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Genetic relationships of *Amaurobioides* (Anyphaenidae) spiders from the southeastern coast of New Zealand

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Abstract

Members of the genus *Amaurobioides* construct silk retreats in rock crevices of the marine spray zone, a harsh and unusual habitat for spiders. This study expands the distribution records of three morphological species of *Amaurobioides* found on the eastern and southern coasts of New Zealand's South Island and uses mitochondrial DNA to examine their relationships and characterize their dispersal capabilities. Both 16S and ND1 sequences distinguish *A. pletus* found on the northeastern coast from a complex of two southern species comprised of *A. maritimus* from the mainland and *A. picunus* from Stewart Island. Neither 16S DNA nor ND1 protein separates these southern species. However, ND1 parsimony and likelihood analyses place 10 of 11 Stewart Island specimens in a clade of low support that nests deeply within *A. maritimus*. A nested haplotype analysis characterizes *A. maritimus* and *A. picunus* populations as having restricted gene flow/dispersal but with some long distance dispersal. Genetic distances between *A. pletus* and the *A. maritimus-A. picunus* complex indicate a Pliocene origin, whereas distances between *A. maritimus* and *A. picunus* suggest a Pleistocene divergence.

Key words: *Amaurobioides maritimus*, *Amaurobioides picunus*, *Amaurobioides pletus*, mitochondrial DNA, nested haplotype analysis, biogeography

Introduction

Spiders of the genus *Amaurobioides* Pickard-Cambridge (family Anyphaenidae) live in the marine spray zone, where they construct silk retreats in rock crevices at or slightly below mean high tide level (Figs. 1; Forster 1970; Forster & Forster 1999; BDO personal observations). In New Zealand, these spiders are typically found on rock outcrops, although large boulders also provide suitable habitat (BDO unpublished observations). Consequently, soft sandstone that erodes easily or beaches of sand or gravel act as barriers to their dispersal. *Amaurobioides* are known from only a few sites, even along the eastern and southern coasts of New Zealand's South Island (SI), where collecting has been most extensive. Before the current study, *A. pletus* Forster was known only from Akaroa (Fig. 2), *A. maritimus* Cambridge only from Brighton, Black Head, and two sites a few km north of these, and *A. picunus* Forster only from Halfmoon Bay and adjacent Horseshoe Bay on the northeast coast of Stewart Island.

There are no conspicuous ecological differences among *Amaurobioides* species (BDO unpublished observations), although *A. maritimus* and *A. picunus* adults are larger than those of *A. pletus* (Forster 1970; BDO unpublished observations). Nothing is known about the mode or frequency of dispersal in *Amaurobioides*. After depositing an egg sac within her retreat, a female seals the retreat opening with silk and remains inside until spiderlings emerge from the sac (Forster 1970; BDO unpublished observations). Spiderlings live in the maternal retreat with the female for an undetermined period of time after they emerge from an egg sac (BDO

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personal observations), suggesting that early dispersal, where ballooning might be expected to be more common, may be limited. The small number of mature males reported by Forster (1970), present in the Otago Museum, and collected by BDO may indicate that males spend more time away from their retreats or travel longer distances than females and, consequently, suffer higher mortality.

New Zealand's biogeography bears the imprint of geological events during the Oligocene, Pliocene, and Pleistocene that created physical barriers to the dispersal of plants and animals. During the Oligocene (32 Ma) erosion and rising sea levels restricted landmass to small areas in the south of the SI and the north central region of the North Island (NI) (Suggate 1978; Cooper & Cooper 1995; Trewick & Morgan-Richards 2005). Land area expanded during the Miocene and from 15–10 Ma formed a single slender island, which was again divided during the early Pliocene (5 Ma) into islands roughly the size of the present NI and SI (Suggate 1978; Morgan-Richards *et al.* 2000; Trewick & Wallis 2001). During the Pleistocene (1.8 Ma–14,000), sea level dropped, connecting NI, SI, and Stewart Islands (Fleming 1979; Thornton 1985). On the SI, extensive glaciation drastically reduced the habitable area and altered climate and vegetation, impacting the diversity and distribution of many terrestrial invertebrates (Trewick 2000; Trewick & Wallis 2001; Neiman & Lively 2004; Trewick & Morgan-Richards 2005).

The objectives of this study were to: 1. More fully characterize the distributions of *A. pletus* and *A, maritimus* by collecting specimens from unsampled localities, 2. Characterize differences among *A. pletus, A, maritimus*, and *A. picunus*, 3. Infer relationships among these species, and 4. Estimate their times of divergence. Forster (1970) distinguished *A, pletus, A. maritimus, and A. picunus* by genitalic differences, which suggest that the latter two species are closely related. However, the small differences in these morphological features, the small number of specimens available for study, and the variability of these features suggest that a re-examination of these species is warranted (R. Forster, conversation with BDO, January, 2000). As demonstrated by studies cited above, molecular data are well suited to these tasks. In this study we use two mitochondrial genes, 16S and NADH dehydrogenase subunit ND1, both of which have been employed successfully to reconstruct the phylogenies of spider populations and species (Hedin 1997a, b; Gillespie 1999; Hedin & Maddison 2001; Bond *et al.* 2001; Masta & Maddison 2002; Maddison & Hedin 2003; Vink & Paterson 2003; Griffiths *et al.* 2005; Garb & Gillespie 2006).

Materials and methods

This study is based on specimens collected by BDO from 16 localities (Table 1, Fig. 2) under permits issued by the New Zealand Department of Conservation. Most were collected during January and February 2000, but those from Taylor's Mistake, Camp Bay, and Tumbledown Bay were collected during January 2004. With one exception, each specimen was prodded from its retreat with a small wire, collected in a glass vial, and preserved in 95% ethanol. One specimen from Stewart Island (the single specimen that shared ND1 haplotype N9, with mainland sites) was found dead on the shore in a partially dried condition. All specimens will be deposited in New Zealand's Otago and Canterbury Museums.

DNA was extracted from two legs of each specimen using a Puregene DNA isolation kit from Gentra Systems, Inc. We used the PCR primers and thermocycler parameters of Hedin (1997a, b) to amplify a 425 bp portion of the 16S mitochondrial gene and the entire 429 bp ND1 subunit of the mitochondrial NADH dehydrogenase. Prior to sequencing, we prepared most PCR products by electrophoresis on agarose gels, staining bands with EtBr, excising bands, and cleaning them with a QIAquick® gel extraction kit. The PCR products of specimens collected at Taylor's Mistake, Camp Bay, and Tumbledown Bay were cleaned with a QIAquick® PCR purification kit. We then performed cycle sequencing with Biosystems' Big Dye Terminator™ chemistry, ran products on agarose gels, stained them with EtBr, and excised bands. After cleaning this labeled product with a Qiagene Gel Kit, we read DNA sequences with an Applied Biosystems 3100 genetic

TABLE 1. Collecting localities and distributions of the six 16S haplotypes S 1-6 (GenBank DQ356614, DQ356620, DQ356615, DQ356616, DQ356619, DQ356618), the twelve ND1 haplotypes N 1-12 (GenBank DQ355335, DQ355337, DQ355338, DQ356588, DQ355340, DQ355343, DQ356591, DQ356586, DQ355342, DQ355341, DQ355344, DQ356585), and the three ND1 protein haplotypes NP 1-3. Specimen numbers denote the unique collecting numbers given to each specimen.

Map No. and locality	Species	Lat. Long.	Specimen Numbers	S 1	S 2	S 3	S 4	S 5	S 6	N 1	N 2	N 3	N 4	N 5	N 6	N 7	N 8	N 9	N 10	N 11	N 12	ND1 Protein
1. Kaikoura	*A. pletus*	-42.41409	16S: 97, 106, 107	2	1	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	NP 1
		173.70090	ND1: 97, 100, 106																			
2. Paia Point	*A. pletus*	-42.47188	16S: 102, 105	2	-	-	-	-	-	3	-	-	1	-	-	-	-	-	-	-	-	NP 1
		173.53613	ND1: 102-105																			
3. Oaro	*A. pletus*	-42.49654	16S: 111, 113	2	-	-		-	-	3	1	-	-	-	-	-	-	-	-	-	-	NP 1
		173.51990	ND1: 108, 111-113																			
4. Taylor's Mistake	*A. pletus*	-43.58459	16S: 306, 307	2	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	NP 1
		172.77873	ND1: 306, 307																			
5. Camp Bay	*A. pletus*	-43.62146	16S: 311, 312	2	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	NP 1
		172.77946	ND1: 311, 312																			
6. Robinson's Bay	*A. pletus*	-43.76253	16S: 19, 21	2	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	NP 1
		172.95784	ND1: 18, 19, 21																			
7. Akaroa	*A. pletus*	-43.79868	16S: 119, 120	2	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-		-	NP 1
		172.96511	ND1: 119, 121																			
8. Tumbledown Bay	*A. pletus*	-43.85902	16S: 313, 314	2	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	NP 1
		172.76932	ND1: 313, 314																			
9. Shag Point	*A. maritimus*	-45.46859	16S: 22, 24	-	-	2	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	NP 2
		170.82850	ND1: 22-25, 93, 94																			
10. Black Head	*A. maritimus*	-45.92977	16S: 27, 31	-	-	-	2	-	-	-	-	-	-	-	2	-	-	-	-	-	-	NP 2
		170.42926	ND1: 30, 31																			
11. Brighton	*A. maritimus*	-45.94785	16S: 32	-	-	-	1	-	-	-	-	-	-	-	1	1	1	-	-	-	-	NP 2
		170.33537	ND1: 32, 90-92																			NP 3
12. Nugget Point	*A. maritimus*	-46.43103	16S: 40, 41	-	-	-	2	-	-	-	-	-	-	-	3	-	-	1	-	-	-	NP 2
		169.79384	ND1: 37, 38, 40, 41																			
13. Bluff	*A. maritimus*	-46.61165	16S: 47, 52	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	5	-	-	NP 2
		168.35987	ND1: 45, 48, 50, 52, 55																			
14. Stewart Isl., Half Moon Bay	*A. picunus*	-46.89389	16S: 57, 62, 68	-	-	-	3	-	-	-	-	-	-	-	-	-	-	1	-	9	1	NP 2
		168.12896	ND1: 56, 57, 59, 60, 62, 65, 68, 69, 71, 72, 75																			
15. Mullet Bay	*A. maritimus*	-46.33097	16S: 86	-	-	-	-	1	-	-	-	-	-	-	-	-	-	2	1	-	-	NP 2
		167.71840	ND1: 84, 87, 89																			
16. Monkey Island	*A. maritimus*	-46.30009	16S: 79,81	-	-	1	-	-	1	-	-	-	-	-	-	-	-	3	2	-	-	NP 2
		167.72497	ND1: 77, 79-82																			

analyzer, and edited Electropherograms with the EditView program. We aligned the edited sequences with Clustal V (Higgins *et al.* 1996), as implemented by DNA Star, with parameters set as follows: multiple alignment gap penalty 10, gap length penalty 10; pairwise alignment ktuple 2, gap penalty 5, window 4, diagnols 4. No indels were required to align either 16S or ND1 sequences.

Maximum parsimony (MP) analyses (settings: collapse branches if maximum length is zero, ACCTRAN) were performed with Paup* 4.0b10 (Swofford 1998). We used an exhaustive search for the analysis of 16S DNA and a branch and bound search (options: compute via stepwise, addition sequence furthest) for ND1 DNA. Bootstrap settings were: 1000 replicates, full heuristic, retain groups with frequency > 50%, addition sequence simple, starting tree generated by stepwise addition, for multiple swapping trees swap on best only, TBR swapping.

We used MODELTEST 3.7 (Posada & Crandall 1998) to determine the appropriate model and parameters (Posada & Crandall 2001; Posada & Buckley 2004) to use in maximum likelihood (ML) estimations run with PAUP 4.0b10. Maximum likelihood trees were obtained by heuristic searches performed with TBR branch swapping from starting trees obtained by stepwise addition and 200 random addition sequence replicates. Bootstrap values are based on 100 replicates.

FIGURE 1. a. An adult female *A. maritimus* from Black Head. **b**. A silk retreat of *A. pletus* from Akaroa.

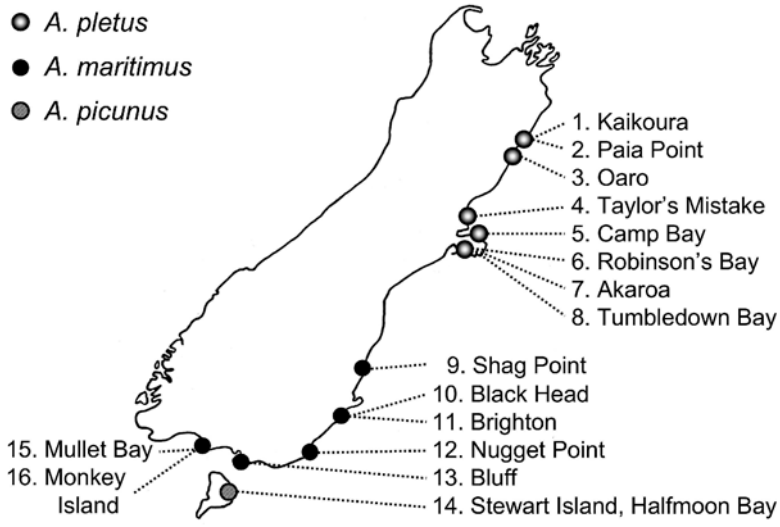

FIGURE 2. Distribution of the localities from which specimens included in this study were collected. Numbers refer to the localities identified in Table 1.

Data were exported from PAUP for use in a Templeton, Crandall, Sing analyses (statistical parsimony analysis; Crandall *et al.* 1994; Templeton 1998; Templeton *et al.* 1992) implemented with the TCS program (Clement *et al.* 2000). As there were no indels in either 16 S or ND1 DNA, the gaps = 5th state or gaps = missing options produced identical networks. A 95% confidence limit was used for the inclusion of haplotypes in a network.

We performed a nested clade haplotype analysis (Templeton *et al.* 1987, 1995; Crandall 1994, 1996) using the GeoDis 2.4 program and its November 2005 inference key (Posada & Templeton 1999–2005). We restricted this analysis to the ND1 haplotypes of *A. maritimus* and *A. picunus*, as they were included in the same network and provided a sufficiently complex network for a meaningful analysis. For this analysis, we used shoreline distances between localities measured with Image J (Image J 2006) from an enlarged digital map of the South Island. The distances from Half Moon Bay, Stewart Island to all localities pass through Bluff, whose straight-line distance from Half Moon Bay is 37 km.

Results

Six 16S haplotypes were represented among 32 specimens and 12 ND1 haplotypes were represented among 61 specimens (Figs. 3, 4). Tables 2 and 3 present the pairwise Kimura 2-parameter (K2P; Kimura 1980) and uncorrected *p* distances between these haplotypes. GenBank assigned a 132 amino acid sequence to each ND1 DNA haplotype. Only three proteins were represented among these haplotypes, one for *A. pletus*, one for *A. maritimus* and *A. picunus*, and one for a single *A. maritimus* specimen from Brighton (Fig. 2).

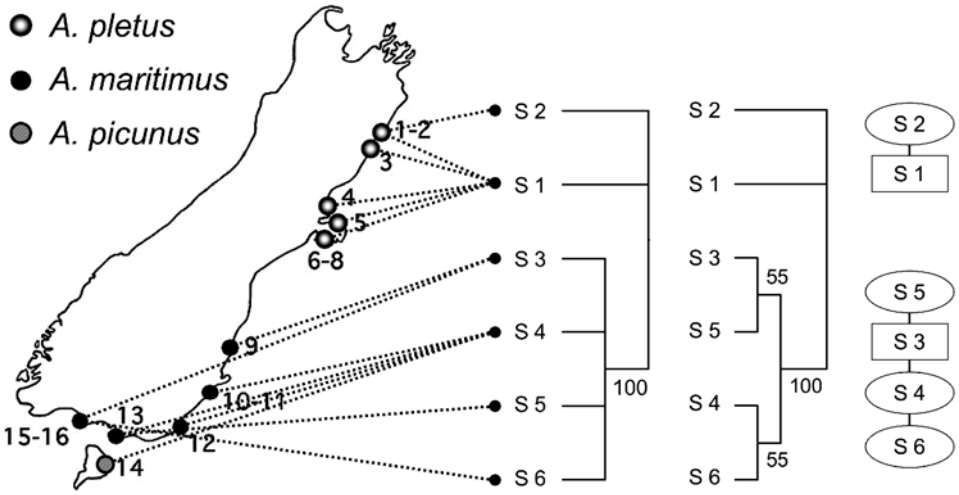

FIGURE 3. Maximum parsimony cladogram (left), maximum likelihood tree (center), and TCS network of 425 base pairs (9 of them parsimony-informative) of six 16S DNA haplotypes. The cladogram is a strict consensus of three trees, each with CI = 0.93, RI = 0.90 (rescaled = 0.84). Parsimony bootstrap values are based on 1000 replicates and likelihood bootstrap values on 100 replicates.

MODELTEST selected two Tamura-Nei models (Tamura & Nei 1993): TRN+1 for 16S and TRN for ND1. The parameters for 16S were: base frequencies: A = 0.4431, C = 0.1067, G = 0.1086, T = 0.3416; Rate matrix: A–G = 1.8741, C–T= 39.7716, all others = 1.0000; proportion of invariable sites = 0.8837, variable sites (G); equal rates for all sites. The parameters for ND1 were: base frequencies: A = 0.4057, C = 0.1445, G = 0.0803, T = 0.3695; rate matrix: A–G= 2.2065, C–T = 27.3047, all others = 1.0000; proportion of invariable sites = 0, variable sites (G), equal rates for all sites.

The unrooted 16S and ND1 MP and ML trees (Figs. 3, 4) each united the two southern species, *A. maritimus* and *A. picunus*, in a clade with 100% bootstrap support. In the 16S trees, Stewart Island specimens shared

the most common haplotype (S4) with four other mainland sites. Although the 16S ML tree further subdivides the southern clade, these two subclades have equivocal support. Ten of the 11 specimens from Stewart Island had ND1 haplotypes N11 and N12, which are restricted to the island and form a clade with minimal bootstrap support. The eleventh specimen (the individual found dead) shared haplotype N9 with three mainland localities. TCS networks show nearly identical patterns to those described above, each retaining only *A. maritimus* and *A. picunus* within the same network at a 95% confidence interval. The two *A. pletus* 16 S haplotypes (S1–S2) are separated from the four *A. maritimus-A. picunus* haplotypes (S3–S6) by a mean K2P of 2.397% ± 0.065 standard error (SE). The two unique *A. picunus* ND1 haplotypes (N11–N12) are separated from the two *A. maritimus* haplotypes in their subclade (N6–N7) by a mean K2P of 0.468% ± 0.096 SE. The four *A. pletus* ND1 haplotypes (N1–N4) are separated from the eight *A. maritimusi-A. picunus* haplotypes (N5–N12) by a mean K2P of 9.263 ± 0.092 SE. The two *A. maritimus* ND1 protein haplotypes differed by a single amino acid (uncorrected $p = 0.00758$) and the two widely distributed NP1 and NP2 haplotypes differed by 6 amino acids (uncorrected $p = 0.04545$).

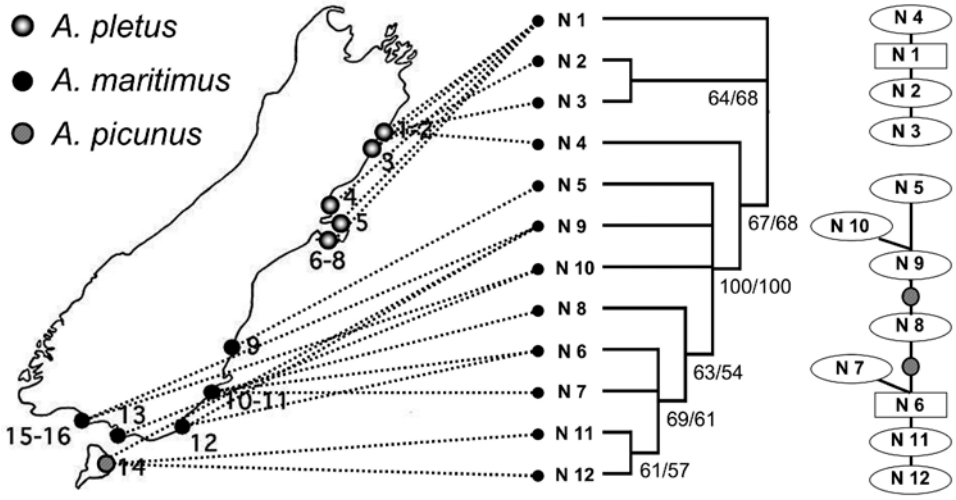

FIGURE 4. Maximum parsimony cladogram and identical maximum likelihood tree (left) and TCS network of 429 base pairs (39 of them parsimony-informative) of 12 ND1 DNA haplotypes. The cladogram is a strict consensus of two trees, each with CI = 0.96, RI = 0.98 (rescaled = 0.94). Parsimony bootstrap values (left) are based on 1000 replicates and likelihood bootstrap values (right) on 100 replicates. Small, shaded circles in the TCS network represent inferred, missing haplotypes.

TABLE 2. Pairwise distances between 16 S haplotypes. Kimura 2-parameter values are given in the upper triangle and uncorrected *p* values in the lower triangle.

	S1	S2	S3	S4	S5	S6
S1		0.00236	0.02397	0.02397	0.02642	0.02642
S2	0.00235		0.02152	0.02152	0.02397	0.02397
S3	0.02353	0.02118		0.00236	0.00236	0.00473
S4	0.02353	0.02118	0.00235		0.00473	0.00473
S5	0.02588	0.02353	0.00235	0.00471		0.00711
S6	0.02588	0.02353	0.00471	0.00235	0.00706	

The nested cladogram shown in Figure 5 differs slightly from the ND1 network of Figure 4. Clade 1-1 is comprised of three haplotypes that are represented at a total of 8 localities and clade 1-2 is comprised of five haplotypes that are represented at a total of five localities. Haplotypes N8, N11, and N12 of clade 1-2 are each

present at only a single locality and haplotypes N11 and N12 are both present only at locality 14. This makes it inappropriate to have a nested clade comprised of haplotype N8 alone, haplotype N11 alone, or haplotype N12 alone. Therefore, we combined haplotype N8 with haplotype N6 to form a single clade within nested clade 1-2 and haplotypes N11 and N12 to form another clade within nested clade 1-2. The permutational contingency tests for clades 1-1 and 1-2 and for the total cladogram each had a *P* value of 0.0000 and each moved through the inference key couplets as follows: 1, 2, 3, 5, and 6: "insufficient genetic resolution to discriminate between range expansion/colonization and restricted dispersal/gene flow." Couplet 7 then concluded that the ND1 nested cladogram was explained by "restricted gene flow/dispersal but with some long distance dispersal".

TABLE 3. Pairwise distances between ND1 haplotypes. Kimura 2-parameter values are given in the upper triangle and uncorrected *p* values in the lower triangle.

	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12
N1		0.00234	0.00468	0.00234	0.08787	0.09338	0.09616	0.09062	0.08514	0.08787	0.09616	0.09357
N2	0.00233		0.00234	0.00468	0.09062	0.09616	0.09895	0.09338	0.08787	0.09062	0.09895	0.09636
N3	0.00466	0.00233		0.00704	0.09062	0.09895	0.10176	0.09616	0.09062	0.09338	0.10176	0.09916
N4	0.00233	0.00466	0.00699		0.08514	0.09062	0.09338	0.08787	0.08242	0.08514	0.09338	0.09080
N5	0.08159	0.08392	0.08625	0.07925		0.01179	0.01419	0.00704	0.00234	0.00468	0.01419	0.01655
N6	0.08625	0.08858	0.09091	0.08392	0.01166		0.00234	0.00468	0.00941	0.00704	0.00234	0.00468
N7	0.08858	0.09091	0.09324	0.08625	0.01399	0.00233		0.00704	0.01179	0.00941	0.00468	0.00703
N8	0.08392	0.08625	0.08858	0.08159	0.00699	0.00466	0.00699		0.01179	0.00704	0.00704	0.00939
N9	0.07925	0.08159	0.08392	0.07692	0.00233	0.00932	0.01166	0.00466		0.00234	0.01179	0.01416
N10	0.08159	0.08392	0.08625	0.07925	0.00466	0.00699	0.00932	0.00699	0.00233		0.00941	0.01177
N11	0.08858	0.09091	0.09324	0.08625	0.01399	0.00233	0.00466	0.00699	0.01166	0.00932		0.00234
N12	0.08625	0.08858	0.09091	0.08392	0.01632	0.00466	0.00699	0.00932	0.01399	0.01166	0.00233	

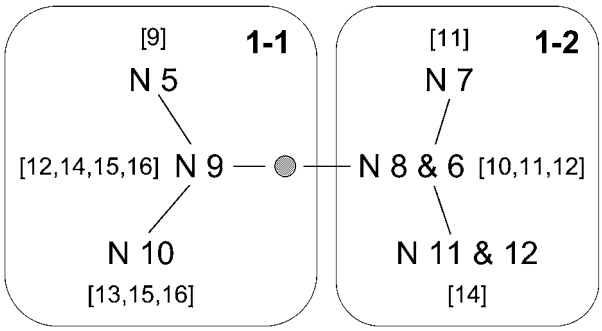

FIGURE 5. ND1 nested cladogram based on the TCS diagram shown in Fig. 4, showing relationships between haplotypes N1 – 12 and the localities at which these haplotypes are found as smaller numbers in brackets.

Discussion

Specimens collected during the course of this study extend the known ranges of *A. pletus* and *A. maritimus*. The range of *A. pletus* extends from the previously known Kiakoura locality southward to Akaroa and Tumbledown bay on the south shore of Bank's Peninsula (Fig. 2). A long stretch of coast that appears to be comprised of predominately sand and gravel beaches separates Bank's Peninsula from the rocky shores of Shag Point, the northern-most record of *A. maritimus*. The range of *A. maritimus* extends along the remainder of the eastern and southern coast to Monkey Island, where gravel beaches extend westward to the end of road access

to the coast and into the Fiordlands beyond.

Both 16S and ND1 genes are useful in recovering relationships among *Amaurobioides*. As shown in other studies (e.g., Hedin 1997b; Hedin & Maddison 2001), ND1 evolves more rapidly than 16S and, therefore, provides more resolution. The mean K2P distance of 9.263% that separates the ND1 sequences of *A. pletus* from the *A. maritimus-A. pletus* complex attests to the distinctness of these species. This distance is similar to or greater than that observed among New Zealand onychophorans and insects (Trewick 2000; Trewick & Wallis 2001), about twice that observed for the oldest clades of Hawaiian tetragnathid spiders (Gillispie 1999), and almost ten times the greatest distance that separates populations of New Zealand's two widow spider species (Griffiths *et al.* 2005). A rate of 2% (e.g., Brown *et al.* 1979; DeSalle *et al.* 1987; Juan, *et al.* 1995; Gillespie 1999; Trewick & Morgan-Richards 2005) or 2.3% (e.g., Brower 1994; Trewick & Wallis 2001; Garb & Gillespie 2006) mitochondrial DNA sequence divergence per million years is often used to estimate the coalescence time of clades. This indicates that *A. pletus* and the *A. maritimus-A. pletus* complex diverged 4.0–4.6 Ma.

This date suggests an early to middle Pliocene origin for these species through geographical isolation on the NI and SI, respectively. When these islands were joined during the Pleistocene, *A. pletus* probably invaded the SI, migrating southward as far as Bank's Peninsula. The 200 km of shore that separates *A. pletus* and *A. maritimus* corresponds roughly to the SI's central or Canterbury region of low endemicity. This region is often referred to as the "beech-gap" because there is disjunction in the distribution of many species, including *Nothofagus* species, in this region (Burrows 1965; Heads 1998; Trewick & Wallis 2001). During the Pleistocene, glaciers from the Southern Alps extended nearly halfway across this narrow portion of the SI, producing a large outwash aggregate field that extended to the coast (Fleming 1979; Trewick & Wallis 2001). Elevated by Miocene volcanism (Thornton, 1985), Bank's Peninsula (Fig. 2, localities 5–8) was probably less affected by these events and may have served as a refuge for *Amaurobioides*. Although animal and plant species have colonized this central zone in the 14,000 years since the glaciers receded, it appears to remain a formidable barrier to the dispersal of *Amaurobioides*.

The species status of *A. picunus* is not well supported by our analysis. Neither 16S DNA nor ND1 proteins distinguished a Stewart Island clade. Although, 10 of the 11 specimens from Stewart Island have the unique N11 and N12 haplotypes, these haplotypes form a clade that nests deeply within *A. maritimus* and has low bootstrap support. If Stewart Island haplotypes represent a distinct lineage, the mean K2P distance of 0.468% that separates it from the most closely related mainland haplotpes (N6 & N7) indicates a separation of 0.2–0.23 Ma. Like Stewart Island populations of the freshwater fish *Galaxias gollumoides* McDowall & Chadderton that share haplotypes with populations in Southland rivers (Walters *et al.* 2001), Stewart Island *Amaurobioides* are closely allied with mainland populations. This contrasts with the 7% sequence divergence that separates the endemic Stewart Island giant weta, *Deinacrida carinata* Salmon, found in lowland vegetation from the more wide-spread mainland *D. connectens* Ander, which lives under rocks in the alpine zone (Trewick & Morgan-Richards 2005).

Although *Amaurobioides* spiders are restricted to the marine shore, their distribution appears to conform to biogeogrpahic regions established for terrestrial animals and plants. These comprise a southern Otago-Southland region, a central Canterbury region of low endemism, a northeastern Marlborough region, and a northwestern Nelson region. (Trewick & Wallis 2001; Neiman & Lively 2004). Specimens collected more recently from the northern and western coasts of the SI and from the NI will allow us to determine if *A. pletus* is confined to the Marlborough region or if it extends into the Nelson region. This material will also allow us to more fully characterize the distribution and diversity of New Zealand *Amaurobioides* and to delineate species using molecular and morphological characters.

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