Mechanisms generating biological diversity in the genus *Platypleura* Amyot & Serville, 1843 (Hemiptera: Cicadidae) in southern Africa: implications of a preliminary molecular phylogeny

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Truly understanding biological diversity requires a move from descriptive studies to mechanistic interpretations based on comparative biology and a thorough recognition of the natural history of the focal organisms. A useful step in such comparative studies is the generation of a phylogeny, so that one can assess the phylogenetic independence of the focal taxa and trace the evolutionary significance of their characteristics. As a preliminary to such studies on the platypleurine cicada genus *Platypleura*, we sequenced 498 bases of the cytochrome oxidase I (*COI*) gene from thirteen African species. To circumvent problems with outgroup selection, we also included sequences from representatives of the platypleurine genera *Brevisiana, Capcicada, Munza, Oxypleura, Severiana,* and *Systophlochius,* all of the subtribe Platypleuriti, and two species of the genus *Ugada,* of the subtribe Hainanosemiiti. The resulting phylogenies support the synonymization of the monotypic genus *Systophlochius* with the widespread, speciose genus *Platypleura*; confirm the placement of *Platypleura* sp. 7 in that genus; and confirm the independence of *Capcicada* and *Platypleura.* Although the preliminary phylogeny lacks strong support at many nodes, it suggests that three radiations of *Platypleura* have occurred in southern Africa and that there was progressive southward speciation of these radiations. A novel modification of the ancestral area analysis further suggests that the group has an ancestral association with acacias but there were five independent speciation events associated with host-switching. These insights can be summarized by a general hypothesis that the mechanisms underlying platypleurine biodiversity in southern Africa involve two ancient vicariance events and subsequent speciation by vicariance, switching of plant associations, and changes of habitat preferences. We offer this example to illustrate how analysis of preliminary data can help to generate hypothetico-deductive research hypotheses, to provoke interest in testing these hypotheses, and to illustrate the utility of phylogenies beyond systematics.

Introduction

If we are truly to understand biological diversity, we need to develop beyond merely descriptive studies to mechanistic explanations based on comparative biology and a rigorous appreciation of the natural history of the focal organisms. Cicadas provide interesting model animals for mechanistic studies of the origins of biological diversity through evolutionary studies of communication systems, insect–plant associations and biogeography.[1–3] A useful (but not mandatory[4,5]) step in such comparative studies is the generation of a phylogeny, so that one can assess the phylogenetic independence of the focal taxa, because the significance of biological differences between species may be confounded by the length of time they have had to diverge.[5]

Good target groups for comparative studies are at least moderately speciose and taxonomically well understood, with a relatively well-known natural history that shows variation in traits that are likely to affect the generation of diversity, for example, by increasing rates of genetic change in populations. The cicada genus, *Platypleura* Amyot & Serville 1843, is a good candidate for such study. It contains about 20 species in southern Africa,[6,7] and much is known about their reproductive behaviour and plant associations.[2,8–13] What remains to be assessed is their phylogenetic relationships. Unfortunately, the morphology of the members of this genus provides few phylogenetically informative characters, and other sources of characters are needed. In this study, we present the results of a preliminary phylogenetic analyses of a data set comprising partial sequences of the mitochondrial cytochrome oxidase I (*COI*) gene. We use this phylogeny to formulate hypotheses about the historical biogeography and plant associations of the genus.

Materials and methods

DNA sequences

Tymbal muscle tissue was collected in 96% alcohol from 12 of the 20 southern African species of *Platypleura* (Table 1). We also included material from the Kenyan species, *Platypleura gowdei* Distant 1914, and six of the other 18 genera of the subtribe Platypleuriti (Table 1), and from two species of the genus, *Ugada* Distant 1904, of the subtribe Hainanosemiiti (Table 1). The latter genus served as outgroup to root the resulting trees.

DNA was extracted from the tissues by means of a Chelex 100 extraction protocol.[14] Small pieces of tissue (*c.* 2 mm^3) were sliced finely using a sterile scalpel blade, and placed in 1.5 ml Eppendorf tubes containing 5% Chelex extraction buffer [150 μl of 20% Chelex 100 solution, 450 μl TE (10 mM Tris, 1 mM EDTA) and 0.1% sodium azide]. Samples were incubated in a water bath at 60°C for one to two hours, and then denatured at 100°C in a boiling-water bath for 15 minutes. Samples were then centrifuged at 13 000 rpm for 1 minute, and the supernatant removed for subsequent use in PCR amplifications.

A portion of the *COI* gene was amplified using the primers C1-J-2195 and TL2-N23014.[15] Owing to some difficulties in amplifying some taxa, an internal primer (Cicada F2: 5′-CAT CAT ATA TTT AST GKT GG-3′) was designed and subsequently used for PCR and sequencing. Successful PCR amplifications were detected by electrophoresing 5 μl PCR product and 5 μl tracking dye in a 1% agarose gel, stained with ethidium bromide and visualized using a UV trans-illuminator. The PCR products were purified using the QIAGEN QIA quick purification kit. Sequencing reactions for both strands were done using the ABI Big Dye Sequencing kit versions 2 and (later) 3.1, according to the manufacturer's instructions. Most samples were sequenced using an ABI 377 automatic sequencer at the Central Analytical Facility (CAF), University of Stellenbosch. Two species were sequenced on an ABI 3100 genetic analyser (Rhodes University). Sequence trace files were checked and edited using Sequencher version 3.01 (Gene Codes Corporation). The sequence data were then imported into DAPSA version 4.7[16] and aligned manually.

Four different methods of phylogenetic analysis were undertaken: Maximum Parsimony (MP), Maximum Likelihood (ML), Neighbor-Joining (NJ), and the recently developed Bayesian Inference (BI). Three of the analyses (MP, ML and NJ) were conducted using PAUP* v4.0b8.[17] The BI analysis was conducted using MrBayes v3.0b4.[18] The MP analysis used the FULL HEURISTIC search option with TBR branch swapping, and tree space was searched to completion. The NJ tree construction algorithm[19] was applied to a matrix of similarities obtained using the Jukes-Cantor correction.[20] Bootstrap support values for both these analy-

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Table. 1. Voucher, GenBank numbers and host plant details of the species included in this study. All host plants in bold belong to the family Leguminosae.

Taxon	Voucher no.	GenBank no.	Adult plant association
Subtribe Platypleuriti			
Brevisiana brevis (Walker, 1850)	MHV 067	AY821501	***Acacia karroo, Acacia*** spp.
Capcicada decora (Germar, 1834)	MHV 011	AY821498	*Protea* spp.
Munza laticlavia (Stål, 1858)	MHV 005	AY821502	***Acacia mellifera***
Oxypleura quadraticollis (Butler, 1874)	—	AY821497	***Colophospermum mopane***
Platypleura capensis (Linnaeus, 1764)	MHV 010	AY821504	*Metalasia maricata, Brachylaena discolor*
chalybaea Villet, 1989	MHV 039	AY821505	*Euphorbia triangularis*
deusta (Thunberg, 1822)	MHV 115	AY821507	*Leucosidea sericea, Cliffortia* sp.
haglundi Stål, 1866	MHV 077	AY821508	***Acacia karroo, Acacia*** spp., *Dichrostachys cinerea*
gowdeyi Distant, 1914	MHV 187	AY821515	***Acacia gerrardii***
hirtipennis (Germar, 1834)	MHV 008	AY821513	***Acacia karroo***
cf. *hirtipennis*	MHV 014	AY821506	Acacia karroo
plumosa (Germar, 1834)	MHV 040	AY821509	***Acacia karroo***
sp. 1	MHV 051	AY821510	*Protea* sp.
sp. 4	MHV 024	AY821511	*Acacia karroo*
sp. 7	MHV 183	AY821514	***Acacia karroo***
stridula (Linnaeus, 1758)	MHV 025	AY821512	?*Salix* sp., various exotic trees
wahlbergi Stål, 1855	MHV 065	AY821503	***Acacia karroo***
Severiana severini (Distant, 1893)	MHV 035	AY821499	***Delonix regia***
Platypleura techowi Schumacher, 1913 (= *Systophlochius palochius* Villet 1989 stat. nov.)	MHV 068	AY821500	***Acacia karroo***, **Acacia** spp., ***Cassia*** sp.
Subtribe Hainanosemiiti			
Ugada giovanninae Boulard, 1972	MHV 116	AY821496	Unknown
sp. 1	MHV 110	AY821495	Unknown

ses were obtained using 1000 replicates. For the ML analysis, we first employed MODELTEST v3.04[21] to identify the best-fitting model of DNA substitution. PAUP was then used to find the most likely trees under this model. Support for this topology was assessed by 100 bootstrap replications. The BI analysis was conducted for 1 000 000 generations using random starting trees, with four chains (one hot, three cold), sampling every 50 generations. The first 10% of the resultant trees was considered as 'burn-in' and discarded.

Plant associations and geographical distributions

Plant associations and geographical distributions of the species of *Platypleura* were obtained from a database of over 1200 collecting events kept by M.H.V. The database includes specimen data from the Albany Museum (Grahamstown), the Australian Museum (Sydney), the Bulawayo National Museum (Bulawayo), the Durban Museum of Science (Durban), the Humboldt Museum (Berlin), the Natal Museum (Pietermaritzburg), the National Collection of Insects (Pretoria), the National Museum of Natural History (Paris), the Natural History Museum (London), the South African Museum (Cape Town), the Transvaal Museum (Pretoria), the private collections of Isak Coetzer, Rudi Mijburgh, Renzo Perissinotto and Richard Steven, and various publications listed in the catalogues of Metcalf,[6,22] Duffels and van der Laan,[7] and Sanborn and Villet (unpubl.).

To develop a hypothesis about the ancestral host plant species, we used a modification of ancestral area analysis, implemented using the dispersal–vicariance software DIVA.[23] Normally, this software is used to determine ancestral areas in biogeographic studies, but its application to resolving ancestral plant associations is equally valid; conceptually, we simply substituted associations with host plants for associations with land areas. DIVA requires a fully dichotomized tree, so the fully resolved NJ topology was recreated using MacClade version 3[24] imported into DIVA. The maximum number of ancestral areas was constrained to three ('MAXAREAS = 3'). Two analyses were conducted: one in which associations with *Acacia karroo* and *A. gerrardii* were considered as distinct, and one in which they were viewed as a single '*Acacia*' association.

Results and discussion

Phylogenetic analyses

The final dataset comprised 498 bases, which aligned readily, without the need for the use of gaps corresponding to insertion or deletion events. This alignment contained 208 variable characters (41.7%) and 132 (26%) potentially phylogenetically informative sites. The sequence of *Platypleura hirtipennis* was incomplete, missing the last 105 base pairs. The sequences are deposited in GenBank under the catalogue numbers listed in Table 1.

Parsimony analysis yielded nine equally parsimonious trees, and their consensus tree (Fig. 1) showed only moderate agreement between them. The consistency and retention indices were low (ci = 0.452; ri = 0.406), which reflected the relatively high tree length (485 steps) relative to the number of potentially informative sites (132 sites). Only four branches received bootstrap support, and only two of these had better than 70% support.

MODELTEST chose the TVM + I + G model using the Akaike information criterion.[25] Using this model, the maximum likelihood analysis found two equally likely trees, the difference between which was in the order of terminals arising from the polytomy in *Platypleura* (Fig. 2). Six branches received bootstrap support, and only three of these had support of 70% or better.

Neighbor-Joining distance analysis using the Jukes-Cantor correction produced a phylogram (Fig. 3) with seven branches supported by bootstrap analysis, of which four received support greater than 70%.

Bayesian analysis resulted in a dendrogram (Fig. 4) with eleven branches supported by posterior probabilities above 50%, of which six had posterior probabilities greater than 70%.

Taxon sampling is unlikely to be the primary cause of poor resolution of the *Platypleura* clade, because 12 of the 20 southern African species of *Platypleura* were included in the study. The large divergence between morphologically cryptic sister species like *P. capensis* and *P. stridula*[26] or *P. plumosa* and *P. hirtipennis*[12,27] cannot be a result of taxon sampling. The pattern of long terminal branches and short internal nodes (Figs 2–4) supports another hypothesis, involving an ancient period of rapid speciation. This situation has been found in lineages of flies and beetles that have undergone rapid bursts of speciation, even with molecular data sets containing longer sequences and more genes, and supplemented with morphological characters (e.g. refs 28–30). If this were the case, it would also explain why morphological data have been unsuccessful in resolving the phylogeny of these cicadas, and suggests that more molecular data will not materially alter the situation.[29] We thus recognize that the interpretations that

follow are somewhat limited, but view the hypotheses that we describe as suitable starting points for further analysis and debate.

Systematics

Despite these limitations, this preliminary analysis clarifies our taxonomic understanding of *Platypleura* in southern Africa by resolving three taxonomic problems. First, the relationship between *Systophlochius* and *Platypleura* can be clarified. All of the analyses provided good evidence that (*Systophlochius* + *Platypleura*) is a monophyletic clade, with bootstrap values above 66% and a posterior probability of 100% (Figs 1–4). Because the recognition of *Systophlochius* as a distinct genus renders *Platypleura* paraphyletic in the MP and NJ analyses (Figs 1 and 3), the genera are synonymized here. The morphology of the male genitalia is important in defining platypleurine genera,[31] but those of *Systophlochius*[26] are unlike those characteristic of *Platypleura*,[26,27,31] which led to its original erection.[26] However, the colour pattern of the wings of *Systophlochius* is like that of most *Platypleura* species. The genitalia of *P. plumosa, P. hirtipennis* and *Platypleura* sp. 7 are also aberrant for their genus[12] but they are still regarded as species of *Platypleura,* which underscores the problems posed in using few and particular characters in this group of cicadas.

Furthermore, one of us (M.H.V.) has examined the type material of *Platypleura divisa* var. *techowi* Schumacher, 1913, and *P. divisa* Germar, 1834, in the Humboldt Museum, Berlin, and found that the former is not synonymous with the latter as concluded by Schumacher,[32] but that it is a senior synonym of *S. palochius* Villet, 1989. Thus,

Platypleura Amyot & Serville 1834
= *Systophlochius* Villet 1989 (**syn. nov.**)

Platypleura techowi Schumacher 1913 (**stat. nov.**)
= *Platypleura divisa* var. *techowi* Schumacher 1913
= *Systophlochius palochius* Villet 1989 (**syn. nov.**).

Second, *Platypleura* sp. 7 shares the slightly aberrant male genital morphology of *P. plumosa* and *P. hirtipennis,*[27] but completely lacks the wing pigmentation characteristic of the genus. This analysis confirms that the genitalia provide a reliable phylogenetic character in this context, and facilitates the imminent description of this species by resolving its generic affinity. This example and that of *P. techowi* illustrate the point that one cannot consistently rely on a limited suite of morphological characters to inform taxonomic decisions about ranks. While genitalia are useful in one case, and wing pigmentation in another, it is only comprehensive phylogenetic analysis that allows the indication of relationship (through the creation of monophyletic taxa) at ranks above the species level.

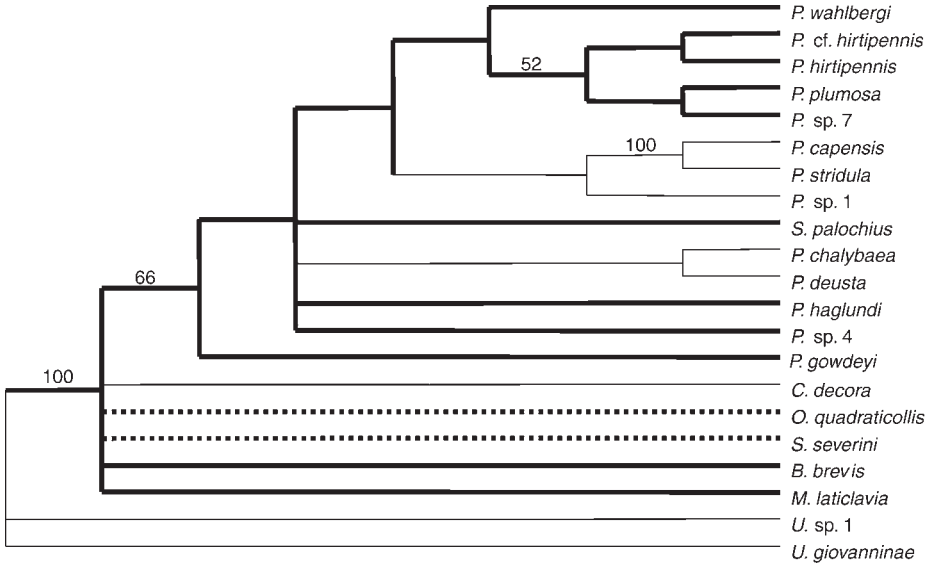

Fig. 1. Strict consensus tree of nine most parsimonious trees for partial sequences of the *COI* gene of southern African cicadas of the tribe Platypleurini. Length = 485 steps, ci = 0.452, ri = 0.406. Numbers indicate bootstrap support for vertices with bootstrap support greater than 50%. *Acacia*-associated lineages are indicated with bold lines; lineages that feed on other leguminous trees are indicated with dotted lines.

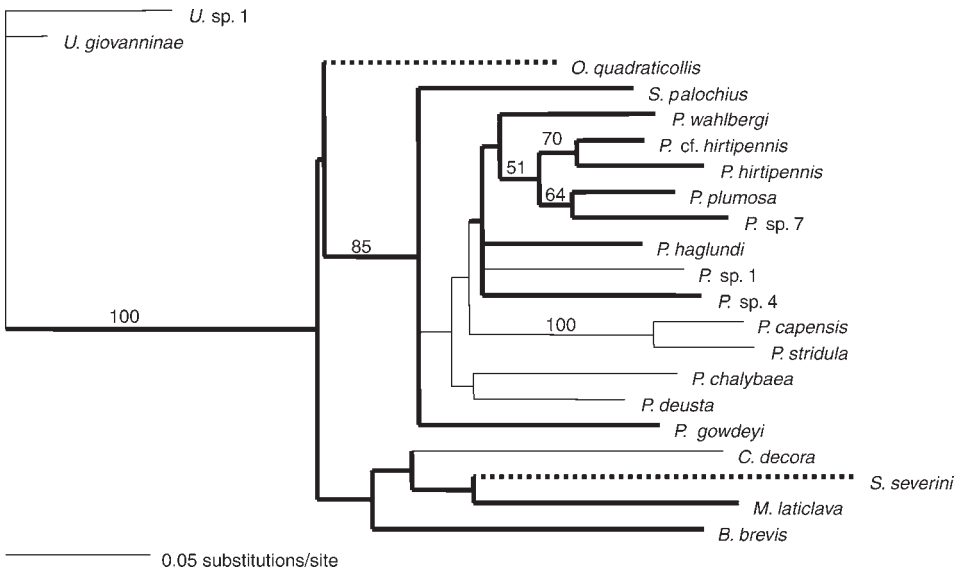

Fig. 2. Maximum likelihood analysis for partial sequences of the *COI* gene of southern African cicadas of the tribe Platypleurini. Numbers indicate bootstrap support for vertices with bootstrap support greater than 50%. *Acacia*-associated lineages are indicated with bold lines; lineages that feed on other leguminous trees are indicated with dotted lines.

Finally, these analyses supported the erection of the genus *Capcicada.* Although it is superficially similar to *Platypleura* in morphology and wing pattern, its male genitalia are distinctive and there was no other evidence of a close relationship between them. Here, male genitalia are validated as a useful phylogenetic character by complementary molecular analysis.

The phylogenies offered no consistent resolution amongst the genera. The sister group of *Platypleura* consistently contained *Oxypleura,* but other taxa were sometimes also included and the relationships are not strongly supported.

Within *Platypleura,* there was consistent support for the clades (*P. capensis* + *P. stridula*) and ((*P. plumosa* + *P.* sp. 7) + (*P. hirtipennis* + *P.* cf. *hirtipennis*)). The clade (*P. deusta* + *P. chalybaea*) was supported by Bayesian analysis and appeared in all of the

other analyses. A few other relationships within the genus were consistently resolved (Figs 1–4), but not supported by bootstrap analysis or posterior probability. For instance, *P. gowdeyi* was always placed at the base of its genus, although sometimes in a polytomy. In half of the analyses, *P. wahlbergi* was the sister group to the *P. plumosa/P. hirtipennis* group (Figs 1, 2), and the remaining analyses did not substantially contradict this. The (*P. capensis* + *P. stridula*) clade was consistently associated with a group that included the *P. plumosa/P. hirtipennis* group and excluded the (*P. deusta* + *P. chalybaea*) clade. All of these trends are reflected in the Neighbor-Joining tree (Fig. 3), which was the preferred topology for the genus for this reason.

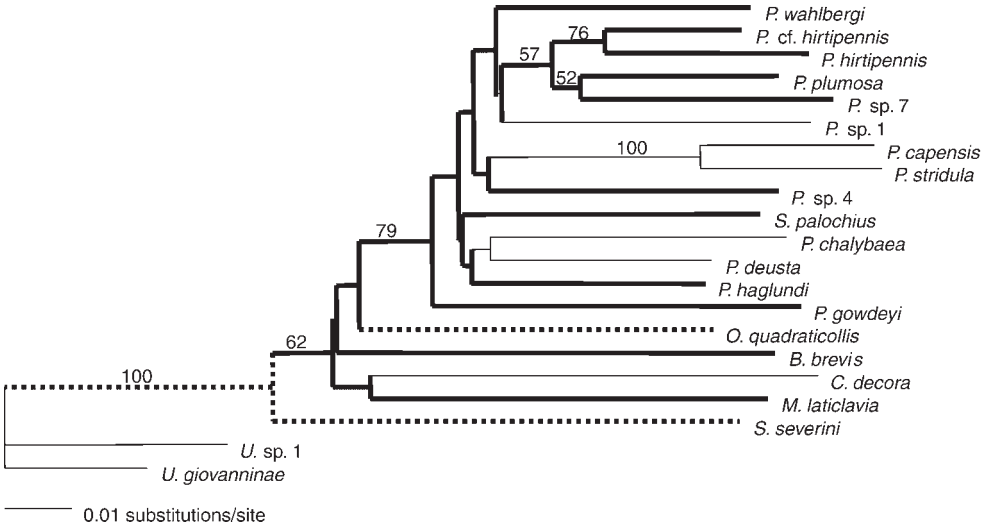

Fig. 3. Jukes-Cantor Neighbor-Joining analysis for partial sequences of the *COI* gene of southern African cicadas of the tribe Platypleurini. Numbers indicate bootstrap support for vertices with bootstrap support greater than 50%. *Acacia*-associated lineages are indicated with bold lines; lineages that feed on other leguminous trees are indicated with dotted lines.

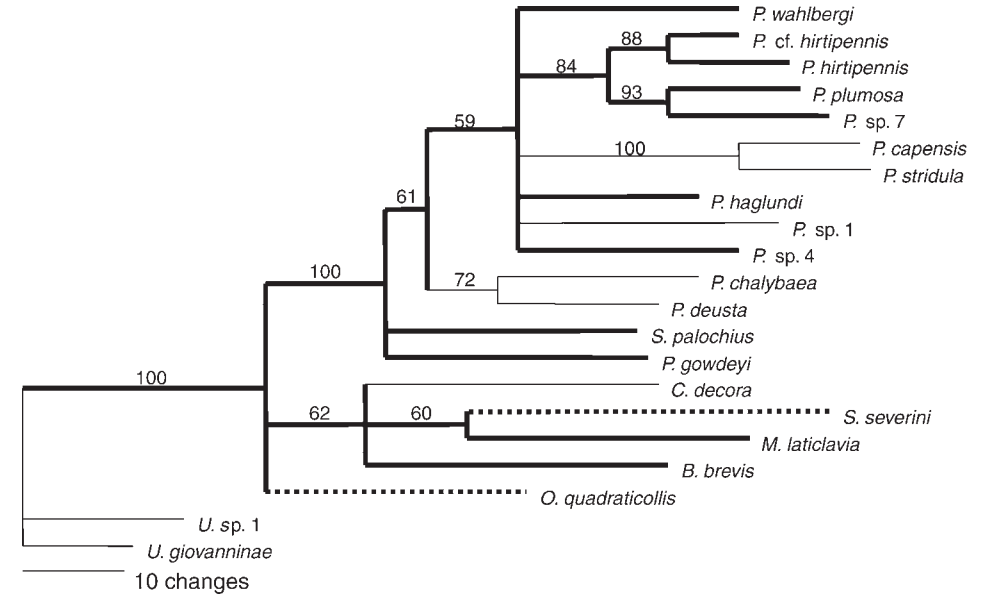

Fig. 4. Bayesian inference analysis for partial sequences of the *COI* gene of southern African cicadas of the tribe Platypleurini. Numbers shown above branches are posterior probabilities for vertices with posterior probabilities greater than 0.5. *Acacia*-associated lineages are indicated with bold lines; lineages that feed on other leguminous trees are indicated with dotted lines.

Plant associations

Apart from their taxonomic uses, phylograms and cladograms have other significant heuristic applications in understanding biological diversity. For instance, when assessing hypotheses of adaptations that might generate diversity, it is crucial to know which conditions are derived, and which derived conditions are phylogenetically independent. Obligate plant associations are one such feature that is likely to shape insect diversification; the majority of *Platypleura* species are quite specific in their plant associations, and several live only on *Acacia karroo* (Fabaceae/Leguminosae). However, it is not immediately clear whether the association of southern African platypleurines with *Acacia* trees has arisen more than once.

When considering the two species of *Acacia* to be separate associations, DIVA determined the ancestral association to be with *Acacia gerrardii* and/or *Acacia karroo,* with five switches to other hosts (viewed by DIVA as dispersal events). If the two species of *Acacia* were considered as a single genus-level association, then DIVA unambiguously resolved this genus as the ancestral association of *Platypleura. Platypleura* sp. 1, *P. deusta, P. chalybaea, P. capensis* and *P. stridula* have derived associations with the Proteaceae, Rosaceae, Euphorbiaceae, Asteraceae and various exotic trees, respectively. Mapping the plant associations of the other genera of Platypleuriti onto the various dendrograms (Figs 1–4) suggests that their ancestral association was with leguminous trees, particularly of the genus *Acacia.* Amongst these links, species of *Munza* and *Brevisiana* are associated with *Acacia* trees, while at least some species of *Oxypleura* and *Severiana* are associated with leguminous tree species of other genera.

The switches to alternative associations include a range of plant families, and there is no clear correlation to plant phylogeny, which is not expected, since the taxonomic radiations of the groups occurred on different evolutionary time scales. Instead, it is more likely a factor of which plants were most commonly available in the vegetation. Thus, in the Eastern Cape province, *Euphorbia,* which is a common genus of the thicket biome, is utilized by *Platypleura chalybaea* as a host, despite the presence of latex in specialized lacticifers. Similarly, *Platypleura capensis* utilizes the common woody Asteraceae genera *Brachylaena* and *Metalasia.* The host shift of *P. stridula* to a preference of exotic trees must be recent, and its original host is not yet known, but might be an indigenous *Salix* (M.H.V., pers. obs.).

Historical biogeography

A third use of cladograms in understanding the origins of biodiversity is in generating hypotheses about the historical biogeography of a group. In the case of the platypleurine

cicadas, *Ugada* is West African, while the other genera included in this study (Table 1) are essentially savanna taxa. *Platypleura* occurs in the region between Kenya, Angola and South Africa.[6,7] When the preferred dendrogram for the genus (Fig. 3) is mapped onto the geographical distributions of the taxa by rotating its branches on their nodes, we can generate a hypothesis about the events underlying their speciation (Fig. 5). This allows the postulation of two ancient vicariance events that founded three clades within the genus *Platypleura,* corresponding to northern, central and Cape radiations, arising from a southward expansion of the genus. The northern radiation includes *S. palochius, P. haglundi,* and (*P. deusta* + *P. chalybaea*); the central radiation includes {*P. wahlbergi* + [(*P. plumosa* + *P.* sp. 7) + (*P. hirtipennis* + *P.* cf. *hirtipennis*)]}; and the southern radiation includes (*P. capensis* + *P. stridula*). Within each clade, there is also at least some evidence of southward radiation, although it is not as compelling in the central clade.

Speciation subsequent to the vicariance events that created the three radiations could have been driven by three mechanisms. The first is further vicariance, as suggested in the {*P. wahlbergi* + [(*P. plumosa* + *P.* sp. 7) + (*P. hirtipennis* + *P.* cf. *hirtipennis*)]} clade. These species share the same plant association (*Acacia karroo*), and similar habitat preferences (arid thornveld). *P.* sp. 7 occurs along the most arid parts of the Orange River, while *P. plumosa* and *P. hirtipennis* occur parapatrically in the Eastern Cape (Fig. 5, ref. 27). This pattern is not dissimilar to that found for the rock agama, *Agama atra,*[33] the rock rabbit, *Pronolagus rupestris*[34], and the gecko, *Pachydactylus rugosus.*[35] Matthee and Flemming[33] consider a number of possible causes for this disjunction, but their preferred explanation is one of temperature fluctuations and rainfall cycles in southern Africa during the Pleistocene.[36] The action of such climate perturbations could have caused rapid isolation and subsequent speciation, a scenario in keeping with the short branch lengths observed at the internal nodes within the genus (Figs 2–4). Linder[37] comments in some detail on the effect of recent glacial extremes on the expansion and contraction of certain vegetation types (especially the fynbos and forest biomes) in the southern regions of the continent, but these events (over the last 20 000 years) are probably too recent to be correlated with the phylogeny of these cicadas, which have long terminal branches (Figs 2–4), indicating an age greater than this.

In some platypleurine species speciation may also be linked to changes in habitat preferences and plant associations that generally occur simultaneously. For example, the southwestward speciation of *Platypleura capensis* and *P. stridula* is linked to dispersal into the winter rainfall region and the formation of new plant associations (Fig. 5). Although not included in this study, the cryptic sister species *P. divisa* and *P. maytenophila* provide an example of speciation involving a change in habitat but not plant association,[8] showing that these speciation mechanisms need not be linked. Because only twelve of the 20 species of *Platypleura* that occur in the region have been included, further hypothesizing about mechanisms of speciation should await a complete sample.

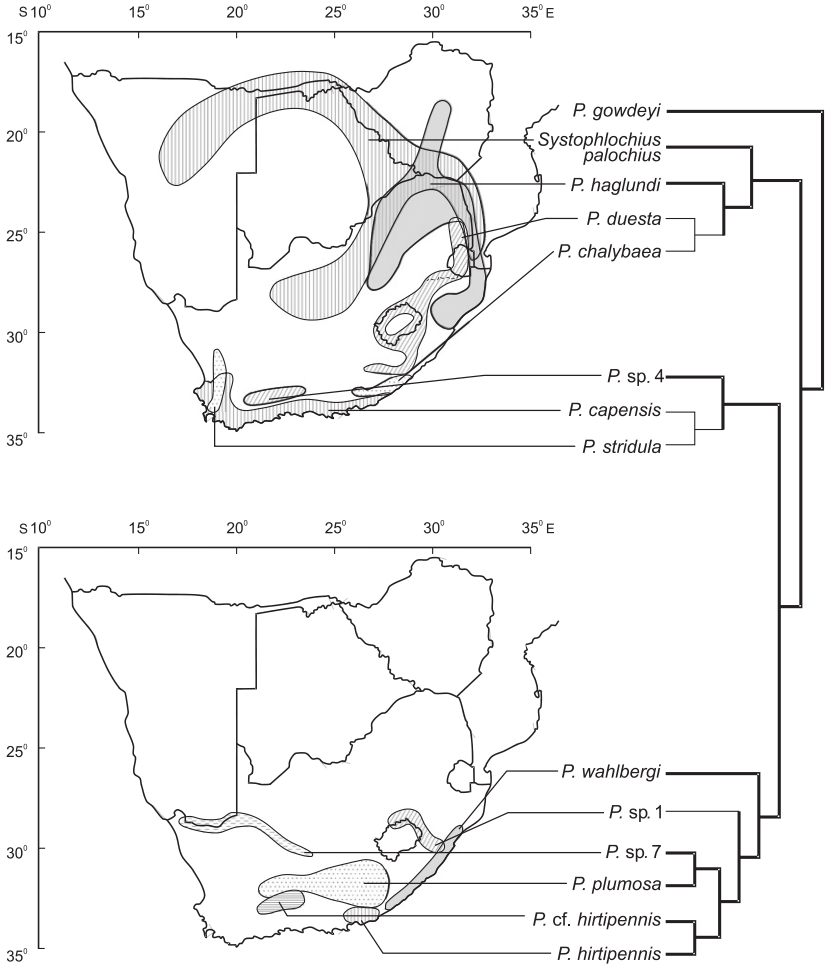

Fig. 5. Jukes-Cantor Neighbor-Joining dendrogram matched against the geographical distributions of 12 southern African species of *Platypleura. Platypleura gowdeyi* is an East African species that occurs outside the map region. *Acacia*-associated lineages are indicated with bold lines.

Conclusion

We are aware of the provisional nature of the hypotheses we generated here from preliminary and partially ambiguous results, but hope that we have provoked interest in testing these hypotheses. More crucially, our analyses illustrate our general point that phylogenies are central to developing hypotheses about the mechanisms generating real-world case studies of biological diversity. This argument is illustrated at the species level in this study, but it underlies the analysis of syndromes of mating behaviour in cicadas that has shown why some African cicada tribes show high rates of endemism and speciation, while others follow the opposite trend.[2] Together, these two studies exemplify the general power of phylogeny to explain biological diversity at a spectrum of taxonomic levels.

Once knowledge of a phenomenon moves from the descriptive to the explanatory, one can begin to predict the effects that changes will have on the phenomenon. In the case of biological diversity, such predictive ability might allow the identification of taxa that are most at risk from particular environmental changes, thereby contributing to the rationale for environmental management.

S. Howis (Rhodes University) assisted in generating some of the sequences and J. Deckert (Humboldt Museum, Berlin) provided access to type specimens in his care. The study received financial support from Rhodes University and the National Research Foundation.

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