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Shape and sound reveal genetic cohesion not speciation in the New Zealand orthopteran, *Hemiandrus pallitarsis*, despite high mitochondrial DNA divergence

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Levels of neutral mitochondrial (mt)DNA sequence diversity play a prominent role in alpha taxonomy, with divergence thresholds of approximately 3% widely being accepted as indicative of species differences. However, a number of studies of New Zealand invertebrates conclude that intraspecific mtDNA divergences are commonly much higher. We investigated the diversity and distribution of mitochondrial cytochrome oxidase I DNA sequences from populations of the New Zealand ground weta *Hemiandrus pallitarsis* with respect to variation among ten morphological characters and analysis of acoustic signalling associated with mate recognition. We looked for correlation between neutral mtDNA sequence variation and potential indicators of species differences. Despite high genetic distances (> 8.0% uncorrected) among mtDNA haplotypes in this species, morphological and male precopulation signals refute a hypothesis that multiple species exist. Other possible explanations for high genetic diversity, including accidental sampling of nuclear paralogues and elevated substitution rate, are not supported, whereas there is evidence for stable population size through time. We conclude that generalizations about genetic diversity and species status are overly simplistic. In some circumstances, notably southern hemisphere invertebrate taxa, species cohesion appears to have been retained throughout the Pleistocene, indicating the persistence of a relatively high population size. *Biological Journal of the Linnean Society*, 2012, **105**, 169–186.

ADDITIONAL KEYWORDS: DNA barcoding – genetic diversity – insect signalling – phylogeography – Pliocene – population size – speciation.

INTRODUCTION

Molecular phylogenetic studies at the species level reconstruct plausible evolutionary hypotheses depicting lineage formation through time. As neutral genetic changes accumulate with the passage of time, levels of genetic divergence have been used to aid alpha taxonomy (Avise, 1974; Avise & Aquadro, 1982). However, it is also well recognized that the determination and definition of closely-related species is problematic, primarily because speciation does not occur at a fixed rate. Variation in the speed and process of new species formation has fuelled debate about speciation (Mallet, 1995; De Queiroz, 2007) and has resulted in the formulation of a plethora of species concepts to suit various instances (Mayden, 1997).

Although the process of speciation is poorly understood in most lineages, the use of genetic difference as an indicator of taxonomic status has experienced a renaissance (Hebert, Ratnasingham & DeWaard, 2003; Hebert *et al*., 2004a), despite the remaining problem of what constitutes a relevant threshold of genetic difference. Numerous studies have now demonstrated that a value of 2–3% fairly consistently correlates with many existing taxonomies, yielding optimism that short neutral DNA sequences can generally provide a marker for taxonomy (Hebert *et al*.,

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2004b; Ward *et al*., 2005; Hajibabaei *et al*., 2006; Lefébure *et al*., 2006). Although several commentators have identified situations whereby short DNA sequences might mislead taxonomy (Meier *et al*., 2006; Elias *et al*., 2007), a more fundamental problem concerns unexpectedly high genetic distances that are encountered within some species. Naive employment of genetic distance in new taxonomy risks concealing the very processes involved in the evolution of species that are of most interest (Rubinoff, Cameron & Will, 2006; Trewick, 2008).

In New Zealand, mitochondrial (mt)DNA phylogeographical studies of the cricket-like insects, weta, found intraspecific sequence diversity to be much higher than typically observed in invertebrate taxonomy and phylogeographical studies, the majority of which deal with Northern Hemisphere taxa (e.g. 3.9–9.5% uncorrected distance; Trewick, Wallis & Morgan-Richards, 2000; Morgan-Richards, Trewick & Wallis, 2001; King, Kennedy & Wallis, 2003; Trewick & Morgan-Richards, 2005; c.f. Ball *et al*., 2005; Robinson *et al*., 2009). Mitochondrial cytochrome oxidase I (COI) genetic distances three or four times the value commonly applied in DNA barcoding studies to delimit species (Hebert *et al*., 2003) have been reported in other New Zealand invertebrates as well. Forest, alpine, and aquatic insect taxa all provide examples of high intraspecific mtDNA distances (Trewick *et al.*, 2011).

High genetic diversity could be indicative of poor taxonomy; specifically, insufficient taxon splitting (i.e. hitherto unrecognized species). In some instances, this has proved to be the case, and inferences based on mtDNA sequence have been corroborated by other data supporting species status (Trewick, 2000; Hebert *et al*., 2004a). More commonly, however, cladogenesis and high mtDNA sequence distances (usually of allopatric populations) are taken as direct indicators for species differences (Gleeson, Howitt & Ling, 1999; Williams, Ormerod & Bruford, 2006). Reviews of phylogeographical studies in New Zealand (Wallis & Trewick, 2009; Trewick *et al*., 2011) indicate that high intraspecific mtDNA diversity is by no means the rule, so perhaps lineage-specific constraints on speciation or unusually high mutation rates explain these high values. Alternatively, the explanation may lie in the broad differences in biotic composition, spatial scale, and environmental histories of New Zealand taxa compared to parts of the world that have furnished most equivalent studies. Generalizations about the relationship between neutral sequence diversity and species partitioning are likely based on observations of predominantly Northern Hemisphere invertebrates where intense biotic turnover occurred as recently as 100–20 kya in the last glacial maximum (LGM) of the Pleistocene (Hewitt, 2000). The distributions of organisms in New Zealand, on the other hand, may have been influenced less by Pleistocene climate cycling, retaining instead the signature of earlier (Pliocene) events. Although New Zealand is small compared to northern hemisphere landscapes, it is large by island standards (Neall & Trewick, 2008), and was not subjected to polar glaciation during cooling in the Pleistocene (Alloway *et al*., 2007). Rather, its land area was increased when sea levels were lower by virtue of its placement on a largely submerged continent (Trewick, Campbell & Paterson, 2007). However, New Zealand's position on the margins of two tectonic plates has resulted in major geophysical activity during the Pliocene and Pleistocene. In particular, much of southern North Island of today was below sea level in Pliocene time, emerging in the Pleistocene, accompanied by growth of the southern North Island axial ranges (Trewick & Bland, 2011). The North Island was not glaciated but was subjected to a latitudinal climate and vegetation gradient, with much of the southern area characterized by shrubland and grassland rather than forest (Alloway *et al*., 2007; Trewick *et al*., 2011). The size and distribution of land in the central/southern North Island also reflected changes in sea level as a response to changes in global ice volume during Pleistocene time (Naish, 1997; Alloway *et al*., 2007). These resulted in unification of the main New Zealand islands during the last glacial maximum. Additionally, the biology of central/southern North Island was likely influenced by volcanic eruptions over the past 5 Myr with the last major eruption only approximately 1875 years ago, which deposited pyroclastic rock over 20 000 km^2 of central North Island and ash over 30 000 km^2 of eastern North Island (Trewick & Bland, 2011).

In the present study, we examine and contrast three types of variation in the weta *Hemiandrus pallitarsis* (Orthoptera: Anostostomatidae), in the context of the New Zealand North Island landscape. Preliminary genetic analysis indicated a high level of genetic diversity among individuals from different locations (Pratt, Morgan-Richards & Trewick, 2008). We investigated the extent and distribution of neutral mtDNA sequence diversity and sought concordance of independent traits and markers that would provide evidence of distinct species using the genotypic cluster species definition (Avise & Ball, 1990; Mallet, 1995). Existing research has noted morphological differences among some populations of this species (Johns, 2001; Gwynne, 2005) and so morphometric variation in ten continuous characters (eight from males) was documented to allow the identification of clusters that shared common mtDNA lineages and

distinct morphology. Putative cryptic species based on mtDNA clades were explicitly tested by use of variation in male precopulation vibratory signals, a character that is likely associated with mate recognition (Gwynne, 2004). We contrast male vibratory signals among three populations (i.e. two from the same mtDNA clade and one from a distinct mtDNA clade) to differentiate between putative intra- and interspecific variation. We examine four possible explanations because we seek to understand high intraspecific genetic diversity: cryptic species, nuclear-mitochondrial pseudogenes (*numts*), substitution rate, and population size.

MATERIAL AND METHODS

New Zealand ground weta

The Anostostomatidae Saussure, 1859 (Johns, 1997) are a family of ensiferan Orthoptera with a predominantly southern hemispheric distribution, known in New Zealand as weta. *Hemiandrus* (ground weta) is the most speciose genera within the New Zealand anostostomatids. There are nine valid *Hemiandrus* species (Johns, 1997; Jewell, 2007), and a further 28 undescribed species, the names of which are disclaimed as 'not available' as per Article 8.3 of the International Code of Zoological Nomenclature (1999) (Johns, 2001). Johns (2001) has identified key diagnostic characters and given these undescribed species tag-names (in quotation marks), which we refer to where necessary.

Ground weta are small to medium (length 12–45 mm), nocturnal, flightless crickets, which conceal themselves in burrows during the day (Barrett, 1991). They are found throughout the New Zealand archipelago, from the subantarctic Snares Island to Northland. Of the seven putative species with short ovipositors, five have narrow ranges in northern South Island (*Hemiandrus* 'vicinus', *H.* 'promontorius', *H.* 'onokis', *H.* 'horomaka', *H.* 'turgidulus') and two (*H. pallitarsis*, and *H. bilobatus*) are restricted to North Island (Johns, 2001). The species of interest in this study is *H. pallitarsis*, whose wide range is sympatric with the narrow range of *H. bilobatus* in the southern tip of North Island (Johns, 2001; Pratt *et al*., 2008). Female *H. pallitarsis* possess an unusual and distinctive accessory organ on the sixth abdominal sternite (Johns, 2001; Gwynne, 2002). During copulation, males attach to this accessory organ, in a lock-and-key fashion, and remain engaged until both the sperm ampulla and spermatophylax (nuptial gift) are transferred to the female. This accessory organ has probably evolved under sexual selection to acquire these nuptial gifts from males (Gwynne, 2005). Although these weta do not produce audible sound and lack tympanal organs (ears), local mate attraction involves vibratory signals, produced by drumming of the abdomen on a suitable substrate such as a plant leaf or stem (Gwynne, 2004). Drumming patterns differ between sexes and are likely be species specific (Gwynne, 2004).

Sampling

Hemiandrus pallitarsis individuals were obtained from 20 locations in North Island, New Zealand, throughout the species' range (Fig. 1; see also the Supporting information, Table S1). Weta were hand collected by searching the ground, low growing plants and tree trunks after dark by torchlight. Additional specimens were obtained from museum collections. Specimens used for morphological and genetic analyses were initially frozen at –20 °C and subsequently preserved in 95% ethanol. Weta collected were identified as *H. pallitarsis* using a combination of morphological characters: single foretibial spine, three prolateral spines and four retrolateral spines on the mesotibia, 70% cover of fine pilosity on the fourth maxillary palps, and bare third maxillary palps (Johns, 2001). Weta were deemed to be adult on the basis that in the penultimate instar in males, the falci (hook-like structures) on the last tergite are simple rounded knobs, whereas, in adults, the falci become blackened hooks, and adult females have reduced or absent apical styles on the ovipositor and well developed accessory organ (Johns, 2001).

Specimens from all locations were used in phylogeographical analyses of mtDNA sequences, and 168 adults from ten locations were subjected to morphometric analysis (Fig. 1; see also the Supporting information, Fig. S1, Table S2). Specimens of *H. bilobatus*, *H.* 'vicinus', *H.* 'promontorius', *H.* 'onokis' and *H.* 'horomaka' were obtained as suitable samples for the phylogenetic outgroup based on morphology (Johns, 2001), and multigene phylogenetics (Pratt *et al*., 2008).

Molecular data

MtDNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

Muscle tissue from the hind femur of one leg of each specimen was dissected out and stored at –20 °C. Total genomic DNA was extracted using a salting-out extraction method (Sunnucks & Hales, 1996; Trewick & Morgan-Richards, 2005). An approximately 1400 bp fragment comprising most of the COI gene was targeted for amplification and sequencing. PCR was performed using universal insect mtDNA primers LCO1490 (Folmer *et al*., 1994) and L2-N-3014 (Simon

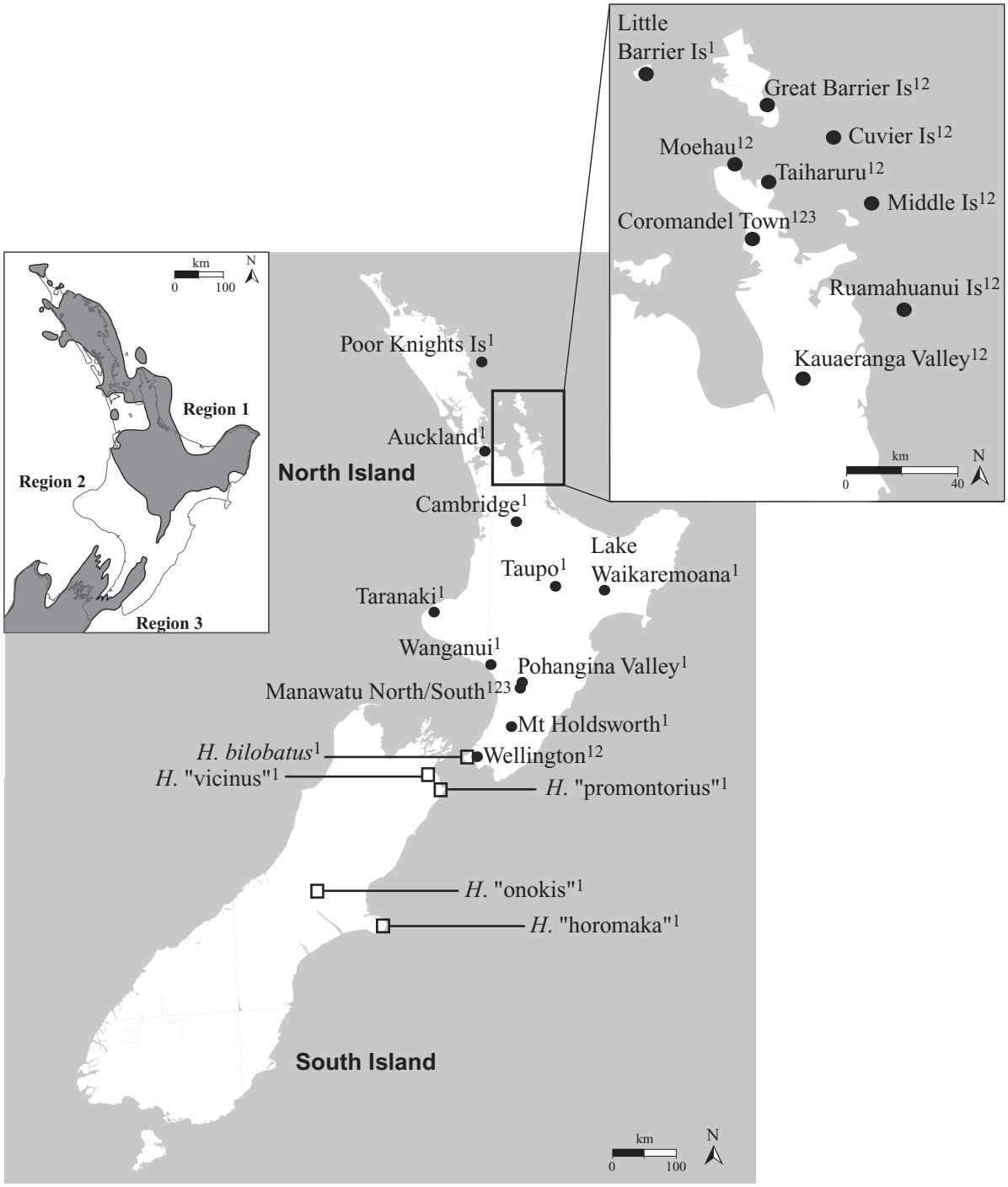

Figure 1. Collection sites for *Hemiandrus pallitarsis* (●) and outgroup species (□) in New Zealand showing locations with genetic data (1), morphological data (2), and acoustic data (3). Insets show collection sites in the Coromandel Peninsula and hypothesized distribution of land (above sea level: Regions 1 and 3; below sea level: Region 2) during the early Pliocene (*sensu* Trewick & Bland, 2011).

et al., 1994) under the cycle conditions: 94 °C for 2 min; 38 cycles (35 cycles for the smaller fragment) of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 m 30 s; and 72 °C for 8 min. For DNA samples that did not yield this fragment, a smaller, overlapping an approximately 700-bp region of COI was amplified using the primers LCO1490 and HCO2198 (Folmer *et al.*, 1994).

PCR products of the expected size were purified for sequencing using the SAP/EXO1 digest protocol (USB Corp.). Purified PCR products were sequenced using terminal primers with BigDye™ Terminator chemistry (Perkin-Elmer Applied Biosystems) and analyzed on an AB13730 genetic analyser (Applied Biosystems Inc.). DNA sequence reads were verified and aligned using SEQUENCHER, version 4.2 (Gene Codes Corp.) and SE-AL, version 2.0a11 (Rambaut, 1996). Unique haplotypes were identified using MACCLADE, version 4.0 (Maddison & Maddison, 1999). Nuclear-mitochondrial pseudogene (numts) sequences that are common in insect and other eukaryote genomes (Bensasson, Zhang & Hewitt, 2000; Richly & Leister, 2004; Pamilo, Viljakainen & Vihavainen, 2007) were identified through comparison of multiple PCR products, comparison of overlapping gene sequence fragments obtained from individuals using a combination of universal and novel primers, identification of unexpected insertions/deletions, stop codons, frameshifts, and abnormal basal placement of nuclear homologues within phylogenetic trees compared to other sequences from the same location (Bensasson *et al*., 2001). In all cases of suspected numts, two different sequences were obtained from the same individual and the nuclear copy was then excluded from the data set.

Phylogenetic analysis

Phylogenetic analyses using maximum likelihood (ML) were implemented in PAUP* 4.0b10 (Swofford, 2002) and a Bayesian approach implemented in MrBayes, version 3.1.2 (Ronquist & Huelsenbeck, 2005). To determine the most appropriate model of DNA evolution, log-likelihood scores were generated and used to conduct an Akaike information criterion test in MODELTEST, version 3.06 (Posada & Crandall, 1998). To investigate the monophyly of *H. pallitarsis* with respect to other *Hemiandrus* species, an alignment of 1393 bp of mtDNA COI was analyzed, with ten individuals representing the five closest known relatives of *H. pallitarsis* as an outgroup. Intraspecific phylogenetic analyses were performed using a COI alignment of 622 bp ($N = 102$) and the Bayesian approached as outlined below. ML was run with the model and parameters obtained using MODELTEST, version 3.06 – TVM+I+Γ model (-ln $L = 3057.3407$), with a gamma distribution shape parameter of 0.5777, 0.5548 proportion of invariable sites, and base frequencies of A = 0.26410, C = 0.25660, G = 0.16600, and T = 0.31330, and 500 bootstrap replicates. Bayesian analyses were performed using GTR (*nst* = 6) models, with two independent simultaneous runs for six million generations with three heated chains that were sampled every 1000 generations. Consensus tree and credibility values for each node were obtained after removal of burnin trees after examination of output statistics (10–20% of trees).

Population genetic analysis

The partitioning of genetic variability among populations and among groups of populations was defined by analysis of molecular variance (AMOVA; Excoffier, Smouse & Quattro, 1992) using ARLEQUIN, version 3.1 (Excoffier, Laval & Schneider, 2005). Pairwise estimates of genetic differentiation between populations were examined using Φ_{ST}, an analogue of F_{ST} that incorporates haplotype frequency and similarity (Excoffier *et al*., 1992). To determine the possible origins of diversification in *H. pallitarsis*, thirteen populations were partitioned into regions inferred to have been above and below sea level during the Pliocene (Fig. 1; see also the Supporting information, Table S1). Our expectation is that greater genetic diversity is likely to exist in regions where populations have existed the longest, with only a subset of this diversity being carried by range expansion into younger regions (Hewitt, 1999; Avise, 2000). Therefore, we predicted greater genetic diversity in Region 1 based on a hypothesis of 'north to south' expansion, or in Region 3 based on the 'south to north' prediction. To test for a correlation between genetic and geographical distances (expected under isolation-by-distance), euclidean geographical distances (km) among all population pairs were linearly regressed against their Φ_{ST} values. Mantel's test (Mantel, 1967) was performed using ARLEQUIN with 10 000 randomizations to assess the significance of distance correlations.

The global molecular clock hypothesis was tested using Tajima's relative rate test (Tajima, 1993) in MEGA, version 4.0.2 (Tamura *et al*., 2007). This test considers three sequences, one of which is an outgroup. Under the molecular clock hypothesis $E(n_{ijk})$ must equal $E(n_{ijk})$ (where n_{ijk} is the observed number of sites in which sequences 1, 2, and 3 have nucleotides i, j, and k), irrespective of the substitution model and whether or not the substitution rate varies with the site. If this hypothesis is rejected, the molecular clock hypothesis can be rejected for this set of sequences, implying disparity in substitution rates among lineages examined. Tajima's relative rate test was applied to sequences 1393 bp long, with *H. bilobatus* as the outgroup. Molecular clock rates of between 1.4% and 2.6% sequence divergence per million years were used to estimate the probable range of divergence times of lineages within *H. pallitarsis*. This range of calibration rates were derived from geologically time-constrained arthropod mitochondrial data (Brower, 1994; Folmer *et al*., 1994).

Past population demographics

Past population demographics were inferred using several methods. Fu's F_S (Fu, 1997) and Tajima's D (Tajima, 1989) neutrality tests were performed in ARLEQUIN to test for site departures from a non-random model such as directional or balancing selection, demographic expansion or contraction, or genetic hitchhiking. Significant negative values of both F_S and D can be indicative of demographic expansion when applied to neutral markers (Aris-Brosou & Excoffier, 1996). Mismatch analysis (Rogers & Harpending, 1992) was conducted using absolute pairwise sequence differences among *H. pallitarsis* haplotypes under a model of sudden population expansion in ARLEQUIN, version 3.1. Harpending's (1994) Raggedness Index (r) was calculated and its significance assessed with 10 000 rounds of parametric bootstrapping. The Bayesian Skyline Plot method (Drummond *et al*., 2005) was implemented in BEAST, version 1.5.4 (Drummond & Rambaut, 2007) to estimate changes in population size through time. This approach accommodates genealogical uncertainty using Markov chain Monte Carlo (MCMC) integration under a coalescent model, and generates a posterior probability distribution for the effective population size (N_e) through time. The evolutionary model suggested by Modeltest was used and the substitution rate was fixed at 0.0115 (Brower, 1994). Two independent runs with MCMC chain length of 50 million were performed, sampling every 1000 generations and with a burn-in of 10%. Results from the two runs were inspected to ensure ESS values were greater than 1000, then combined and re-sampled with LOGCOMBINER, version 1.5.4, and a skyline plot was generated in TRACER, version 1.5 (Rambaut & Drummond, 2007).

Morphometric data

Morphological structures of 95 adult male and 73 adult female *H. pallitarsis* were measured (see the Supporting information, Fig. S1, Table S2) with digital callipers (AIA) accurate to 0.01 mm. Traits measured were: pronotum length (Pr), metafemur length (Mf), fastigium width (the distance between lateral ocelli; Fa), head width (Hw), head length (Hl), distance from fastigium to hind coxa (used as a proxy for body length, BL, as the abdomen usually distorts during preservation), length of subgenital plate (LSg), and anterior width of subgenital plate (WSg). The female accessory organ was dissected, mounted on a microscope slide, and measured using a stereomicroscope (Nikon Alphaphot YS) with an eyepiece graticule. The mean length of the left and right tines in the fork of the accessory organ (LAO), and width of the accessory organ at the base of the fork (WAO) were measured for all individuals except those from Great Barrier Island (i.e. museum specimens that could not be dissected) and Wellington (i.e. too few specimens).

Morphological analysis

Because a paired t-test confirmed the observation that females are significantly larger than males (t-test, $P < 0.001$), male and female morphometrics were analyzed separately. The data were log-transformed because several traits did not follow a normal distribution (Anderson–Darling normality test; $P > 0.10$). Variation of morphological traits among populations was analyzed using both univariate and multivariate methods. Principal component analyses were conducted using SPSS, version 16.0 (SPSS Inc.). One-way analysis of variance (ANOVA) was used to compare patterns of variation between populations using the principal component (PC) scores. Tukey's post-hoc test was used to determine which populations differed significantly.

Drumming bout data

Drumming patterns (vibrational signalling) were compared between two major mtDNA haplogroups, and also between morphogroups, allowing us to differentiate between concordance of sexual signalling with either mtDNA lineages or morphological similarity. Weta from three locations were chosen to explicitly test whether mtDNA clades represent cryptic species (Fig. 2). Two populations from mtDNA clade I (Coromandel and Manawatu-North) were compared with one population from mtDNA clade II

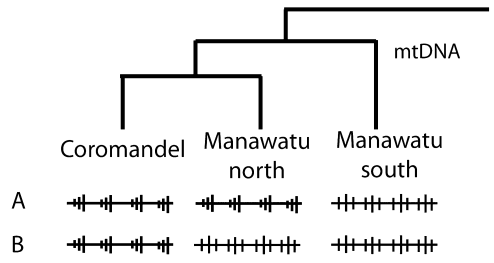

Figure 2. Evidence from male pre-copulatory vibatory signals of *Hemiandrus pallitarsis* are used to distinguish between two hypotheses. A, if the mitochodrial (mt)DNA sequence variation represents distinct species we expect populations from the same mtDNA clade (Coromandel and Manawatu-north) to be more similar to each other than either is to populations from an alternate mtDNA clade (Manawatu-south). B, if the mtDNA sequence variation is intraspecific then we expect populations geographically adjacent (Manawatu-north and Manawatu-south) to be more similar to each other than either is to a distant population (Coromandel).

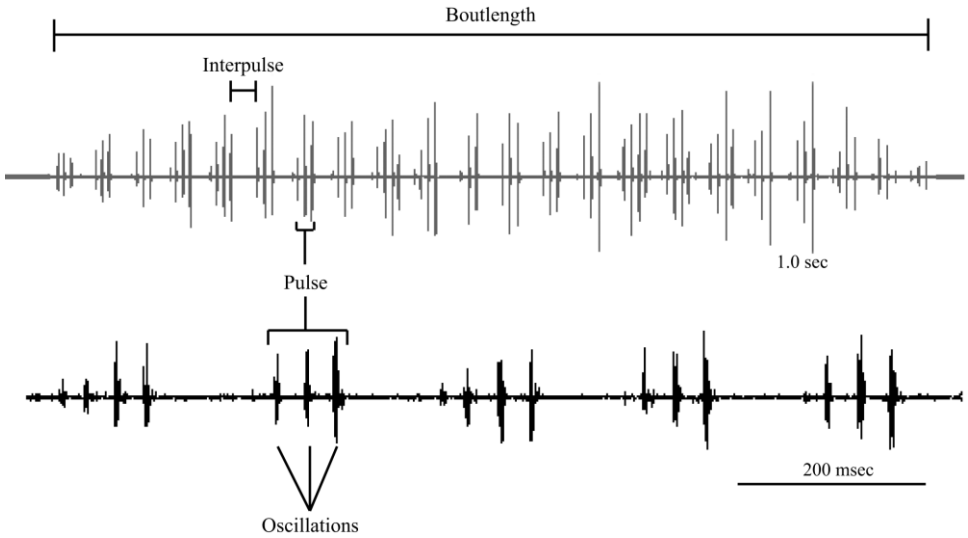

Figure 3. Oscillogram of a single drumming bout (pre-copulatory vibatory signal) produced by a *Hemiandrus pallitarsis* male showing measurements taken: top trace shows bout length, inter-pulse interval, and number of pulses; bottom trace shows five pulses from within the drumming bout, indicating the number of oscillations within each pulse.

(Manawutu-South; see Results). If mtDNA clades represent distinct species (Mallet, 1995), we predicted that male precopulation vibratory signals would be more similar within mtDNA clades than between (Fig. 2A). Reinforcement or reproductive character displacement would increase the distinction between adjacent populations in the Manawatu (collection sites are approximately 7 km apart). However, if variation in mtDNA is intraspecific, we expect the two Manawatu populations to have similar male precopulation vibratory signal, which may differ significantly from the morphologically distinctive weta at Coromandel (Fig. 2B).

Male *H. pallitarsis* drumming was recorded in the field within 10–30 min of capture. Each weta was placed onto a Piezo film (19 × 25 mm) contact pickup (http://windworld.com/) connected to a digital acoustic recorder (Olympus VN-2100PC) under a small enclosure. After approximately 30 min on the recording device, each weta was marked on the pronotum with white correction fluid (BiC Wite-Out) to prevent accidental resampling, and then released. Because calling-song/drumming rate has been found to have a linear relationship with temperature in other orthopterans (e.g. Jerusalem crickets; Weissman, 2001), the ambient air temperature was measured using a digital thermometer placed at the base of the recording device at the beginning of each recording session.

Drumming recordings were transferred into the sound analysis software, AUDACITY, version 1.2.6 (http://audacity.sourceforge.net/). All drumming bouts were amplified and the drumming parameters measured directly from the oscillogram were: (1) total drumming bout duration in seconds (Bout length); (2) number of pulses within a drumming bout (Pulses); (3) mean number of oscillations within one pulse (Oscillations); (4) inter-pulse interval in seconds (Inter-pulse); and (5) the number of pulses per second (Pulse rate; Fig. 3).

Drumming bout analysis

Intra- and inter-male variation and population variation was analyzed using the statistical software MLWIN, version 2.02 (Rasbash *et al*., 2005). A hierarchical model, with multiple drumming observations within specimens, was used to test whether drumming parameters differ significantly between the three populations sampled: Coromandel, Manawatu-North, and Manawatu-South. For each of the five drumming parameters, the equation employed was: $Y_{ij} = \beta_{0i} + \beta_{1j} + \beta_{2j} + \beta_{3temp}$, where Y_{ij} = each drumming parameter (e.g. bout length, pulses, etc.), i = individual level, j = observations within an individual level, β_{0i} = reference population mean + an error value of mean observations within an individual, β_{1j} = difference in mean value between population 1 and reference population, β_{2j} = difference in mean value between population 2 and reference population, and β_{3temp} = mean temperature – population mean. Parameters in the model were deemed significant if the sum of two times the SE was less than the parameter estimate. The same method was employed to determine the significance of within individual variation. Significant dummy variables for the populations were taken as evidence of differences between the populations (Suits, 1957).

RESULTS

Molecular results

Co-amplification of mitochondrial and nuclear copies (*numts*) of COI were identified in seven weta from six locations. These individuals had more than one DNA sequence from a single PCR product revealed as two bases at single nucleotide sites (double peaks on the chromatogram). We identified one sequence as a nuclear copy using serially diluted DNA extractions, DNA translation, and their placement on a phylogenetic tree as sister to the main *H. pallitarsis* clade, with short terminal branches. We developed two primers (nu-CU 5′-TCT GGT TTT TTG GCC ACC CAG AAG; nu-Tkt 5′-TGA TTT TTC GGT CAT CCG GAA GTG) used in conjunction with L2-N-3014 (Simon *et al*., 1994) to amplify the evolutionary conservative nuclear copy and used this to identify weta with *numts*. Nuclear DNA sequences were excluded from the study and alternative primer combinations used to amplify from the weta.

Two datasets of cytochrome oxidase DNA sequences were analyzed for the ground weta and all sequences submitted to GenBank (Accessions EU676740, EU676768, EU676771, EU676777, EU676779, EU676788, EU676789, EU676794, EU676797, and JF895542–JF895608). The first dataset comprised sequences from 24 individuals of *H. pallitarsis* from 12 locations, and five outgroup species: two *H.* 'onokis' individuals, one *H.* 'horomaka', three *H. bilobatus*, three *H.* 'promontorius', and one *H.* 'vicinus'. This alignment of 34 haplotypes, each 1393 bp, consisted of 406 (28.4%) variable, and 353 (24.7%) parsimony-informative positions. Approximately 84% of all substitutions were at third-codon positions, 13% of the variable sites occurred at the first-codon position, and 2% were found at the second-codon position. ML and separate Bayesian runs [GTR (*nst* = 6) models] produced consistent results, and analysis using HKY (*nst* = 2) models also produced similar topology. The topology among *H. pallitarsis* haplotypes was similar in all analyses with or without an outgroup and for the two sequence data sets.

Phylogenetic analysis of the 1393 bp alignment showed that the 24 *H. pallitarsis* individuals formed a monophyletic group (posterior probability = 0.98), which was sister to a clade comprising Canterbury *H.* 'onokis' and Banks Peninsula *H.* 'horomaka' (Fig. 4). Interestingly, *H. bilobatus* which is sympatric with *H. pallitarsis* in Wellington, North Island, is sister to *H.* 'promontorius' from Cape Campbell, South Island. The Marlborough Sounds species, *H.* 'vicinus', is sister to Wellington *H. bilobatus* and Cape Campbell *H.* 'promontorius'. Sequences from different individuals of each species form monophyletic clades, although there is a greater DNA sequence diversity within *H. pallitarsis* than there is between either *H. bilobatus* and *H.* 'promontorius', or *H.* 'onokis' and *H.* 'horomaka'. Within *H. pallitarsis*, those individuals from Wellington are sister to the rest of *H. pallitarsis*.

The second mtDNA sequence dataset excluded all outgroup sequences but included sequences from a further 78 *H. pallitarsis* individuals from eight additional locations, giving an alignment of 102 *H. pallitarsis* from 19 locations, which was 622 bp in length. This alignment contained 131 variable sites (20%), of which 117 (18%) were parsimony informative. Approximately 89% of all substitutions were at third-codon positions, and only 10% of the variable sites occurred at the first-codon position; no substitutions were found at the second-codon position. All but two substitutions were synonymous mutations resulting in no amino acid change. Fifty-eight unique haplotypes were detected, with no haplotype-sharing among sampling sites. Maximum likelihood (single optimal tree, -ln $L = 2619.5477$) and Bayesian analyses of the 58 haplotypes resulted in trees of near identical topology, and consistent with that from Bayesian analysis of the 1393 bp alignment (Fig. 4; see also the Supporting information, Fig. S2). All analyses revealed three main clades within *H. pallitarsis*: a predominantly northern clade including individuals from Pohangina Valley, Wanganui, and some from Manawatu (Clade I); a central clade containing individuals from Taranaki, Taupo, Cambridge, and remaining individuals from Manawatu (Clade II); and a Wellington clade (Clade III). Bootstrap resampling with ML and the Bayesian credibility values all indicated strong support for these three main clades (97/100/70; 0.99/1/0.98, respectively; see Supporting information, Fig. S2). Whether using Neighbour-joining of HKY distances or Bayesian analysis with a GTR+I+Γ model, the main structure of unrooted networks was the same (Fig. 5). Manawatu was the only area sampled that yielded haplotypes in two of these clades; individuals collected at Manawatu North had Clade I haplotypes, whereas individuals from Manawatu South had Clade II haplotypes (Fig. 5; see also the Supporting information, Fig. S2). This intriguing pattern was not an artefact of collection, DNA extraction, PCR or sequencing events. Sampling sites within Manawatu were separated by < 7 km and the Manawatu river but differ genetically by 0.061–0.077 (uncorrected *p*-distance) and ML distances of 0.088–0.13 (TVM+I+Γ; see the Supporting information, Table S3). There is also strong support for the genetic separation of geographically proximate populations within Wellington (Clade III) – uncorrected *p*-distances up to 0.051 (0.068 TVM+I+Γ) between populations separated by approximately 7 km.

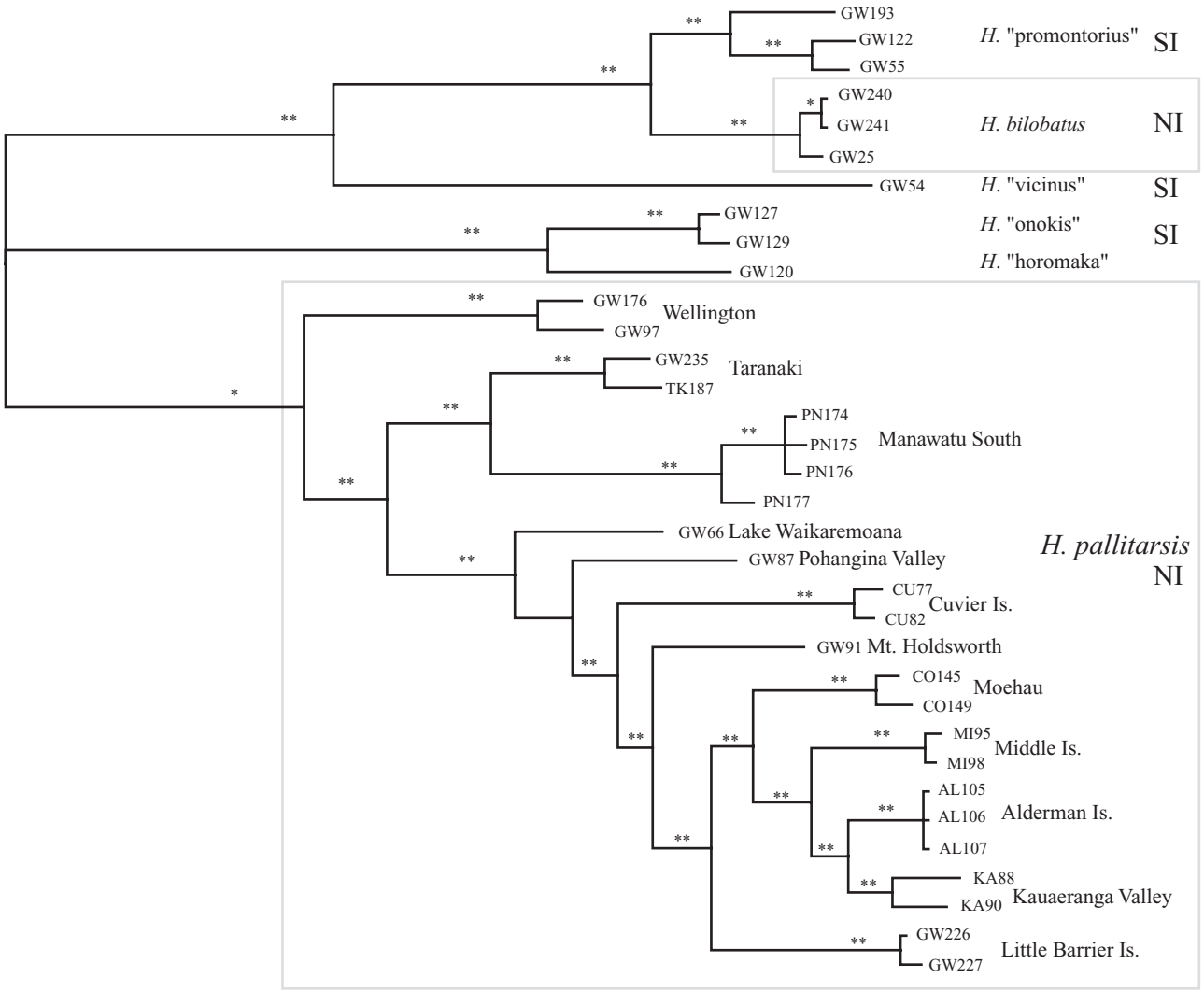

Figure 4. Bayesian consensus network based on 1393 bp of mitochondrial DNA (COI) showing the evolutionary relationships among short-ovipositor ground weta of New Zealand: *Hemiandrus pallitarsis* (large grey box), *Hemiandrus bilobatus* (small grey box), *Hemiandrus* 'onokis', *Hemiandrus* 'horomaka', *Hemiandrus* 'promontorius', and *Hemiandrus* 'vicinus'. Node stability is indicated by posterior probabilities, * > 0.95, ** > 0.99. NI, North Island; SI, South Island.

Genetic distances

For the long COI dataset with representatives from six species, uncorrected *p* genetic distances reached a maximum of 16.4%. Most pairwise distances (96%) between these species were greater than 10.5%. However, nine pairwise distances between *H. bilobatus* and *H.* 'promontorius', and two between *H.* 'horomaka' and *H.* 'onokis', were less than 4.5%. By contrast, uncorrected pairwise genetic distance in *H. pallitarsis* reached 9.0% between weta from Taupo and Wanganui (representing mtDNA clades I and II). MtDNA distances between clades I and III (within *H. pallitarsis*) reached a maximum of 8.5% (*p*-distance; mean 7.3%) and between clades II and III the greatest genetic distance was 8.0% (*p*-distance; mean 6.9%). However, a more complex general time reversible nucleotide substitution submodel (TVM+I+Γ) probably provides a more realistic estimate consistent with the expectation of a nonlinear relationship between true and observed distances. The ML (TVM+I+Γ) distances within *H. pallitarsis* reached a maximum of 0.16 (see the Supporting information, Table S3)

Population structure

Of the 58 *H. pallitarsis* COI haplotypes (among 102 individual weta), 54 were useful for population genetic analyses. Four localities with a sample size of one were excluded (Great Barrier Island, Cambridge, Poor Knights Island, Pohangina Valley). Nucleotide diversity (π) ranged from 0–0.043 (mean = 0.0083, SD = 0.0124) and haplotype diversity (h) ranged from

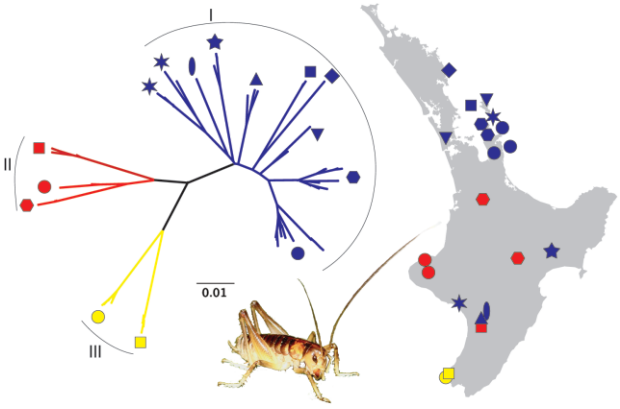

Figure 5. Spatial distribution of genetic diversity in the ground weta *Hemiandrus pallitarsis* in New Zealand based on unrooted Neighbour-joining network of 622 bp alignment of mitochondrial DNA (COI). Three clades (I, II, III) identified in Bayesian and maximum likelihood analyses (Figs 4; see also the Supporting information, Fig. S2) are indicated in blue, red, and yellow, respectively. Symbols show the spatial location of samples within lineages resolved by Bayesian analysis (see the Supporting information, Fig. S2).

zero, where all individuals in a population have the same haplotype, to one, where each individual in a population has a unique haplotype (mean ± SD: 0.5985 ± 0.4036; Table 1). There were four populations where a single haplotype was found (Auckland, Taiharuru, Ruamahuanui Island, Taupo), resulting in no haplotypic variation ($h = 0$). Two populations (Little Barrier Island, Lake Waikaremoana) revealed unique haplotypes for each individual sequenced ($h = 1$). Haplotypes from each site were always part of the same clade (and frequently sister; see the Supporting information, Fig. S2).

Overall, genetic diversity was highly structured. AMOVA revealed that 95 of 105 (90.5%) pairwise population Φ_{ST} comparisons were significant greater than zero ($P \leq 0.05$), with a mean of 0.833 (range 0.42–1.00). AMOVA also revealed 79.6% of the molecular variance was partitioned among populations, whereas only 20.4% ($P \leq 0.001$) was partitioned within populations. Where the 13 populations were partitioned into three regions, using a hypothesis of land above and below sea level during the Pliocene (Fig. 1), highest diversity was found in Region 3 (Wellington), followed by Region 2 (populations below sea level), whereas populations occupying northern half of the NI (Region 1) had the lowest haplotype and nucleotide diversity (Table 1). With a hypothesis of population range expansion through leading edge colonization, this pattern of genetic diversity implies range expansion from the south to the north. However, a Mantel test for matrix correlation between genetic differentiation and geographical distance was not significant (Z-score = 19188459, $r = -0.340197$, $P = > 0.05$), suggesting *H. pallitarsis* mtDNA does not fit an isolation-by-distance model.

Molecular clock

Tajima's relative rate test showed no evidence of rate variation between lineages and therefore we could not reject a molecular clock hypothesis ($P > 0.05$). Applying a broad range of putative molecular clock rates between 1.4% and 2.6% sequence divergence per million years, the three clades within *H. pallitarsis* appear to have diverged during the Pliocene (2.0–6.4 Mya). On this basis, the two northern clades (I and II) diverged from the Wellington clade (III) and each other approximately 2.2–6.1 Mya. Should this rate be appropriate for *Hemiandrus* COI, we could infer that the mtDNA lineages now present in populations in Manawatu North and Manawatu South diverged during the Pliocene. However, judging from palaeogeographical evidence, they are unlikely to have diverged in the Manawatu because this region was below sea level (Fig. 1, inset) (Trewick & Bland, 2011). In the Coromandel, the populations on islands east of the peninsula are inferred as having diverged from the Coromandel Peninsula populations during the Pleistocene (Cuvier Island: 0.95–2.64 Mya; Middle Island: 0.68–2.06 Mya; Ruamahuanui Island: 0.43–1.15 Mya), although all these were not islands when joined to Coromandel Peninsula during lowered sea level as little as 20 000 years ago.

Past population demographics

Tajima's D-values were not significant for any of the populations analyzed, and Fu's F_S was significant and negative for just one population (Table 1). When all haplotypes were combined, the neutrality tests were also not significant ($D = 0.677$, $P = 0.798$; $F_S = -4.166$, $P = 0.206$), providing no evidence of selection on this locus, nor of population expansion. A mismatch distribution for *H. pallitarsis* mtDNA haplotypes using pairwise genetic distances was 'ragged', which is typical of populations that are stable through time (see the Supporting information, Fig. S3). Harpending's r of 0.0038 ($P = 0.12$) also provides no evidence for recent population expansion. The Bayesian Skyline Plot also supported a relatively stable population over the last approximately 3.5 million years with only a small increase in population size during the late Pleistocene (see the Supporting information, Fig. S4).

MORPHOMETRIC RESULTS

All morphomentric analyses of males and females had variables with positive loadings on the first principal component (PC1), indicating that scores from PC1 can

Table 1. Summary statistics and molecular indices for the New Zealand ground weta *Hemiandrus pallitarsis*

Collection location (number of sample locations)	*N*	*K*	*h*	π	*D*	F_S
Poor Knights Island* (1)	1	1	–	–	–	–
Great Barrier Island* (1)	1	1	–	–	–	–
Little Barrier Island (1)	2	2	1	0.003	0	0.693
Auckland (1)	6	1	0	0	0	–
Moehau (2)	8	5	0.742	0.004	–0.0764	0.086
Cuvier Island (1)	9	4	0.583	0.002	–0.91	–0.286
Taiharuru (1)	5	1	0	0	0	–
Middle Island (1)	9	4	0.583	0.001	–0.9361	–1.417
Coromandel Town (2)	8	5	0.893	0.006	0.7968	0.336
Ruamahuanui Island (1)	8	1	0	0	0	–
Kauraeranga Valley (2)	8	7	0.964	0.008	–0.1409	–1.794
Cambridge* (1)	1	1	–	–	–	–
Taupo (1)	2	1	0	0	0	–
Lake Waikaremoana (1)	3	3	1	0.01	–0.0002	0.588
Taranaki (2)	5	2	0.6	0.001	1.2247	0.626
Wanganui (1)	3	2	0.667	0.016	0	4.174
Manawatu (4)	9	8	0.972	0.043	1.7262	0.794
Pohangina Valley* (1)	1	1	–	–	–	–
Wellington (3)	9	8	0.972	0.029	1.6903	0.116
Region 1	**64**	**35**	**0.577**	**0.004**	–	–
Region 2	**15**	**12**	**0.736**	**0.024**	–	–
Region 3	**9**	**8**	**0.972**	**0.029**	–	–
Total (25)	**88**	**55**	**0.632**	**0.009**	**1.2912**	

Number of individuals collected (*N*), number of haplotypes (*K*), haplotype diversity (*h*), nucleotide diversity (π), Tajima's *D*, and Fu's *F*s.

*Removed from population-level analyses as a result of a sample size of one. Regions 1, 2, and 3 are the area above (1 and 3) and below (2) sea level during the Pliocene (Fig. 1 inset).

be used as general descriptors of body size. Scores from the second principal component (PC2) were negative for some of the variables and positive for others, indicating PC2 describes variation in shape (data not shown). For male weta, PC1 and PC2 explained 68.3% and 11.2% of the total variance, respectively (Fig. 6A). For the female weta, these two components accounted for 51.8% and 12.9% of the total variance, respectively (Fig. 6B). Head measurements and body length loaded negatively on PC2 for each sex; therefore, low PC2 scores describe weta with large heads and a relatively long thorax but short legs and small subgenital plates. Additionally, female weta with larger heads also tend to have shorter and narrower accessory organs. One-way ANOVA on PC1 scores revealed significant differences in size among locations for both males and females ($P < 0.001$). Post-hoc Tukey's test identified weta from Manawatu as being significantly smaller than weta from all other locations ($P < 0.05$). Additionally, male weta from Kauaeranga Valley were significantly larger than those from Manawatu, Great Barrier Island, Moehau, and Coromandel Town ($P < 0.05$).

Drumming bout results

In total, 78 drumming bouts were recorded from 25 adult male *H. pallitarsis*. The number of individual weta recorded at each site (*N*) varied, as did the temperature range: Coromandel, $N = 12$ (17.5–20.3 °C); Manawatu-North, $N = 5$ (13.9–16.8 °C); and Manawatu-South, $N = 8$ (13.9–18.8 °C). In all cases, there was as much variation among an individual's drumming bouts as there was between individuals in a population (after correcting for temperature). However, all of the drumming components revealed significant differences between Coromandel and Manawatu-North, and all components except for the inter-pulse interval showed significant differences between Coromandel and Manawatu-South. Three of the five drumming components measured revealed no significant difference between the two Manawatu populations, which represent two distinct mtDNA clades. Drumming bouts in Coromandel were longer, contained more pulses, and subsequently had higher pulse rates than either of the two Manawatu populations, although they had fewer oscillations in each

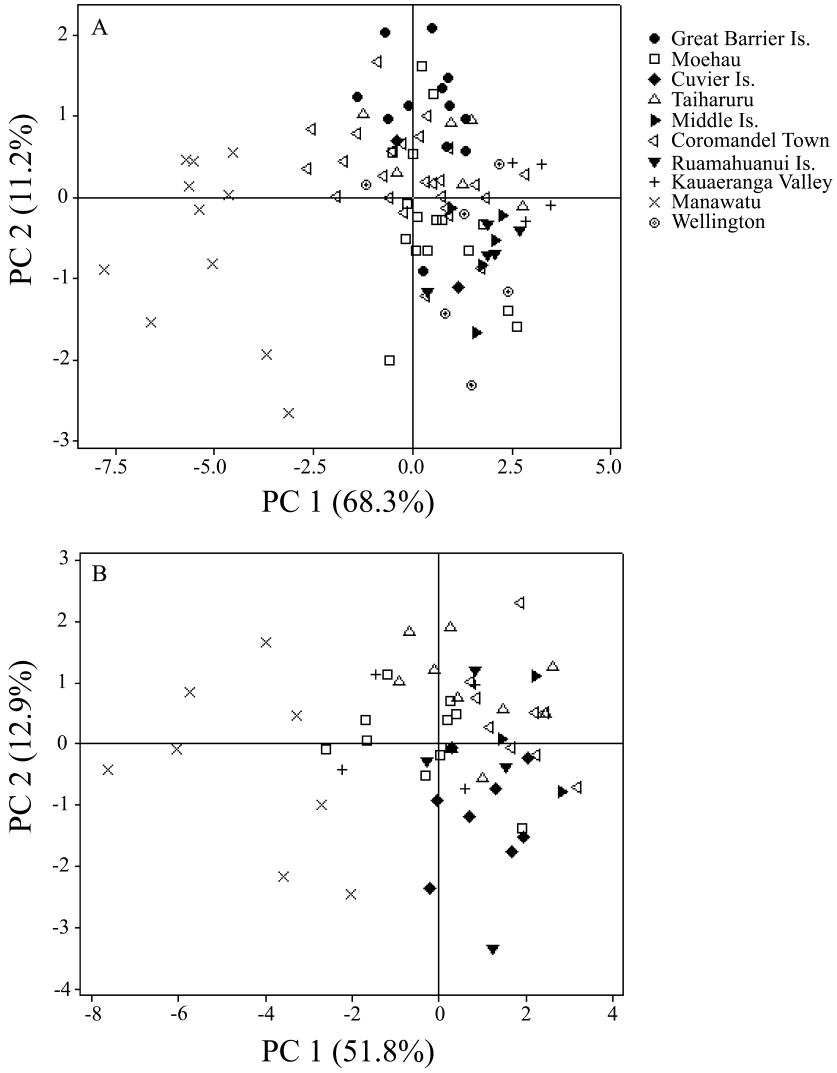

Figure 6. Principal component (PC) analyses on eight morphological traits of adult male *Hemiandrus pallitarsis* (A) and ten morphological traits of adult female *H. pallitarsis* (B). Percent of total variation is marked on each axis.

pulse and a shorter inter-pulse interval (Table 2). Greater variation between the Coromandel male precopulation vibratory signals and the two Manawatu populations was observed than between the two Manawatu populations, as expected if the mtDNA variation was intraspecific (Fig. 2B).

DISCUSSION

Genetic diversity

In a global context, the level of genetic divergence found within *H. pallitarsis* mitochondrial DNA (COI; up to 9.0% uncorrected) is high, more typical of interspecific divergence in invertebrates studied in the Northern Hemisphere (Ball *et al.*, 2005; Robinson *et al.*, 2009). This level is, however, consistent with studies of a number of other New Zealand invertebrates, including other species of Anostostomatid weta. For example, uncorrected intraspecific COI genetic distances are 9.5% in Auckland tree weta *Hemideina thoracica* (Morgan-Richards *et al.*, 2001), 7.6% in alpine scree weta *Deinacrida connectens* (Trewick *et al.*, 2000), and between 5.3% and 7.1% for several other tree and giant weta species (King *et al.*, 2003; Trewick & Morgan-Richards, 2005). Several explanations might account for this high mitochondrial sequence diversity.

Cryptic species

A simplistic inference from observations of high sequence distances (and an expectation of DNA barcoding) is the existence of numerous, hitherto unrecognized (i.e. cryptic) species (Bickford *et al.*, 2007).

Table 2. Comparison of male precopulation signals from three populations of New Zealand ground weta *Hemiandrus pallitarsis*, showing mean ± SE values for five drumming-bout components

	Drumming component				
	Bout length (s)	Pulses	Oscillations	Inter-pulse (s)	Pulse rate (pulses/s)
Coromandel (C)	3.29 (0.087)	20.56 (0.346)	3.30 (0.054)	0.09 (0.002)	6.29 (0.068)
Manawatu-North (MN)	2.67 (0.260)	15.27 (1.037)	3.62 (0.084)	0.11 (0.006)	5.55 (0.204)
Manawatu-South (MS)	2.69 (0.155)	16.62 (0.619)	3.69 (0.057)	0.09 (0.004)	5.98 (0.122)
Temperature increase by 1 °C	–0.278	–0.591	–0.024	–0.003	0.211
Significance*	C – MN MS	C – MN MS	C – MN MS	MN – C MS	C – MN MS

*Populations separated by a dash (–) differ significantly.

However, the establishment of separate species that have any biological meaning demands multiple characters (Mallet, 1995; De Queiroz, 2007; Roe & Sperling, 2007). Without this, taxonomy is simply a discrete classification system divorced from an evolutionary context that offers little to modern biology (Trewick, 2008). For example, within New Zealand Onychophora (velvet worms), high genetic diversity concordant at mitochondrial and nuclear loci between sympatric populations strongly supports the existence of morphologically cryptic species (Trewick, 2000). By contrast, high mtDNA sequence diversity within New Zealand tree weta was discordant with patterns of parapatric karyotype and allozyme variation, indicating ongoing gene flow and therefore not correlated with cryptic species (Morgan-Richards, 1997; Morgan-Richards *et al*., 2001). Used in isolation, mtDNA sequence diversity in *H. pallitarsis*, following DNA barcoding practices, suggests the presence of several cryptic species. However, patterns of morphometric and drumming bout variation are discordant with the patterns of genetic diversity as is expected of intraspecific variation (Mallet, 1995). Notably, two distinct mtDNA clades were identified in adjacent Manawatu locations, although there was no significant difference in body size or shape and little difference in male precopulation vibratory signals (bout length, pulse, oscillation) of the weta they represent. Had the mtDNA clades identified cryptic species, one would have expected mate recognition signals to be under divergent selection in the Manawatu where two distinct mtDNA lineages are parapatric (and possibly sympatric). The likely result would be significant differences in male drumming (used to attract mates) and female accessory organs (used to receive nuptial gifts during copulation). Conversely, male precopulation vibratory signals differed significantly for all five components compared between two populations in the same mtDNA clade. Similarly, populations from Wellington are genetically differentiated from populations on the Coromandel Peninsula, although body size and shape in these populations do not differ. Discordance of patterns among characters within *H. pallitarsis* populations provides evidence of cohesion rather than multiple cryptic species. By contrast, congeneric species identified using discrete morphological characters, such as *H. bilobatus* and *H.* 'promontorius' (Johns, 2001) each formed mtDNA monophyletic clades concordant with morphology. There is no variation in *H. pallitarsis*, of the morphological characters used for alpha taxonomy in other ground weta.

Substitution rate and *numts*

Higher than expected intraspecific genetic distance could result from lineage specific acceleration of the rate of mitochondrial evolution as has been postulated but not proven for other species with exceptionally high levels of mitochondrial divergence (Edmands, 2001; Boyer, Baker & Giribet, 2007). In *H. pallitarsis*, the distribution of mutations at the first-, second-, and third-codon positions (10%, 0%, and 89%, respectively) are not obviously different from those found in other invertebrate studies (e.g. third codon: 76–84%, second codon: 1.6–4.5%, first codon: 9.4–19.75% Trewick *et al*., 2000; Chinn & Gemmell, 2004), and relative rate tests including outgroup *Hemiandrus* species with smaller ranges and lower levels of intraspecific diversity found no evidence of lineage specific rate variation. Therefore, there is no evidence that the rate of COI evolution in *H. pallitarsis* differs significantly from that of related taxa exhibiting less variation.

Apparently high genetic distances could be artefacts of unrecognized co-amplification of nuclear mitochondrial pseudogenes (*numts*) and the accumulation of mutational changes since integration. Failure to detect *numts* can affect DNA barcoding studies and may lead to incorrect species delimitation (Song *et al*., 2008), although rate of molecular evolution in the

nuclear genome is usually slower than that within the mitochondrial genome (Bensasson *et al*., 2001). In the present study, *numt* sequences were carefully identified and excluded and cannot therefore explain the high genetic diversity within *H. pallitarsis*.

Population size

A fundamental expectation of neutral theory is that levels of genetic diversity are directly associated with population size and history (Kimura, 1968; Rowe & Beebee, 2007). Thus, the simplest explanation for high sequence diversity and significant population structuring within *H. pallitarsis* is long-term demographic stability. Within *H. pallitarsis*, neutrality tests, mismatch distribution, and Bayesian Skyline analysis all failed to support a model of recent population expansion, and AMOVA indicated populations were highly spatially structured. If differences in the depth of coalescence, and therefore DNA sequence diversity, exist among organisms that all conform to the same assumptions about mutation rate (intrinsic processes), then an extrinsic explanation is required.

Some New Zealand phylogeographical studies provide strong evidence for the type of refugia and range expansion patterns that are typical of northern hemisphere systems (e.g. *Clitarchus* stick insects; Morgan-Richards, Trewick & Stringer, 2010), whereas others indicate that species ranges persisted through climate cycles (*Asplenium* fern; Shepherd, Perrie & Brownsey, 2007; see also Trewick *et al*., 2011). Because wholesale ablation of habitable environments did not occur in New Zealand, perhaps phylogeographical structuring of different taxa reflects their relative dependence on different habitat and vegetation types? Today, *H. pallitarsis* occurs under forest cover as well as in areas that have been cleared of forest, indicating that the species could have survived in regions denuded of high vegetation during glaciations.

Any influence of Pleistocene glacial cycling on populations must overlay the substantial impact of a changing landscape resulting from tectonic and volcanic activity throughout the Pliocene and Pleistocene as southern North Island emerged from the sea (Trewick & Bland, 2011). It is therefore possible that *H. pallitarsis*, as well as some other New Zealand invertebrates, have maintained relatively large populations throughout the Pleistocene (compared to population sizes in the Northern Hemisphere at the same time), and thus carry a signature of Pliocene phylogeography.

Phylogeography

Genetic diversity among populations is expected to be lower in more recently colonized populations as a result of leading edge range expansion (Hewitt, 1999; Excoffier, Foll & Petit, 2009). Population genetic analysis revealed Region 3 (i.e. southern populations from the Wellington region) had the highest level of genetic diversity and Region 1 (i.e. northern North Island) had the lowest (Table 1). Bayesian analysis of the longer data mtDNA set found *H. pallitarsis* from Wellington were sister to all the other individuals sequenced (Fig. 4). These results suggest range expansion has occurred in a 'south to north' direction, as inferred for New Zealand freshwater crayfishes (Apte, Smith & Wallis, 2007), and cave weta (Goldberg, Trewick & Paterson, 2008), although this may be a signature much older than the last glacial climate cycle (Trewick *et al*., 2011). At no spatial scale did *H. pallitarsis* show genetic isolation-by-distance. Geographical barriers that may have influenced the distributional patterns of *H. pallitarsis* by reducing gene flow are likely to date into the Pliocene, given our estimates of genetic distance. Land area and topography in North Island New Zealand has changed extensively in this time fame (Trewick & Bland, 2011). Much of the southern part of North Island was below sea level and the Wellington region was connected to Marlborough (now northern South Island). The extensive separation of Wellington from proto-North Island likely limited gene flow between northern and southern populations. However, the phylogeographical pattern found in *H. pallitarsis* is not that straightforward. The occupation of the Manawtu by two distinct lineages implies at least two separate expansion fronts, possibly from different directions and at different times (Fig. 5). Occupation of this area is implausible until tectonic forces resulted in land emerged during late Pleistocene time (Trewick & Bland, 2011). Pre-existing lineages likely evolved outside this area, possibly on either side of former sea straits in the region (Fig. 1). The current phylogeographical pattern was probably also influenced by volcanic activity in central North Island that destroyed a large area of forest and removed insect populations (Morgan-Richards, Trewick & Wallis, 2000; Trewick & Bland, 2011).

Conclusions

Using the approach of Avise & Ball (1990) and Mallet (1995), we recognize species when independent characters and genetic markers are concordant. Thus, we conclude that the New Zealand ground weta, *H. pallitarsis*, is a single widespread species, rather varied both genetically and morphologically, although lacking clusters of populations with identifying and concordant characters. Although high sequence diversity among populations provides an appealing basis for partitioning, we found that variation in male

precopulatory mating behaviour and morphology were not consistent with reproductive isolation among mtDNA clades. The high intraspecific diversity found in *H. pallitarsis* matches that reported in other New Zealand Anostostomatid weta (Trewick & Morgan-Richards, 2004, 2005) and invertebrates (Trewick *et al*., 2011), as well as Australian leaf-litter invertebrates (Garrick *et al*., 2004). These observations contrast with intraspecific genetic distances typically reported between insect species, many of which were strongly influenced by climate change in the Pleistocene. Not only is the depth of intraspecific genetic diversity in New Zealand exceptional, but also its spatial distribution cannot be (exclusively) attributed to latitudinal range expansion following the LGM. A lesser influence of Pleistocene climate cycling on a lineage means that a signature of older Pliocene evolution is revealed. Our data lend support to arguments that simplistic comparisons of genetic distances can mislead taxonomy.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Morphometric characters measured for *Hemiandrus pallitarsis* specimens. Sg, length subgenital plate (a,b); MxSg, maximum width subgenital plate (a,b); LAO1 and LAO2, length of right and left tine of the accessory organ fork, respectively (c); WAO, width of accessory organ (c); H1, head length (d); Hw, head width (d); Fa, fastigium width (d); BI, distance between third tergite and anterior fastigium (e); Pr, length of pronotum (e); Mf, metafemur (f).

Figure S2. A Bayesian consensus network of *Hemiandrus pallitarsis* population relationships based on molecular analysis of 622 bp mitochondrial DNA sequence data. An arrow indicates position of root in analyses that included outgroup *Hemiandrus* species. Node support is indicated by an asterisk (*) if its posterior probability was > 0.95 and maximum likelihood bootstrap value was > 75%. Terminal labels indicate specimen code, sampling location, and sample size before identical sequences were removed from analysis. Codes are as in Table S1.

Figure S3. Using absolute pairwise differences under a model of sudden population expansion, ragged mismatch distribution is indicative of a stable population of the New Zealand ground weta *Hemiandrus pallitarsis*, observed (open circles, dashed line) and expected (solid line) mitochondrial DNA mismatch distribution.

Figure S4. Bayesian demographic reconstruction inferred for *Hemiandrus pallitarsis* with BEAST, where *NeT* is the product of the effective population size and generation time. The solid outline shows the 0.95 posterior intervals for the population size change through time and the graph line shows the median population size.

Table S1. Sampling locations for *Hemiandrus* specimens. *Individuals used in Bayesian analysis of the entire COI gene fragment. †Locations above sea level during the early Pliocene (Trewick & Bland, 2011).

Table S2. Body measurements (mm) of *Hemiandrus pallitarsis* specimens ($N = 168$). F, Female; M, Male.

Table S3. Matrix of pairwise genetic distance among New Zealand ground weta *Hemiandrus pallitarsis*, based on 622 bp of mitochondrial (mt)DNA (COI) using TVM+I+Γ. The three main mtDNA clades are indicated with boxes.

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