

Phylogenetic relationships of the New Zealand red admiral butterfly subspecies.

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Abstract

The red admiral or kahukura (*Vanessa gonerilla* (Lepidoptera: Nymphalidae)), is a once-common, endemic butterfly of New Zealand. Two subspecies are recognised, *Vanessa gonerilla gonerilla*, widespread throughout the North and South Island, as well as some nearby offshore islands, and *Vanessa gonerilla ida*, restricted to the Chatham Islands. There is anecdotal evidence that suggests there is no gene flow occurring between the subspecies. The taxonomic split of *V. g. gonerilla* and *V. g. ida* is largely based upon consistent slight differences in morphology and behaviour. This research aims to characterise the genetic variation and to accompany previous phenotypic analysis and identify any genetic boundaries between the subspecies. Two specimens of *V. g. ida* were collected from the Chatham Islands, DNA was isolated from these and a 645bp section of the mitochondrial COI gene was sequenced. Reference sequences of *Vanessa gonerilla* were obtained from GenBank, and compared with our sequence. A single nucleotide point mutation (SNP) was found in Chatham Island specimens, which was not found in any of the reference sequences. Unfortunately, location and subspecies data were not available for the reference sequences sourced from GenBank, limiting the usefulness of this data in the analysis. The next stage of this research will be to obtain additional specimens, from both the Chatham Islands and mainland New Zealand, and to sequence additional gene regions, which will give a more detailed picture of the variations which occur between these subspecies.

Objectives of study

- Investigate whether the morphologically distinct subspecies of Red admiral butterfly *V. g. gonerilla* and *V. g. ida* are supported by genetic data from mitochondrial sequences, gene regions CO1 and 16S.
- Build a phylogeographic structure of the subspecies, testing the validity of subspecies using monophyly as a criterion based on genetic data, and the degree of gene flow between the subspecies.

The study species

Vanessa gonerilla gonerilla Fabricius, 1775 occupies forest habitats or open areas on mainland New Zealand where there is access to its larval host plant *Urtica ferox* G.Forst. This species has a lifespan of approximately 9 months for over-wintering generations, and 4 – 6 months for the summer generations with peak sightings of all life stages occurring between September and April. Imago *V. g. gonerilla* possess a wingspan of 50mm – 60mm, with males being smaller than females. Colour on the underside of wings varies considerably from mottled brown to mottled pale fawn accompanied by a striking blue eyespot bracketed by white and red arcs. Top side of the forewings are mostly black with a red band running from the front edge. White spots fringed with blue are present near the forewing tips. Rear wings are dark reddish brown with a red patch containing four black circles which contain a white/blue centre (plate 1.).

Vanessa gonerilla ida Alfen, 1899 is found only in the Chatham Islands. Physical appearance of *V. g. ida* is similar to that of *V. g. gonerilla*, however, subtle differences in hindwing shape and colour differentiate the two. *V. g. ida* possess a wider red patch and more rounded hindwings with less pronounced scalloping indentations between the veins. Ovum, larvae, and pupa stages of both variations lack distinguishable features and therefore are not able to be set apart. Larval host plant of *V. g. ida* is *Urtica australis* Hook.f.



Plate 1. Upper and lower surface of *V. gonerilla gonerilla* (above) and *V. gonerilla ida* (below) Gibbs, 1980.

Subspecies

Subspecies are taxonomic units used to formally describe intraspecific geographic variation in morphological traits (Patten, 2015). Mayr and Ashlock (1991) define subspecies as “a collection of populations occupying a distinct breeding range and are diagnosably distinct from other such populations”, a widely accepted definition, yet in practice highly subjective (Patten, 2015). What these units represent in evolutionary terms, and whether they can be used as reliably useful units in conservation, evolutionary theory, biology, and taxonomy, is also debated (Kodandaramaiah et al, 2012). It is common practice in taxonomic revisions for several closely related taxa to be lumped together as subspecies, or subspecies to be elevated to the level of species if they are deemed worthy. It follows that various taxa currently considered subspecies might represent a genuine species in their own right (Kodandaramaiah et al, 2012). From a taxonomic perspective, the intraspecific category representing diverging lineages within a species, possibly under various stages of speciation, is often the subspecies (Patten, 2015). Phylogenetic methods are increasingly being employed in identifying geographic variation within a species and drawing taxonomic boundaries in subspecies taxonomy (Patten, 2015), however, morphological variation is also needed, as genetic differentiation by itself, is insufficient evidence to diagnose a subspecies (Mousseau and Sikes 2011).

Methods

DNA was extracted using a Qiagen Blood and Tissue DNA isolation kit. A 645bp section of the COI was PCR amplified and sequenced by Massey Genome service. The resulting sequences were then aligned to obtain a consensus sequence for *V. g. ida*. This was then compared to the 3 *V. g. gonerilla* COI sequences that are currently available on GenBank to check for variations between these reference sequences and our consensus. The records for the samples sourced from GenBank do not state which subspecies they belong to.

Results and discussion

Sequences differences of between 1 and 4 base pairs were detected between the *V.g.ida* consensus sequence and the *V. g. gonerilla* reference sequences retrieved from GenBank. Similar variation was also found between the *V. g. gonerilla* reference sequences, with one of the three sequences having 4 SNPs not shared by the others.

A difference of 4 base pairs between our consensus sequence and the reference sequences is potentially interesting, however, the small number of specimens collected to date and the limited number of sequences available via Genbank make these initial results insignificant. More specimens from both subspecies are needed to generate robust results. The next stage of this research will be to collect and extract DNA from additional specimens from both *V. gonerilla* subspecies. The COI region of these will be sequenced, in addition to the 16S region. Increasing the number of specimens and the number of gene regions examined will give an improved understanding of the variation within, and between, the two subspecies.

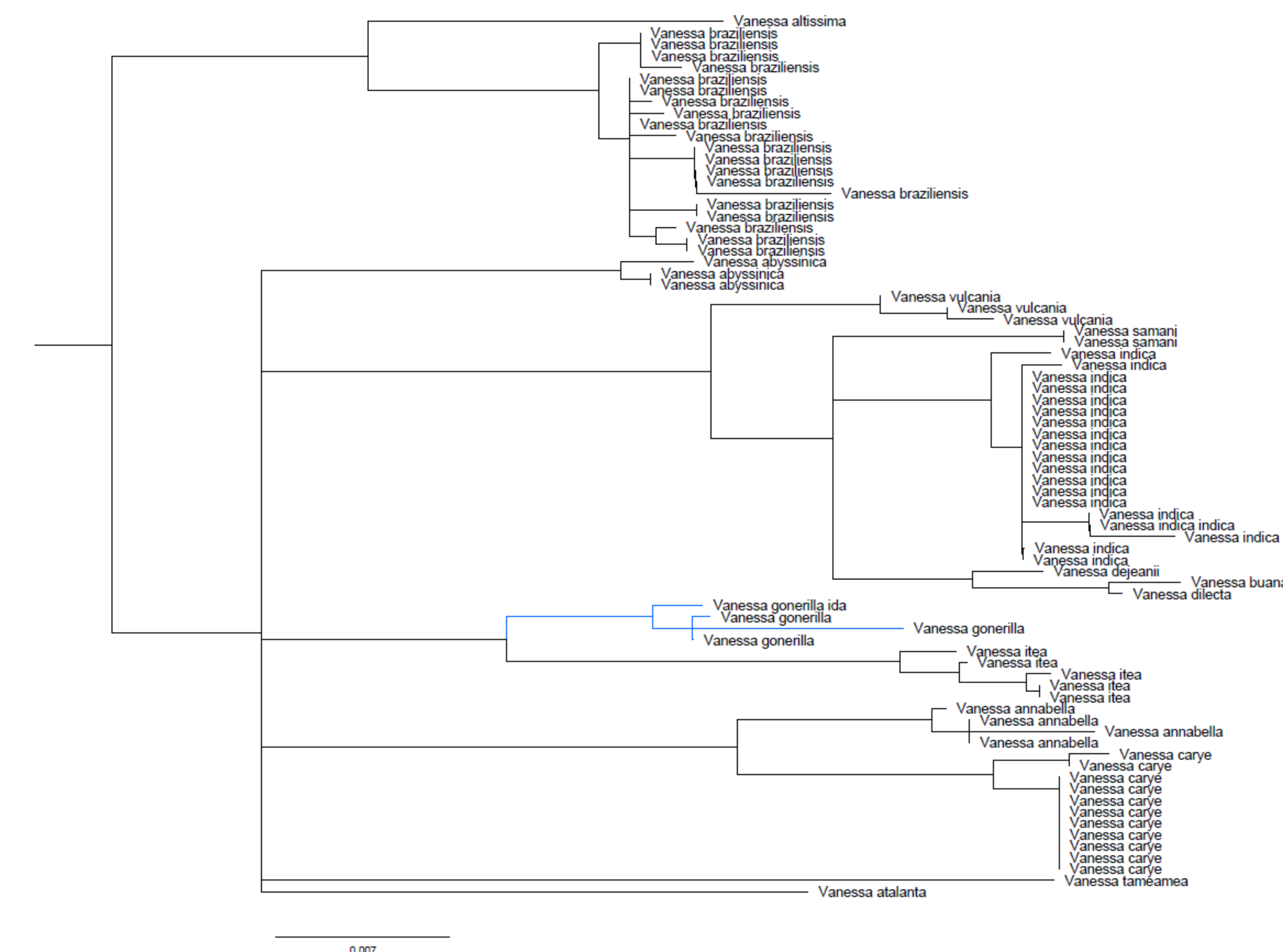


Figure 1. Phylogenetic tree of Vanessa genus

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