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The Molecular Phylogeny of the New Zealand Endemic Genus Hadramphus and the Revival of the Genus Karocolens

Emily D. Fountain [1,2,*], Robert H. Cruickshank [2] and Adrian M. Paterson [2]

[1] Department of Forestry and Wildlife Ecology, University of Wisconsin-Madison, Madison, WI 53706, USA
[2] Department of Pest-Management and Conservation, Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln 7647, New Zealand; bugblokenz@outlook.com (R.H.C.); Adrian.Paterson@lincoln.ac.nz (A.M.P.)
* Correspondence: efountain@wisc.edu; Tel.: +1-7173-4279-59

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Abstract: The delineation of species is important to the fields of evolution, ecology and conservation. The use of only a single line of evidence, e.g., morphology or a single gene sequence, may underestimate or overestimate the level of diversity within a taxon. This problem often occurs when organisms are morphologically similar but genetically different, i.e., for cryptic species. The *Hadramphus* genus contains four endangered, morphologically similar species of weevils, each endemic to a specific New Zealand region (*Hadramphus spinipennis* Chatham Islands, *H. stilbocarpae* Fiordland, *H. tuberculatus* McKenzie Country, *H. pittospori* Poor Knights Islands). The systematic relationships among these species are unclear. We used samples from these species and a closely related genus, *Lyperobius huttoni*, to obtain data from the mitochondrial gene cytochrome c oxidase subunit I and the nuclear gene internal transcribe spacer 2. In addition to the multi-locus coalescent approach, we modelled morphological characteristics combined with the genetic data. We found that *H. spinipennis*, *H. tuberculatus* and *H. stilbocarpae* were a closely related clade. Despite a strong morphological similarity, *Hadramphus pittospori* was found to be genetically distinct from the other *Hadramphus* species, which supports the resurrection of the monotypic genus *Karocolens* for this species.

Keywords: *Aciphylla*; Chatham Islands; integrative taxonomy; Poor Knights Islands; weevils

1. Introduction

Accurate species delineation is fundamental to the fields of ecology and evolution and is particularly important in conservation. Effective conservation relies on each species being a well-defined taxonomic unit for preservation purposes, or for species diversity in general. As an indicator for biodiversity hotspots, the correct identification of species is fundamental to conservation [1].

Taxonomic units can be determined using multiple types of data. In particular, the use of integrative taxonomy is recognised as a robust method for delimitating species [2]. Integrative taxonomy allows different types of methods and data, such as genetic, morphological, behavioral, and developmental, to be combined in a synergetic way to more completely identify the boundaries among taxa [3].

Although integrative taxonomy is a strong method for identifying species, the approach is limited when dealing with cryptic and allopatric species [4]. Species delimitation that uses morphological characteristics, even when using an integrative approach, can be subjective and based on ambiguous and hard to determine traits [4]. Previously established phylogenies based on strictly morphological characteristics have been found, at times, to be based on questionable synapomorphies [5]. Phenotypic

characteristics can vary within a single species, and this can be misleading when building phylogenies based on traits such as colour and size. The New Zealand endemic widow spider was previously separated into two distinct species, *Latrodectus katipo* and *L. atritus*, based on colour morph. Molecular methods determined that the widow spider is a single species, *L. katipo*, and that the colour variation correlated with mean annual temperature [6]. When identifying cryptic or allopatric species, combining genetic data with coalescent theory provides a quantitative assessment of species status [7].

The use of multispecies coalescent models to estimate genetic structure that provide information on species trees from multilocus genetic data has provided researchers with a strong tool to identify species events, understand evolutionary processes and determine relationships among taxa [4,8]. For example, a molecular reconstruction of scorpions using 5025 genes reconstructed a basal topology completely different from traditional morphological taxonomy, changing the understanding of scorpion evolution [9]. Coalescent methods have become increasingly important in conservation. For example, Vuataz et al. [10] used a coalescent approach on freshwater insects from Madagascar and found evidence for considerably more endemic (and threatened) species than expected. Likewise, Rutchsmann et al. [11] found an additional 11 mayfly species when they used a coalescent approach on the Canary Islands. Microendemicity has been detected using a molecular coalescence approach in Balkan trichopterans [12]. All of these approaches suggest that we have often underestimated the level of divergence using past methods.

New Zealand has a high diversity of invertebrate species, many of which have been in decline since the arrival of humans [13]. A large proportion of New Zealand's invertebrates have yet to be described, and even fewer have been genetically analysed. The evolutionary divergence of insects on islands isolated from neighbouring continents results in high levels of endemism [14–16]. As an oceanic island, New Zealand is considered one of the world's biodiversity hotspots, with a large proportion of endemic species [17]. By offering greater insight into the evolutionary history and taxonomic diversity of invertebrates, coalescent methods may help to identify and conserve declining species in New Zealand, inform conservation decisions, and help protect an international biodiversity hotspot.

Hadramphus Broun, 1911 is an endemic genus of weevils (Coleoptera: Curculionidae) in the tribe Molytini that consists of four species: *H. tuberculatus* (Pascoe, 1877), *H. spinipennis* Broun, 1911, *H. stilbocarpae* Kuschel, 1971, and *H. pittospori* (Kuschel, 1987). These weevils are some of the largest in the world for this tribe and are characterized by rounded tubercles on their backs [18]. All four species are of conservation concern due to their limited population numbers or a reduced range compared to their historical distribution.

The Canterbury knobbled weevil, *H. tuberculatus*, is perhaps the rarest beetle species in the genus, consisting of only one known population. Last sighted in the 1922, it was presumed extinct by the late 1990s. However, in 2004 it was rediscovered at Burkes Pass Scenic Reserve, Mackenzie County [19]. *Hadramphus tuberculatus* is the only species in the genus to be found in subalpine grasslands rather than coastal habitats. Based on evidence from Holocene fossils and museum records, *H. tuberculatus* was once distributed throughout the Canterbury Plains and surrounding hills but is now confined to only one small reserve [20]. The host-plants for *H. tuberculatus* were historically thought to be *Aciphylla subflabellata* and *A. glaucescens*, but currently it is found feeding on *A. aurea* [18,21]. Captive rearing studies show that *H. tuberculatus* is somewhat plastic in host-plant choice and can successfully feed on *A. aurea*, *A. subflabellata* and *A. dieffenbachii* [22].

Found in the Chatham Islands, *H. spinipennis* is currently confined to Rangatira (South East) Island, Mangere Island, and Little Mangere Island, although museum records show the species was once present on Pitt Island as well [18]. It is a coastal species and is often found on rocky coastal cliffs on its host-plant *Aciphylla dieffenbachii.* Due to its range restriction and loss of populations in the past, *H. spinipennis* is considered endangered. Although extensive studies on ecology and population dynamics were done by Schöps [23] who suggested that the remaining populations were healthy, a recent study by Fountain [24] suggests the population on Rangatira is declining.

Hadramphus stilbocarpae was once widespread on several islands in Fiordland and the sub-Antarctic, but has undergone population decline, due mainly to the introduction of rats. It is currently found on Resolution Island, Puysegur Point, Bird Island, The Snares (North East Island and Broughton Island) [18] and a small population was translocated to Breaksea Island in 1991 [25]. Population decline and the continual threat from rats have led to this species being classified as endangered. *Hadramphus stilbocarpae* is confined to coastal areas and is found on the host-plants *Anisotome lyalli* and *Stilbocarpa robusta*.

Discovered in 1981, *H. pittospori* is endemic to the Poor Knights Islands [18,26]. The protected status of the island groups means that the weevil was automatically classified as a protected species. The weevil is usually found in coastal lowlands on its host plant, *Pittosporum crassifolium*. *Hadramphus pittospori* was originally placed into its own genus, *Karocolens,* by Kuschel [26]. Kuschel noted that *H. pittospori* was closely related to the genus *Hadramphus* but key morphological differences, such as prothorax and elytra shape, and differing host-plant family, Pittosporacea instead of Apiaceae or Araliaceae, placed the weevil in its own genus. Craw [18] synonymised the genus *Karocolens* with *Hadramphus* as a number of morphological traits defining *Karocolens* were found to be shared with the other *Hadramphus* species.

Few genetic studies have been conducted on the *Hadramphus* genus [24,27]. In this study, we construct the first molecular phylogeny for *Hadramphus* using mitochondrial and nuclear genes. A member of its sister genus, *Lyperobius huttoni* Pascoe, 1876, is used as an outgroup based on its positioning in the morphological phylogenetic tree constructed by Craw [18]. We examine whether *H. pittospori* correctly belongs to the genus *Hadramphus* or if the genus *Karocolens* should be reinstated.

2. Methods and Methods

2.1. Specimen Collection

Hadramphus tuberculatus, *H. spinipennis*, *H. stibocarpae* and *L. huttoni* were collected by pitfall trapping and visual searches of their host-plants. For each captured weevil, we collected a tarsal clip from the mesothoracic leg using ethanol-sterilised scissors. We then returned the weevil to the host-plant it was found on, or in the case of pitfall trapping, to the nearest host-plant. Tarsal clips were stored in 100% ethanol at −20 °C until DNA extraction. For *H. pittospori*, a weevil was collected from Poor Knights Islands and stored in ethanol. We also sampled an additional museum specimen of *H. pittospori* from the New Zealand Arthropod Collection (NZAC) collected on Aorangi Island in 1981. Seventy-one *H. tuberculatus* tarsal clips were collected from Burkes Pass Scenic Reserve between October and February in 2007, 2009, 2010, and 2011; six *L. huttoni* tarsal clips were collected in the Mackenzie Basin in November 2009; 15 *H. spinipennis* tarsal clips were collected from Rangatira (South East) Island in February 2010 and 13 tarsal clips from Mangere Island in February 2011 [24]; two *H. stilbocarpae* tarsal clips were collected from Breaksea Island from in January 2010; and *H. pittospori* was collected 17 December 2009.

2.2. DNA Extraction, Amplification and Sequencing

We cut each tarsal clip into several pieces using a sterile scalpel blade and then transferred them to a sterile 1.7 mL microcentrifuge tube. For *H. pittospori*, we made a pin hole in the thorax, and for the museum specimen we removed the pin from the thorax and the whole body was submerged in lysis buffer (Supplementary Material 1). A Qiagen DNeasy Blood and Tissue Kit (Qiagen, catalogue # 69504) was used for DNA extraction following the manufacturer's spin-column protocol for animal tissues and for the museum specimen a QIAmp Investigator Kit (Qiagen, catalogue # 56504) was used for DNA extraction. We amplified the mitochondrial gene cytochrome *c* oxidase subunit I (COI) and the nuclear gene internal transcribe spacer 2 (ITS2) by PCR. Each PCR reaction consisted of 0.25 mM of dNTPs, 0.02 unit/μL of polymerase (*i*-taq, iNtRON Biotechnologies), 0.2 μM of each primer, 1 × PCR buffer, 2.5 μL DNA extraction, and deionized water to bring the total reaction volume to 25 μL. The PCR

reaction for the museum specimen of *H. pittospori* included an additional 1 mM $MgCl_2$, 5 µg/µL BSA and the amount of DNA extraction was increased to 5 µL.

For COI, we used the primer set LCO1490 and HCO2198 (656 base pairs) [28]. The PCR cycle consisted of one step at 94 °C for 3 min followed by 35 cycles of 94 °C for 45 s, 45 °C for 45 s and 72 °C for 1 min 20 s, then with a final step at 72 °C for 5 min. Positive results for *H. pittospori* could not be obtained from the primers LCO and HCO, so we amplified a smaller fragment of COI using MLepF1 and LepR1 (426 base pairs) [29]. The same PCR reagents and parameters were used for this primer set. For ITS2, primers, PCR mix and parameters followed the protocol in Fountain et al. [24]. We sequenced PCR products using 0.8 µM of primer, Big Dye version 3.1 (Applied Biosystems, Warrington, Cheshire, UK) and the following thermal regime: 96 °C for 1 min followed by 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. Sequence products were resolved in an AVANT 3100 (ABI) capillary sequencer. We BLAST [30] searched the results to confirm sequence identity. All sequences were submitted to GenBank, with accession numbers MH536680–MH536727 and MH537666–MH537735 (Supplementary Material 2).

2.3. Data Analysis

We analysed the chromatograms of 100 COI sequences and 48 ITS2 sequences. An additional 15 COI sequences and 25 ITS2 sequences previously published for *H. spinipennis* sequences [24] were included for a total of 115 COI sequences and 73 ITS2 sequences. We built sequence alignments using MEGA 7.0.26 [31]. All COI sequences were visually aligned and the ITS2 sequences were auto-aligned in WebPrank [32]. For COI and ITS2, we calculated nucleotide composition and pairwise distances in MEGA 7.0.26 and nucleotide diversity for each species in DnaSP 6.10.03 [33]. Maximum likelihood (ML) trees were built in MEGA 7.0.26 [31] with all sites including gaps and an extensive SPR ML heuristic method for tree inference. The best fit evolutionary models for the ML analyses were found using the AIC with jModelTest 2.1.1 [34], which identified the transition (TIM) model as the optimal model for COI and the symmetrical (SYM + G) model [35] with gamma distribution as the optimal model for ITS2. For all Bayesian phylogenetic analyses, we used StarBEAST2 [36] implemented in BEAST 2.4.7 [37]. To assign taxon groups for the species tree, we used current species taxonomy based on morphological characteristics [18], with *L. huttoni* as an outgroup. Evolutionary models were determined through model averaging using a reversible jump MCMC implemented in BModelTest [38] as part of the BEAST2 package. We checked effective sampling sizes and convergence using Tracer 1.6 [39]. Four independent runs of each analysis were conducted and then combined in Log Combiner 2.4.7. We compared the performance of strict and uncorrelated relaxed lognormal clock models using path sampling [40]. For the species tree, we combined the trees from the four independent runs in Log Combiner 2.4.7 using a 20% burn-in for each run. A maximum clade credibility tree was compiled in TreeAnnotator 2.4.7 after discarding the initial 10% burn-in and we visualized the tree in FigTree 1.4.3 [41].

To determine tree topology for each gene tree and the taxonomic placement of each species, we first conducted StarBEAST2 analyses for each gene tree separately using all sequences. For ITS2, we were unable to amplify a fragment for *H. pittospori, and* so this species was not included in the ITS2 analysis. Both Bayesian gene trees converged on the same topology, with the last common ancestor of *H. pittospori* and the other *Hadramphus* species being considerably earlier than the last common ancestor of the remaining three *Hadramphus* species in COI, and *H. tuberculatus* and *H. stilbocarpae* shared a more recent common ancestor than with *H. spinipennis* in both COI and ITS2.

The multi-locus dataset was reduced to 72 individuals that had a sequence for both COI and ITS2. For *H. pittospori*, the ITS2 sequence was replaced by question marks to represent unknown nucleotides. In addition to a species phylogeny based on DNA sequences, we also conducted phylogenetic analysis combining morphological traits and DNA sequences to determine how morphology influenced the placement of *H. pittospori*. We conducted two analyses using the morphological descriptions of Craw [18] and Kuschel [26,42]. For both Craw and Kushel datasets, we included 13 morphological traits

(Supplementary Material 3) and analysed the morphological traits under the Lewis MK model [43]. We then conducted path-sampling analyses and calculated the Bayes factor to determine support for species delimitation. For all final analyses, we used an uncorrelated relaxed lognormal clock prior to the species tree. We estimated the clock rates for COI and ITS2 and used a lognormal prior with the clock rate for COI based on the mutation rate of Polyphaga [44] (M = 0.02 S = 0.8 and M = 0.001 S = 0.9, respectively). The COI and ITS2 gene trees were unlinked, and each run consisted of 100,000,000 generations, sampling every 3500 for analyses of only DNA sequences, and 130,000,000 generations, sampling every 4500 for morphological analyses.

3. Results

In total, 115 extractions for COI and 73 extractions for ITS2 were successfully amplified and sequenced. For COI, all sequences were trimmed to 426 bp and the ITS2 sequences were 419–452 bp. Nucleotide diversity in COI within *H. pittospori* was the highest in comparison to the others' within-species diversity (Table 1). *Hadramphus tuberculatus* had the most individuals sequenced for COI and also the greatest number of haplotypes when compared to the other species (Table 1). Interspecific enetic distances ranged from 0.052–0.289, with the largest divergence between *H. pittospori* and *L. huttoni* (Table 2). The intraspecific genetic distances ranged from 0.001–0.010 (Table 2). The COI ML tree supports the high intraspecific nucleotide diversity in *H. pittospori* (Figure 1A). For *L. huttoni*, *H. spinipennis*, and *H. tuberculatus*, the majority of branch tips were collapsed due to low bootstrap support, and the remaining branches within each species were shorter than 0.01 (Figure 1A). The ITS2 ML consensus tree had higher intraspecific variation compared to the COI ML tree, and the tree was mainly concordant with the COI ML tree, except for paraphyly in *H. stilbocarpae* (Figure 1B).

An examination of log-likelihood in Tracer 1.6 indicated that the MCMC chains reached convergence and all effective sampling size (ESS) values reached above 200. The uncorrelated lognormal clock for the species tree was the best supported clock model and was used in all analyses. The StarBEAST2 analyses (individual genes and multi-coalescent approaches) supported the divergence of *H. pittspori* much earlier than the subsequent divergence of the remaining *Hadramphus* species at 22.76 (1.01–60.68) million years ago (MYA) (Figure 2). *Hadramphus spinipennis* was the sister taxon of the pair *H. tuberculatus* and *H. stilbocarpae* in the analyses (Figure 2).

Table 1. Estimates of diversity in 426 bp fragment of *c* oxidase subunit I (COI) for each species including sample size (N), number (#) of haplotypes, number of polymorphic sites and nucleotide diversity (π) with standard deviation (STD).

Species	N	# Haplotypes	# Polymorphic Sites	Π (STD)
huttoni	10	2	1	0.001 (0.0002)
pittospori	2	2	5	0.012 (0.0059)
spinipennis	28	4	3	0.001 (0.0003)
tuberculatus	71	7	6	0.002 (0.0003)
stilbocarpae	2	2	3	0.007 (0.0035)

Table 2. Mean genetic distances for a 426 bp fragment of COI calculated using the Kimura-2-parameter model are shown along the bottom diagonal. Standard errors, shown above the bold diagonal, were obtained by bootstrapping (10,000 replicates). The mean intraspecific distance is presented along the diagonal in bold.

Species	*huttoni*	*pittospori*	*spinipennis*	*tuberculatus*	*stilbocarpae*
huttoni	**0.001**	0.029	0.024	0.024	0.025
pittospori	0.289	**0.010**	0.027	0.028	0.028
spinipennis	0.210	0.270	**0.007**	0.011	0.013
tuberculatus	0.210	0.270	0.052	**0.002**	0.012
stilbocarpae	0.210	0.287	0.071	0.061	**0.001**

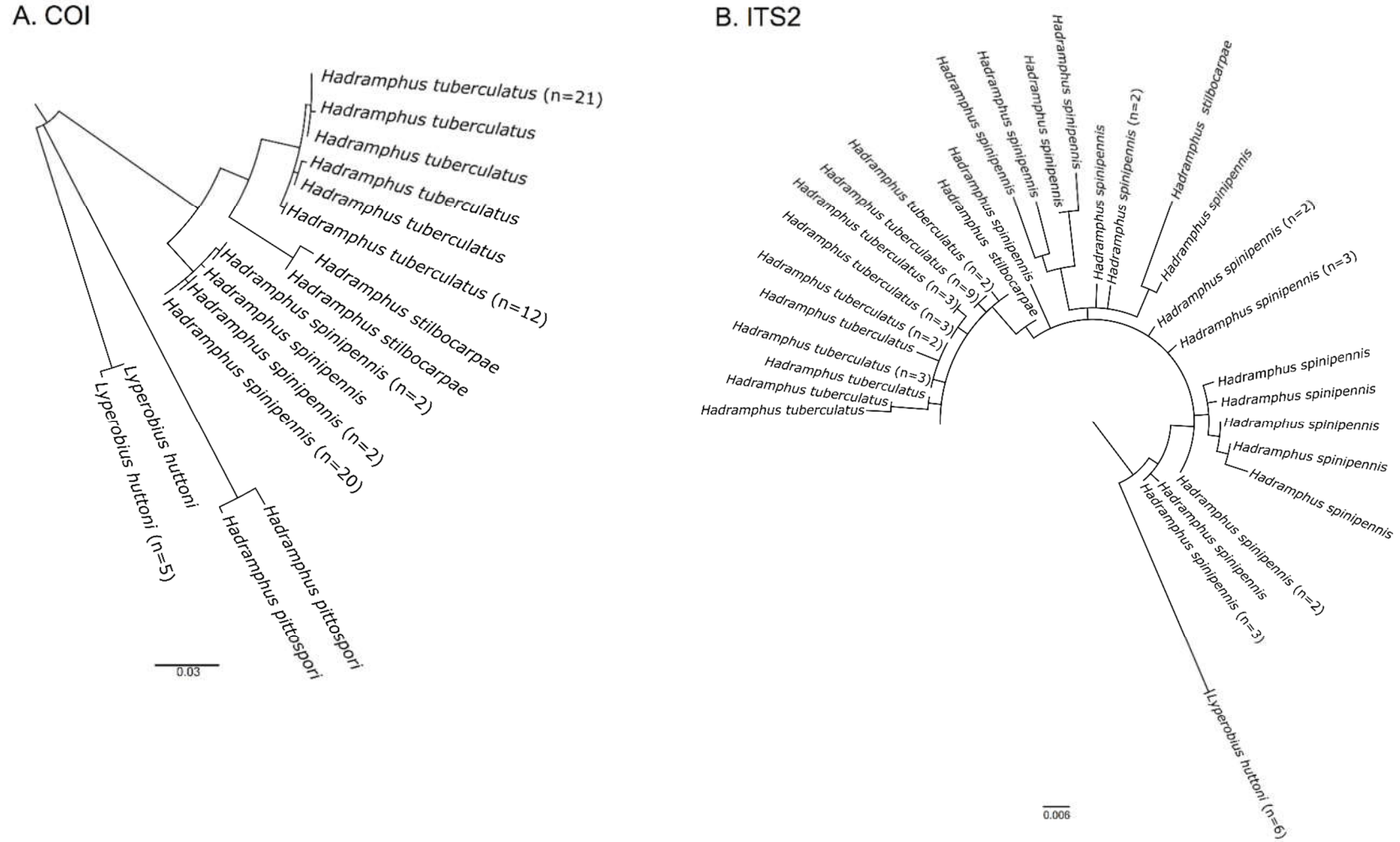

Figure 1. Maximum likelihood bootstrap consensus trees inferred from 1000 replicates for (**A**) cytochrome *c* oxidase subunit I (COI) and (**B**) internal transcribe spacer 2 (ITS2). Nodes with a lower than 50% bootstrap are collapsed. For branches with zero length, the number of samples with identical sequences is listed in parentheses after the species name.

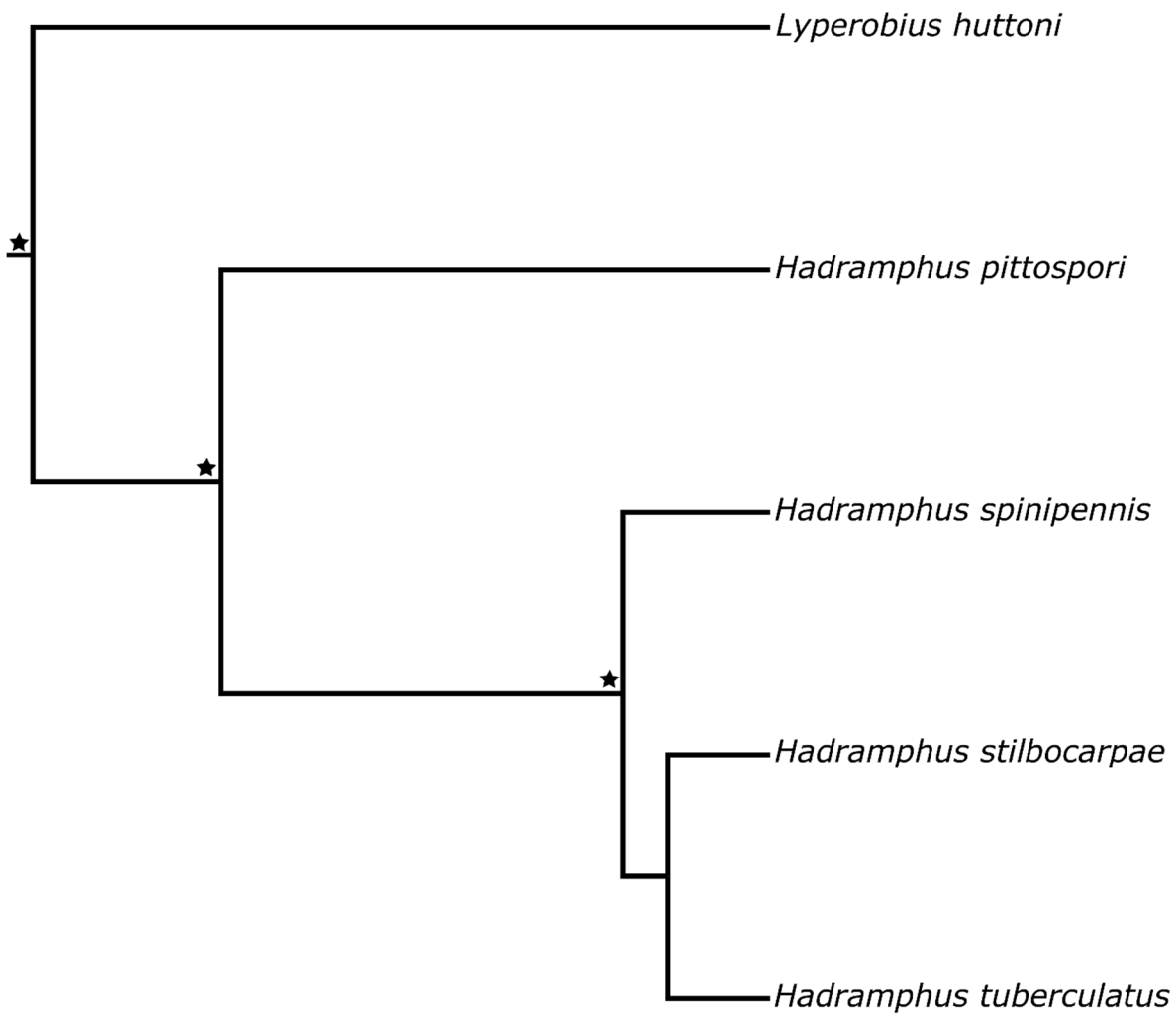

Figure 2. Multi-locus coalescent species tree built from COI and ITS2 gene trees. The stars represent nodes supported with a posterior probability of one.

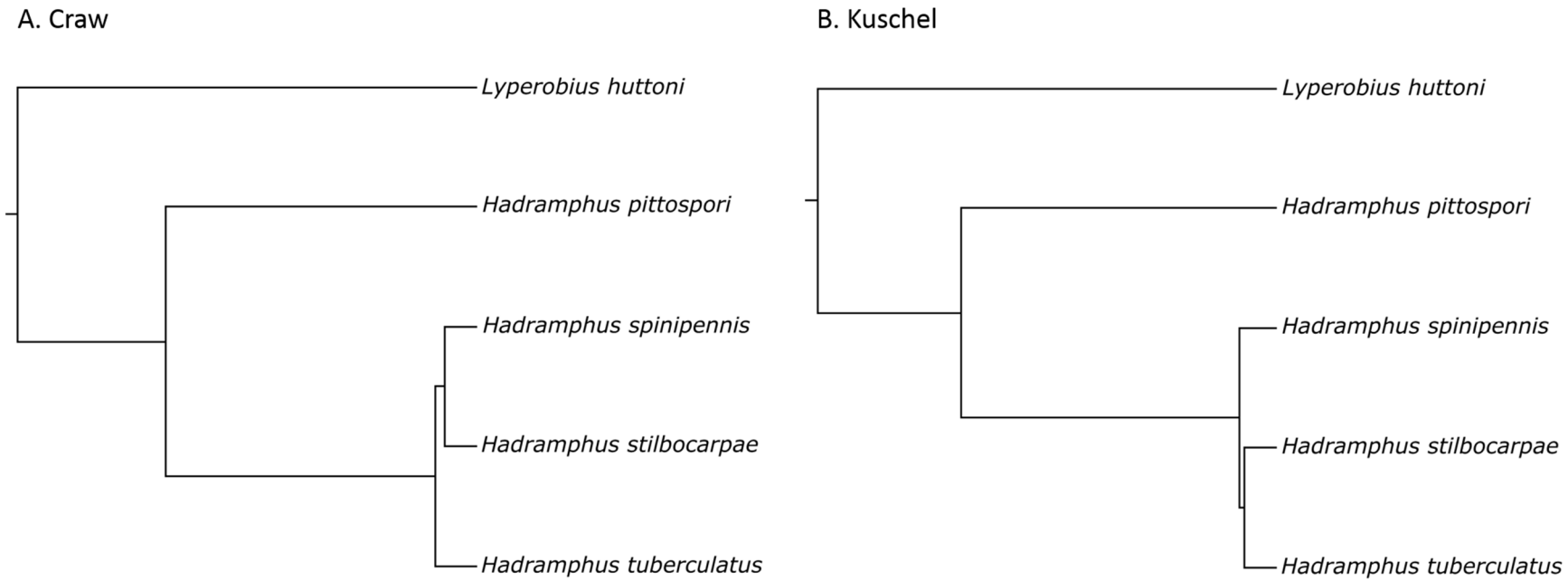

Figure 3. Multi-locus coalescent species trees with morphological characteristics included in the analysis: (**A**) morphological characteristics based on Craw [18], (**B**) morphological characteristics based on Kuschel [26,42]. All nodes except for the most recent, *stilbocarpae* and *spinipennis* (**A**) and *tuberculatus* and *stilbocarpae* (**B**), are supported with posterior probabilities of one.

In the morphological analysis, the species' trees converged on two different topologies (Figure 3). Both the Craw and Kuschel species trees supported the placement of *H. pittospori* as the sister group of the remaining three *Hadramphus* species (posterior probability = 1). The results of the path sampling analysis supported the species delimitations of the Kuschel phylogeny (marginal L estimate = −2990.32 for Kuschel and −2996.07 for Craw).

4. Discussion

We present the first phylogenetic species tree for the endemic genus *Hadramphus* using a multilocus coalescent approach. When combining morphological characteristics, we found support for the taxonomic classification for *H. pittospori* originally suggested by Kuschel [26].

Despite sequencing only two specimens of *H. pittospori*, the nucleotide diversity within the species was greater when compared to the within-species diversity of the other species. The high diversity within the species may be a result of differences in the time of collection. DNA was extracted and amplified from one specimen shortly after collection in 2009, whereas the other specimen was collected in 1981. Captive rearing of *H. pittspori* has shown that the weevil takes over a year from hatching to pupate into an adult and will survive 2–3 years as an adult [45], suggesting a possible generation time of 1 year. Therefore, at least 30 generations passed between the collection of the *H. pittospori* samples, allowing for multiple mutations to occur and possibly resulting in the high genetic diversity between the two samples. Although DNA damage is common in historical samples, we do not feel this contributed to the differences in the sequences. Each sequence returned had an open-reading frame, which is expected for COI, and all samples were amplified and sequenced three times with each replicate returning identical results, suggesting no deamination or oxidative damage [46]. The high nucleotide diversity in *H. pittospori* may also be a result of the fine scale population structure as one specimen was collected from Aorangi Island and the other specimen was collected from the Poor Knights Islands, with no specific island identified. Increasing the sample size in future studies will help to determine if the nucleotide diversity is a result of population structure or a relic of small sample size or historical sampling. Overall, the within-species nucleotide diversity is consistent with other large-bodied, flightless weevils (e.g., Galápagos weevils, [47]).

The COI genetic distance between *H. pittospori* and the other three *Hadramphus* species ranged from 0.27–0.29, which was greater than the distance of *H. stilbocarpae, H. spinipennis,* and *H. tuberculatus* to the outgroup *L. huttoni* (0.20–0.21). The genetic distance amongst these three species ranged from 0.05–0.07, suggesting that *H. pittospori* is as distantly related as another genus to the other *Hadramphus* species. One of the thresholds in DNA barcoding is that the genetic divergence between species should be 10 times greater than the within-species divergence [48]. Given this criterion, the divergence between *H. pittspori* and the other species in *Hadramphus* is at least 20 times greater than the within-species divergence, further supporting the distant relationship of *H. pittospori* to the rest of *Hadramphus*.

The COI and ITS2 ML gene trees were mostly congruent, except for *H. stilbocarpae*, being paraphyletic in ITS2. Nonmonophyly in closely related species is well documented in insects [49] and the slower mutation rates in nuclear genes may result in incomplete lineage sorting in recently diverged species. The COI gene tree from the StarBEAST analysis matches the ML COI tree; however, that in the Bayesian ITS2 tree, *H. stilbocarpae*, was not paraphyletic. The most recent common ancestor for *H. pittospori* and the other three *Hadramphus* species was during the late Oligocene–early Miocene period and the most recent common ancestor for *H. spinipennis*, *H.stilbocarpae* and *H. tuberculatus* was in the late Miocene (6.12 MYA), suggesting that the *H. pittospori* lineage has diverged from the rest of *Hadramphus* for an evolutionary long period. Although we used the molecular rate for COI for polyphaga [44], rates of molecular evolution can differ between lineages and across timescales [50]; therefore, the common ancestor times should be viewed with caution. Although the lack of taxon sampling makes the resolution of taxonomy difficult [51], our study provides insight into the phylogenetic structure of the genus *Hadramphus*, which is currently recognized to contain only four species. Future studies would benefit from wider taxon sampling of Molytini in New Zealand and the

use of fossil or geological dating, such as the rise of Poor Knights Islands and the Chatham Islands, for calibration.

The taxonomic tree based on morphological characters presented by Craw [18] placed all four *Hadramphus* species into a well-supported clade; however, the genetic data does not fully support the morphological character tree. Originally, *H. pittospori* was placed into its own genus, *Karocolens* [26], until it was incorporated into *Hadramphus* in 1999 [18]. The genetic phylogeny suggests that *H. pittospori* may not be part of the genus *Hadramphus* due to the large amount of genetic variation between *H. pittospori* and the other *Hadramphus* species. Indeed, the amount of genetic variation between *H. pittospori* and *Hadramphus* is similar to that between *Lyperobius* and *Hadramphus* and suggests that *H. pittospori* may not belong in the *Hadramphus* genus. Depending on which morphological characters are chosen for phylogenetic analysis, the traits may be biased depending on whether the characters of interest are chosen to be included or removed from the data [52]. In Craw [18], he states there are some unique characters remaining that separate *H. pittospori* from other *Hadramphus* species, such as the prothorax being as, or nearly as, long as it is wide and diverging into a straight line. The remaining characteristics separating *H. pittospori* may be more important to the evolutionary history of the species and should be analysed in more detail. Indeed, when modelling the morphological characteristics with the genetic data, those characters considered important by Kuschel [26] are supported over those emphasised by Craw [18].

The phylogenetic relationship between *H. tuberculatus*, *H. spinipennis* and *H. stilbocarpae* in the genetic trees differs from that of the morphology-based tree in which *H. spinipennis* and *H. stilbocarpae* were considered sister taxa. Morphological characteristics, such as low median rostral carina and a distinctive tubercle on interval 3 of the elytra declivity [18], placed *H. spinipennis* and *H. stilbocarpae* as sister species. Goldberg and Trewick [27] reported *H. spinipennis* and *H. tuberculatus* to be sister taxa based on the results of their COI Bayesian tree. In our study, *H. tuberculatus* is shown to be a sister taxon to *H. stilbocarpae*; however, the relationship is only supported by a posterior probability of 0.71. A difference in phylogenetic trees inferred from morphological and molecular data has been reported for other species in New Zealand. For example, the beetle genus *Prodontria* showed marked differences in its molecular and morphological phylogenetic trees [53]. In particular, the sister-species relationship of the group *P. modesta* and *P. lewisi* to *P. capito* was unsupported.

This study provides clarification on the taxonomic groupings in the genus *Hadramphus*. The phylogeny provides an indication of evolutionary relationships that were not predicted based on morphology alone. Based on the genetic data and the supported morphological description of *H. pittospori* by Kuschel [26], we are reinstating the genus *Karocolens*, consisting of a single species, *K. pittospori*, as originally described by Kuschel [26]. The four species in *Hadramphus* range from protected to critically endangered and are all managed by the Department of Conservation. By combining the evolutionary history of species with their taxonomy rather than relying on morphology alone, more evidence-based decisions can be made regarding their conservation status.

Supplementary Materials: The following are available online at http://www.mdpi.com/1424-2818/10/3/88/s1. Supplementary Material 1: Additional methods used for the extraction of DNA from the museum specimen of *H. pittospori*. Supplementary Material 2: A list of GenBank accession numbers for sequences obtained from GenBank and also the accession numbers for the new sequences from this study. Supplementary Material 3: The morphological characteristics defined by Craw [18] and Kuschel [26] used in the Bayesian analyses.

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