position of *frigidus* within the genus and it would appear that its nearest relatives are *kennicotti* and *bowditchi*. The last two species belong to different species groups and *frigidus*, to our knowledge, has never been assigned to one. It is possible that *bowditchi*'s involvement may be spurious since we were only able to obtain sequences for two genes (Appendix 1). Both *frigida* and *kennicotti* are boreal species with overlapping ranges (Vickery 1987); however, there is nothing else in the early writings that would have predicted a connection between them.

From what was stated above regarding the "primitiveness" of the species group Marginatus, one might expect the latter to be basal to all species of *Melanoplus*. Species of Marginatus, along with the other taxa in clade E are certainly basal to clades A to D, but with rather weak support (52%), although, as pointed out above, moving E from its present position significantly lowers the likelihood. In any case, basal to all species (with 87% support) is a species that is not part of Marginatus, *M. lakinus* (Scudder). Statistically, moving *lakinus* from its basal position to a more internal one significantly lowered the likelihood, except in one instance in which *lakinus* and cluster E were reversed. The p-level, however, was just above the 5% mark at 7%. In the study by Knowles and Otte (2000) *lakinus* also emerged as basal to *Melanoplus*, depending on the method of tree construction and choice of outgroup.

If present results are confirmed with the inclusion of additional taxa and other sequences, in particular those of nuclear genes, the validity of many species groups or series will have to be questioned. Monophyly of certain other species groups of *Melanoplus* was similarly disputed by Knowles and Otte (2000). Such taxonomic units may be useful from an identification point of view, but it should not be expected that they necessarily reflect phylogeny. In their molecular phylogenetic analysis of the order Orthoptera, Rowell and Flook (1998) pointed out that if a reliable molecular phylogeny exists, but is discordant with the outcome of morphological studies, the most likely explanation is that many anatomical traits relied upon by taxonomists are subject to convergent evolution. Assuming our analysis accurately mirrors phylogeny, then convergence could easily explain the joining together of, for example, *bivittatus* and *yarrowi* or the various members in Scudder's (1898) Collinus species series. We have already alluded to the possible role of sexual selection underlying changes in genitalic characters that may have accompanied (caused?) the massive radiation in the early history of *Melanoplus*. Presumably, these changes took place during the short internodes depicting bouts of rapid evolution (Fig. 2).

But what of other morphological traits, commonly used to identify species, that we suspect of having evolved convergently? If morphological changes and speciation are decoupled phenomena, as some would suggest (*e.g.*, Larson 1989), then one reasonable scenario is that nongenitalic morphological traits evolved somewhere along the various lineages postradiation. If we consider, for instance, species pairs *Bohemanella* and *kennicotti*, *gladstoni* and *aspasmus* or *aridus* and *Phoetaliotes* in the NJ phylogram, it will be noticed that each pair is connected by relatively long branches which could allow sufficient opportunity for the accumulation of independent morphological changes. Such changes may have resulted in convergence (*e.g.*, in species group Bivittatus) or simply autapomorphy (*e.g.*, in *Phoetaliotes*). In contrast, others (*e.g.*, Arnqvist & Thornhill 1998) have argued that genitalic and nongenitalic characters are correlated and influenced by a common array of genes. In this case we would have to conclude that at least some of the nongenitalic traits may have also evolved during the short bursts of speciation. These different viewpoints are admittedly based on different sets of organisms. There is, therefore, all the more reason to undertake detailed analyses of both genitalic and nongenitalic traits in *Melanoplus*, perhaps with the aid of scanning electron microscopy (Dakin 1987) and more modern multivariate techniques such as landmark-based morphometrics (Bookstein 1991). Traits could then be mapped onto a reliable phylogeny to ascertain where changes took place and whether the two types of characters are interrelated.

Table 2 lists, in increasing order, nucleotide diversities for those species with sample sizes > 1. Additional sequenced specimens are included to provide as large a sample as possible. Many factors can influence diversity levels, such as population size and time since divergence, modulated perhaps by selection and gene flow. Although *M. sanguinipes* is probably the most widely distributed species and, at times, an outbreak species (Vickery & Kevan 1983), its diversity is at the lower end of the range. In terms of time of origin, application of the molecular clock would suggest that, on average, the "alleles" for this species shared a common ancestor about 490,000 y ago. This relatively short time may possibly account for the absence of lineage sorting that accompanied the evolution of *sanguinipes* and *femurrubrum* from their common, presumably polymorphic, ancestor. Such "sorting out" may well have already occurred during events leading to the evolution of the monophyletic, somewhat older taxa, *dawsoni*, *Bohemanella* and *confusus*, which are two to three times as diverse as *sanguinipes*. Another monophyletic species is *bivittatus*, with a diversity only slightly larger than that of *sanguinipes*. It remains a possibility, however, that *bivittatus* may prove not to be monophyletic, with the inclusion of additional specimens of other species in clade C.

The species *infantilis* (the least diverse and monophyletic) and *devastator* (most diverse and not monophyletic) are somewhat enigmatic. A recent population increase might possibly account for the observed coalescence of specimens of *M. infantilis*, although this species has not, at least in agricultural times, exhibited outbreak tendencies. With respect to *devastator*, there would seem to be two groups. The first, consisting of *devastator* 1 and 2, clusters with the subspecies *defectus* of *M. sanguinipes* (mean distance of 0.80 ± 0.16 %) and the second, consisting of *devastator* 3 and 4, is basal to all species, although unresolved, within the large cluster A. The large nucleotide diversity of *devastator* is mostly the result of the extraordinary branch length of *devastator* 3 and of the internode connecting the latter and *devastator* 4 to their root. This pair is clearly much older, whereas *devastator* 1 and 2 are more recently evolved, sharing a common ancestor with the two *defectus* subspecies of *sanguinipes* about 400,000 y ago. As with *sanguinipes* and *femurrubrum*, there may not have been sufficient time for lineage sorting. The situation involving *packardii* and *foedus* is similar, with three "alleles" of *packardii* having fairly old lineages and another sharing a common ancestor with *foedus*.

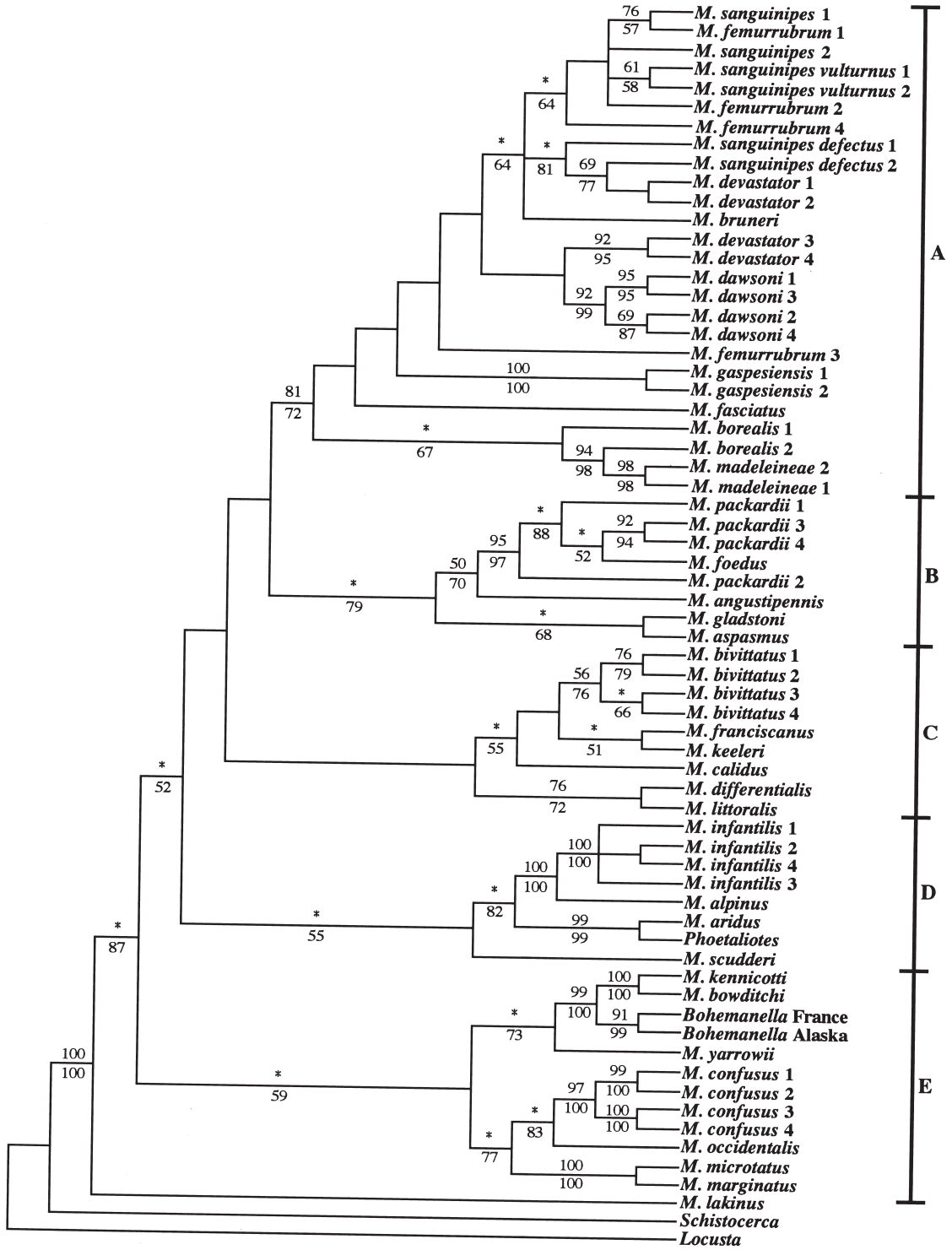

Fig. 1. Consensus wMP tree. Bootstrap values (±50%) for unweighted parsimony are indicated above and below branches respectively; a * signifies values < 50%. Unlabelled branches are considered unresolved using both methods.

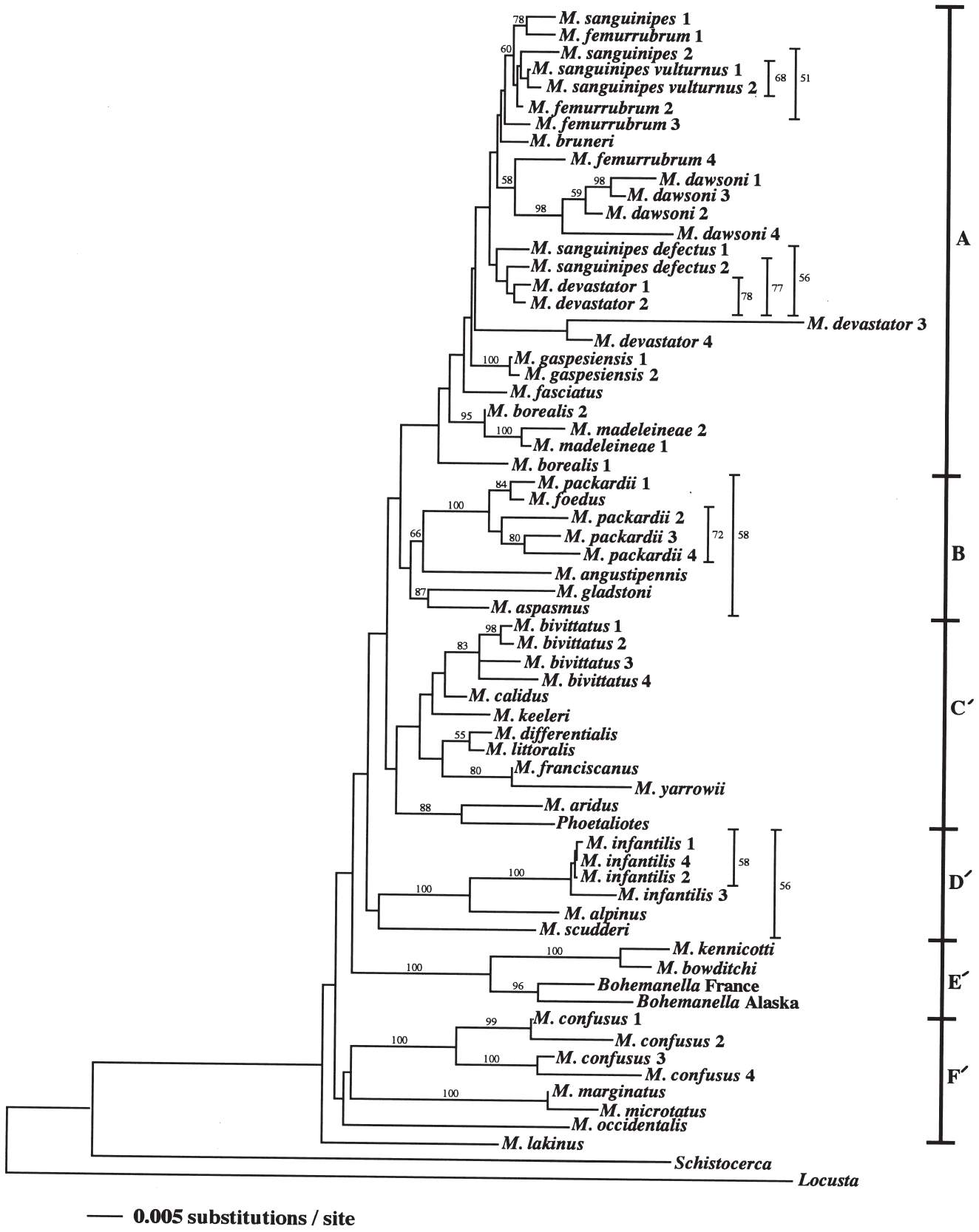

Fig. 2. NJ phylogram. Bootstrap values (± 50%) are indicated above branches.

Table 2. Percent nucleotide diversities ± $s_{\bar{x}}$.

Species	% Diversity ± $s_{\bar{x}}$ (n)
infantilis	0.39 ± 0.10 (4)
gaspesiensis	0.70 ± 0.12 (9)
sanguinipes	0.97 ± 0.13 (9)
madeleineae	1.01 ± 0.13 (10)
bivittatus	1.12 ± 0.16 (4)
femurrubrum	1.27 ± 0.18 (5)
borealis	1.28 ± 0.16 (10)
packardii	1.67 ± 0.27 (4)
dawsoni	1.81 ± 0.26 (4)
frigidus	2.63 ± 0.34 (6)
confusus	2.94 ± 0.31 (4)
devastator	3.29 ± 0.35 (4)

The North American southwest has been proposed as a major place of origin for the tribe Melanoplini (Rehn 1954, 1958; Vickery 1987), given the distribution of extant taxa. In this study, *lakinus* is a southwestern species and emerges basally to all *Melanoplus*. Within clades B and D, southwestern species *aspasmus* and *aridus* occupy basal positions. This may also be true of *devastator* (3 and 4) with respect to clade A and the pair (*marginatus*, *microtatus*) with respect to a small clade within E. If these indications are confirmed with further study, then we would have to conclude that there must have been several incursions from this region of North America.

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Appendix 1. Species sources and accession numbers.

Species	Source	Accession Numbers (cytb, COII, ND2, COI)
Locusta migratoria	GenBank	X80245[a]
Schistocerca gregaria	SFU	AF145491, M83966[b], AF227278, AF260532
Bohemanella frigida	UMI	AF227306[c], AF227307[c], AF227308[c], -
Bohemanella frigida	URF	AF227297[c], AF227298[c], AF227299[c], AF317193
Melanoplus alpinus	MU	AF145558[d], AF145559[d], AF317466, AF317467
Melanoplus angustipennis	UR	AF145511[d], AF145512[d], AF317180, AF317181
Melanoplus aridus	UR	AF317176, AF317177, AF317178, AF317179
Melanoplus aspasmus	UMI	AF145562[d], AF145563[d], AF317468, AF317469
Melanoplus bivittatus 1	UR	AF145523[d], AF145524[d], AF227282[c], AF260535[e]
Melanoplus bivittatus 2	UR	AF317148, AF317149, AF317150, AF317151
Melanoplus bivittatus 3	UR	AY083397, AY083398, AY083399, AY083400
Melanoplus bivittatus 4	UR	AY083401, AY083402, AY083403, AY083404
Melanoplus borealis 1	MU	AF317438[f], AF317439[f], AF317440[f], AF317441[f]
Melanoplus borealis 2	UR	AY063147f, AY063148f, AY063149f, AY063150f
Melanoplus bowditchi	UMI	AF145551[d], -, AY083455, -
Melanoplus bruneri	UR	AF145555[d], AF145556[d], AF317188, AF317189
Melanoplus calidus	UMI	AF145538[d], AY083444, -, -
Melanoplus confusus 1	UR	AF145496[d], AF145497[d], AF317156, AF317157
Melanoplus confusus 2	UR	AF317158, AF317159, AF317160, AF317161
Melanoplus confusus 3	UR	AY083413, AY083414, AY083415, AY083416
Melanoplus confusus 4	UR	AY083417, AY083418, AY083419, AY083420
Melanoplus dawsoni 1	UR	AF145514[d], AF145515[d], AF317436, AF317437
Melanoplus dawsoni 2	UR	AY063135[f], AY063136[f], AY063137[f], AY063138[f]
Melanoplus dawsoni 3	UR	AY083436, AY083437, AY083438, AY083439
Melanoplus dawsoni 4	UR	AY083440, AY083441, AY083442, AY083443

"-" = no sequence
a = Flook *et al.* 1995, b = Liu & Beckenbach 1992, c = Litzenberger & Chapco 2001a, d = Chapco *et al.* 1999, e = Chapco *et al.* 2001, f = Chapco & Litzenberger 2002, g = Litzenberger & Chapco 2001b

Melanoplus devastator 1	UR	AF145505d, AF145506d, AF317162, AF317163
Melanoplus devastator 2	UR	AF317164, AF317165, AF317166, AF317167
Melanoplus devastator 3	UR	AY083421, AY083422, AY083423, AY083424
Melanoplus devastator 4	UR	AY083425, AY083426, AY083427, AY083428
Melanoplus differentialis	UR	AF145546d, AF145547d, AF317434, AF317435
Melanoplus fasciatus	UR	AF317184, AF317185, AF317186, AF317187
Melanoplus femurrubrum 1	UR	AF145526d, AF145527d, AF317138, AF317139
Melanoplus femurrubrum 2	UR	AF317140, AF317141, AF317142, AF317143
Melanoplus femurrubrum 3	UR	AF317144, AF317145, AF317146, AF317147
Melanoplus femurrubrum 4	UR	AY083393, AY083394, AY083395, AY083396
Melanoplus foedus	UR	AF145531d, AF145532d, AF317191, AF317192
Melanoplus franciscanus	UMI	AF145534d, -, -, -
Melanoplus gaspesiensis 1	UR	AY063175f, AY063176f, AY063177f, AY063178f
Melanoplus gaspesiensis 2	UR	AY063179f, AY063180f, AY063181f, AY063182f
Melanoplus gladstoni	UR	AF145517d, AF145518d, AF317182, AF317183
Melanoplus infantilis 1	UR	AF145520d, AF145521d, AF227281c, AF260536e
Melanoplus infantilis 2	UR	AF317152, AF317153, AF317154, AF317155
Melanoplus infantilis 3	UR	AY083405, AY083406, AY083407, AY083408
Melanoplus infantilis 4	UR	AY083409, AY083410, AY083411, AY083412
Melanoplus keeleri	UR	AF145553d, AY083449, AY083450, AY083451
Melanoplus kennicotti	UR	AF145529d, AF317431, AF317432, AF317433
Melanoplus lakinus	UR	AF317172, AF317173, AF317174, AF317175
Melanoplus littoralis	UMI	AF145542d, AY083445, -, -
Melanoplus madeleineae 1	UR	AY063199f, AY063200f, AY063201f, AY063202f
Melanoplus madeleineae 2	UR	AY063191f, AY063192f, AY063193f, AY063194f
Melanoplus marginatus	UMI	AF145560d, AF145561d, AF227286c, AY004209g
Melanoplus microtatus	UMI	AF227283c, AF227284c, AF227285c, AF317190
Melanoplus occidentalis	UMI	AF145549d, AY083452, AY083453, AY083454
Melanoplus packardii 1	UR	AF145508d, AF145509d, AF227280, AF260534
Melanoplus packardii 2	UR	AF317168, AF317169, AF317170, AF317171
Melanoplus packardii 3	UR	AY083429, AY083430, AY083431, AY083432
Melanoplus packardii 4	UR	AY083433, AY083434, -, AY083435
Melanoplus sanguinipes 1	UR	AF145499d, AF145500d, AF227279c, AF260533e
Melanoplus sanguinipes 2	UR	AF317130, AF317131, AF317132, AF317133
Melanoplus sanguinipes defectus 1	UR	AY083385, AY083386, AY083387, AY083388
Melanoplus sanguinipes defectus 2	UR	AY083389, AY083390, AY083391, AY083392
Melanoplus sanguinipes vulturnus 1	UR	AY083377, AY083378, AY083379, AY083380
Melanoplus sanguinipes vulturnus 2	UR	AY083381, AY083382, AY083383, AY083384
Melanoplus scudderi	SHSU	AF145502d, AF145503d, AF260537e, AF260538e
Melanoplus yarrowii	UMI	AF145544d, AY083446, AY083447, AY083448
Phoetaliotes nebrascensis	UR	AF260552e, AF260553e, AF260539e, AF260540e