

Biogeography of Sulawesi grasshoppers, genus *Chitaura*, using DNA sequence data

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Abstract

A molecular phylogeny is presented for part of the genus *Chitaura* (Orthoptera: Acrididae) based on DNA sequence data from the mitochondrial cytochrome oxidase 1 locus. *Chitaura* has at least 20 parapatrically distributed colour forms in Sulawesi and the Moluccas. While the status of these forms is uncertain, they behave as genetically isolated species where they are in contact. Molecular data are consistent with speciation *in situ* with isolation mainly due to wet rainforest on mountain ridges. *Chitaura* has apparently been diversifying within Sulawesi for 7–14 Ma and reached the Moluccas from North Sulawesi some 2.5–5 Ma ago.

Introduction

Grasshoppers in the genus *Chitaura* I. Bolivar, 1918 (Orthoptera: Acrididae) are flightless, brightly-coloured inhabitants of forest edge, stream-side and light gap habitats in primary and secondary forest. The genus has 10 described species from Sulawesi, 9 of which are endemic and one occurs also in the Moluccas. Outside Sulawesi, the genus is recorded from southern India, Java, Bali, Palawan and several Moluccan islands (Ambon, Haruku, Saparua, Seram, and the Kai Islands) but has only one or two species in each locality. The closely related, fully winged genus *Oxytauchira* Ramme, 1941 has a single representative in Sulawesi (Monk and Butlin, 1990).

Within Sulawesi, the *Chitaura* species are apparently parapatric, suggesting speciation *in situ* as a result of island or habitat fragmentation in the past. This pattern is found in several other taxa. It has been documented most clearly in the

Sulawesi macaques but has also been described in pond-skaters, cicadas, carpenter bees, butterflies, limacodid moths and tiger beetles (Whitten *et al.*, 1987; Ciani *et al.*, 1988; Knight and Holloway, 1990; Cassola, 1996). If these current distributions do reflect past land or habitat islands, they should be broadly coincident across taxa and related to topographic features unless the patterns have been obscured by subsequent dispersal and/or hybridisation. The phylogenetic relationships of the species or populations may reflect the history of vicariance within taxa.

Thus *Chitaura* provides a model for the within-island diversification which is a major contributor to the high proportion of endemic species in the Sulawesi fauna in general. However, it is difficult to relate patterns of present-day distribution, or phylogenetic relationships, to past geological or climatic events without an independent timescale. The 'molecular clock' (Avice, 1994) provides an opportunity to determine *relative* timings of branching events with confidence, and *absolute* timings within the limits set by the available calibrations. Here we describe a phylogeny for *Chitaura* based on mitochondrial DNA sequence data from 27 individuals representing seven of the described species and up to nine additional species (plus one individual of *Oxytauchira gracilia* and an outgroup). This is the first molecular phylogeny for a SE Asian insect group. Unfortunately, there is currently no morphologically-based phylogenetic hypothesis for the genus, and species definitions are in need of revision, as discussed below.

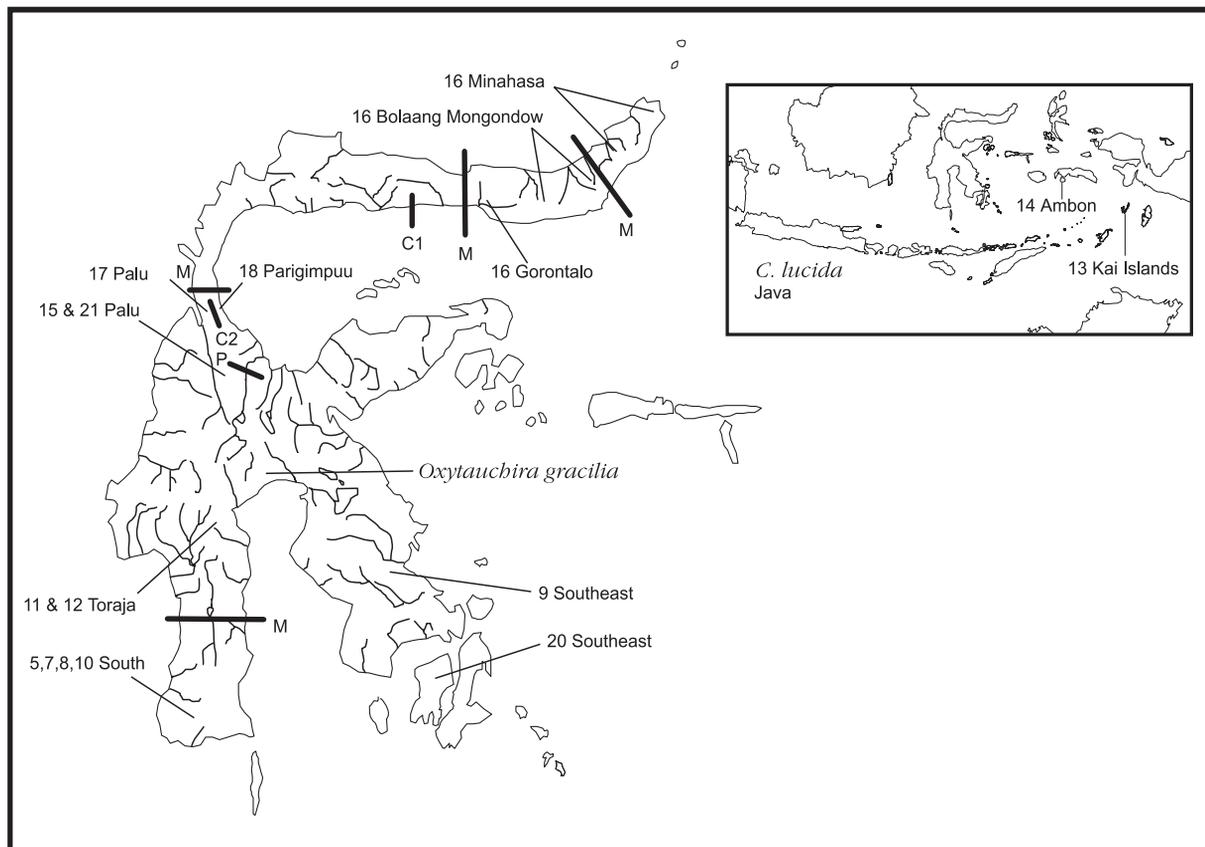


Fig. 1. Locations of samples included in the molecular analysis and positions of contact zones. Contact zones are marked with heavy lines for *Chbitaura* (C1 between *Chbitaura* 16 and 18 and C2 between *Chbitaura* 18 and 17) in relation to those in macaques (M) and one in the pond-skater *Philomera* (P) (see text for references). Although parapatric distributions have been described for other taxa, contact zones have yet to be identified. Inset: Part of SE Asia showing the locations of *Chbitaura* samples from outside Sulawesi.

Distribution and contact zones

The currently described species are defined, often from only a few specimens, on the basis of the distinctive adult coloration (Willemse, 1956; Hollis, 1975). Our extensive collections from Sulawesi (Fig. 1) suggest that the actual number of endemic colour forms is at least 20. For present purposes, these forms are identified by numbers, some of which are tentatively equated with described species (Fig. 2). In more than 50 collecting sites, we have always found only one form, confirming that distributions are parapatric. However, the geographical extent of individual forms apparently varies widely. At least four, and possibly seven, forms exist in the Gunung Lompobatang area of South Sulawesi, while a single colour form is found over the entire eastern half of North Sulawesi. Given this

taxonomic uncertainty, the molecular phylogeny should be interpreted as a phylogeny of individuals rather than species.

Defining the extent and status of these forms depends on the identification of contact zones. To date, we have identified two such zones (Fig. 1). In both cases there is an abrupt transition over a distance of about 200 m, coincident for several colour pattern elements and with no intermediates detected. In north Sulawesi, *Chbitaura* 16 (= *C. brachyptera* I. Bolivar) has hind femora that are red proximally fading to greenish-yellow distally and without spots. The thoracic sternites are red. These and other colour pattern characteristics distinguish it from *Chbitaura* 18 whose hind femora are orange-yellow with three distinctive black markings and whose thoracic sternites are white with narrow dark margins. The two forms have been found

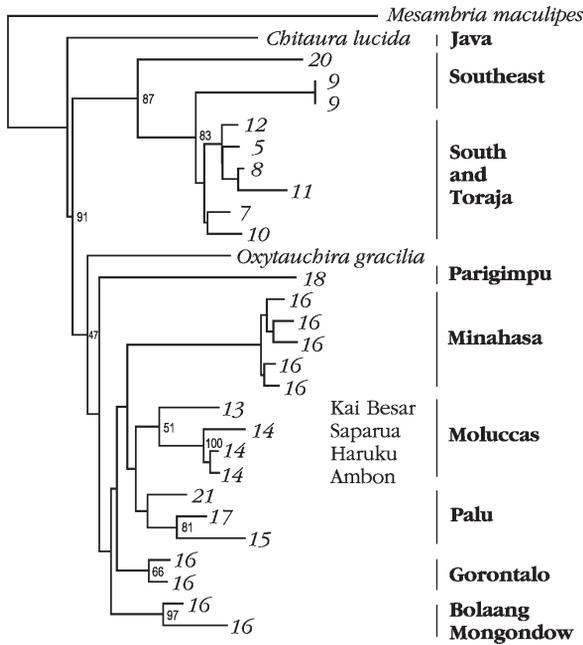


Fig.2. Neighbour-joining tree based on Jukes-Cantor distances between sequences from the mitochondrial CO1 locus, rooted by outgroup. The tree topology includes all of the well supported nodes in a Wagner parsimony tree. Bootstrap values from the parsimony tree are included on these nodes. Tentative associations of numbered colour forms with described species are as follows: 5 - *C. elegans*; 7 - *C. vidua*; 10 - *C. poecila*; 13 - *C. striata*; 14 - *C. moluccensis*; 16 - *C. brachyptera*. The remaining colour forms do not correspond to any of the descriptions in Willemse (1956).

on either side of a small river near Tilamuta, west of Gorontalo. No site has been found with both forms present. The differences between forms *Cbitaura* 18 and 17 are less striking but, nevertheless, include several apparently independent pattern elements on the legs, head, thoracic sternites and abdomen. The forms occur together in a small, recently cut clearing where the main Palu-Gorontalo road crosses the mountains of the isthmus north of Palu. Despite occurring together, no intermediates have been found. These observations strongly suggest genetic independence between the taxa and are most readily explained by secondary contact following allopatric divergence.

Although the two contact zones are in the same general areas as contacts in macaques, there are substantial differences. The *Cbitaura* 18/16 contact in North Sulawesi is displaced some 50 km west of a macaque contact zone which is located at the lowland area around

Gorontalo (Ciani *et al.*, 1988). The *Cbitaura* 18/17 contact near Palu (Fig.1) is orientated east-west across the mountain ridge rather than north-south along the isthmus. In this respect it is more similar to the contact zone in *Ptilomera* pond-skaters (Polhemus and Polhemus, 1990).

Molecular analysis

A molecular phylogenetic analysis has been conducted on 29 individuals using 290 base pairs of DNA sequence from the mitochondrial cytochrome oxidase 1 (CO1) locus. DNA sequence was obtained from either dried or ethanol-preserved specimens. DNA was purified from a single hind femur by a silica-based extraction method and then the sequence of interest was amplified by the Polymerase Chain Reaction (PCR). The amplified DNA was manually sequenced directly from the PCR product following separation of the two strands. Details of these methods are given in Walton *et al.* (1997) and general descriptions can be found in Avise (1994). Mitochondrial DNA was chosen because of its maternal inheritance and high copy number, and because many 'universal' PCR primers are available. The CO1 locus has been amplified previously from a range of insect taxa which aids interpretation of sequence data (Lunt *et al.*, 1996).

The resulting sequence data can be analysed in two ways: cladistically, treating the 290 base-pairs as characters with four possible states corresponding to the four nucleotides of DNA, or phenetically, by calculating distances based on the proportion of bases that differ between each pair of sequences. Here we consider the results of the distance-based methods, which have the advantage of providing divergence time estimates, but the major features of the phylogenetic relationships inferred from cladistic approaches are concordant with these results (Walton *et al.*, 1997). Divergence time estimates assume a 'molecular clock', that is they assume that, for a given locus, sequence divergence accumulates at a steady rate and that this rate is similar in related taxa (Avise, 1994).

Twenty-three individuals from 13 colour forms from within Sulawesi, 4 Moluccan individuals, *Cbitaura lucida* from Java, and an outgroup were included in this analysis (Figs.1 and 2). The analysis (Fig.2) demonstrates that the Sulawesi species are monophyletic and that the Moluccan species fall within the Sulawesi clade. This includes the geographically isolated Kai Is-

land species which has the most distinctive colour pattern (lateral white or yellow stripes on the head and thorax, present in all other Sulawesi and Moluccan forms, are replaced by a broad central stripe). Within Sulawesi, relationships are strongly patterned geographically, with a deep division between South + SE Sulawesi and North Sulawesi, in particular. This geographic pattern reinforces the implication of speciation *in situ*. The Moluccan species fall within the North Sulawesi clade suggesting colonisation from this part of the island, perhaps most probably from the Banggai peninsula which we have been unable to sample so far. Surprisingly, *Oxytauchira gracilia* falls within the Sulawesi *Chitaura* clade suggesting that a revision of generic boundaries is necessary.

Within colour forms, it is only for *Chitaura 16* (= *C. brachyptera*) that we have sequenced sufficient individuals to make inferences about their status. Genetically similar individuals are clearly grouped geographically but distances between these groups are substantial, reaching as much as 10%. This is greater than the distances between some other colour forms and greater than the distances between well-differentiated species in other taxa (Avice, 1994), including other grasshoppers (Hewitt, 1996). It seems likely that cryptic species, separated by contact zones, exist within this colour form which is one of the most widespread.

Maximum sequence divergence within the Sulawesi clade is 14%. This figure can be used to provide a minimum estimate for the timescale of diversification within the island of Sulawesi by comparison with calibrations derived from other insects. Using a range of 1-2% divergence per million years for mitochondrial DNA coding regions (Avice, 1994) the timescale is 7 to 14 Ma. Maximum divergence within the Moluccan clade is 4.5% and this is only slightly less than the minimum divergence between Moluccan and North Sulawesi specimens. Thus, colonisation of the Moluccas appear to have taken place 2.5 to 5 Ma ago.

Within Sulawesi, the major branches in the phylogenetic tree fit better with an hypothesis of vicariance due to mountain barriers than an alternative hypothesis based on inundation of lowland areas, or break-up of forest. For example, taxa from the Gunung Lompobatang and Toraja areas are closely related despite separation by the lowland area near Parepare where a macaque contact zone is located (Fig.1). This is probably the only area to have been inundated by the sea during the Pleistocene. Breakup of

rainforest during drier climatic intervals might explain the positions of other macaque contact zones. However, the large-scale distribution of *Chitaura* (present in Java, Sulawesi, the Moluccas and Palawan, absent in Sumatra, Borneo and peninsular Malaysia) suggests that it is excluded from perennially wet forests and occurs only in drier monsoon forest habitats. Thus dry intervals may not have been as important an influence on distribution of *Chitaura* as they were for macaques. Forms 17 and 18 are separated only by a narrow mountain chain, yet are quite distinct in DNA sequence. Form 16 includes several divergent clades in the molecular tree including the Gorontalo and Bolaang Mongondow samples from opposite ends of the same mountain block. These patterns are consistent with persistent separation by wet rainforest on mountains. Contact 17/18 north of Palu may be a recent consequence of deforestation along the road.

Conclusions

Our molecular data for *Chitaura* are consistent with the presence of the genus on the island since its formation by rifting of South Sulawesi away from Borneo some time more than 10 Ma ago (Hall, 1998 this volume). They do not place any upper limit on this figure since populations on the island may have contracted at any time post-colonisation. However, this may be viewed as a lower limit, albeit with quite large potential error because of the reliance on external calibration of the molecular clock. It is interesting that the divergence between Javan and Sulawesi individuals is only slightly greater than the divergence within Sulawesi. This suggests a relatively homogeneous population across the Sunda shelf from Java, through Borneo to Sulawesi (and Palawan?) around 10-15 Ma ago. Presumably *Chitaura* has since been excluded from Borneo by climatic change.

Within Sulawesi, the distribution patterns and relationships are not concordant either with the macaque distributions or with expected positions of separate islands in the past. They are more consistent with the positions of current extrinsic barriers to gene exchange. The large number of forms on the single isolated Gunung Lompobatang, and the molecular divergence within form 16 in North Sulawesi, are suggestive of divergence across local mountain barriers. Although *Chitaura* can be found at high elevations (at least 2000 m) they are most common by

larger water courses or at forest edges. Thus forested mountain ridges may represent substantial barriers to dispersal. The deep separation of some of the forms within the phylogenetic tree suggests that these barriers have been stable for at least 5 Ma. The Moluccan islands appear to have been colonised from North Sulawesi or the Banggai peninsula relatively recently (2.5-5 Ma ago). This is consistent with recent geological reconstructions which suggest that these islands have only been above water and close to Sulawesi on approximately this timescale (Hall, 1998 this volume).

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