Contrasting patterns of population structure and demographic history in cryptic species of Bostrychia intricata (rhodomelaceae, rhodophyta) from New Zealand

Journal:	*Journal of Phycology*
Manuscript ID:	JPY-14-248-ART
Manuscript Type:	Regular Article
Date Submitted by the Author:	16-Dec-2014
Complete List of Authors:	Muangmai, Narongrit; Victoria University of Wellington, Fraser, Ceridwen; Australian National University, Fenner School of Environmental and Society Zuccarello, Giuseppe; Victoria University of Wellington, School of Biological Sciences
Keywords:	cytochrome c oxidase subunit I (COI), demography, phylogeography, phylogenetics, population genetics, macroalgae, red algae

CONTRASTING PATTERNS OF POPULATION STRUCTURE AND DEMOGRAPHIC HISTORY IN CRYPTIC SPECIES OF *BOSTRYCHIA INTRICATA* (RHODOMELACEAE, RHODOPHYTA) FROM NEW ZEALAND[1]

Narongrit Muangmai[2]

School of Biological Sciences, Victoria University of Wellington, Wellington 6041, New Zealand

Ceridwen I. Fraser

Fenner School of Environmental and Society, ANU College of Medicine, Biology & Environment, Australian National University, Canberra ACT 2601, Australia

and

Giuseppe C. Zuccarello

School of Biological Sciences, Victoria University of Wellington, Wellington 6041, New Zealand

[2]e-mail: seaweed_53@hotmail.com

Running title: Phylogeography of *B. intricata* in New Zealand

ABSTRACT

Spatial patterns of genetic diversity provide insight into the demography and history of species. Morphologically similar but genetically distinct 'cryptic' species are increasingly being recognized in marine organisms through molecular analyses. Such species are, on closer inspection, often discovered to display contrasting life histories or occasionally minor morphological differences; molecular tools can thus be useful indicators of diversity. *Bostrychia intricata,* a marine red alga, is widely distributed throughout the Southern Hemisphere, and comprises many cryptic species. We used mitochondrial COI sequences to assess the genetic variation, population genetic structure and demographic history of *B. intricata* in New Zealand. Our results supported the existence of three cryptic species of *B. intricata* (N2, N4 and N5) in New Zealand. Cryptic species N4 showed a higher genetic diversity and wider distribution than the other two species, which were only found in the North Island and northern South Island. Our analyses showed low to moderate genetic differentiation among eastern North Island populations for cryptic species N2, but high differentiation among North and South Island populations for N4, suggesting different levels of gene flow between populations of these cryptic species. Data also indicated that N2 has recently undergone population expansion, probably since the Last Glacial Maximum (LGM), while the higher genetic diversity in N4 populations suggests persistence *in situ* through the LGM. The contrasting population structures and inferred demographic histories of these species highlight that life history can vary greatly even among morphologically indistinguishable taxa.

Key index words: cytochrome c oxidase subunit I (COI); demography, phylogeography; phylogenetics; population genetics, macroalgae; red algae

50

51 *Abbreviations*: AMOVA, analysis of molecular variation; COI, cytochrome c oxidase

52 I gene; LGM, Last Glacial Maximum; NZ, New Zealand; SAMOVA, spatial analysis

53 of molecular variance

54

INTRODUCTION

Present-day patterns of genetic diversity can allow inference of dispersal and connectivity among marine populations, and can contribute to our understanding of how historical climatic events, oceanographic conditions and tectonic processes have influenced the evolution and demographic history of marine organisms (Hewitt 2004, Lomolino et al. 2006, Hemmer-Hansen et al. 2007, Fraser et al. 2012). In the high latitudes of the Southern Hemisphere, numerous recent studies have shed light on the diversity, distribution and biogeographic patterns of marine populations (reviewed by Allcock and Strugnell 2012, Fraser et al. 2012). Phylogeographic studies have provided strong evidence for significant population structure in numerous marine organisms (Brante et al. 2012, Le Port and Lavery 2012, Fraser et al. 2013). Such structure can be used to infer how species, and populations within species, have responded to past processes such as climate change. For example, higher genetic diversity in low versus high latitude populations has been interpreted to reflect postglacial recolonisation of higher latitude areas following recession of ice at the LGM (e.g. crustaceans: Nikula et al. 2010; kelp: Fraser et al. 2009, 2010, Macaya and Zuccarello 2010a, b).

New Zealand (NZ) consists of two main islands, the North and South Islands, which have striking geological and environmental differences, such as northern volcanoes and southern glaciated regions. The complex geographical and oceanographic (Fig S1) systems have resulted in phylogeographic structure in both terrestrial and marine taxa (Ross et al. 2009, Wallis and Trewick 2009). Several phylogeographic studies of marine species have detected deep genetic splits between northern/southern and eastern/western geographical regions (Apte and Gardner 2002, Sharyn et al. 2006,

Ross et al. 2009, 2012) (Fig S1). In contrast, other studies have detected little or no phylogeographic structure around New Zealand, indicating broad population connectivity in some groups (Smith et al. 2002, Waters and Roy 2003). Inconsistency of phylogeographic patterns observed among marine taxa in New Zealand suggests that different species response in different ways, possibly due to species-specific dispersal ability, reproductive strategies or species' and demographic history.

The phylogeographic structure of several brown seaweeds, e.g., *Carpophyllum maschalocarpum* (Turner) Greville, *Durvillaea antarctica* (Chamisso) Hariot, *Macrocystis pyrifera* (Linnaeus) C. Agardh, has previously been investigated in NZ (Fraser et al. 2009, Macaya and Zuccarello 2010a, Buchanan and Zuccarello 2012). These studies, which relied largely on data from mitochondrial markers, demonstrated spatial heterogeneity in genetic diversity among seaweed populations from the North Island, with a transition region between the bottom of the North Island and the top of the South Island (Fraser et al. 2009, Buchanan and Zuccarello 2012). In addition, genetic evidence indicated that the distribution and population connectivity of these buoyant brown seaweeds has been strongly influenced by surface ocean circulation patterns (e.g. Antarctic Circumpolar Currents – ACC) and historical events (e.g., the LGM) (Fraser et al. 2009, Macaya and Zuccarello 2010a, Buchanan and Zuccarello 2012, Collins et al. 2010). Whereas population structure and dispersal patterns of brown seaweeds in New Zealand have been quite well studied, research on other groups of seaweed – especially non-buoyant taxa with limited dispersal potential – has been relatively uncommon. Although non-buoyant taxa should theoretically be less capable of long-distance dispersal than robust, buoyant taxa, and might therefore be expected to show stronger phylogeographic structure (Fraser et al. 2013), dispersal

105 capacity alone is not always a good predictor of population connectivity (Waters et al.
106 2013).
107
108 The genus *Bostrychia* Montagne is a filamentous red alga of the family
109 Rhodomelaceae, order Ceramiales, and currently contains ~19 species, which are
110 widely distributed in tropical and temperate regions (King and Puttock 1989,
111 Zuccarello and West 2006). *Bostrychia* has been used as a model system to study
112 evolution, speciation processes, and population connectivity (review in Zuccarello
113 and West 2011). For example, studies on genetic diversity of *Bostrychia radicans*
114 (Montagne) Montagne and *B. moritziana* (Sonder ex Kützing) J. Agardh show that
115 these two morphospecies consist of seven non-interbreeding genealogical lineages,
116 suggesting cryptic species (Zuccarello et al. 1999b; Zuccarello and West 2003), some
117 of which occur in sympatry (Zuccarello and West 2003, Zuccarello et al. 2006,
118 Zuccarello et al. 2011). However, while the phylogenetic diversity and
119 phylogeography of the warm-temperate *Bostrychia* species have been well studied,
120 diversity and distribution patterns of *Bostrychia* species endemic to the Southern
121 Hemisphere are still poorly documented.
122
123 The most widespread *Bostrychia* species throughout the Southern Hemisphere is *B.*
124 *intricata* (Bory de Saint-Vincent) Montagne (King and Puttock 1989, Zuccarello and
125 West 2008). This species is normally found in clumps in the upper intertidal either on
126 shaded rocks (King & Puttock 1989) or logs (Fraser et al. 2013). Phylogenetic
127 research using plastid-encoded Rubisco spacer sequences of *B. intricata* has indicated
128 high levels of genetic diversity within this species, suggesting the presence of
129 multiple cryptic species (Zuccarello and West 2008). More recent research on the

evolution of *Bostrychia* species endemic to the Southern Hemisphere based on phylogenetic analyses and species delimitation methods using three different molecular markers indicated that eight cryptic species (N1–N8) should be recognized within *B. intricata*, three of which (N2, N4 and N5) occur in NZ (Muangmai et al. 2014). Additionally, a preliminary study on the phylogeography of *B. intricata* from the high latitudes of the Southern Hemisphere (New Zealand, southern South America and some sub-Antarctic islands) indicated strong phylogeographic structure within this species, although some lineages showed evidence of recent long-distance, trans-oceanic dispersal (Fraser et al. 2013).

Despite the broad-scale studies of phylogenetic diversity and phylogeography of *B. intricata* in the Southern Hemisphere, our knowledge of how far these cryptic species differ in aspects of their genetic diversity, connectivity and history are still limited. By investigating the phylogeography and population structure of cryptic *B. intricata* species in NZ based on partial COI sequences, we test the hypotheses that (i) different cryptic species showed significant differences in genetic diversity and demographical history, and (ii) the observed differences in phylogeographic patterns of these cryptic species were associated with the historical events and changing environments.

MATERIALS AND METHODS

Algal sampling

Specimens of the morphospecies *B. intricata* were collected along the coasts of the North and South Islands of NZ in 2011–2012. Details of locations and sample sizes for 43 populations (NZ1–NZ43) are listed in Table 1. This morphospecies normally formed patches on shaded rocks in the upper intertidal. To avoid collecting the same

155 individual, algal samples were randomly collected from patches which were at least
156 0.5 m apart. Algal specimens were preserved with silica gel in the field. All
157 specimens were identified based on previous species descriptions (e.g. King and
158 Puttock 1989, Zuccarello and West 2008). For DNA analyses, algal samples were
159 rinsed with autoclaved seawater to remove any sand and dirt, and then the apical
160 portions were used for DNA isolation.
161
162 *DNA Extraction, PCR and Sequencing*
163 DNA was extracted using a modified Chelex method (Zuccarello et al. 1999a). We
164 chose the short fragment of cytochrome c oxidase subunit I (COI, mitochondrial
165 DNA) as an appropriate molecular marker for this population study because it showed
166 the greatest level of genetic variation in discriminating cryptic species within *B.*
167 *intricata* when compared to other makers (Muangmai et al. 2014). PCR amplification
168 and sequencing of COI was performed using two sets of primer: GazF1 and GazR2
169 (Saunders 2005) or BstCF2 and BstCR2 (Fraser et al. 2013). The PCR reaction profile
170 followed Saunders (2005) or Fraser et al. (2013). PCR amplification was checked by
171 electrophoresis on a 1% agarose gel, and PCR products were subsequently purified
172 using ExoSAP-IT (USB, Cleveland, OH, USA). Purified PCR products were
173 sequenced commercially (Macrogen Inc., Seoul, Korea).
174
175 *Alignment of DNA sequence and data analyses*
176 All DNA sequences were edited and aligned using Geneious 6.0 software
177 (Biomatters, http://www.geneious.com) and then manually checked. For phylogenetic
178 analyses, the data set included haplotypes obtained from this study and additional
179 sequences retrieved from GenBank (Table S1). Phylogenetic relationships were

determined using Maximum Likelihood (ML) and Bayesian Inference (BI), and two sequences of *B. arbuscula and B. gracilis* were used as outgroups following Muangmai et al. (2014). DNA substitution models were determined using Kakusan 4 (Tanabe 2011). ML analyses were performed in raxmlGUI v1.3 (Silvestro and Michalak 2012) with the GTR + I + R under the option 'ML + thorough bootstrap', and bootstrapping values were calculated from 1,000 pseudoreplicates. BI analyses were conducted with MrBayes v3.2 (Ronquist et al. 2012) under the best model indicated by BIC (K80 + G to the codon position 1 and HKY85 + G to the codon position 2 and 3). Two runs of Markov Chain Monte Carlo (MCMC) were performed for 2,000,000 generations, sampling every 100 generations, and the first 25% of saved trees were discarded as burn-in. ML and BI trees were edited with the program FigTree v1.3.1 (Rambaut 2009).

Haplotype analysis was performed using the data set that included all sequences generated in this study and haplotypes from Fraser et al. (2013). The genetic diversity indices, including number of haplotypes (*H*), number of segregating sites (*S*), haplotype diversity (*Hd*) and nucleotide diversity (π), for each population were assessed using DnaSP v5.10.01 (Librado and Rozas 2009). Statistical parsimony networks were constructed using TCS 1.21 (Clement et al. 2000) to observe the relationships among haplotypes.

Cryptic species were defined based on phylogenetic and species delimitation methods (Muangmai et al. 2014), and population structure and demographic history were separately analyzed for each major lineage. For population genetic analysis, populations with a sample size of eight individuals or more were selected (Felsenstein

2006), and two populations from a previous study (Fraser et al. 2013) were included (here coded as populations NZ44 from Brighton, South Island, and NZ46 from Stewart Island: see Table S1). Pairwise fixation index (F_{ST}) values between populations were calculated using Arlequin v 3.5.1.3 (Excoffier and Lischer 2010). The significance of F_{ST} values was estimated by 1023 random permutations (Schneider et al. 2000). Population structure was further analyzed using the SAMOVA algorithm (Dupanloup et al. 2002) to define groups of populations based on the combined information between geographic distances and genetic variation, implemented in SPADS 1.0 (Dellicour and Mardulyn 2014). The criteria for SAMOVA analysis were set as the number of groups (K) ranging from 2 to 10, and 10,000 runs of iterations with 10 repetitions. The optimal number of K was considered based on a maximum or plateau of F_{CT} value. Furthermore, populations were partitioned into the biogeographic regions described by Apte and Gardner (2002) and Shears et al. (2008), as eastern north, western north, eastern south and western south regions (Fig. S1), and population differentiation among these four regions was subsequently tested using the hierarchical analysis of molecular variance (AMOVA) in Arlequin, with significance determined by 10,000 permutations.

Historic population demography was determined using three different methods: statistical tests of neutrality, mismatch distribution and the estimation of time to the most recent common ancestor. Tajima's *D* (Tajima 1989) and Fu's F_S tests were used to test for deviation from selective neutrality, and these analyses were carried out using DnaSP. Analyses of mismatch distribution were performed in Arlequin with 1000 bootstrap replicates. This method can indicate past population expansion by mode shape: unimodal for a recent population expansion and multimodal for a

stationary population at demographic equilibrium (Harpending 1994). The time to most recent common ancestor (TMRCA) was performed using BEAST v2.0.2 (Drummond et al 2012). Mutation rates for COI were estimated following Muangmai et al (2014) with 0.13 – 0.15 substitutions per site per million years. Data were partitioned by codon, and substitution models were set as for the phylogenetic analyses. The MCMC analyses were achieved with four independent runs for 20 million generation under the assumptions of an uncorrelated lognormal relaxed clock and a Yule model prior. The initial 25% of saved trees were removed as the burn-in, and a maximum credibility tree based on the remainder was produced using TreeAnnotator v2.0.2 (part of the BEAST v2.0.2 package). The time-calibrated tree with 95% highest posterior density was visualized in FigTree.

RESULTS

Genetic diversity and distribution

Partial COI sequences of 376 bps were successfully obtained from 384 samples of 43 populations of *B. intricata* around NZ (Fig. 1, Table 1). Genetic distance among these sequences ranged from 0.2% to 12.8%. Phylogenetic trees obtained from ML and BI analyses were almost completely topologically congruent, and supported the hypothesis that three different cryptic species of *B. intricata*: N2, N4 and N5, referred to in Muangmai et al. (2014) occur in NZ (Fig. S2). Of the 384 samples, most of the samples (250) were from species N4, while another 128 and six samples belonged to species N2 and N5 respectively. A total of 31 different haplotypes were identified (10 for N2, 20 for N4 and 2 for N5) (Fig. 2, Table 1). Haplotype and genetic diversity indices of the three cryptic species in each population are presented in Table 1. Haplotype diversity (*Hd*) ranged from 0.35 to 0.68 for species N2 and from 0.20 to

255 0.83 for species N4 (Table 1). Nucleotide diversity (π) was relatively low for all
256 species, varying from 0.0009 to 0.0042 for species N2, and from 0.0005 to 0.0139 for
257 species N4 (Table 1).
258
259 The most widely distributed cryptic species of *B. intricata* in NZ was N4, which was
260 recorded in 37 populations (Fig. 1). In contrast, species N2 and N5 were restricted to
261 the North Island and top of the South Island (Fig. 1). Species N2 was detected in 19
262 populations, whereas species N5 was rare and only found in three populations (NZ16,
263 NZ19 and NZ27) around Cook Strait, the strait between the North and South Islands.
264 Although two different species were found to coexist at quite a few (12) sites, only
265 one site (Moa Point population: NZ12) had all three species occurring in sympatry
266 (Fig. 1).
267
268 Haplotype networks constructed for the partial COI dataset (including NZ haplotypes
269 from Fraser et al. 2013) are presented in Fig. 2. Species N2 consisted of 11 haplotypes
270 (1–4 bp differences), and its haplotype network was star-like with a central, common
271 (71%) haplotype, 2A, occurring across nearly all populations where this species was
272 found (Fig. 2). Haplotypes 2K and 2G were only detected from populations on the
273 east coast of the North Island, while haplotype 2I was shared among populations from
274 the top of the South Island (Fig. 1, Table 1). The seven other haplotypes were found
275 only in single populations (Table 1). Cryptic species N4 showed the highest diversity,
276 comprising 28 haplotypes (1–16 bp difference) with complex relationships (Fig. 2).
277 Several common haplotypes were detected, for example 4E1, 4A and 4V. 4E1 was the
278 most abundant haplotype, which occurred in 22 of 46 populations around NZ,
279 accounting for 44% of the samples for this species. The haplotype 4A was commonly

found in populations on the east coast of the South Island (14%), while haplotype 4V was shared across populations around the west coast of the South Island (8%)(Fig.1 and Table 1). Another eight haplotypes of N4 were observed in at least two populations, whereas the remaining 17 haplotypes were only found in single populations (Fig. 1, Table1). Cryptic species N5 had low diversity, containing only two different haplotypes, 5A and 5B, (2 bp differences), although far fewer samples of this species were found than for the other two species. Haplotype 5A was found in populations from the southern North Island (NZ12 and NZ16), while 5B was only found in one population, NZ27, from the top of South Island (Fig. 1, Table 1).

Population structure

Population differentiation was separately analyzed for cryptic species N2 (9 populations) and N4 (21 populations). Population pairwise F_{ST} values indicated the significant genetic differentiation among some populations of cryptic species N2 (Table S2). The F_{ST} analyses also showed that the genetic differentiations among the geographically distant populations were relatively low or not significant (Table S2). For example, the Titirangi Bay population (NZ 26) from the top of the South Island was weakly, but significantly genetic differentiated from populations at the bottom of the North Island (Red rock (NZ18) and Moa Point (NZ12)), less than 80 kilometers away, but not significantly different from more distant populations from the upper eastern North Island (Maraetai Bay (NZ5) and Waihau Bay (NZ6), more than 1000 kilometers away) (Table S2). In contrast, species N4 showed highly significant population differentiation among some areas (Table S3). Genetic differentiation between proximate populations was also observed on the west coast of the South Island. For example, the Gentle Annie population (NZ40) was significantly

differentiated from the nearby population (less than 40 km away) of Gibson's Beach (NZ41) as well as the more distant population (more than 900 km away) of Waipatiki Beach (NZ8) (Table S3).

Population structure analyses based on the SAMOVA algorithm showed that 9 populations of cryptic species N2 were clustered into two groups: Castle Point population (group 1; NZ10) and eastern North Island and top of South Island (group 2; NZ5, NZ6, NZ7, NZ9, NZ11, NZ12, NZ18 and NZ26) (maximum $F_{CT} = 0.336$, $P < 0.05$ at K = 2), whereas 21 populations of cryptic species N4 were assigned to six differentiated groups ($F_{CT} = 0.824$, $P < 0.05$ at K = 6) (Fig. 3). The six groups of cryptic species N4 proposed by SAMOVA were: west coast of North Island and top of South Island (group 1; NZ3, NZ13, NZ17, NZ30, NZ32, NZ33, NZ34, NZ36 and NZ37), east coast of North Island (group 2: NZ8), east coast of South Island (group 3: NZ21, and group 4: NZ 23, NZ24, NZ44 and NZ46) and west coast of South Island (group 5; NZ41, NZ42 and NZ43 and group 6; NZ38 and NZ 39) (Fig. 3). Furthermore, AMOVA analysis of species N4 using the grouping scheme based on four major biogeographic regions proposed by Apte and Gardner (2002) and Shears et al. (2008) (Fig. S1) indicated that 67.57% of the genetic variation occurred among groups of western and eastern North Island and western and eastern South Island ($F_{CT} = 0.621$, $P < 0.01$), while lower levels of genetic variation (14.83%) existed among populations within groups ($F_{SC} = 0.542$, $P < 0.01$) (Table S4).

Demographic history and dating analyses

The Tajima's D and Fu neutrality tests were used to observe historical population expansions for all populations of cryptic species N2 and N4 of *B. intricata*.

Significantly negative values of both Tajima's D and Fu tests (D = -1.38, $P < 0.05$; F_S= -3.78, $P < 0.05$) were observed for species N2, indicating a recent population expansion. By contrast, the Tajima's D and Fu tests were non-significant (D = 0.55, $P < 0.05$; F_S= 1.26, P = 0.41) for species N4, indicating demographically stable populations for this species. Similarly, the mismatch distribution for species N2 was unimodal, supporting a hypothesis of expanding populations, whereas cryptic species N4 showed a multimodal distribution, suggesting more stable populations (Fig. S3). Different patterns of historic population demography in these two cryptic species of *B. intricata* were further supported by the TMRCA analyses. Diversified among species was inferred to have occurred in the late Pleistocene (< 0.2 million years ago) (Fig. S4). The diversification of cryptic species N2 appears to have occurred between 55,000 – 12,000 years ago, while the diversification of cryptic species N4 occurred earlier, around 190,000 – 80,000 years ago (Fig S4). Cryptic species N5 seemed to be recently evolved, around 20,000 – 2,000 years ago (Fig. S4).

DISCUSSION

Our molecular analyses indicated different levels of genetic variation and distribution patterns among the three cryptic species of *B. intricata* in New Zealand, suggesting that each has experienced a different demographic history. Our broad-scale analyses, with samples from 43 sites around the country, strongly support the occurrence of three cryptic species within *B. intricata* in NZ, as previously indicated by Muangmai et al. (2014); although this earlier study used far fewer samples from NZ. The differences in sample size could occasionally yield the same number of cryptic species; however the large samples are still necessary to precisely assess the genetic diversity and phylogeographic pattern of algal cryptic species (Zuccarello et al. 2006,

Zuccarello et al. 2011). Cryptic species have been detected in other *Bostrychia* species (Zuccarello and West 2003, 2006, Muangmai et al. 2014) and in other red algal genera (e.g., *Porteria hornemannii*, Payo et al. 2013 and *Spyridia filamentosa*, Zuccarello et al. 2002). Previous phylogeographic studies of algae in NZ have indicated genetic differences between northern and southern regions. High levels of genetic variation were found in North Island populations of the brown macroalgae *C. maschalocarpum* and *D. antarctica*, whereas relatively low genetic variation was encountered in the South Island for *C. maschalocarpum*, *D. antarctica* and *M. pyrifera* (Fraser et al. 2009, Macaya and Zuccarello 2010a, Buchanan and Zuccarello 2012). In rough accordance with these findings, we detected three cryptic species (N2, N4 and N5) in northern New Zealand, whereas only one cryptic species (N4) was found in southern New Zealand (Fig. 2). Nonetheless, considerable genetic diversity was detected within and among populations of species N4 in southern New Zealand. High levels of genetic heterogeneity have previously been observed in a brown alga, *Adenocystis utricularis* (Bory) Skottsberg, from the eastern coast of the South Island (Fraser et al. 2013), and it has been proposed that both *A. utricularis* and *B. intricata* may be less susceptible to population decline during glacial periods than the larger kelps (Fraser et al. 2013).

Distributions of cryptic species N2 and N5 was mostly confined in North Island and top of the South Island, as this pattern detected by the samples of their populations being restricted north of Cape Campbell (east coast) and Golden Bay (west coast) of the South Island (Fig. S1). Many phylogeographic studies of marine taxa in NZ have previously demonstrated that the contemporary upwelling or ocean currents around Cape Campbell and Golden Bay form a significant biogeographic barrier between the

northern and southern biogeographic province (Fig. S1), this was found, for example, in greenshell mussel (Apte and Gardner 2002), seastars (Spocer and Roy 2002, Ayers and Waters 2005) and limpets (Goldstien et al. 2006). It is possible that the restricted northern distribution observed in *B. intricata* species N2 and N5 is due to this north-south biogeographic break and the inability of these species to establish past this barrier. However, previous population studies of NZ seaweed showed no evidence for this north-south split, for example, the brown alga *Carpophyllum maschalocarpum* exhibited a population disjunction in the middle of the North Island (Buchanan and Zuccarello 2012). The observed incongruent pattern of species distribution may be attributable to the differences in life history, dispersal and adaptive capacity of the various cryptic species

Present-day patterns of high levels of genetic diversity can indicate population stability (Grant and Bowen 1998). The differences in genetic diversity and distributions between cryptic *B. intricata* species may be that the three species originated and evolved at different times in the past. Our dating analyses indicated that species N4 is older than either species N2 or N5. If cryptic species N4 arose early, it would have had more time to accumulate mutations than the other cryptic species, and would have had a longer time to adapt to conditions around NZ. It may also have benefited from a relative absence of competition; assuming that all three cryptic species share similar habitat requirements, the later-evolving lineages will have had to compete with existing populations of species N4 for resources.

Another non-mutually exclusive possibility is that these cryptic species have responded differently to historical events and regional climate change, and this

promoted the disparity in genetic composition and distribution patterns between them. The LGM is a well-known historic event that played an important role in shaping population diversity and connectivity of marine taxa in the Southern Hemisphere (Fraser et al. 2012), and its main impacts were approximately 27,000 – 18,000 years ago (Suggate and Almond 2005). Some studies indicated that the LGM had a significant impact on the genetic diversity and structure of algal species. For example, the observed genetic homogeneity of some brown seaweeds (*Carpophyllum maschalocarpum* and *Durvillea antarctica*) from low latitude populations (South Island of New Zealand and sub-Antarctic Island) suggest that these algae may have be removed by ice scouring during the LGM or changes in water temperature and then recolonized in the post-glaciation period (Fraser et al. 2009, Buchanan and Zuccarello 2012). The LGM is likely to have facilitated the patterns of genetic diversity and distribution within cryptic *B. intricata* species. Of the three different cryptic *B. intricata* species, we found that the origin of species N4 (180,000 – 90,000 years ago) predated the LGM, and this cryptic species seemed to have survived the glaciation period while retaining its genetic diversity, as indicated by the high-level genetic diversity and wide distribution in both North and South Islands. On the other hand, the diversifications of cryptic species N2 (55,000 – 12,000 years ago) and N5 (20,000 – 2,000 years ago) could have existed during the LGM or have post-dated the LGM. The low genetic diversity and more limited distribution observed in cryptic species N2 and N5 could be explained by two possibilities. Firstly, cryptic species N2 and N5 were eliminated from southern areas, and populations in the north contracted, and later were prevented from moving southward after the LGM; secondly these two species diversified after the LGM and then expanded their populations but were prevented from dispersing past the north-south barrier. Both scenarios suggest the

influences of a climate change after glaciation (e.g. warmer seawater in northern New Zealand or rising seawater level at the Cook Strait) on their diversifications and distribution. From these observed patterns, we also hypothesize that the three cryptic species may have different genetic-physiological adaptations and abilities to persistent in changing environments. Further studies on whether these cryptic *B. intricata* species are different in eco-physiological responses (e.g. temperature, exposure), would help to shed light on the mechanism facilitating the genetic differentiation and distribution pattern in the genus *Bostrychia* or even in other marine red algae.

Phylogeographic research on marine macroalgae in the Southern Hemisphere have shown that the pattern of population differentiation and connectivity have relied on dispersal potential (Fraser et al. 2009, Macaya and Zuccarello 2010a), habitat availability and density (Montecinos et al. 2012) and the effect of historical events and environmental changes (Fraser et al. 2009, Macaya and Zuccarello 2010b, Buchanan and Zuccarello 2012). For examples, kelp species (e.g. *Durvillea antarctica* and *Macrocystis pyrifera*), with high dispersal potential, have demonstrated a higher level of population connectivity than other marine algae with low effective dispersal potential (e.g. *Mazzaella laminarioides*) (Fraser et al. 2009, Macaya and Zuccarello 2010b, Montecinos et al. 2012). In *B. intricata*, we detected disparate patterns of population structure between two cryptic species, suggesting higher level of gene flow in cryptic species N2 than N4, and this pattern is likely be related to their differences in the extent of dispersal frequency. Our results in population and dating analyses, which indicate that cryptic species N2 has expanded more recently than species N4, may also explain the different patterns observed in cryptic species N2 and N4. We suggest that the difference in evolutionary and demographic history of

455 species could be another possibility to account for the differences in population
456 structure and connectivity of cryptic algal species.
457
458 Our SAMOVA results support the major biogeographic provinces previously
459 proposed for NZ, with northern/southern biogeographic provinces (*B. intricata* N4
460 groupings 1, 2, 6 versus 3, 4, 5) (Apte and Gardner 2002, Sharyn et al. 2006) and a
461 genetic split between western/eastern regions, especially on the South Island (*B.*
462 *intricata* N4 groupings 5,6 versus 3, 4) (Jones et al. 2008, Veale & Lavery 2012) (Fig.
463 3). However, SAMOVA analyses for cryptic species N2 and N4 suggested that these
464 groupings were partially incongruent with the 11 bioregions as proposed by Shears et
465 al. (2008), with some groupings spanning several of the proposed regions, especially
466 on the North Island. For example, we found evidence for connectivity between the
467 northeastern and the Portland regions on the North Island for cryptic species N2
468 (group 1, Fig. 3) and the Raglan region and the Abel region on the North Island for
469 cryptic species N4 (group 1, Fig. 3). Our mitochondrial data may not, however, have
470 been able to detect all biogeographic detail; more rapidly-evolving markers (such as
471 microsatellites) could be used in future studies to assess whether fine-scale structure
472 or population connectivity occurs in these regions.
473
474 In conclusion, our data clearly show the occurrence of three cryptic species of *B.*
475 *intricata* (N2, N4 and N5) in NZ. We have not been able to find any morphological
476 difference between these species. This is very common in *Bostrychia* species that do
477 not have many morphological characters to investigate (Zuccarello and West 2003).
478 These cryptic species substantially differ in the level of genetic diversity, distribution
479 pattern and demographic histories. While a single marker may not provide all the

evidence of a species history, variable organellar markers do give one level of understanding of a species history, and are commonly used when nuclear markers have not been developed. These findings highlight that different pattern of species history can be quite substantial in species that are morphologically indistinguishable (cryptic species). Future research should be carried out on physiological analyses to assess whether these species differ at a non-morpholigcal levels.

ACKNOWLEDGMENTS

For help with collections, we thank: Wendy Nelson, Mike Wilcox, Christian Boedeker and Maren Preuss. We also thank John West for useful discussion and Joe Buchanan for molecular analyses help. Special thanks the Victoria University of Wellington for providing a scholarship to NM.

493 REFERENCES

494 Allcock, A. L. & Strugnell J. M. 2012. Southern Ocean diversity: new paradigms
495 from molecular ecology. *Trends Ecol. Evol*. 27:520–8.
496 Apte, S. & Gardner, J. P. A. 2002. Population subdivision in the New Zealand
497 greenshell mussel (*Perna canaliculus*) inferred from single-strand
498 conformation polymorphism analysis of mitochondrial DNA. *Mol. Ecol*.
499 11:1617–35.
500 Ayers, K. L. & Waters, J. M. 2005. Marine biogeographic disjunction in central New
501 Zealand. *Mar. Biol.* 147:1045–52.
502 Brante, A., Fernández, M. & Viard, F. 2012. Phylogeography and biogeography
503 concordance in the marine gastropod *Crepipatella dilatata* (Calyptraeidae)
504 along the southeastern Pacific coast. *J. Hered*. 103:630–7.
505 Buchanan, J. & Zuccarello, G. C. 2012. Decoupling of short- and long-distance
506 dispersal pathways in the endemic New Zealand seaweed *Carpophyllum*
507 *maschalocarpum* (Phaeophyta, Fucales). *J. Phycol*. 48:518–29.
508 Clement, M., Posada, D. & Crandall, K. 2000. TCS: a computer program to estimate
509 gene genealogies. *Mol. Ecol*. 9: 1657–60.
510 Collins, C. J., Fraser, C. I., Ashcroft, A. & Waters, J. M. 2010. Asymmetric dispersal
511 of southern bull□kelp (*Durvillaea antarctica*) adults in coastal New Zealand:
512 testing an oceanographic hypothesis. *Mol. Ecol*. 19:4572–80.
513 Dellicour, S. & Mardulyn, P. 2014. SPADS 1.0: a toolbox to perform spatial analyses
514 on DNA sequence data sets. *Mol. Ecol. Resour*. 14: 647–51.
515 Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. 2012. Bayesian
516 phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* 29: 1969–73.
517 Dupanloup, I., Schneider, S. & Excoffier, L. 2002. A simulated annealing approach to

define the genetic structure of populations. *Mol. Ecol*. 11:2571–81.

Excoffier, L. & Lischer, H.E. L. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour*. 10: 564–7.

Felsenstein, J. 2006. Accuracy of coalescent likelihood estimates: Do we need more sites, more sequences, or more loci? *Mol. Biol. Evol*. 23: 691–700.

Fraser, C. I., Nikula, R., Ruzzante, D. E. & Waters, J. M. 2012. Poleward bound: biological impacts of Southern Hemisphere glaciation. *Trends Ecol. Evol*. 27:462–71.

Fraser, C. I., Nikula, R., Spencer, H. G., & Waters, J. M. 2009. Kelp genes reveal effects of subantarctic sea ice during the Last Glacial Maximum. *Proc. Natl. Acad. Sci. USA.* 106: 3249–53.

Fraser, C. I., Nikula, R. & Waters, J. M. 2011. Oceanic rafting by a coastal community. *Proc. Biol. Sci.* 278:649–55.

Fraser, C. I, Zuccarello, G. C., Spencer, H. G., Salvatore, L. C., Garcia, G. R. & Waters J. M. 2013. Genetic affinities between trans-oceanic of non-buoyant macroalgae in the high latitudes of the Southern Hemisphere. *PLOS One* 8:e69138.

Grant, W. S. & Bowen, B. W. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Heredity* 89:415–26.

Goldstien, S. J., Schiel, D. R. & Gemmell N. J. 2006. Comparative phylogeography of coastal limpets across a marine disjunction in New Zealand. *Mol. Ecol.* 15:3259–68.

Harpending, H.C. 1994. Signature of ancient population growth in a low-resolution

543 mitochondrial DNA mismatch distribution. *Hum. Biol.* 66:591–600.
544 Hemmer-Hansen, J., Nielsen, E. E., Gronkjaer, P. & Loeschcke, V. 2007.
545 Evolutionary mechanisms shaping the genetic population structure of marine
546 fishes; lessons from the European flounder (*Platichthys flesus* L.). *Mol. Ecol*.
547 16:3104–18.
548 Hewitt, G. M. 2004. Genetic consequences of climatic oscillations in the Quaternary.
549 *Philos. Trans. R. Soc. Lond. B* 359:183–95.
550 Jone, T. C., Gemmill C. E. C. & Pilditch, C. A. 2008. Genetic variability of New
551 Zealand seagrass (*Zostera muelleri*) assessed at multiple spatial scale. *Aqua.*
552 *Bot.* 88: 39–46.
553 King, R. J. & Puttock, C. F. 1989. Morphology and taxonomy of *Bostrychia* and
554 *Stictosiphonia* (Rhodomelaceae / Rhodophyta). *Austral. Syst. Bot.* 2:1–73.
555 Le Port, A. & Lavery, S. 2012. Population structure and phylogeography of the short-
556 tailed stingray, *Dasyatis brevicaudata* (Hutton 1875), in the Southern
557 Hemisphere. *J. Hered.* 103:174–85.
558 Librado, P. & Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis
559 of DNA polymorphism data. *Bioinformatics* 25**:** 1451–2.
560 Lomolino, M. V., Riddle B. R. & Brown, J. H. 2006. *Biogeography,* third edition.
561 Sinauer Association Inc., Sunderland, Massachusetts. 845pp.
562 Macaya, E. C. & Zucarello, G. C. 2010a. DNA barcoding and genetic divergence in
563 the giant kelp *Macrocystis* (Laminariales). *J. Phycol*. 46:736-42.
564 Macaya, E. C. & Zucarello, G. C. 2010b. Genetic structure of the giant kelp
565 *Macrocystis pyrifera* along the southeastern Pacific. *Mar. Ecol. Prog. Ser*.
566 420:103–12.
567 Montecinos, A., Broitman, B. R., Faugeron, S., Haye, P. A., Tellier, F. & Guillemin,

M. 2012. Species replacement along a linear coastal habitat: phylogeography and speciation in the red alga *Mazzaella laminarioides* along the south east pacific. *BMC Evol. Biol.* 12:97.

Muangmai N, West, J. A. & Zuccarello G. C. 2014. Evolution of four Southern Hemisphere *Bostrychia* (Rhodomelaceae, Rhodophyta) species: phylogeny,species delimitation and divergence times. *Phycologia* 53:593–601..

Nikula, R., Fraser, C. I., Spancer, H. G. & Waters, J. M. 2010. Circumpolar dispersal by rafting in two subantarctic kelp-dwelling crustaceans. *Mar. Ecol. Prog. Ser*. 405:221–30.

Payo D. A., Leliaert F., Verbruggen H., D'hondt S., Calumpong H. P. & De Clerck O. 2013. Extensive cryptic species diversity and fine scale-scale endemism in the marine red alga *Portieria* in the Philippines. *P. Roy. Soc. B. – Biol. Sci.* 280: 20122660.

Rambaut, A. 2009. FigTree. v1.3.1. Available at: http://tree.bio.ed.ac.uk/software (last accessed 27 March 2014).

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. 2012. MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Syst. Biol.* 61: 539–42.

Ross, P. M., Hogg, I. D., Pilditch, C. A. & Lundquist, C. J. 2009. Phylogeography of New Zealand's coastal benthos. *N. Z. J. Mar. Freshw*. *Res.* 43:1009–27.

Ross, P. M., Hogg, I. D., Pilditch, C. A., Lundquist, C. J. & Wilkins, R. J. 2012.

Population genetic structure of the New Zealand estuarine clam *Austrovenus stutchburyi* (Bivalvia: Veneridae) reveals population subdivision and partial congruence with biogeographic boundaries. *Estuar. Coast*. 35:143–54.

Saunders, G. W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Phil. Trans. R. Soc.* B 360: 1879–88.

Schneider, S., Roessli, D. & Excoffier, L 2000. *Arlequin: a software for population genetic data*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Sharyn, J. G., Schiel, D. R. & Gemmell, N. J. 2006. Comparative phylogeography of coastal limpets across a marine disjunction in New Zealand. *Mol. Ecol.* 15:3259–68.

Shears, N. T., Smith, F., Babcock, R. C., Duffy, C. A. & Villouta, E. 2008. Evaluation of biogeographic classification schemes for conservation planning: application to New Zealand's coastal marine environment. *Conserv. Biol*. 22:467–81.

Silvestro, D. & Michalak I. 2012. raxmlGUI: a graphical front-end for RAxML. *Org. Divers. Evol.* 12: 335–7.

Smith, P. J., McMillan, P. J., Bull, B., McVeagh, S. M., Gaffney, P. M., & Chow, S. 2002. Genetic and meristic variation in black and smooth oreos in the New Zealand Exclusive Economic Zone. *N. Z. J. Mar. Freshw. Res*. 36: 737–50.

Spocer, R. & Roy, M. S. 2002. Phylogeographic analysis of the brooding brittle star *Amphipholis squamata* (echinodermata) along the coast of New Zealand reveals high cryptic genetic variation and cryptic dispersal potential. *Evolution* 56:1954–67.

Suggate, R. P. & Almond P. C. 2005. The Last Glacial Maximum (LGM) in western

South Island, New Zealand: implications for the global LGM and MIS 2. *Quat. Sci. Rev.* 24:16–7.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585–95.

Tanabe, A. S. 2011. Kakusan4 and Aminosan: two programs for comparing nonpartitioned, proportional, and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Mol. Ecol. Resour.* 11:914–21.

Veale, A. J. & Lavery S. D. 2012. The population genetic structure of the intertidal waratah anemone (*Actinia tenebrosa*) around New Zealand. *N. Z. J. Mar. Freshw. Res.* 46:523–36.

Wallis, G. P. & Trewick, S. A. 2009. New Zealand phylogeography: evolution on a small continent. *Mol. Ecol.* 18:3548–80.

Water, J. M. & Roy, M. S. 2003. Marine biogeography of southern Australia: phylogeographical structure in a temperate sea-star. *J. Biogeor*. 30:1787–96.

Waters, J. M., Fraser, C. I. & Hewitt G. M. 2013. Founder takes all: density-dependent processes structure biodiversity. *Trends Ecol. Evol*. 28:78–85.

Zuccarello, G. C., Buchanan, J. & West, J. A. 2006. Increased sampling for inferring phylogeographic patterns in *Bostrychia radicans*/*Bostrychia moritziana* in the eastern USA. *J. Phycol*. 42:1349–52.

Zuccarello, G. C., Buchanan, J., West, J. A. & Pedroche, F. F. 2011. Genetic diversity of the mangrove-associated alga *Bostrychia radicans*/*B. moritziana* (Ceramiales, Rhodophyta) from southern Central America. *Phycol. Res*. 59:98–104.

Zuccarello, G. C., Sandercock, B. & West, J. A. 2002. Diversity within red algal

641 species: variation in world-wide samples of *Spyridia filamentosa*
642 (Ceramiaceae) and *Murrayella periclados* (Rhodomelaceae) using DNA
643 markers and breeding studies. *Eur. J. Phycol.* 37: 403–18.
644 Zuccarello, G. C. & West, J. A. 2003. Multiple cryptic species: molecular diversity
645 and reproductive isolation in the *Bostrychia radicans / B. moritziana* complex
646 (Rhodomelaceae, Rhodophyta) with focus on North American isolates. *J.*
647 *Phycol.* 39: 948–59.
648 Zuccarello, G. C. & West, J. A. 2006. Molecular phylogeny of the subfamily
649 Bostrychioideae (Ceramiales, Rhodophyta): Subsuming *Stictosiphonia* and
650 highlighting polyphyly in species of *Bostrychia*. *Phycologia* 45:24–36.
651 Zuccarello, G. C. & West, J. A. 2008. *Bostrychia* (Rhodomelaceae, Rhodophyta)
652 species of New Zealand, and relationships in the Southern Hemisphere. *N. Z.*
653 *J. Mar. Freshw. Res*. 42:315–24.
654 Zuccarello, G. C. & West, J. A. 2011. Insights into evolution and speciation in the red
655 alga *Bostrychia*: 15 years of research. *Algae* 26:3–14.
656 Zuccarello G. C., West J. A., Kamiya M. & King R. J. 1999a. A rapid method to score
657 plastid haplotypes in red seaweeds and its use in determining parental
658 inheritance of plastid in the red alga *Bostrychia* (Ceramiales). *Hydrobiologia*
659 401: 207–14.
660 Zuccarello, G. C., West, J. A. & King, R. J. 1999b. Evolutionary divergence in the
661 *Bostrychia moritziana/B. radicans* complex (Rhodomelaceae, Rhodophyta):
662 molecular and hybridisation data. *Phycologia* 38: 234–44.
663
664

Table 1. Sampling sites of cryptic species N2, N4 and N5 of *B. intricata* in New Zealand and genetic diversity indices. Code= Population code, *N*= number of samples, *H*= number of haplotypes, *S*= number of segregating sites, *Hd*= haplotype diversity, π= nucleotide diversity

Code	Sampling site	Coordinates	*N*	Haplotypes Present	*H*	*S*	*Hd*	π
North Island								
NZ1	Casnell Island, Leigh	36°29'14.21"S 174°43'37.24"E	4	N2 = 2A(4)	1	0	0.00	0.0000
NZ2	Sandspit, Snells beach, Leigh	36°24'13.82"S 174°44'09.76"E	4	N2 = 2A(4)	1	0	0.00	0.0000
NZ3	Waitemata Harbour, Auckland	36°50'28.93"S 174°43'49.74"E	10	N4 = 4E8(4), 4E9(3), 4E10(2), 4E11(1)	4	7	0.77	0.0069
NZ4	Kaikoura Island, Huaraki Gulf	36°10'29.40"S 175°19'35.56"E	1	N4 = 4E8(1)	1	0	0.00	0.0000
NZ5	Tekaha, Maraetai Bay, East Cape	37°43'36.19"S 177°41'27.83"E	10	N2 = 2A(10)	1	0	0.00	0.0000

NZ6	Waihau Bay, East Cape	37°31'11.63"S 177°55'17.27"E	10	N2 = 2A(5), 2D(2), 2K(3)	3	2	0.68	0.0021
NZ7	Lottin Point, East Cape	37°32'57.05"S 178°08'03.06"E	10	N2 = 2A(4), 2G(1), 2K(5)	3	2	0.64	0.0020
NZ8	Waipatiki Beach, Hawke Bay	39°18'01.79"S 176°58'43.22"E	10	N4 = 4R(8), 4S(2)	2	1	0.36	0.0009
NZ9	Porangahua beach, Hawke Bay	40°18'02.65"S 176°40'15.49"E	10	N2 = 2A(8), 2E(2)	2	1	0.48	0.0012
NZ10	Castle Point, Wellington	40°54'05.15"S 176°13'48.61"E	10	N2 = 2F(4), 2G(6)	2	3	0.53	0.0042
NZ11	Cape Palliser, Wellington	41°36'45.48"S 175°17'50.83"E	12	N2 = 2A(10)	N2 = 1	N2 = 0	N2 = 0.00	N2 = 0.0000
				N4 = 4E1(2)	N4 = 1	N4 = 0	N4 = 0.00	N4 = 0.0000
NZ12	Moa Pint, Wellington	41°20'40.78"S 174°48'35.34"E	19	N2 = 2A(14)	N2 = 1	N2 = 0	N2 = 0.00	N2 = 0.0000
				N4 = 4E1(3)	N4 = 1	N4 = 0	N4 = 0.00	N4 = 0.0000
				N5 = 5A(2)	N5 = 1	N5 = 0	N5 = 0.00	N5 = 0.0000

NZ13	Manukau Harbour, Auckland	36°55'52.43"S 174°45'18.74"E	11	N4 = 4E1(7), 4E7(3), 4E8(1)	3	2	0.61	0.0016
NZ14	New Plymount, Taranaki	39°03'21.67"S 174°03'35.31"E	6	N2 = 2A(3) N4 = 4E1(3)	N2 = 1 N4 = 1	N2 = 0 N4 = 0	N2 = 0.00 N4 = 0.00	N2 = 0.0000 N4 = 0.0000
NZ15	Cape Egmont, Taranaki	39°16'26.79"S 173°45'09.52"E	8	N2 = 2A (1) N4 = 4E1(4), 4E5(2), 4E6(1)	N2 = 1 N4 = 3	N2 = 0 N4 = 2	N2 = 0.00 N4 = 0.66	N2 = 0.0000 N4 = 0.0023
NZ16	Kapita Coast	41°01'21.19"S 174°54'26.21"E	6	N2 = 2A(4) N5 = 5A(2)	N2 = 1 N5 = 1	N2 = 0 N5 = 0	N2 = 0.00 N5 = 0.00	N2 = 0.0000 N5 = 0.0000
NZ17	Titahi Bay, Porirua	41°06'20.79"S 174°49'24.49"E	10	N4 = 2E1(10)	1	0	0.00	0.0000
NZ18	Red Rock, Wellington	41°20'56.38"S 174°44'27.25"E	17	N2 = 2A(11), 2J(6)	2	1	0.48	0.0012
South Island								
NZ19	Hakahaka Bay, Picton,	41°17'58.22"S 174°06'50.76"E	8	N2 = 2A(2) N4 = 4E1(6)	N2 = 1 N4 = 1	N2 = 0 N4 = 0	N2 = 0.00 N4 = 0.00	N2 = 0.0000 N4 = 0.0000

NZ20	Paparoa Point, Kaikoura	42°14'10.51"S 173°50'48.81"E	4	N4 = 4Q(4)	1	0	0.00	0.0000
NZ21	Halfmoon Bay, Kaikoura	42°15'40.11"S 173°48'39.65"E	10	N4 = 4Q(10)	1	0	0.00	0.0000
NZ22	Port Levy, Bank Peninsula	43°38'52.49"S 172°49'10.36"E	6	N4 = 4A (6)	1	0	0.00	0.0000
NZ23	Pigeon Bay, Bank Peninsula	43°40'34.11"S 172°53'27.58"E	10	N4 = 4A(8), 4E1(2)	2	3	0.36	0.0028
NZ24	French Farm Bay, Akaroa, Bank Peninsula	43°46'21.43"S 172°54'50.99"E	10	N4 = 4A(5), 4E1(4), 4P(1)	3	7	0.64	0.0064
NZ25	Dunedin	45°53'13.70"S 170°30'44.72"E	4	N4 = 4A(4)	1	0	0.00	0.0000
NZ26	Titirangi Bay, Havelock	41°01'08.73"S 174°07'57.36"E	12	N2 = 2A(8), 2C(2) N4 = 4E1(2)	N2 = 2 N4 = 1	N2 = 1 N4 = 0	N2 = 0.35 N4 = 0	N2 = 0.0009 N4 = 0.0000
NZ27	Kenupuru Bay,	41°11'42.31"S	5	N4 = 4E1(4)	N4 = 1	N4 = 0	N4 = 0.00	N4 = 0.0000

	Havelock	174°04'28.01"E		N5 = 5B(1)	N5 = 1	N5 = 0	N5 = 0.00	N5 = 0.0000
NZ28	Te Mahia Bay, Havelock	41°12'57.31"S 173°58'16.61"E	1	N4 = 4E1(1)	1	0	0.00	0.0000
NZ29	Double Cove, Marlborough	41°14'00.40"S 174°00'55.51"E	6	N2 = 2A(3)	N2 = 1	N2 = 0	N2 = 0.00	N2 = 0.0000
				N4 = 4E1(3)	N4 = 1	N4 = 0	N4 = 0.00	N4 = 0.0000
NZ30	Okiwi Bay, Marlborough	41°06'08.72"S 173°39'30.96"E	11	N2 = 2I(1)	N2 = 1	N2 = 0	N2 = 0.00	N2 = 0.0000
				N4 = 4E1(9), 4E3(1)	N4 = 2	N4 = 1	N4 = 0.20	N4 = 0.0005
NZ31	Cable Bay, Nelson	41°09'18.93"S 173°25'03.61"E	8	N2 = 2H(1), 2I(1)	N2 = 2	N2 = 1	N2 = 0.66	N2 = 0.0017
				N4 = 4E1(2), 4E4(1), 4O(1)	N4 = 3	N4 = 10	N4 = 0.83	N4 = 0.0139
NZ32	Burton, Nelson	41°19'10.52"S 173°10'28.72"E	13	N2 = 2A(1)	N2 = 1	N2 = 0	N2 = 0.00	N2 = 0.0000
				N4 = 4E1(12)	N4 = 1	N4 = 0	N4 = 0.00	N4 = 0.0000
NZ33	Astrolabe, Sandy Bay, Abel Tasman NP	40°59'46.93"S 173°00'40.86"E	10	N4 = 4E1(10)	1	0	0.00	0.0000
NZ34	Coquille Bay, Abel Tasman National Park	40°59'21.04"S 173°01'49.51"E	10	N4 = 4E1(10)	1	0	0.00	0.0000

NZ35	Tinline Bay, Abel Tasman National Park	40°59'25.25"S 173°01'40.51"E	4	N4 = 4E1(4)	1	0	0.00	0.0000
NZ36	Wainui Bay, Tasman	40°48'13.01"S 172°57'11.13"E	11	N4 = 4E1(11)	1	0	0.00	0.0000
NZ37	Pohara, Tasman	40°49'47.53"S 172°53'32.16"E	11	N4 = 4E1(10), 4E2(1)	2	1	0.51	0.0013
NZ38	Whararíki Beach, Puponga	40°30'02.63"S 172°40'52.24"E	10	N4 = 4T(7), 4V(2), 4Y(1)	3	2	0.51	0.0018
NZ39	Whanganui Inlet, Tasman	40°34'52.61"S 172°37'47.46"E	12	N4 = 4E1(7), 4V(5)	2	9	0.62	0.0132
NZ40	Gentle Antie Seaside, Westport	41°30'21.81"S 171°56'46.86"E	10	N4 = 4W(10)	1	0	0.00	0.0000
NZ41	Gibsob's Beach, Cape Foulwind, Westport	41°44'53.13"S 171°28'16.06"E	10	N4 = 4V(7), 4X(3)	2	1	0.46	0.0012
NZ42	Tauranga Bay,	41°46'25.31"S	10	N4 = 4T(4), 4V(6)	2	1	0.53	0.0014

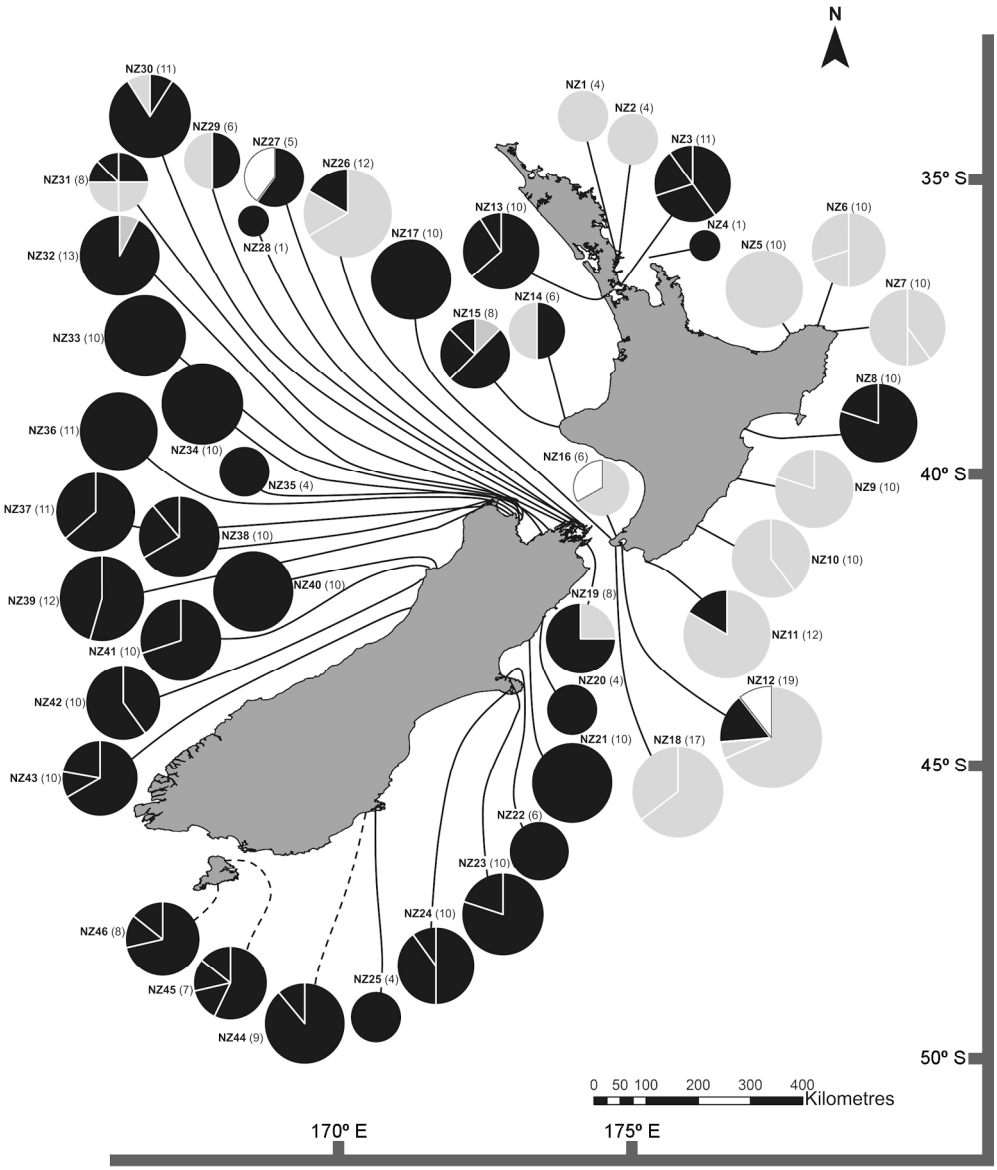
	Westport	171°27'17.71"E						
NZ43	Charleston, North of Woodpacker Bay	42°00'01.32"S 171°23'44.46"E	10	N4 = 4T(7), 4U(1), 4V(2)	3	3	0.60	0.0023
South Island – additional data from Fraser et al. (2013)								
NZ44	Brighton, Dunedin	45°56'54.91"S 170°20'12.72"E	9	N4 = 4A(8), 4N(1)	2	8	0.22	0.0048
NZ45	Stewart Island: Ringaringa	45°54'09.07"S 168°08'41.10"E	7	N4 = 4A(4), 4B(1), 4C(1), 4F(1)	4	3	0.64	0.0023
NZ46	Stewart Island: The Neck	45°55'25.37"S 168°11'26.95"E	8	N4 = 4A(6), 4B(1), 4C(1)	3	2	0.52	0.0015

Figure Legends

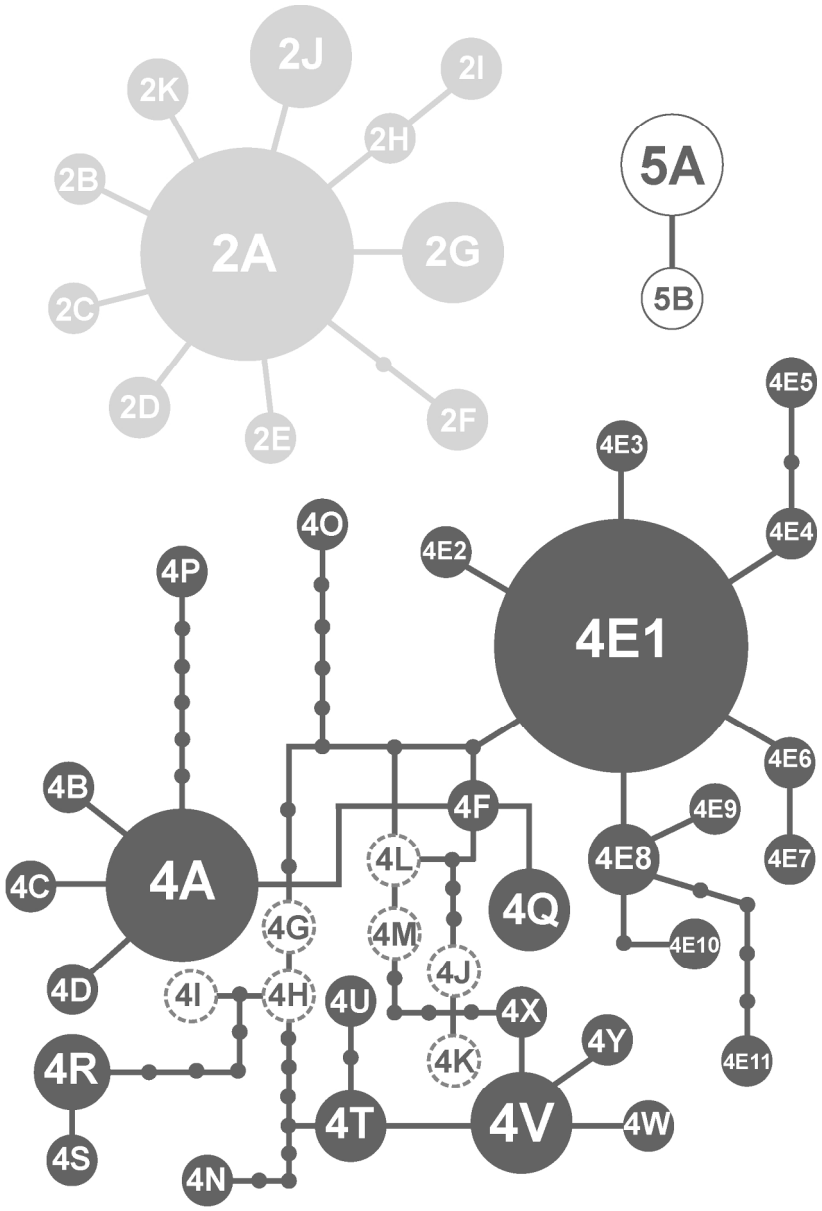
Fig. 1. Distribution of three cryptic *Bostrychia intricata* species based on COI data. Each pie chart shows the proportion of cryptic species and their haplotypes. Colors represent different cryptic species: Grey: species N2; Black: species N4; and White: species N5. Population codes and sample sizes are indicated next to the pie charts (see also Table 1).

Fig. 2. COI haplotype networks for cryptic *Bostrychia intricata* species (N2, N4, N5) obtained from the TCS analyses. Colors represent the different cryptic species as indicated in Fig. 1. Solid circles correspond to haplotypes found in this study, and dashed circles correspond to haplotypes from Fraser et al. (2013). Small circles represent inferred missing or extinct haplotypes.

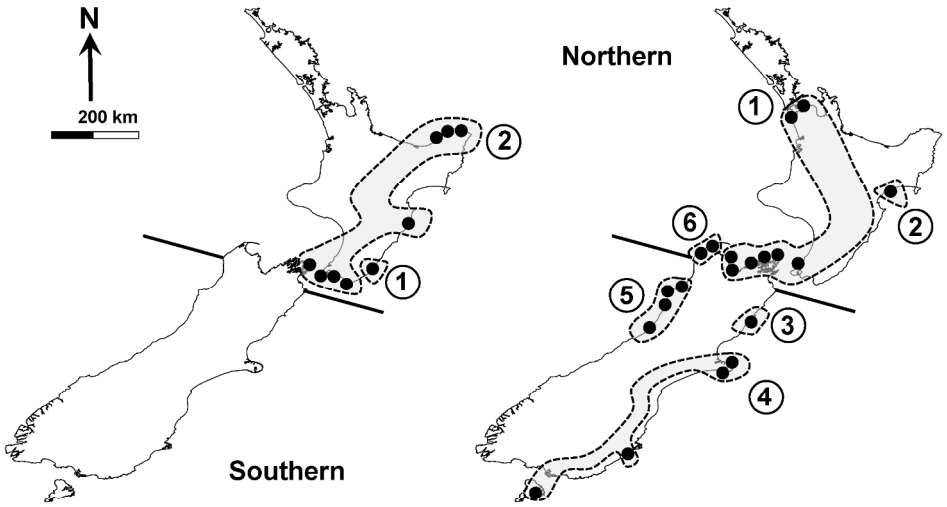
Fig. 3. Cluster analyses base on SAMOVA algorithm for cryptic *Bostrychia intricata* species N2 (A) and N4 (B). Small circles represent the populations sampled in this analysis (see Fig 1 and Table 1). Shaded areas show population grouping designated by K=2 for cryptic species N2 (group 1 and 2) and K=6 for cryptic species N4 (group 1 – 6). Solid black lines show the separation of the northern and southern biogeographic provinces of New Zealand according to Apte and Gardner (2002).



185x220mm (300 x 300 DPI)



160x236mm (300 x 300 DPI)



A) Cryptic species N2 B) Cryptic species N4

240x151mm (300 x 300 DPI)

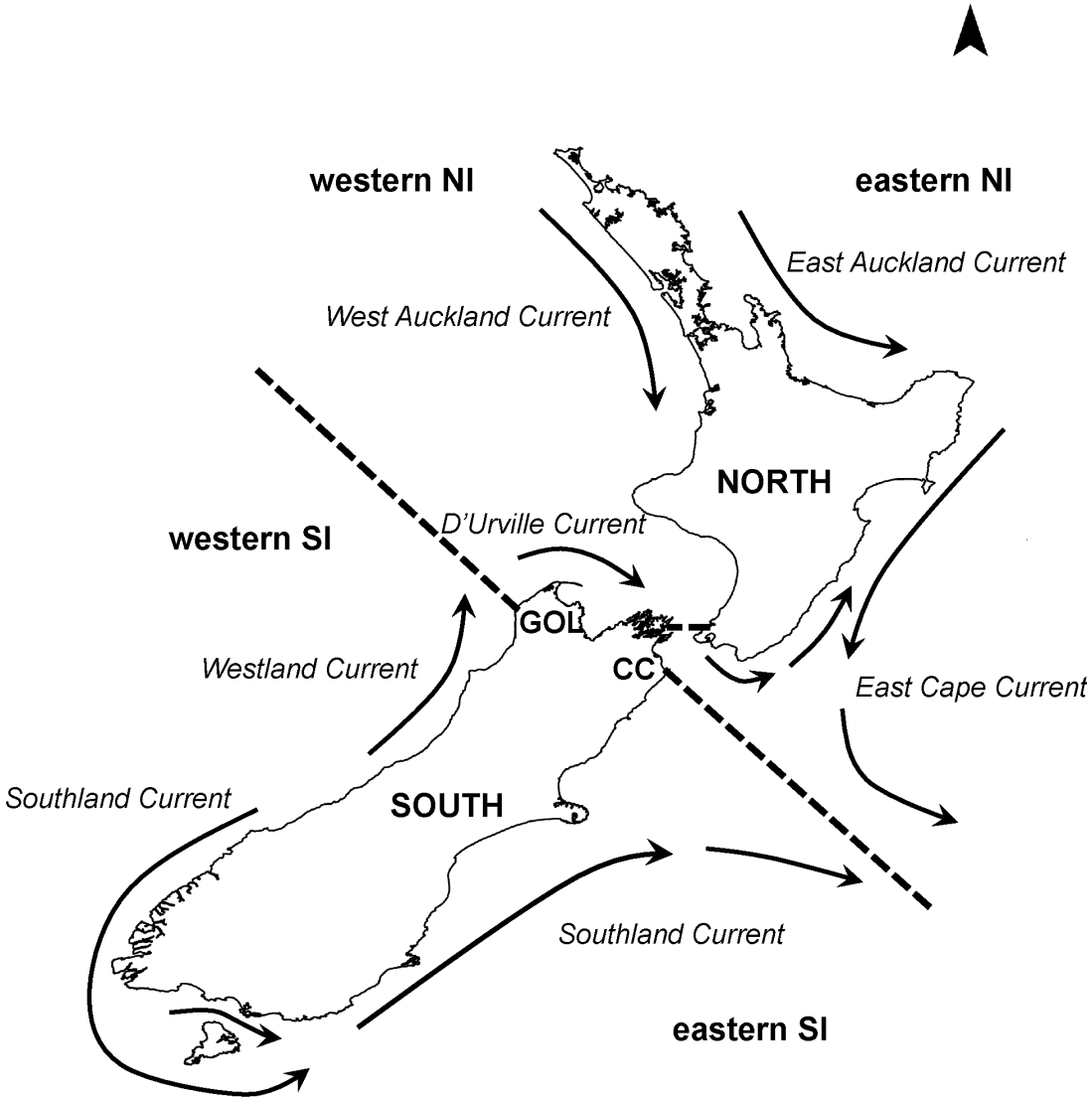


Fig. S1. Map of New Zealand showing regional hydrographic conditions. The north/south and west/east splits described by Apte & Gardner (2002) and Shears et al. (2008) are separated by dashed line; western NI, western SI, eastern NI and eastern SI. CC: Cape Campbell; GOL: Golden Bay; NI: North Island and SI: South Island.

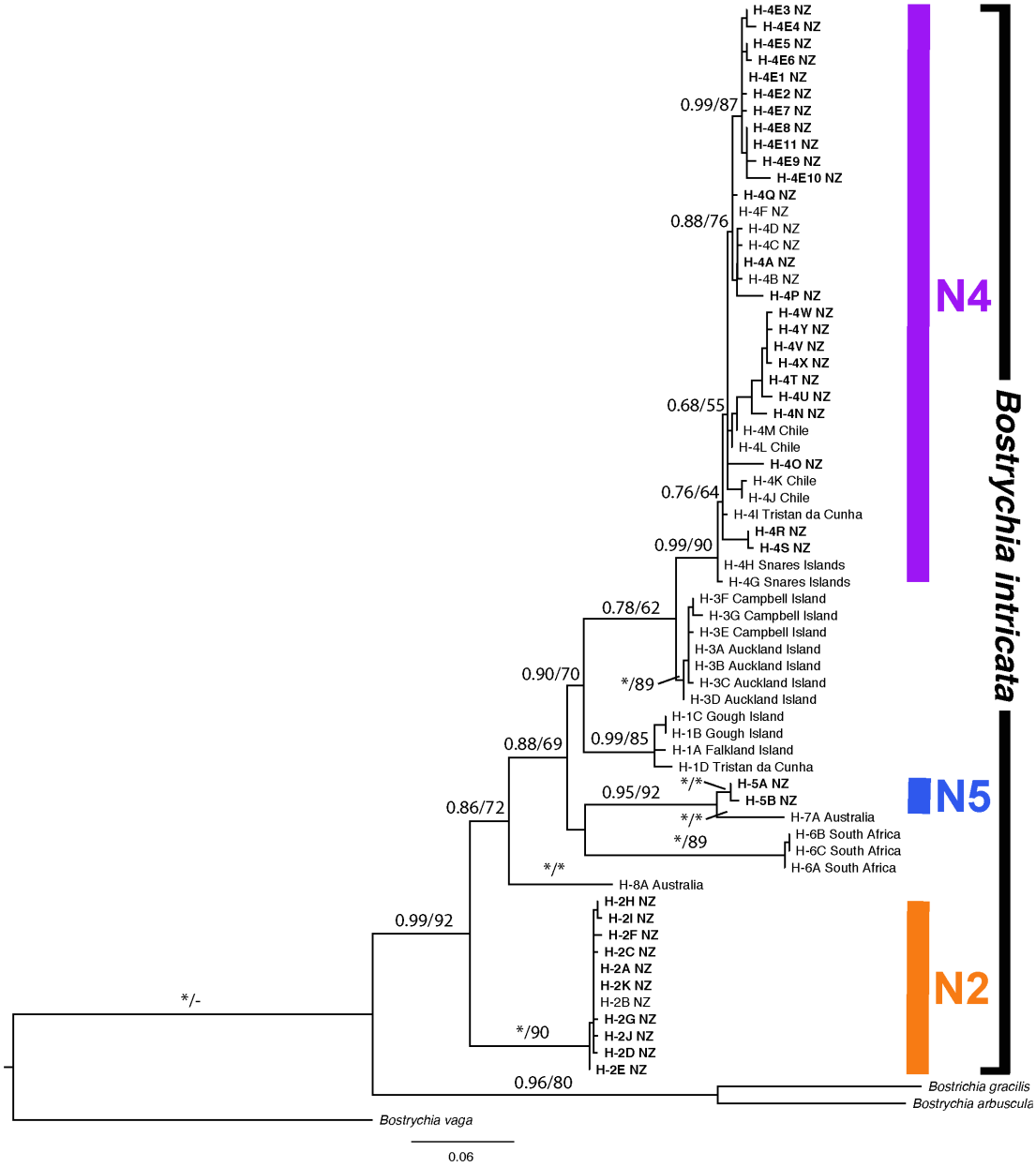


Fig. S2. Phylogenetic tree inferred from Bayesian Inference analyses of COI data set for *Bostrychia intricata*. Support values at each node are bootstrap values from ML bootstrap (left) and Bayesian Posterior Probability (right). Asterisk (*) indicates full support (100%, 1.0) in both analyses and a hyphen (-) indicates no support. Bold letters at each branch tip refer to haplotypes found in this study. H: Haplotype; NZ: New Zealand.

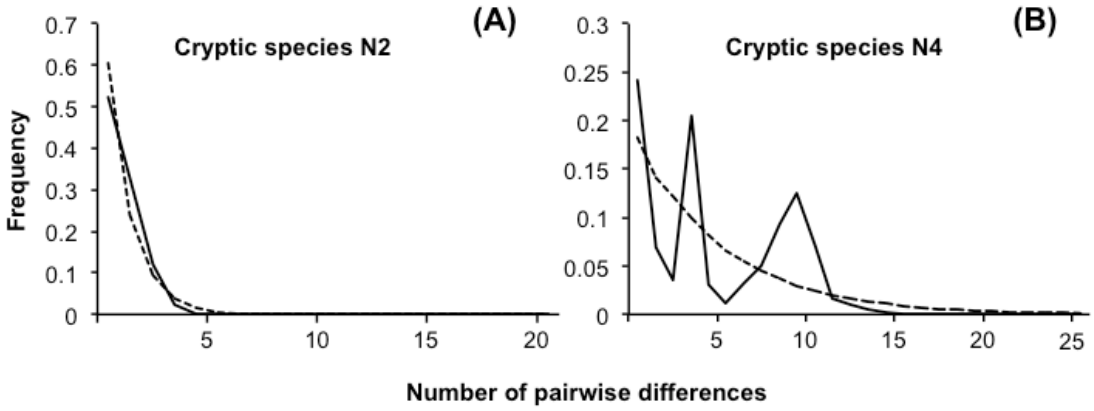


Fig. S3. Mismatch distribution of cryptic *Bostrychia intricata* species N2 (A) and N4 (B) based on COI sequences. Dashed lines indicate the expected distributions under a recent expansion model, and solid lines indicates the observed distributions. Bold letters at each branch tip refer to haplotypes found in this study. H: Haplotype.

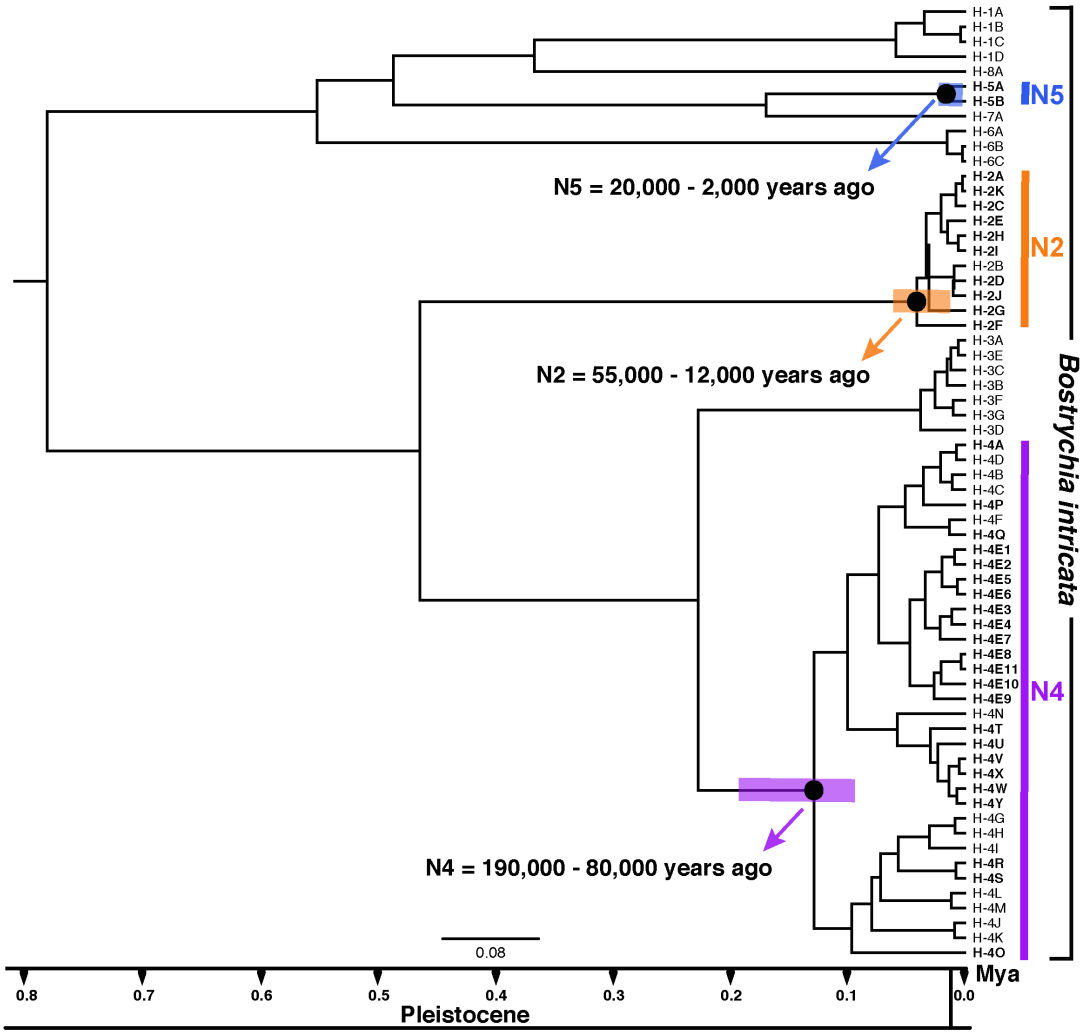


Fig. S4. Bayesian tree for *Bostrychia intricata* reconstructed using BEAST under a relaxed clock model of the COI sequences. Bars show 95% highest posterior densities of divergences dates and scale bar are in million years. Black dots at node indicate the support of > 95% Bayesian posterior probabilities.

Table S1. Samples used for the phylogenetic analysis, and haplotype network, including location of sample, COI haplotype designation and Genbank Accession Number.

Taxon	Location	Haplotype	Accession Number
Bostrychia intricata	Falkland Island	1A	JN881519
	Gough Island	1B	JN881518
	Gough Island	1C	JN881517
	Tristan da Cunha	1D	JN881516
	Casnell Island, Leigh, North Island, New Zealand	2A	**NEW**
	Moa Point, Wellington, North Island, New Zealand	2B	JN881543
	Titirangi Bay, Havelock, South Island, New Zealand	2C	**NEW**
	Waihau Bay, East Cape, North Island, New Zealand	2D	**NEW**
	Porangahua Beach, Hawke Bay, North Island, New Zealand	2E	**NEW**
	Castle Point, North Island, New Zealand	2F	**NEW**
	Castle Point, North Island, New Zealand	2G	**NEW**
	Cabel Bay, Nelson, South Island, New Zealand	2H	**NEW**
	Cabel Bay, Nelson, South Island, New Zealand	2I	**NEW**
	Red Rock, Wellington, North Island, New Zealand	2J	**NEW**

	Waihau Bay, East Cape, North Island, New Zealand	2K	**NEW**
	Campbell Island	3A	JN881535
	Auckland Island	3B	JN881533
	Auckland Island	3C	JN881534
	Auckland Island	3D	JN881532
	Canpbell Island	3E	JN881539
	Campbell Island	3F	JN881537
	Campbell Island	3G	JN881536
	New Zealand	4A	JN881529
	New Zealand	4B	JN881526
	New Zealand	4C	JN881527
	New Zealand	4D	JN881528
	Manukau Harbor, Auckland, North Island, New Zealand	4E1	**NEW**
	Pohara, Tasman, South Island, New Zealand	4E2	**NEW**
	Okiwi Bay, Marlborough, South Island, New Zealand	4E3	**NEW**
	Cable Bay, Nelson, South Island, New Zealand	4E4	**NEW**
	Cape Egmont, North Island, New Zealand	4E5	**NEW**

	Cape Egmont, North Island, New Zealand	4E6	**NEW**
	Manukau Harbor, Auckland, North Island, New Zealand	4E7	**NEW**
	Huaraki Gulf, Kaikoura Island, New Zealand	4E8	**NEW**
	Waitemata Harbor, Auckland, North Island, New Zealand	4E9	**NEW**
	Waitemata Harbor, Auckland, North Island, New Zealand	4E10	**NEW**
	Waitemata Harbor, Auckland, North Island, New Zealand	4E11	**NEW**
	New Zealand	4F	JN881525
	Snares Island	4G	JN881523
	Snares Island	4H	JN881522
	Tristan da Cunha	4I	JN881521
	Chile	4J	JN881530
	Chile	4K	JN881541
	Chile	4L	JN881531
	Chile	4M	JN881540
	New Zealand	4N	JN881520
	Cable Bay, Nelson, South Island, New Zealand	4O	**NEW**
	French Farm Bay, Akaroa, Bank Peninsula, South Island, New	4P	**NEW**

	Zealand		
	Paparoa Point, Kaikoura, South Island, New Zealand	4Q	**NEW**
	Waipatiki Beach, Hawke Bay, North Island, New Zealand	4R	**NEW**
	Waipatiki Beach, Hawke Bay, North Island, New Zealand	4S	**NEW**
	Charleston, Westport, South Island, New Zealand	4T	**NEW**
	Charleston, Westport, South Island, New Zealand	4U	**NEW**
	Charleston, Westport, South Island, New Zealand	4V	**NEW**
	Gentle Antie seaside, Westport, South Island, New Zealand	4W	**NEW**
	Gibson Beach, Cape Foulwind, Westport, South Island, New Zealand	4X	**NEW**
	Whahariki Beach, Puponga, South Island, New Zealand	4Y	**NEW**
	Moa Point, Wellington, North Island, New Zealand	5A	KM502804
	Kenupuru Bay, Haverock, South Island, New Zealand	5B	**NEW**
	Kommetjie, Cap Province, South Africa	6A	KM502799
	Kommetjie, Cap Province, South Africa	6B	KM502800
	Umhlanga Rocks KwaZulu Natal, South Africa	6C	KM502801
	Whiskey Bay, Victoria, Australia	7A	KM502805

	Narooma, New South Wales, Australia	8A	KM502806
Bostrychia gracilis	Taranaki, North Island, New Zealand		KM502798
Bostrychia arbuscula	Brighton Beach, South Island, New Zealand		KM502795
Bostrychia vaga	Auckland, North Island, New Zealand		KM502794

Table S2. Population pairwise F_{st} values estimated from COI sequences of *B. intricata* species N2. Statistical significance ($P < 0.05$) indicated by bold type. For population codes refer to Table1- Figure 1.

	North Island								South Island
	NZ5	NZ6	NZ7	NZ9	NZ10	NZ11	NZ12	NZ18	NZ26
NZ5									
NZ6	**0.178**								
NZ7	0.370	0.013							
NZ9	0.111	0.159	**0.306**						
NZ10	**0.429**	**0.362**	**0.374**	**0.389**					
NZ11	0	0.179	**0.370**	0.111	**0.429**				
NZ12	0	**0.231**	**0.430**	0.159	**0.488**	0			
NZ18	**0.243**	**0.253**	**0.367**	**0.228**	**0.453**	0.243	**0.285**		
NZ26	0.111	0.159	**0.305**	0.111	**0.389**	0.111	**0.159**	**0.228**	

Table S3. Population pairwise F_{st} values estimated from COI sequences of *B. intricata* species N 4. Statistical significance ($P < 0.05$) indicated by bold type. Population codes refer to Table S1- Figure 1. NI= North Island; SI: South Island.

	Eastern NI		**Western NI**								**Eastern SI**					**Western SI**					
	NZ3	NZ8	NZ13	NZ17	NZ30	NZ32	NZ33	NZ34	NZ36	NZ37	NZ21	NZ23	NZ24	NZ44	NZ46	NZ38	NZ39	NZ40	NZ41	NZ42	NZ43
NZ3																					
NZ8	**0.850**																				
NZ13	**0.370**	**0.948**																			
NZ17	**0.437**	**0.981**	0.136																		
NZ30	**0.396**	**0.949**	0.082	0																	
NZ32	**0.468**	**0.983**	**0.162**	0	0.019																
NZ33	**0.436**	**0.981**	0.137	0	0	0															
NZ34	**0.436**	**0.981**	0.137	0	0	0	0														
NZ36	**0.453**	**0.982**	0.150	0	0.100	0	0	0													
NZ37	**0.433**	**0.954**	**0.225**	0.285	0.166	**0.314**	0.284	0.285	0.300												
NZ21	**0.723**	**0.978**	**0.904**	**1**	**0.909**	**1**	**1**	**1**	**1**	**0.920**											
NZ23	**0.591**	**0.915**	**0.698**	**0.778**	**0.909**	**0.796**	**0.777**	**0.777**	**0.787**	**0.719**	**0.257**										
NZ24	**0.417**	**0.828**	**0.386**	**0.417**	**0.370**	**0.448**	**0.416**	**0.433**	**0.433**	**0.412**	0.433	0.006									
NZ44	**0.611**	**0.879**	**0.715**	**0.769**	**0.706**	**0.789**	**0.769**	**0.769**	**0.779**	**0.729**	**0.566**	**0.018**	**0.116**								
NZ46	**0.618**	**0.912**	**0.753**	**0.828**	**0.748**	**0.845**	**0.828**	**0.828**	**0.837**	**0.771**	**0.418**	**0.051**	**0.111**	**0.021**							
NZ38	**0.826**	**0.956**	**0.926**	**0.960**	**0.927**	**0.960**	**0.960**	**0.960**	**0.962**	**0.933**	**0.955**	**0.878**	**0.784**	**0.817**	**0.825**						
NZ39	**0.436**	**0.736**	**0.412**	**0.417**	**0.395**	**0.417**	**0.417**	**0.417**	**0.432**	**0.424**	**0.521**	**0.326**	**0.240**	**0.356**	0.654	**0.369**					
NZ40	**0.873**	**0.984**	**0.968**	**1**	**0.971**	**1**	**1**	**1**	**1**	**0.974**	**1**	**0.929**	**0.840**	**0.875**	**0.924**	**0.814**	**0.535**				
NZ41	**0.846**	**0.967**	**0.943**	**0.975**	**0.944**	**0.975**	**0.975**	**0.974**	**0.976**	**0.949**	**0.972**	**0.889**	**0.798**	**0.881**	**0.821**	**0.484**	**0.427**	**0.821**			
NZ42	**0.834**	**0.962**	**0.935**	**0.969**	**0.936**	**0.969**	**0.969**	**0.968**	**0.971**	**0.941**	**0.965**	**0.883**	**0.788**	**0.822**	**0.871**	0.062	**0.375**	**0.810**	**0.286**		
NZ43	**0.818**	**0.947**	**0.916**	**0.949**	**0.917**	**0.945**	**0.949**	**0.949**	**0.952**	**0.923**	**0.942**	**0.866**	**0.775**	**0.802**	**0.855**	0.064	**0.362**	**0.771**	**0.444**	0.054	

Table S4. Analysis of molecular variance (AMOVA) of cryptic species N4 of *B. intircata* from COI sequences. Groups were defined according to four biogeographic regions in NZ, as eastern North Island, western North Island, eastern South Island and western South Island (Table S3). d.f.: degree of freedom. SS: sum of squares. Asterisk: significant value $P < 0.01$.

Source of Variation	**d.f.**	**SS**	**Variance components**	**% Variation**	**Fixation indices**
Among groups	3	343.729	2.16704	67.57	$F_{CT} = 0.67579$*
Among populations	17	91.839	0.47585	14.83	$F_{SC} = 0.54230$*
Within Populations	193	105.839	0.56380	17.58	$F_{ST} = 0.85161$*
Total	213	541.463	3.20669		