Fewer species of *Argosarchus* and *Clitarchus* stick insects (Phasmida, Phasmatinae): evidence from nuclear and mitochondrial DNA sequence data

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The systematics of three genera of New Zealand stick insect in the subfamily Phasmatinae were investigated in light of inconsistencies in morphological variability within and among species. We sequenced a region of the mitochondrial genome, cytochrome oxidase (COI & COII; 1448 bp), and a nuclear marker, the internal transcribed spacers (ITS1 & ITS2; 1804 bp) from 49 stick insects. Mitochondrial DNA sequence divergences among the three genera (*Argosarchus*, *Clitarchus* and *Acanthoxyla*) were relatively high (~12%) but the current taxonomy within genera was not supported. Within the three genera, low levels of genetic divergence were observed at both nuclear and mitochondrial loci, and phylogenetic analyses failed to support reciprocal monophyly of the two species in *Argosarchus* and *Clitarchus*. Sympatric individuals of *Argosarchus spiniger* and *A. horridus* were more closely related to each other than to members of their respective morphospecies from elsewhere. No males were found in the Chatham Island population of *Argosarchus* and although this population has been referred to as *A. schauinslandi*, genetic and morphological evidence does not support its distinction from mainland *Argosarchus*. Likewise, individuals identified as *Clitarchus tuberculatus* were genetically identical, or most similar to, *C. hookeri* from the same or adjacent sites rather then grouping with the stick insects they were morphologically most similar to. Lack of spatial, behavioural or ecological evidence concordant with the described species *A. spiniger*, *A. schauinslandi* and *C. tuberculatus* leads us to infer that these species are synonymies of *A. horridus* and *C. hookeri* respectively. We conclude that *Argosarchus* and *Clitarchus* have each been over-split and actually consist of a single morphologically polymorphic, facultative parthenogenetic species. The genus *Acanthoxyla* with eight described species also has low levels of genetic divergence, similar to those found in *Argosarchus* and *Clitarchus*. A possible hybrid origin of *Acanthoxyla* involving its sister genus *Clitarchus* is implied by sharing of ITS sequence variants, but further sampling is needed before the species status of these obligate parthenogenetic lineages can be resolved. In contrast to some New Zealand Orthoptera, the Phasmatinae show little genetic variation suggesting coalescence in recent times, possibly reflecting lineage sorting in the Pleistocene.

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Introduction

Molecular phylogenetic studies of stick insect genera in Europe (*Bacillus*) and North America (*Timema*) have revealed complex histories and relationships among sexual and parthenogenetic taxa. More than 20 species of *Timema* are known in the vicinity of California, with largely allopatric distributions and species with a high degree of host-plant specialization (Sandoval *et al*. 1998; Law & Crespi 2002a; Nosil *et al*. 2002). High genetic diversity indicates the genus originated some 20 million years ago with an active period of speciation starting about 2.5 Mya and extending into the Pleistocene (Sandoval *et al*. 1998; Law & Crespi 2002b). Similar patterns of diversity

are seen in *Bacillus* in southern Europe (Mantovani *et al*. 2001; Scali *et al*. 2003). Both regions, and their stick insect fauna, were beyond the reach of Pleistocene glaciation, but the onset of climatic fluctuations may have stimulated speciation, which includes the formation of parthenogenetic lineages in both genera.

New Zealand, which has been geologically isolated for some 70 million years, has a temperate climate and escaped the impacts of polar glaciation. Phylogeographical studies of invertebrates have revealed high levels of intraspecific geographical structuring and genetic diversity (Morgan-Richards *et al*. 2001; Trewick *et al*. 2000), cryptic species (Trewick 2000) and evidence of rapid radiations (Chambers *et al*. 2001; Trewick & Wallis 2001; Trewick & Morgan-Richards 2005) associated with Pliocene mountain-building and Pleistocene climate fluctuations. Given that the Phasmidae are primarily tropical insects and the group is at its ecological limits in New Zealand, it is of interest to contrast diversity of the endemic stick insect fauna with that of other regions.

Two subfamilies (Pachymorphinae and Phasmatinae) and nine genera of stick insects are recognized in the New Zealand fauna (Jewell & Brock 2002). These occupy a range of habitats from sea level to above the tree line. Some genera appear to have restricted geographical ranges while others occur throughout the country. In a recent revision of the New Zealand stick insects, Jewell & Brock (2002) made several significant taxonomic changes, although the total number of described species (21) is little changed from Salmon (1991). While it is likely that current research will bring to light hitherto unrecognized species, there are also doubts about the validity of some existing species. Within the Phasmatinae there is a stark contrast between the high morphological diversity of *Acanthoxyla* Uvarov and the other genera that have only one (*Pseudoclitarchus* Salmon) or two species currently recognized (*Argosarchus* Hutton, *Clitarchus* Stål). This contrast is doubly intriguing given that all *Acanthoxyla* spp. are obligate parthenogens whilst the other genera are sexual or facultative parthenogens (i.e. able to reproduce sexually or parthenogenetically). To what extent, if any, are patterns of diversity and speciation in these New Zealand stick insects similar to those of their counterparts in the northern hemisphere, and does the current taxonomy accurately reflect their evolutionary history? Here we use phylogenetic evidence from mitochondrial and nuclear DNA sequences to examine the extent and pattern of genetic diversity within and among species.

Taxonomic background

Argosarchus currently comprises two species: *Argosarchus horridus* (White) and *A. spiniger* (White). Holotypes of these two species described by White (1846) are female and male respectively, but Hutton (1899) proposed their synonymy. Although Salmon (1991) considered the two species to be represented by distinct male and female forms, Jewell & Brock (2002) tentatively concurred with Hutton (1899) in suggesting that the type of *A. spiniger* is the male *A. horridus*, and questioned how many species of *Argosarchus* there really are. According to Salmon (1991), *A. horridus* and *A. spiniger* females differ in the degree of development of thoracic spines and abdominal foliaceous lobes, but it is clear that these characters and coloration vary widely. A third species, *A. schauinslandi* Brunner, was described from specimens collected on mainland New Zealand and the Chatham Islands, but the name has recently been applied explicitly to the Chatham Island population (Dugdale & Emberson 1996). The type of *A. schauinslandi* is lost (Jewell & Brock 2002), and specimens of the Chatham population were seen by neither Salmon (1991) nor Jewell & Brock (2002), but the species was nevertheless judged by Salmon (1991) as a likely synonym of *A. horridus* on the basis of the original descriptions. The Chatham population is of some interest as it is the only stick insect known from this archipelago, situated in the Pacific Ocean some 800 km east of mainland New Zealand, but it is rarely seen (Dugdale & Emberson 1996).

Two species of *Clitarchus* are currently recognized. *Clitarchus hookeri* comprises bright green and pale brown forms, and males and females can be either colour. In many parts of the species-range the two colour morphs are found in sympatry and in copula. Some populations are largely parthenogenetic as they apparently comprise only females. The viability of unfertilized eggs from females in mixed-sex populations was demonstrated by Stringer (1968) and Salmon (1991). Salmon (1991) described a second species, *C. tuberculatus*, which tends to be darker brown than the common brown morph of *C. hookeri* and has a more rugose cuticle than many *C. hookeri*. However, *Clitarchus* tends to be variable in colour, surface texture and development of shallow abdominal lobes — the very characters on which *C. tuberculatus* is based. Stringer (1968) had previously concluded from extensive field observation that 'these forms are found to intergrade so that it is impossible to distinguish them'. Salmon (1991) states that *C. tuberculatus* is known only from females and is presumed to be parthenogenetic. However, although it is much less common, the *C. tuberculatus* morphotype is sympatric with *C. hookeri* throughout its range in both main islands of New Zealand. The paucity of evidence supporting the taxon led Jewell & Brock (2002) to ask whether *C. tuberculatus* is a 'form' of *C. hookeri*.

Eight species of *Acanthoxyla* are recognized (Jewell & Brock 2002). They include four species described by Salmon (1955), but subsequently relegated, along with all other species, to subspecies of *Acanthoxyla prasina* (Salmon 1991). Salmon (1991) justified this re-classification on the basis of the morphological similarity of eggs of *Acanthoxyla*, although he did not apply the same criterion to the status of *C. tuberculatus*

with respect to *C. hookeri*. A separate study of eggs from three species of *Acanthoxyla* supports their specific status (Mantovani & Scali 1987). Jewell & Brock (2002) chose to revert to species status for the eight *Acanthoxyla*, a reflection of the extent of morphological diversity of adult forms and eggs, which is greater than that in any other genus of New Zealand stick insects.

Methods

Sampling

We collected representatives of *Argosarchus*, *Clitarchus* and *Acanthoxyla* from sites around New Zealand to obtain a representative sample of their morphological and geographical diversity (Fig. 1, Table 1). The genera differ in the range of native plants that they consume. *Argosarchus* was found most frequently on *Hoheria populnea*, *Plagianthus betulinus* and *Lophomyrtus bullata*. *Clitarchus* were collected almost exclusively from the two New Zealand tea tree species *Leptospermum scoparium* and *Kunzea ericoides*. *Acanthoxyla* has the most cosmopolitan tastes, including *Metrosideros* spp. (ratas and pohutakawa), *Leptospermum scoparium* and *Kunzea ericoides*, plus exotic species of cedar. In keeping with stick insects world wide, these genera were also found on exotic Rosaceae (bramble, rose). However, within each genus the various forms or species do not have distinct food preferences.

DNA extraction, amplification and sequencing

Muscle tissue from fresh, frozen or alcohol-preserved specimens was removed from a leg for extraction of genomic DNA using the salting-out method (Sunnucks & Hale 1996).

We amplified and sequenced mitochondrial and nuclear DNA. The mitochondrial fragment, comprising the 3′ end of cytochrome oxidase I (COI), tRNA-Leucine, and cytochrome oxidase II (COII), was amplified using primers C1-J-2195 and TK-N3785 (Simon *et al*. 1994). The mtDNA sequences used in the present study therefore span those used in previous studies of stick insects *Bacillus*, *Leptynia* (COII) and *Timema* (3′ COI).

Nuclear sequences comprising the internal transcribed spacers (ITS1 and ITS2) of the rRNA cluster and the intervening 5.8S, were obtained using primers ITS4 and ITS5 (White *et al*. 1990). However, ITS PCR from some *Acanthoxyla* DNA templates gave two products (nominated slow and fast on the basis of migration rates under agarose electrophoresis). We also used a primer (STITS5F) that binds near the 18S/ITS1 junction within an insertion in the slow ITS sequence (Morgan-Richards & Trewick 2005). Amplification with primers ITS4 and STITS5F with DNA templates that had given two products (with ITS4 and ITS5) yielded just one of the expected size. A combination of three primers enabled us to sequence both classes of ITS from such templates.

PCR used standard conditions (Trewick *et al*. 2000). Amplification products were treated to Shrimp Alkaline Phosphotase/Exonuclease I digestion. Cycle sequencing with the PCR primers used Bigdye chemistry (PE) following the manufacturer's protocols, with automated reading on an ABI3730. Consensus sequences were obtained using Sequencher v4.1 (ABI, PE), and aligned using SeAl v2.0a3 (Rambaut 1996).

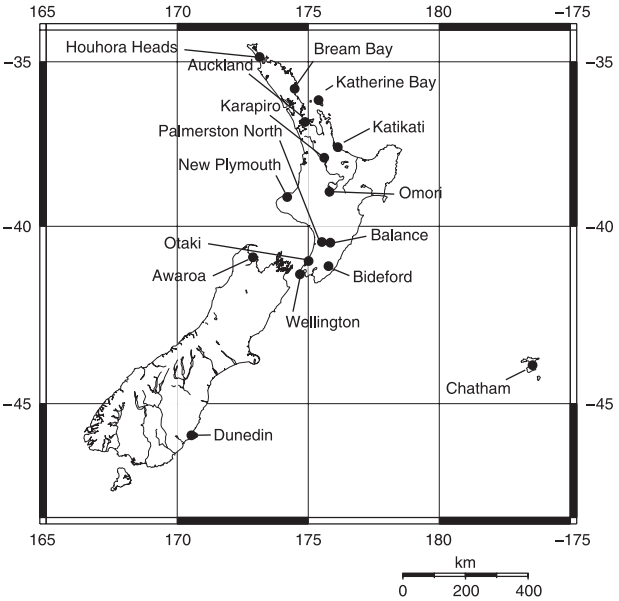

Fig. 1 Sample locations of New Zealand stick insects used in this study.

Data analysis

We searched for evidence of gene conversion in ITS using GENECONV v1.81 (Sawyer 1999), a method that utilizes information from indels in addition to nucleotide sequence and performed well in a comparison of methods (Posada 2002). The global permutation *P*-values < 0.05 (based on BLAST-like global scores with 10 000 replicates) were considered as evidence of gene conversion (or recombination). A multiple comparison correction is built into these *P*-values. For phylogenetic analyses we used Paup *4.0b10 (Swofford 2002) to implement Neighbour-Joining (NJ) and Maximum Parsimony (MP) methods. We used Spectronet (Huber *et al*. 2002) to construct median and consensus networks.

Results

We obtained 44 ITS1-2 and 45 COI-II DNA sequences from a total of 49 individuals representing the three New Zealand phasmatinid genera surveyed (Table 1). Most, but not all, individuals were sequenced for both genes (see Table 1). These data consisted of aligned sequences of 1804 bp and 1448 bp for ITS1-2 and COI-II respectively. The majority of individuals yielded a single unambiguous sequence for each

Table 1 Specimen details with sampling location and species under current taxonomy. Clade groupings for COI-COII and ITS1-2, and sequence variants for ITS1-2 are shown.

Genus	Species	Code	Location	Sex	Colour	COI-COII Clade	ITS1-2 Clade	ITS1-2 Sequence
Argosarchus	*horridus*	Arg. Bal-111	Balance	f		1	III	x
Argosarchus	*horridus*	Arg. Khd-64	Wellington	f		1	III	w
Argosarchus	*horridus*	Arg. Chat-160	Chatham	f		1	III	z
Argosarchus	*horridus*	Arg. Chat-153	Chatham	f		1	III	z
Argosarchus	*horridus*	Arg. Omo-159	Omori	f		1	III	x
Argosarchus	*spiniger*	Arg. Bal-112	Balance	m		1		
Argosarchus	*spiniger*	Arg. Bal-33	Balance	f			III	w
Argosarchus	*spiniger*	Arg. PN-18	Palmerston North	m		1	III	y
Argosarchus	*spiniger*	Arg. Khd-65	Wellington	f		1	III	w
Argosarchus	*spiniger*	Arg. Otr-4	Wellington	m		1	III	w
Argosarchus	*spiniger*	Arg. Otr-5	Wellington	f		1	III	y
Clitarchus	*hookeri*	Ch. How-66	Auckland	f	green	2		
Clitarchus	*hookeri*	Ch. Opa-67	Auckland	f	green	2	II	q
Clitarchus	*hookeri*	Ch. Arw-3	Awaroa	f	brown	2	II	s
Clitarchus	*hookeri*	Ch. Bal-141	Balance	f	green	2	II	r
Clitarchus	*hookeri*	Ch. Bid-144	Bideford	f	green	2	II	r
Clitarchus	*hookeri*	Ch. Bid-20	Bideford	f	brown	2	II	r
Clitarchus	*hookeri*	Ch. HhH-2	Houhora Heads	m	brown	2	II	l
Clitarchus	*hookeri*	Ch. Cam-77	Karapiro	f	green	2		
Clitarchus	*hookeri*	Ch. GB-34	Katherine Bay	f	green		II	o
Clitarchus	*hookeri*	Ch. GB-35	Katherine Bay	m	green	2	II	m
Clitarchus	*hookeri*	Ch. GB-36	Katherine Bay	f	brown		II	p
Clitarchus	*hookeri*	Ch. GB-37	Katherine Bay	m	brown	2	II	n
Clitarchus	*hookeri*	Ch. Ot-2	Otaki	f	green	2		
Clitarchus	*hookeri*	Ch. Ot-1	Otaki	f	brown		II	i
Clitarchus	*hookeri*	Ch. Wn-43	Wellington	f	brown	2	II	
Clitarchus	*hookeri*	Ch. Wn-44	Wellington	f	brown	2	II	
Clitarchus	*hookeri*	Ch. Rn-62	Wellington	f	green	2	II	i
Clitarchus	*hookeri*	Ch. Wil-19	Wellington	f	brown	2	II	i
Clitarchus	*hookeri*	Ch. Wby-45	Wellington	f	green	2	II	j
Clitarchus	*hookeri*	Ch. Kat-173	Katikati	m	green	2	II	u
Clitarchus	*hookeri*	Ch. Kat-171	Katikati			2		
Clitarchus	*hookeri*	Ch. Mil-174	Auckland	f	green	2	II	v
Clitarchus	*tuberculatus*	Ct. Km-170	Katikati	f	dark brown/blue	2	II	v
Clitarchus	*tuberculatus*	Ct. Arw-1	Awaron	f	dark brown	2	II	s
Clitarchus	*tuberculatus*	Ct. Bid-60	Bideford	f	dark brown	2	II	r
Acanthoxyla	*geisovii*	Dun-1	Dunedin	f	green	3	I	e
Acanthoxyla	*huttoni*	Ap. Otr-30	Wellington	f	green	3	I	a
Acanthoxyla	*inermis*	Ap. PN-17	Palmerston North	f	brown	3	II	i
Acanthoxyla	*inermis*	PN Ac-1	Palmerston North	f	brown	3	I & II	e & i
Acanthoxyla	*inermis*	Ax. Km-177	Katikati	f	green	3	I	d
Acanthoxyla	*intermedia*	Ap. PN-2	Palmerston North	f	brown	3	I	g
Acanthoxyla	*intermedia*	Ax. New-175	New Plymouth	f	green	3	II	i
Acanthoxyla	*prasina*	Ap. Opa-103	Auckland	f	green	3	I & II	e & i
Acanthoxyla	*prasina*	Bkf-3	Bream Bay	f	green	3	I & II	e & i
Acanthoxyla	*prasina*	Ax. Dun-148	Dunedin	f	green	3	II	i
Acanthoxyla	*prasina*	Ax. Kat-178	Katikati	f	green	3	II	s
Acanthoxyla	*speciosa*	Ap. Bid-86	Bideford	f	grey	3	I	d
Acanthoxyla	*suteri*	Ax. Opa-137	Auckland	f	green	3	I	f

gene fragment, but three *Acanthoxyla* (PN Ac-1, Ap.Opa-103, Stf-3) gave two ITS1-2 products of different size and sequence (Table 1). Representative sequences have been deposited at GenBank (AY940428-AY940431, AY943645-AY943648).

Phylogenetic analyses using NJ and MP of COI-II sequences revealed three well-supported monophyletic clades congruent with the three genera surveyed. Genetic distances within each clade (genus) were low: maximum (and mean) Kimura 2

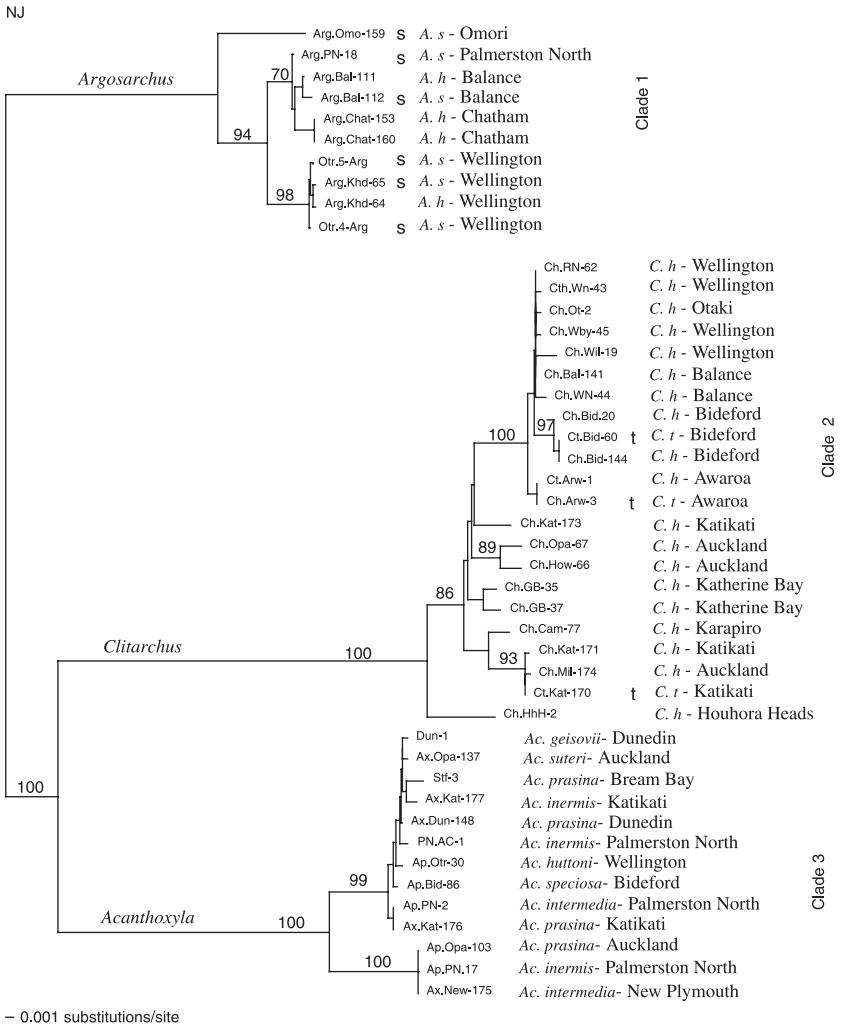

Fig. 2 Neighbour-Joining tree using mtDNA (COI-COII) for Phasmatinae genera *Argosarchus*, *Clitarchus* and *Acanthoxyla*. Numbers on edges are bootstrap scores resulting from 500 replications using MP. Species designations (as two letter codes for *Argosarchus* and *Clitarchus*) and sample locations are shown. For clarity, the placement of taxonomically uncertain species is additionally indicated by s for *Argosarchus spiniger*, and t for *Clitarchus tuberculatus*.

parameter (K2p) distances of 0.024 (0.01), 0.027 (0.013) and 0.023 (0.01) for *Argosarchus*, *Clitarchus* and *Acanthoxyla*, respectively. Placement of some terminal edges within clades varied among analyses but, as expected, all trees indicated support for nodes linking samples with high genetic similarity. In order to express the relative genetic diversity between and within clades we present the results of NJ analysis, but annotate this with the results of boostrap analysis using MP (Fig. 2). Given the low among-haplotype variability within clades (genera) and the consequent likelihood that ancestral states exist within the data, we used networks to better study the fine-scale relationships among haplotypes (Fig. 3). A median network was constructed for *Argosarchus* using Spectronet (Huber *et al*. 2002). In the case of sequences from individuals of *Clitarchus*, where a higher degree of homoplasy was evident, a consensus network was constructed. This network summarizes the patterns of 12 shortest trees yielded by MP analysis implemented in PAUP* using a branch-and-bound search among informative sites only. The maximum K2p genetic distance among genera for COI-II was 0.128.

ITS1-2 sequence variation (within clades) was low, with the following maximum (and mean) K2p distances: clade III 0.0037 (0.0025); clade II 0.0101 (0.0053); clade I 0.006 (0.0031). We found no evidence of recombination among ITS1-2 sequences. Phylogenetic analysis of ITS1-2 sequence variants yielded three well-supported clades having low within-clade genetic diversity as seen with COI-COII. The three groupings of ITS1-2 sequence variants were *Argosarchus* (clade III), *Clitarchus* plus some *Acanthoxyla* (clade II), and *Acanthoxyla* (clade I) (Fig. 4). This pattern indicates a hybrid origin for *Acanthoxyla* and is the subject of ongoing research. The distribution of ITS1-2 sequence variants from *Argosarchus* and *Clitarchus* is not consistent with the current species-level classification (Fig. 4, Table 1). Mitochondrial

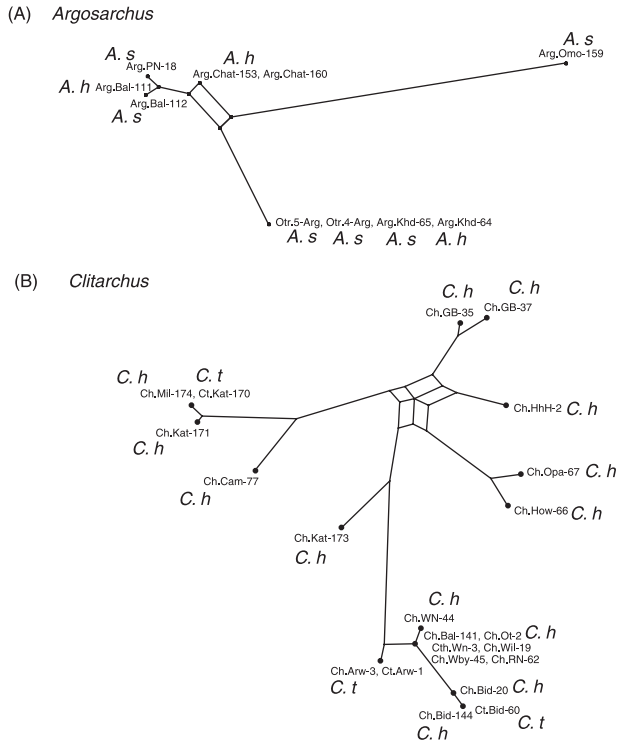

Fig. 3 A, B. Unrooted networks for mtDNA COI-COII sequences for two genera of New Zealand stick insects. Median network for *Argosarchus* (A) and consensus network for *Clitarchus* (B). Sample and species codes (as in Fig. 2) are shown. The nature and extent of phylogenetic signal for alternative splits (conflicts) in the data are indicated by box-like edges.

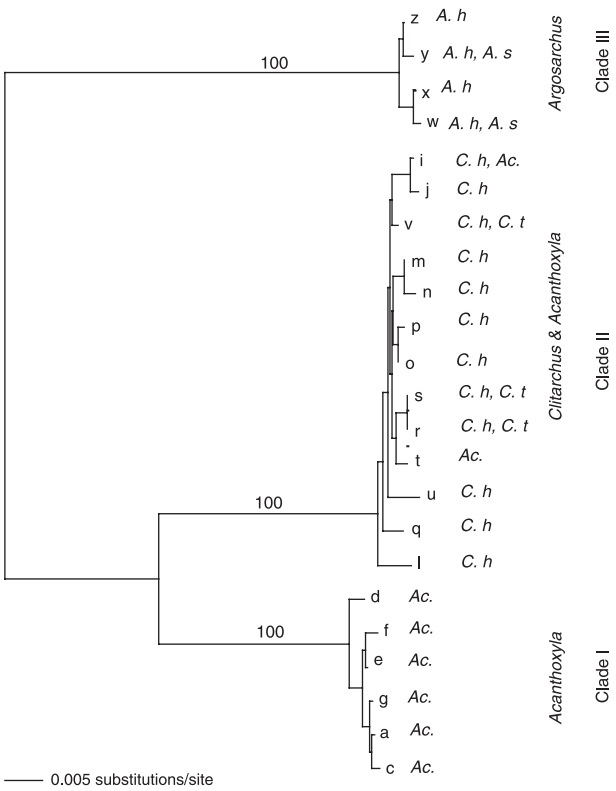

Fig. 4 Neighbour-Joining tree of 23 ITS1-2 sequence variants (a, c, d, etc.) from three New Zealand stick insect genera. Species designations are indicated by two letter codes for *Argosarchus* and *Clitarchus*; *Acanthoxyla* is *Ac*. (see Table 1 for species identification). NB. Although not evident in the NJ tree, variants s and r differ by a 6 bp INDEL.

and nuclear sequence data indicate an absence of species monophyly in each of the three genera.

Discussion

Genetic diversity at both nuclear (internal transcribed spacer) and mitochondrial (cytochrome oxidase) loci was low within each of the three genera/clades (< 3% COI-COII, < 1% ITS1-2). The degree of mitochondrial sequence variation found for each genus was at or below that typically found in insect species (e.g. leaf beetle, Funk 1999; grasshopper, Szymura *et al*. 1996; cicada, Buckley *et al*. 2001; moth, Brown *et al*. 1994). In contrast, some New Zealand Orthoptera have levels of variation far in excess of those encountered in the present study (e.g. scree weta, Trewick *et al*. 2000; tree weta, Morgan-Richards *et al*. 2001). MtDNA sequence diversity within the genera in the present study was similar to within species levels found with COII in European stick insect genera, and much less than between species levels in those same genera (e.g. *Bacillus* spp., Mantovani *et al*. 2001; *Leptynia* spp., Passamonti *et al*. 2004). Similarly, interspecific genetic distances within each of the New Zealand genera were lower than those between sexual and asexual species pairs of *Timema* (Sandoval *et al*. 1998).

However, although there is a general relationship between taxonomic difference and mtDNA sequence diversity (see for example Szymura *et al*. 1996), variance in intraspecific genetic diversity across different groups is high (as noted above). Consequently, although a low level of genetic variation is likely to represent intra- rather than interspecific difference, other evidence is needed to confirm synonymy. Similarly, paraphyly among genetic lineages does not in itself prelude the existence of good biological species, as in the classic example of the brown and polar bears (Talbot & Shields 1996). Phylogeographical structure provides strong evidence of biological or at least historical isolation even where genetic diversity is low (Moritz 1994). In addition, ecological characteristics such as host-plant or habitat specificity can also provide evidence for the existence of distinct taxa in the absence of significant genetic difference on neutral markers (Vogler & DeSalle 1994; Trewick 2001).

Spatial, phylogenetic or ecological structuring consistent with the existence of more than one species of *Argosarchus* and *Clitarchus* is lacking. Within both genera, individuals of the respective species have similar or identical mitochondrial and nuclear DNA sequence variants. Phylogenetic analyses demonstrate that, under the current classification, both genera contain polyphyletic species. A common and likely source of such polyphyly is taxonomic over-splitting (Funk & Omland 2003).

Despite the small number of *Argosarchus* in our survey, we find support in the genetic data for grouping of individuals by geographical proximity rather than morphological similarity. For example, irrespective of morphotype, *Argosarchus* collected in Wellington are genetically more similar to one another than are individuals of the two morphotypes collected at different places. Some *Argosarchus* populations appear to have no males and are therefore presumably parthenogenetic (e.g. only females have been found in populations at Omori and Chatham Island), although neither of the two morphospecies of *Argosarchus* have previously been considered to be obligate parthenogens. Females from all locations reared in captivity produce unfertilized eggs (pers. obs.) We conclude that *Argosarchus horridus* (*sensu lato*) is a polymorphic facultative parthenogen, i.e. some individuals have well developed thoracic spines and abdominal foliaceous lobes and some do not . The different forms are sympatric, consume the same food plants, and are found mating with one another. Not surprisingly, the apparently all-female populations appear to consist of a single morphotype consistent with a clonal mode of reproduction. Further sampling of *A. horridus* across its range in New Zealand would probably reveal additional genetic diversity, but it is already apparent that the Chatham Island population is genetically very close to mainland individuals sampled (0.35% COI-COII; 0.12% ITS1-2). This suggests recent transoceanic colonization of the Chatham Islands, despite their isolated position some 800 km from New Zealand; this is consistent with geological evidence for the recent emergence of these islands (Campbell *et al*. 1994; Campbell 1998).

The similarity of *C. tuberculatus* and *C. hookeri* DNA sequences at mitochondrial and nuclear loci, combined with the lack of monophyly of sequences obtained from these forms, is consistent with them being parts of a single polymorphic taxon (*C. hookeri* by precedent). On both mitochondrial and nuclear markers, three individuals of *C. tuberculatus* were each genetically more similar to individuals of *C. hookeri* collected at adjacent locations than to each other. Despite this small sample size, the phylogenetic relationships provide compelling evidence that *C. tuberculatus* is a form of *C. hookeri*, as suggested by Jewell & Brock (2002). Several forms of *C. hookeri* (*sensu* Salmon 1991) are already recognized, including those that are brown or green, smooth or with fine tubercles on the abdomen and thorax, and to these can be added the darker brown and more rugose form of *C. tuberculatus* as described by Salmon (1991). Stringer (1968) had previously noted the continuous gradation among all of these forms. Salmon (1991) suggested that *C. tuberculatus* was likely to be totally parthenogenetic (i.e. an obligate parthenogen), as he found no males that could be assigned to the species on morphological grounds. However, field and captive observations reveal that *C. hookeri* (even as described by Salmon 1991) is itself a facultative parthenogen (Salmon 1955, 1991; Stringer 1968). For example, some populations around Wellington lack males, and we observed low levels of genetic diversity in this region. It has not been confirmed that females of the *C. tuberculatus* morphotype are able to produce eggs without sex, but it is likely, given that the form is known from male-less populations of *C. hookeri* in Wellington. However, it is also likely that in other parts of the *Clitarchus* range, females of the *C. tuberculatus* form mate with males of one form or other of *C. hookeri* (Stringer 1968). It is therefore reasonable to accept *C. hookeri* (*sensu lato*), including the full range of morphotypes so far described, as a polymorphic facultative parthenogen. No difference in the diet of these species has ever been described or observed (pers. obs.), so these forms have overlapping distributions and diet.

Acanthoxyla provides an intriguing contrast to *Argosarchus* and *Clitarchus*, in that despite being devoid of males and having low mitochondrial genetic diversity, the genus comprises more morphological diversity than the foregoing taxa. *Acanthoxyla* presents special taxonomic issues because all lineages are entirely parthenogenetic: biological and recognition species concepts are therefore not applicable (Mayr 1963; Paterson 1985). Phylogenetic (Cracraft 1983) and cohesion species-concepts (Templeton 1987) can be applied to asexual taxa, but a wider survey of *Acanthoxyla* 'species' is needed to determine whether or not individuals of similar morphotype are monophyletic and have a common ancestry. At present it appears otherwise (Morgan-Richards & Trewick 2005).

The New Zealand Phasmatinae (including *Pseudoclitarchus*) can be considered to comprise three monotypic genera that are sexual (or facultative parthenogens), and paradoxically, one entirely parthenogenetic genus comprises many morphologically diverse taxa. So why is diversity so low? In contrast to the northern hemisphere stick insects, *Timema* and *Bacillus*, and several other endemic New Zealand insects, the relatively wide geographical range, yet low level of taxonomic, ecological and genetic diversity within *Clitarchus* and *Argosarchus* suggests recent intense lineage sorting/extinction followed by range expansion — perhaps in response to Pleistocene climatic fluctuations. In *Clitarchus* (the only genus sufficiently sampled), higher genetic diversity in the northern part of its range and evidence for isolation by distance (Morgan-Richards & Trewick 2005) are consistent with

Pleistocene refugia in the north of New Zealand (i.e. nearer the equator). The extent of forest vegetation in New Zealand was reduced substantially during glacial events, with many plants being restricted to the north (McGlone 1988). It has previously been suggested that while alpine-adapted invertebrates may show high levels of genetic diversity and strong phylogeographical structure, forest-dwelling species might be expected to have lower levels of diversity and less evident spatial structuring (Trewick *et al*. 2000; Trewick & Wallis 2001). These New Zealand stick insects might be expected to be highly susceptible to this effect. The Phasmidae are, after all, a predominantly tropical group of insects and may be at their ecological limits in New Zealand. On a small oceanic landmass, there would have been limited opportunity to escape climate cooling during the Pleistocene. Perhaps, instead of questioning the paucity of diversity, we should be impressed that any stick insects exist in New Zealand at all.

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