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Phylogeography of *Neopurcellia salmoni*, a widespread mite harvestman from the South Island of New Zealand, with the first report of male polymorphism in the suborder Cyphophthalmi (Arachnida: Opiliones)

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Abstract. *Neopurcellia salmoni* Forster, 1948 is a mite harvestman found throughout the forests of the west coast of New Zealand's South Island. This species range is unusually large for the notoriously dispersal-limited Cyphophthalmi, raising the possibility of multiple cryptic species within the lineage. In order to test this hypothesis, we used scanning electron microscopy to examine a large number of individuals from throughout its range, and discovered two distinct male morphotypes distinguished by the presence or absence of dorsal glandular pores. We performed phylogeographic and population genetic analyses using DNA sequence data from the fast-evolving mitochondrial locus cytochrome *c* oxidase I (COI). Tree topologies revealed two well-supported clades within *Neopurcellia* Forster, 1948 occupying non-overlapping geographical regions of the west coast. Molecular dating indicates that these lineages diverged from each other following the Oligocene "drowning" of New Zealand and diversified during the uplift of the Southern Alps. The strong correlation between the evolutionary relationships of lineages within *Neopurcellia* and the geographic distribution of its populations indicates isolation by distance, as expected with dispersal-limited organisms; population genetic analyses confirm strong isolation of populations. However, we discovered that the distribution of male morphotypes does not follow any geographic or phylogenetic pattern. While the presence of two different morphotypes initially suggested multiple *Neopurcellia* species, phylogeographic analysis allowed us to reject this hypothesis. We therefore report here the first known case of male polymorphism in the suborder Cyphophthalmi.

Keywords: Intrasexual polymorphism, alternative reproductive tactic, population genetics, species delimitation, Pettalidae

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Neopurcellia salmoni Forster, 1948 is the most widespread mite harvestman species on the South Island of New Zealand, inhabiting leaf litter habitats in the *Nothofagus* and podocarp forests west of the Southern Alps from Lake Kaniere to Fiordland (Fig. 1) (Boyer & Giribet 2009). The species' broad distribution is particularly unusual given the notoriously limited dispersal capabilities of mite harvestmen, as most species ranges in this group fail to exceed 50 km in any dimension. Therefore, it has been suggested that *N. salmoni* may encompass more than one cryptic species (Boyer & Giribet 2007, 2009). *Metasiro americanus* Davis, 1933, a widespread North American mite harvestman, was recently split into three species following phylogeographic analysis and detailed examination of genitalia (Clouse & Wheeler 2014). Similarly, *Aoraki denticulata* Forster, 1948, the other widespread species of mite harvestmen in New Zealand's South Island, and *Karripurcellia peckorum* Giribet, 2003, a species of pettalid from southwestern Australia, both show highly structured populations and deep genetic divergences suggestive of cryptic species (Boyer et al. 2007; Fernandez & Giribet 2014; Schwentner & Giribet 2018).

In addition to addressing the taxonomic question of whether *Neopurcellia* Forster, 1948 should be split, our analysis allowed us to explore geographic and temporal patterns of diversification within this widespread taxon in light of the turbulent geological history of the South Island. New Zealand is an excellent system for historical biogeographic study, given its isolation from other major land masses and the variety of processes that have shaped its highly endemic biota (Waters & Craw 2006). Once part of the supercontinent Gondwana, New Zealand started drifting away from Antarctica, Australia and South America approximately 80 Ma (McLoughlin 2001; Sanmartin 2002; Giribet & Boyer 2010). *Neopurcellia* is a member of the mite harvestman family Pettalidae, which has a classic Gondwanan distribution with lineages in New Zealand, Australia, South Africa, Madagascar, Chile, and Sri Lanka (Boyer & Giribet 2007). Many textbook examples of Gondwanan vicariant lineages have been the subject of molecular clock dating analyses in recent years, with the result that iconic groups such as the ratite birds and *Nothofagus*, the southern beech, are now understood to have achieved their present-day distribution through trans-oceanic dispersal rather than vicariance (Sanmartín & Ronquist 2004; Cook & Crisp 2005; Goldberg et al. 2008; Mitchell et al. 2014). In contrast, vicariance is still considered the best explanation for many soil invertebrates' presence in New Zealand (Baker et al. 2020; Giribet et al. 2018; Chousou-Polydouri et al. 2019), and Pettalidae has become a well-studied example of a Gondwanan vicariant taxon (Boyer & Giribet 2007; Giribet et al. 2016; Baker et al. 2020).

Throughout the Paleogene, New Zealand's continental crust stretched and thinned, resulting in a gradual marine transgression that peaked in the Oligocene (Cooper & Cooper 1995; Landis et al. 2008). During this period, variously termed the

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"Oligocene marine transgression," "Oligocene drowning," or "Oligocene bottleneck", New Zealand's emergent land area was reduced to an archipelago of low-lying islands corresponding to less than 15% of its current size during maximum inundation, *ca.* 25–22 Ma (Wallis & Jorge 2018). This period ended when increased tectonism along the boundary between the Australian and Pacific plates activated the Alpine Fault in New Zealand, resulting in rapid crustal uplift and the creation of the Southern Alps around 10–5 Ma (Cooper & Cooper 1995). The formation of this mountain chain deeply impacted climate in the South Island, creating a strong precipitation gradient across the island: areas to the west of the Southern Alps experience high precipitation, whereas forests to the east are significantly drier (Gellatly et al. 1988). During the Last Glacial Maximum (LGM), 34–18 ka, vast areas throughout the archipelago became tundra-like, and the Southern Alps experienced extensive glaciation (Marske et al. 2012; Buckley et al. 2015). Vegetation was mostly limited to grass and shrub-dominant communities, with small patches of trees present but uncommon (McGlone et al. 1993). Extant fauna managed to survive the LGM in forest refugia with suitable microclimates (Buckley et al. 2009b); a recent review by Buckley et al. (2015) identified the LGM as one of the most important events in shaping the diversity and distribution of arthropod taxa in New Zealand (e.g., Marra 2008; Buckley et al. 2009a; Marske et al. 2009, 2012; Pons et al. 2011). To date, the effect of these post-Oligocene events on the diversity and biogeography of mite harvestman has not been explored.

We undertook a phylogeographic analysis of *Neopurcellia* with two goals in mind: First, we aimed to test the hypothesis that this currently monotypic genus should be split into multiple species. Integration of molecular and morphological data has revealed near-cryptic species of mite harvestman in other genera (Clouse & Wheeler 2014; Boyer et al. 2015); therefore, we performed careful morphological examination of ninety individuals from across the range of *Neopurcellia* using both light and scanning electron microscopy. Secondly, we undertook an exploration of the biogeographic history of *Neopurcellia,* incorporating a molecular dating approach.

METHODS

Specimen collection.—We collected 89 specimens via leaf-litter sifting and sorting *in situ* from seven localities in the South Island of New Zealand in January of 2019, with permission from the Department of Conservation (National Authorisation Number 38002-RES). Specimens were immediately preserved in 95% ethanol. GPS points and altitude information were taken at each collecting locality. All specimens are accessioned in the Invertebrate Zoology Department of the Harvard Museum of Comparative Zoology and can be searched using the portal MCZBase (online at https://mczbase.mcz.harvard.edu/). Additional specimens collected between 1959 and 2016 were loaned from the Field Museum (FMHD) ($n = 10$), Harvard Museum of Comparative Zoology (MCZ) ($n = 218$), Otago Museum ($n = 4$), and University of Copenhagen Zoological Museum (ZMUC) ($n = 39$) (Table 1). Using only mature individuals, in total, our sample contains 198 specimens from 21 collections. Populations, which we define as all specimens collected at a single locality, range in size from 1 to 32 individuals.

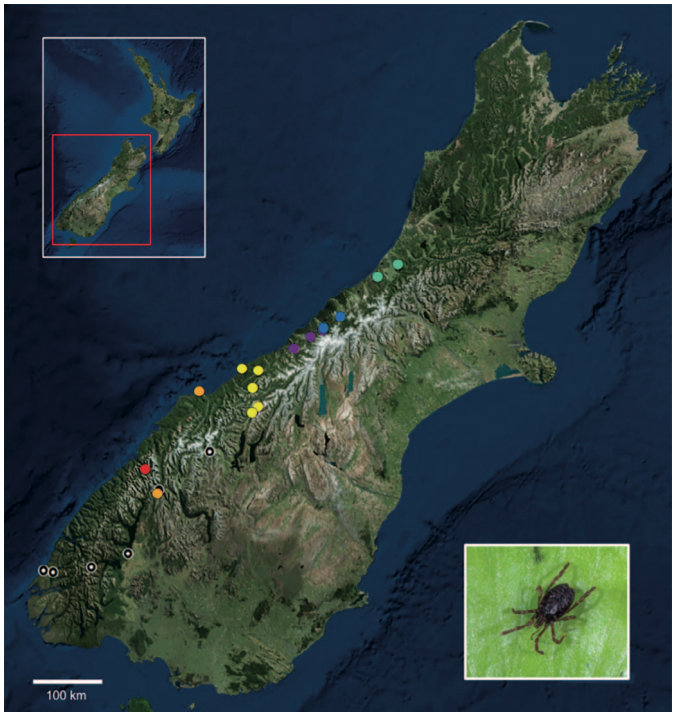

Figure 1.—Distribution of *Neopurcellia salmoni* populations used in this study. Colored points represent localities with DNA sequence data included in this study; black and white points denote populations without molecular data. Inset figures show a map of New Zealand indicating location of the South Island, and the live habitus of *N. salmoni* (MCZ-IZ-152129).

DNA extraction, amplification and sequencing.—We performed DNA extractions using a single leg per specimen with the Qiagen DNeasy Blood and Tissue kit following the manufacturer's protocol. PCR reactions targeted the fast-evolving mitochondrial locus cytochrome *c* oxidase I (COI) using the LCO forward primer and either HCO or HCOout-out reverse primers (Folmer et al. 1994; Schwendinger & Giribet 2005). Each reaction mixture contained 13 μL of sterile water, 2 μL of 2.5 μM primers, one Illustra PuReTaq Ready-To-Go PCR bead tube and 10 μL of genomic DNA. Reactions ran on an Applied Biosciences ABI 2720 Thermal Cycler with the following thermal profile: 5 min at 95°C; 35 cycles of: 30 s at 95°C, 30 s at 45°C and 1.5 min at 72°C; with a final extension of 6 min at 72°C. PCR products were cleaned using Qiagen's QIAquick kit. Samples were sent to Functional Biosciences (Madison, Wisconsin) for Sanger sequencing.

Alignment and phylogenetic analyses.—We quality checked and assembled sequences in Geneious Prime v.2019.0.4, yielding 58 sequences from 17 different localities (GenBank accession numbers MK990740-MK990797). We aligned our new sequences and published COI sequence data for *N. salmoni* (GenBank accession numbers DQ518109, DQ825638, DQ992323-992324, KU207398-207399) using the MAFFT Alignment tool implemented in Geneious with default parameters. COI sequences for 78 Pettalidae (including representatives for the two other endemic genera of New Zealand mite harvestmen), 40 non-pettalid Cyphophthalmi, 1 Dyspnoi, and 1 Eupnoi were used as outgroups for phylogenetic analyses.

Maximum likelihood phylogenetic analysis was performed with W-IQ-TREE v 1.6 (Trifinopoulos et al. 2016) using ModelFinder (Kalyaanamoorthy et al. 2017) to assess the best substitution model. SH-like approximate likelihood ratio tests (SH-aLRT) and ultrafast bootstrapping (UFBoot) were used to assess nodal support (Hoang et al. 2018). We ran two separate analyses under default parameters, one using DNA substitution models and partitioned to separate positions 1 and 2 from position 3, and one using codon substitution models with the invertebrate mitochondrial genetic code specified. We visualized the results in Fig Tree v1.4.4.

Haplotype diversity (h), nucleotide diversity (π), Tajima's D and Fu's F were calculated in DnaSP v6.12 (Rozas et al. 2017) using default parameters. Analyses were run three times: once for all individuals, once for the northern lineage and once for the southern lineage. We calculated AMOVA and pairwise F_{ST} in Arlequin 3.5.2.2 (Excoffier & Lischer 2010). AMOVA was run under three geographic binning schemes: (1) populations were grouped as "northern" or "southern" lineages, (2) populations were grouped according to their phylogeographic lineages (closely related, geographically proximate populations) (Table 1), and (3) populations were not grouped. Populations represented by two or fewer individuals were excluded from AMOVA and pairwise F_{ST} analyses.

Following analysis of COI data, we downloaded and aligned published data for slower-evolving loci sequenced for *Neopurcellia salmoni*, the nuclear 18S and 28S, to assess the possibility of substitutions diagnostic of major lineages within the species (Cook et al. 2010).

Molecular dating.—As there are no known fossil members of *Neopurcellia*, or indeed of any pettalid, we employed a divergence dating strategy based on COI substitution rates. We used DAMBE7 (Xia 2018) to assess substitution saturation levels in each codon position of COI in our full 196-terminal dataset, as well as in a smaller dataset containing only those taxa in the genera *Neopurcellia*, *Aoraki* Boyer & Giribet, 2007, and *Karripurcellia* Giribet, 2003 (76 terminals). These genera were chosen because *Aoraki* (from New Zealand) and *Karripurcellia* (from Western Australia) together constitute the sister group of *Neopurcellia* (Baker et al. 2020). We used this smaller dataset to estimate lineage ages and ran ModelFinder to determine substitution models for each partition, again separating positions 1 and 2 into one partition, and position 3 into its own partition.

To determine the optimal clock model and tree prior, we used an iterative strategy using the stepping-stone Path sampling application of BEAST 2 (Xie et al. 2011). First, we ran three path sampling analyses of 100 steps and 1 million generations using a strict clock, an exponential relaxed clock, and a lognormal relaxed clock, all under a Yule tree prior. We compared their marginal likelihood estimates using Bayes factors. We then fixed the optimal clock model and ran path sampling analyses for the dataset under a Yule, a birth-death, a coalescent constant population, and a coalescent Bayesian skyline tree prior. Again, we compared the marginal likelihood estimates using Bayes factors, and selected the optimal clock model and tree prior to infer branch lengths for a chronogram.

We estimated divergence dates using BEAST v2.5.2 (Bouckaert et al. 2019), specifying separate nucleotide substitution models for each partition based on the results of ModelFinder. Two published COI substitution rates were used to infer branch lengths: one derived from spiders (family Dysderidae; ucld.mean = 0.0199; Bidegaray-Batista & Arnedo 2011), and one from beetles (family Tenebrionidae; ucld.mean = 0.0178; Papadopoulou et al. 2010). These rates constitute the taxonomically closest comparisons available to *Neopurcellia*, and while mitochondrial substitution rates are known to be elevated in Cyphophthalmi (Clouse & Wheeler 2014), we believed that given the shallower nature of this phylogenetic study and low levels of saturation (see Results), this approach would give reasonable date approximations. We ran each clock rate for 200 million generations four separate times. The four runs for each clock rate were combined in LogCombiner, sampling every 5,000 generations with a 10% burn-in for each run. The quality of the BEAST run was assessed using Tracer v.1.7.1 to ensure all parameters had an ESS value of 200 or higher (Rambaut et al. 2018). The four BEAST runs for each clock rate were summarized in TreeAnnotator to generate a maximum clade credibility tree with common ancestor node heights, and imported into FigTree v1.4.4 for visualization.

Morphological analysis.—All 198 individuals were observed using an Olympus SXZ10 dissecting microscope. We identified individuals to sex through observation of the fourth tarsus, which bears an adenostyle claw in males but not in females, and tergite VIII, which forms two lobes lacking ornamentation on the dorsal surface of males but not females. Specimens chosen for imaging presented distinct dorsal morphological features under light microscopy, such as the presence of pores and variation in granule size. A few specimens without distinct dorsal morphological features were also imaged for comparison. Each specimen had all of the appendages dissected off and the body was mounted in dorsal view on a stub prior to coating with Au-Pd alloy on a Denton Vacuum Desk III sputter coater. We imaged the dorsal side of specimens using a JEOL JSM-6610LV scanning electron microscope. In total, we imaged 10 male and 2 female specimens from 8 localities.

RESULTS

Molecular phylogenetics.—IQ-TREE's ModelFinder selected GTR+F+I+G4 as the best nucleotide substitution model for partition 1 (positions 1 and 2), and HKY+F+G4 as the best substitution model for partition 2 (position 3) using the BIC. Analysis using codon substitution models identified GY+F+G4 as the best model, also using the BIC. Monophyly of *Neopurcellia* was supported by bootstrap values of 99–100 in all analyses (Fig. 2, and Fig. S3 available in Supplementary File 1, online at https://doi.org/10.1636/JoA-S-20-003.s1). Within the genus, two lineages were retrieved, corresponding to the northern and southern regions of the west coast (Figs. 1, 2, S3). The northern lineage (represented by cool colors: turquoise, blue, purple) ranges from Lake Kaniere to Westland Tai Poutini National Park and accounts for eight of the sequenced populations (Figs. 1, 2, S3). This lineage can be further broken down into two well-supported clades corresponding to different geographic areas: Lake Kaniere and Totoara Saddle (turquoise) + Franz Josef Glacier (blue), and Fox Glacier (purple) (Figs. 1, 2, S3). Both IQ-TREE analyses retrieved the same relationships within the northern lineage (Figs. 2, S3) with high support values. The southern lineage (represented by warm colors: red, orange, yellow) ranges from

Table 1.—Specimens examined, sorted by locality from North to South. Collections are deposited at the Harvard Museum of Comparative Zoology (MCZ), the University of Copenhagen Zoological Museum (ZMUC), the Field Museum (FMHD), and the Otago Museum.

Area (Figure 1)	Museum ID	Locality	Latitude	Longitude	Collection Date
Turquoise	MCZ IZ-134740	Lake Kaniere	42.8035°S	171.1288°E	1/27/2006
	MCZ IZ-133840	Totara Saddle Road	42.9180°S	170.8723°E	1/27/2006
Blue	MCZ IZ-29303	Whataroa River Road	43.2858°S	170.4010°E	1/18/2014
	MCZ IZ-29581, MCZ IZ-152168	Terrace Walk, Franz Josef	43.3909°S	170.1826°E	1/18/2014, 1/17/19
Purple	MCZ IZ-144625	Alex Knob Track, Westland Tai Poutini NP	43.4115°S	170.1774°E	1/17/2016
	MCZ IZ-133839	Minnehaha Walk, Fox Glacier	43.4687°S	170.0813°E	2/7/2003
	MCZ IZ-152159	Copland Track, Westland Tai Poutini NP	43.5777°S	169.8155°E	1/17/2019
Yellow	MCZ IZ-152145	Swamp Forest Walk, Ship Creek	43.7611°S	169.1501°E	1/16/2019
	MCZ IZ-134741	North entrance to Haast-Paringa Track	43.7790°S	169.3595°E	1/20/2011
	MCZ IZ-29317	Roaring Billy Falls, Te Wahipoumanu	43.9380°S	169.2882°E	2/15/2008
	MCZ IZ-134739	Haast Pass	44.1078°S	169.3553°E	2/8/2003
	MCZ IZ-143225	Blue Pools, Haast Pass Highway	44.1648°S	169.2766°E	1/18/2006
Orange	MCZ IZ-152152	Wharekai-Te Kou Walk, Jackson Bay	43.9691°S	168.6101°E	1/16/2019
	MCZ IZ-29213, MCZ IZ-152128	Lake Gunn	44.8900°S	168.0842°E	1/30/2012, 1/13/2019
Red	MCZ IZ-152129	Milford Sound Lookout Track	44.6732°S	167.9263°E	1/14/2019
No Molecular Data	ZMUC NZ11-9c, 9f	Copland Track along Copland River Valley	43.5768°S	169.8144°E	1/15/2011
	ZMUC NZ11-15b	NE of Makaora, Camerons Creek Valley	44.1561°S	169.3054°E	1/21/2011
	Otago SPM001758	Saltwater Creek	44.4174°S	171.2422°E	9/29/1966
	FMHD 85-474	Mount Aspiring	44.5125°S	168.7450°E	1/11/1985
	ZMUC NZ11-21a	Lake Gunn	44.8583°S	168.1014°E	1/25/2011
	Otago SPM001293	Mount Grey	45.5500°S	167.2500°E	2/14/1959
Total					

Haast-Paringa to Lake Gunn and accounts for nine of the sequenced populations. It can be further broken down into three well-supported clades: Milford Sound (red), Jackson Bay-Lake Gunn (orange) and Haast-Paringa (yellow) (Figs. 1, 2, S3). The analysis using a codon substitution model retrieved a nearly identical topology to that obtained by the nucleotide model, with the exception being the placement of the orange clade (Jackson Bay-Lake Gunn) composed of individuals from collections MCZ IZ-29213, MCZ IZ-152128 and MCZ IZ-152152. While the nucleotide model-based tree retrieved the orange and red (Milford Sound) lineages as a monophyletic group, the codon model-based tree retrieved orange and yellow (Haast-Paringa) lineages as a monophyletic group with red as the sister group (Fig. S3), though neither topology was well supported.

DnaSP identified a total of 34 unique haplotypes for *Neopurcellia salmoni* (Table 2). The vast majority of haplotypes were unique to individual populations, with populations

Table 2.—Absolute haplotype frequencies for each population studied. See Table 1 for key to localities represented by MCZ ID numbers. Bold type denotes haplotypes shared between populations.

	Haplotypes															
MCZ ID	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16
134740	1															
133840		1														
29303			1	**2**												
29581/152168				**3**	7											
144625						1	1									
133839								1								
152159									2	2	1	1				
152145													1	1	1	
134741																1
29317																
134739																
143225																
152152																
152129																
29213/152128																

Table 1.—Extended.

GenBank Accession No.	Glandular Males	Non-glandular Males	Females	Total
DQ992324	-	-	1	1
DQ992323	-	-	1	1
MK990795-990797	4	-	3	7
MK990785-990794	9	4	1	14
MK990783-990784	-	2	2	4
DQ518109, MK990782	1	2	5	8
MK990776-990781	-	5	2	7
MK990755-990757	2	-	1	3
KU207398, MK990762, 990770	-	9	11	20
KU207399, MK990758	3	4	6	13
DQ825638, MK990769	5	-	7	12
MK990759-990761, 990763-990768	8	-	5	13
MK990746-990754	12	1	15	28
MK990740-990745	3	-	6	9
MK990771-990775	3	-	2	5
	-	2	1	3
	16	-	16	32
	-	2	1	3
	7	-	3	10
	-	3	1	4
	-	1	-	1
	73	35	90	198

often having more than one haplotype. Two haplotypes were shared across populations in the same sublineage: H4 (IZ-29303 and IZ-152168) and H20 (IZ-134741, IZ-29317 and IZ-143225). Populations with shared haplotypes were separated by linear distances of 21.2 km and up to 45.3 km, respectively. Genetic indices are summarized in Table 3. Haplotypic diversity was high for *Neopurcellia* in aggregate (0.962), with southern lineage individuals exhibiting higher haplotypic diversity (0.943) than those in the north (0.880). Tajima's D and Fu's F values were positive, which likely does not indicate rapid population expansion; however, all values were statistically nonsignificant ($P > 0.10$). Pairwise F_{ST} values are summarized in Table 4, with the majority of values being greater than 0.80, indicative of high population structure. Average p-distances between populations ranged from 0.007% (localities ~15km apart) to 7.3% (localities ~75km apart).

AMOVA results are summarized in Table 5. When populations were grouped either as "northern" or "southern", 57.53% of variation originated between groups, 37.08% between populations within each group, and 5.39% within populations. When populations were grouped by the smaller phylogeographic lineages, 79.55% of variation originated between lineages, 13.73% between populations within each lineage, and 6.72% within populations. When populations were not grouped, 92.59% of variation originated between populations, and 7.41% within populations. Fixation indices were high and statistically significant ($P = 0.0000$) for all groupings.

The slower-evolving loci 18S and 28S did not exhibit substitutions diagnostic of the two main lineages discovered with our COI dataset. Three published 18S sequences are available for northern lineage *N. salmoni* (GenBank accession numbers DQ825593, EU673611-2) and three for southern (DQ517998, KU207244-5); all six sequences are identical. Two published 28S sequences are available for northern lineage *N. salmoni* (DQ518037, EU673651) and three for southern (EU673650.1, KU2072799-300). Fewer than 10 mutations were found in >2000bp of 28S, each of which was unique to a single individual.

Molecular dating.—DAMBE indicated there was substantial saturation in the full 196-terminal dataset at all three positions, but found little saturation at all positions in the 76-terminal dataset restricted to *Neopurcellia*, *Aoraki*, and *Karripurcellia* (Tables S1-S6 in Supplementary File 1, online at https://doi.org/10.1636/JoA-S-20-003.s1). ModelFinder selected TIM2+G4 and TN+G4 as the optimal site substitution models for partitions 1 and 2 of this dataset, respectively. Path sampling analyses identified a lognormal relaxed clock and

Table 2.—Extended.

Haplotypes																	
H17	H18	H19	H20	H21	H22	H23	H24	H25	H26	H27	H28	H29	H30	H31	H32	H33	H34
1			**1**														
			2														
	1	1															
			3	4	1	1											
							1	1	1	1	1	1	1	2			
															5		
																5	1

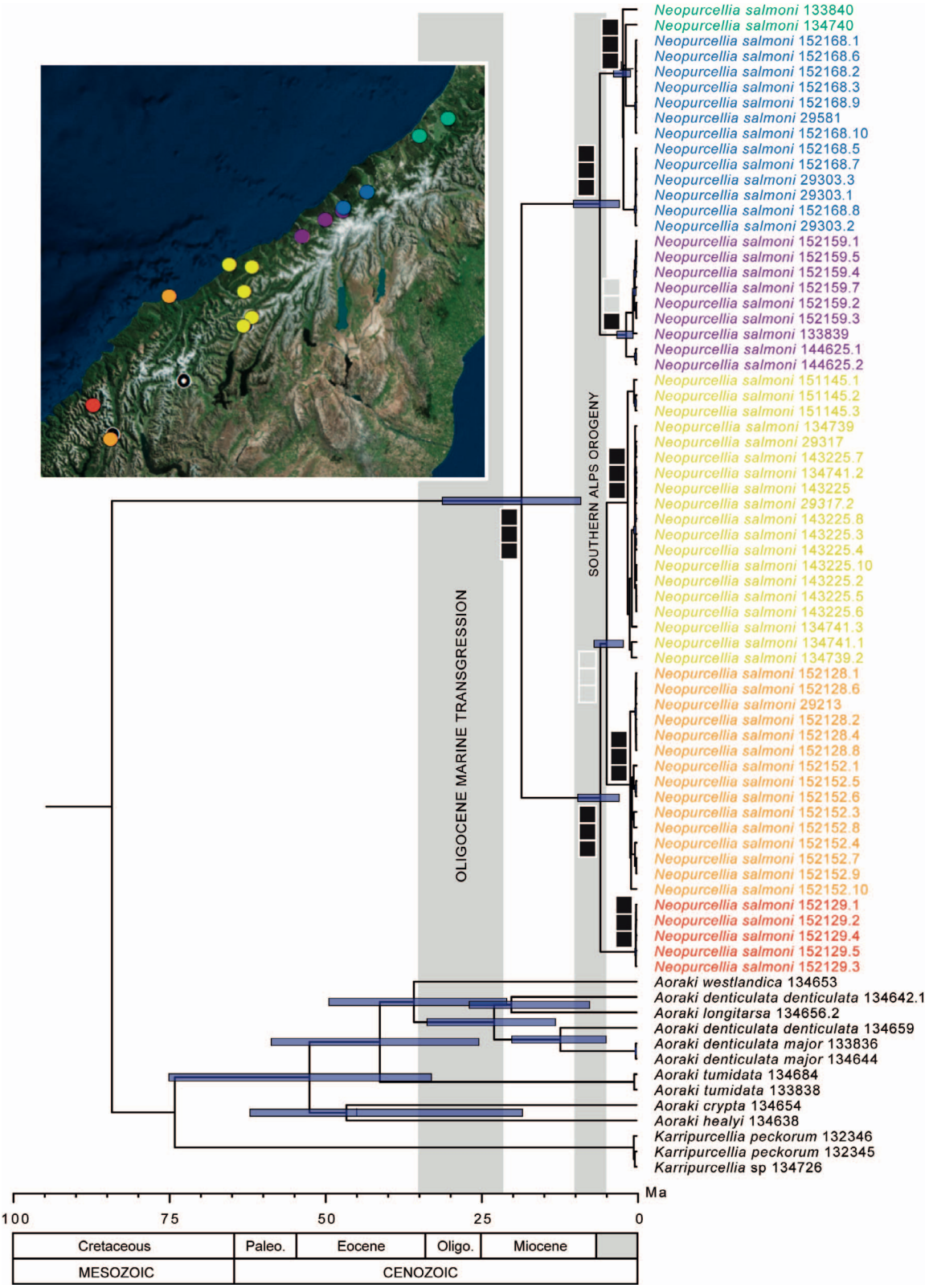

Figure 2.—Ultrametric tree based on analysis of COI sequences in BEAST, using a substitution rate derived from spiders. Inset map shows *Neopurcellia salmoni* localities represented in the tree (see also Figure 1). Terminal colors correspond to locality colors in inset map and Figure 1. Numbers following species names are Harvard Museum of Comparative Zoology Invertebrate Zoology accession numbers. Boxes at nodes indicate support values; from top to bottom: IQ-TREE result: SH-aLRT (grey = below 80%; black = above 80%), IQ-TREE result: UFBoot support (grey = below 95%, black = above 95%), and BEAST result: Bayesian posterior probability (grey = below 95%, black = above 95%). Shaded box in geologic timescale represents Pliocene, Pleistocene, and Holocene.

Table 3.—Genetic indices for *Neopurcellia* populations in this study.

	Number individuals	Number haplotypes	Haplotype diversity (h)	Nucleotide diversity (π)	Tajima's D	Fu's F
All	63	34	0.962 ± 0.010	0.07562 ± 0.00240	1.05975 (p>0.10)	3.485 (p>0.10)
Northern	24	14	0.880 ± 0.048	0.03602 ± 0.00206	0.87272 (p>0.10)	4.607 (p>0.10)
Southern	39	24	0.943 ± 0.020	0.03960 ± 0.00221	0.23827 (p>0.10)	1.521 (p>0.10)

coalescent skyline tree as the optimal priors, with decisive support from Bayes factors (BF = 34.01 compared to the next-best model's marginal *L* estimate, Table S7 in Supplementary File 1, online at https://doi.org/10.1636/JoA-S-20-003.s1). BEAST analyses from both clock rates recovered virtually identical topologies, as well as similar dating estimates for the divergence and diversification events of *Neopurcellia* (Fig. 2 and Figs. S1, S2 available in Supplementary File 1, online at https://doi.org/10.1636/JoA-S-20-003.s1). According to the spider-derived rate, the genus is estimated to have diverged from its from its sister group *ca*. 84 Ma (95% HPD: 127–49 Ma), while the beetle-derived rate finds that *Neopurcellia* diverged from its sister group *ca*. 94 Ma (95% HPD: 142–56 Ma). The split of *Neopurcellia* into the northern and southern lineages is estimated to have occurred *ca*. 18 Ma (95% HPD: 31–9 Ma) for the spider rate and *ca*. 21 Ma (95% HPD: 35–10 Ma) for the beetle rate, with diversification of the two main lineages both taking place *ca*. 6 Ma according to both clock rates (95% HPD across both analyses: 11.3–2.7 Ma) (Fig. 2). These ages, for *Neopurcellia* as well as its divergence from its sister group, are somewhat different than those found in previous studies—Baker et al. (2020) recovered a younger age for *Neopurcellia* at 10.4 Ma (95% HPD: 17.9–3.9 Ma), and an older divergence from *Aoraki* + *Karripurcellia* at 146 Ma (95% HPD: 174.5–117.8 Ma). This discrepancy is likely caused by our use of phylogenetically distant substitution rates, which do not reflect the accelerated mitochondrial rates of Cyphophthalmi.

Morphology.—We found a surprising level of within-species variation in the dorsal morphology of *Neopurcellia salmoni*. Female specimens have uniformly finely granular bodies, no modification to posterior tergites, and tergal segmentation distinguished by linear transverse sulci lacking any ornamentation. Male specimens present a clear case of polymorphism (Fig. 3). The non-glandular morphotype (Fig. 3A) has been previously described by Forster (1948) and revisited by Boyer & Giribet (2007). It presents a non-uniform ornamentation of the dorsal opisthosoma with finer granules on tergites I–V and larger granules on tergites VI and VII (Fig. 4A). Tergal segmentation is distinguished by transverse linear sulci lacking any ornamentation. The glandular morphotype (Fig. 4B–D) has not been described previously. Tergites I and II have finer granules uniformly distributed; however, tergites III–VII bear larger granules and pores in their respective sulci. Tergites III–V bear large granules on the anterior half of the tergite and finer granules on the posterior half of the tergite. Larger granules are accompanied by pores distributed in a seemingly random manner and appear to be formed by the fusion of smaller granules (Fig. 4D). The presence of pores is unusual as it has not been described in the dorsal morphology of any of the members of Cyphophthalmi. There is some degree of variation within the glandular morphotype in one of the populations (ZMUC 11-15b) where some specimens exhibit less prominent larger granules and a reduced number of pores (Figs. 3B, C, 4B, C). However, this slight variation seems to be in regard to size rather than presence, as all observed glandular morphotypes present larger granules and pores on tergites III–VII, indicating a dimorphism rather than continuous variation.

There was no geographic or phylogenetic pattern in the distribution of male morphotypes. None of the tree topologies supported a monophyletic distribution of glandular males within populations. Pores and large granules were present in both northern and southern lineages and across its widespread range independent of the locality's elevation and date of collection. While most populations presented only one of the male morphotypes, this trend was not true for all. Out of 19 sampled populations with male specimens in them, four presented males of both morphotypes, eight had glandular males only and seven had non-glandular males only (Table 1).

Table 4.—Pairwise F_{ST} values (top half) and geographic distances in km (bottom half) between populations. Localities with two or fewer sequenced individuals were excluded. See Table 1 for key to the numbered localities.

	IZ-29303	IZ-29581 IZ-152168	IZ-152159	IZ-152145	IZ-134741	IZ-143225	IZ-152152	IZ-29213 IZ-152128	IZ-152129
IZ-29303	-	0.650	0.953	0.970	0.917	0.989	0.954	0.994	0.996
IZ-29581/IZ-152168	21.2	-	0.850	0.920	0.865	0.937	0.903	0.942	0.941
IZ-152159	57.4	36.2	-	0.953	0.896	0.976	0.939	0.982	0.982
IZ-152145	113.9	92.8	57.3	-	0.467	0.826	0.863	0.951	0.947
IZ-134741	100.4	79.2	43.0	16.9	-	0.188	0.737	0.851	0.838
IZ-143225	133.2	112.8	78.4	46.0	43.4	-	0.896	0.984	0.986
IZ-152152	162.5	142.1	106.2	49.1	63.7	57.4	-	0.749	0.918
IZ-29213/IZ-152128	257.5	236.6	201.2	151.6	160.0	124.4	110.7	-	0.996
IZ-152129	251.0	230.1	194.7	140.8	151.6	121.3	95.4	27.2	-

Table 5.—AMOVA results. Localities with two or fewer sequenced individuals were excluded from the analysis.

		Sum of squares	Percentage of variation	Fixation indices
Northern and Southern	variance among groups	645.12	57.53	FCT: 0.57530*
	variance among populations within groups	571.39	37.08	FSC: 0.87315*
	variance within population	92.23	5.39	FST: 0.94613*
Sub-lineages	variance among groups	1123.73	79.55	FCT: 0.79551*
	variance among populations within groups	92.77	13.73	FSC: 0.67118*
	variance within population	92.23	6.72	FST: 0.93276*
No grouping	variance among populations	1216.51	92.59	-
	variance within populations	92.23	7.41	FST: 0.92591*

* = significant with $p < 0.05$

Glandular morphotypes were also observed at a higher frequency than the non-glandular morphotype, accounting for 67.6% ($n = 73$) and 32.4% ($n = 35$) of all male specimens respectively.

DISCUSSION

Phylogeography and population genetics.—*Neopurcellia salmoni* is an unusual mite harvestman species, presenting male polymorphism unique within the suborder as well as one of the largest species ranges known for these animals. Most Cyphophthalmi species are known from fewer than five localities and their ranges tend not exceed more than fifty kilometers across, yet *N. salmoni* spans a linear transect of over 400 km (Boyer & Giribet 2007). Within this large range, we found a strong correlation between evolutionary relatedness of *Neopurcellia* and the geographic distribution of populations. That is, our analyses indicate that monophyletic groups tend to correspond to adjacent geographic regions, as is expected in dispersal-limited organisms (Figs. 1, 2) (e.g., Boyer et al. 2015; Harms 2018; Schwentner & Giribet 2018). A paucity of shared haplotypes between populations and high F_{ST} values (Tables 2–5) confirm that gene flow is minimal.

The iconic *Nothofagus* southern beech forests of New Zealand's South Island have a patchy distribution, consisting of two very large disjunct forested areas in the western half of the landmass, in addition to many small isolated patches. One of the large expanses of forest extends south of the Haast area all the way to the southwest tip of the island; the other has its southern limit around Greymouth and extends up to the northern coast (Fig. 1). These two forested areas are separated by a 150 km stretch known as the "Westland beech-gap," which lacks beech trees and a number of insect taxa (Haase et al. 2007; Trewick & Wallis 2007; Buckley et al. 2015). We found that *Neopurcellia* is comprised of two lineages, one of which is found throughout the contiguous forests to the south

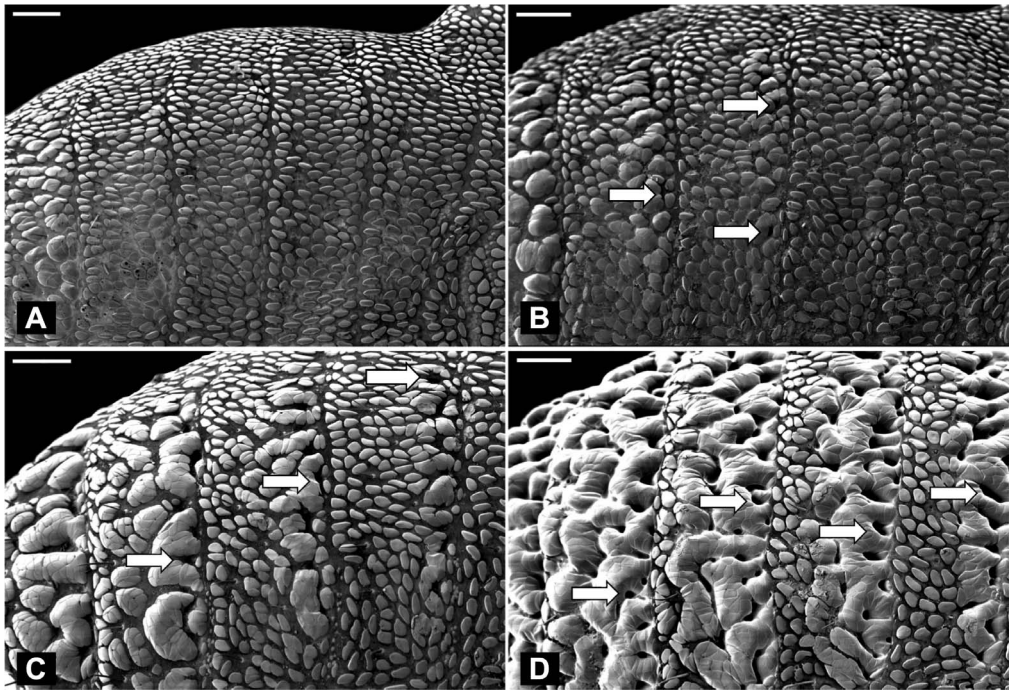

Figure 3.—Scanning electron micrograph illustrating details of granulation and presence of glandular pores on the dorsal opisthosoma of *Neopurcellia salmoni* males: A. Non-glandular (MCZ IZ-134741). B-D. Glandular. Nearly every glandular male examined resembled the individual represented in D (MCZ IZ-133839); only one population contained males of the types represented in B and C (ZMUC 11-15b). Arrows in B-D highlight the presence of pores. Scale bar: 100μm.

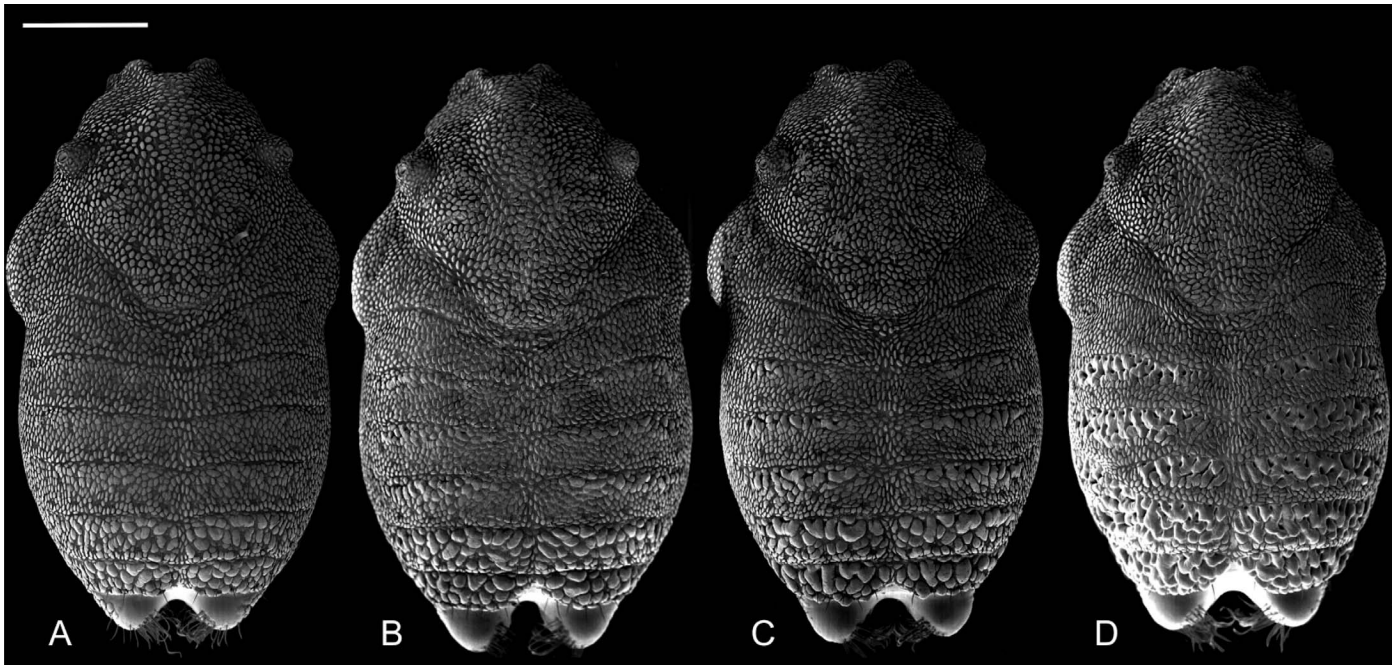

Figure 4.—Scanning electron micrographs illustrating *Neopurcellia salmoni* males from Fig. 3 in dorsal view: A. Non-glandular (MCZ IZ-134741). B-D. Glandular. Nearly every glandular male examined resembled the individual represented in D (MCZ IZ-133839); only one population contained males of the types represented in B and C (ZMUC 11-15b). Scale bar: 500μm.

of the beech-gap (red, orange, and yellow in Figs. 1, 2) and one that is found in small patches of forest within the beech gap (turquoise, blue, and purple in Figs. 1, 2). In order to understand which historical geological processes might have shaped this phylogeographic pattern, we estimated the ages of these two lineages.

Molecular clock estimates.—Relaxed molecular clock estimates indicate that *Neopurcellia* diverged from its sister group in the Late Cretaceous. It diversified into a northern and southern lineage during the Miocene, *ca*. 18–21 Ma, following the Oligocene marine transgression (*ca*. 35–22 Ma) (Fig. 2); it is plausible that the expansion of land area during this time contributed to the initial diversification of *Neopurcellia*. Diversification of populations within each of the northern and southern phylogeographic lineages began around 6.6–5.8 Ma, coinciding with the uplift of the Southern Alps (10–5 Ma) (Fig. 2). This orogenic event shifted the precipitation gradient of the South Island, creating areas of high rainfall along the west coast, where *Neopurcellia* is found, and drier areas to the East. Ecological niche modeling has indicated that moisture is a primary determinant of habitat suitability for mite harvestmen (Boyer et al. 2016). Given that *Neopurcellia* diversified during the uplift of the Southern Alps, it seems likely that the expansion of high-moisture forests due to the uplift of axial mountain ranges allowed *Neopurcellia* to expand its range to its current impressive extent.

While scientists agree that New Zealand's vegetation cover shifted dramatically during the LGM, the past two decades have seen debate concerning the extent to which woody vegetation persisted during this period (Newnham et al. 2013). Some researchers argue that evidence from the fossil record of beetles suggests there was much more extensive woody vegetation than has been inferred from the pollen record (Marra et al. 2006; Burge & Shulmeister 2007). In response, McGlone, Newnham, and colleagues re-evaluated New Zealand's LGM pollen record and provided a new reconstruction of vegetation cover (McGlone et al. 2010; Newnham et al. 2013). According to the results of their work, much of the current range of *Neopurcellia* was covered by glacial ice or permanent snow, but some unglaciated areas would have persisted. Two small patches of vegetation are reconstructed at the western margin of the ice and snow that covered the Southern Alps and much of Fiordland, one at the far southwest tip of the South Island and another just north of Milford Sound. In addition, a very large vegetated area, extending up the west coast of the South Island and into to the North Island, would have had its southern limit around Paringa. All of these unglaciated areas were covered by a mosaic of shrubland, grassland, and patches of beech and rare conifers (McGlone et al. 2010; Newnham et al. 2013).

Many animals endemic to New Zealand's South Island, including both invertebrates such as stoneflies and vertebrates such as rock wrens, show a north-south phylogeographic split that has been dated to *ca*. 2 Ma, consistent with a model in which allopatric isolation in glacial refugia drove diversification (reviewed by Buckley et al. [2015] and Wallis et al. [2016]). While the north-south split within *Neopurcellia salmoni* coincides geographically with the divergences in these other animal taxa, it predates them by *ca*. 20 Ma. Therefore, it is likely that for *N. salmoni,* LGM refugial areas preserved relictual populations of a lineage that was already highly structured as opposed to driving diversification. In the Australian Wet Tropics, a model system for studying the effect of paleoclimatic fluctuations on the evolution of rainforest biota, both molecular dating and paleoclimatic modeling approaches indicate that in the case of mite

harvestmen, LGM refugia acted as museums rather than cradles of biodiversity (Boyer et al. 2016; Oberski et al. 2018). The role of LGM refugia is expected to differ across taxa with respect to organism size and dispersal ability (Graham et al. 2006); Cyphophthalmi represent an extreme case of dispersal limitation, and we are therefore not surprised that our results differ from patterns seen in other co-distributed groups.

Male polymorphism.—Like all Cyphophthalmi, *Neopurcellia salmoni* is sexually dimorphic; males bear an adenostyle claw on their fourth tarsus, while females do not, and males bear prominent scopular hairs on the posterior of the body (visible in Figure 4) while females do not. Females of the species present uniform morphology, but males were readily categorizable to one of two discrete morphotypes: glandular (Figs. 3B–D, 4B–D) and non-glandular (Figs. 3A, 4A). The vast majority of glandular males resembled the individual in Figs. 3D and 4D. Only one population exhibited noticeable variation within the glandular type (ZMUC 11-15b, Figs. 3B–C, 4B–C). Although we initially suspected that the two morphotypes might represent two species, our phylogeographic analysis indicated that geography, rather than morphology, predicted relatedness, allowing us to reject this hypothesis.

Male polymorphism was not previously known within Cyphophthalmi, but it is well documented in the harvestman suborders Eupnoi and Laniatores (Buzatto & Machado 2014). In three families of Eupnoi, males exhibit discrete morphs with respect to the thickness of their pedipalps or size of chelicerae. In Laniatores, some species present discrete variation in the size and robustness of male chelicerae while other species' males may vary with respect to protuberances or spines on their dorsal scuta. Buzatto et al. (2014) carried out a comparative study of 48 Laniatores species from the family Gonyleptidae and showed that male polymorphism never occurred in sexually monomorphic species, indicating a correlation between sexual dimorphism and male polymorphism in harvestmen. Furthermore, the study demonstrated that male polymorphism has been gained independently multiple times in gonyleptids, suggesting significant evolutionary flexibility in this trait.

Intrasexual polymorphism can be an outcome of strong sexual selection. Males with reduced fitness may develop alternative reproductive tactics (ARTs) often associated with morphological divergences in ornaments and weaponry, especially in arthropods (Painting et al. 2015). Given the correlation between male polymorphisms and ARTs (Buzatto & Machado 2014), it is possible that the intraspecific morphological variation observed in *Neopurcellia salmoni* arose as an alternative mating tactic for less fit males. Cyphophthalmi likely engage in scramble competition polygyny, where mating opportunities are equally available to all and mating occurs several times during an adult's life (Machado & Macías-Ordóñez 2007). The eyes of Cyphophthalmi are typically small, laterally positioned, and simple; it is thought that these animals rely heavily on chemical communication (Willemart & Giribet 2010). Male mite harvestmen commonly bear pores associated with the anal plate or the adenostyle claw of the fourth tarsus, while females typically do not, suggesting a sexual function of the pores and associated glands. Therefore, we speculate that the dorsal glandular openings of *Neopurcellia salmoni* may improve mating success by enhancing chemical signaling by glandular males to potential mates.

It is possible that the absence of male polymorphism in some of the *Neopurcellia* populations is due to a small sample size or the absence of adult males in many of the collections; we do not discard the possibility that the trait is more geographically widespread than reported in this study. The wide distribution of the different morphotypes across both southern and northern lineages suggests this is a morphological adaptation that has proven to be successful or neutral. Unfortunately, none of the specimens examined in this study was preserved in such a way that histological work could be performed; further investigation should include such examination of the structure of these unique features, as well as behavioral studies to determine their function.

Conclusions and future directions.—While the presence of two different male morphs initially suggested multiple *Neopurcellia* species, the seemingly random geographic and phylogenetic distribution of the male morphotypes allows us to reject this hypothesis. Furthermore, any impulse to split *N. salmoni* into multiple species based on genetic data alone is checked by the lack of diagnostic morphological differences among lineages in combination with a lack of fixed variation in slower-evolving nuclear loci. This study presents the first known description of male polymorphism amongst Cyphophthalmi and calls for an extensive survey of other members of Pettalidae for variations in their dorsal morphology in order to determine the prevalence of this intriguing trait.

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SUPPLEMENTAL MATERIALS

Tables S1-S7, Figures S1-S3, available in Supplementary File 1, online at https://doi.org/10.1636/JoA-S-20-003.s1

LITERATURE CITED

Baker CM, Boyer SL, Giribet G. 2020. A well-resolved transcriptomic phylogeny of the mite harvestman family Pettalidae (Arachnida,

Opiliones, Cyphophthalmi) reveals signatures of Gondwanan vicariance. Journal of Biogeography 47:1345–1361.

Baker CM, Sheridan K, Derkarabetian S, Pérez-González A, Vélez S, Giribet G. 2020. Molecular phylogeny and biogeography of the temperate Gondwanan family Triaenonychidae (Opiliones: Laniatores) reveals pre-Gondwanan regionalization, common vicariance, and rare dispersal. *Invertebrate Systematics* 34: 637-660 https://doi.org/10.1071/IS19069

Bidegaray-Batista L, Arnedo MA. 2011. Gone with the plate: the opening of the Western Mediterranean basin drove the diversification of ground-dwelling spiders. *BMC Evolutionary Biology* 11:317.

Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, et al. 2019. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 15:e1006650.

Boyer SL, Giribet G. 2007. A new model Gondwanan taxon: Systematics and biogeography of the harvestmen family Pettalidae (Arachnida, Opiliones, Cyphophthalmi), with a taxonomic revision of genera from Australia and New Zealand. *Cladistics* 23:337–361.

Boyer SL, Giribet G. 2009. Welcome back, New Zealand: Regional biogeography and Gondwanan origin of three endemic genera of mite harvestmen (Arachnida, Opiliones, Cyphophthalmi). *Journal of Biogeography* 36:1084–1099.

Boyer SL, Baker JM, Giribet G. 2007. Deep genetic divergences in *Aoraki denticulata* (Arachnida, Opiliones, Cyphophthalmi): A widespread 'mite harvestman' defies DNA taxonomy. *Molecular Ecology* 16:4999–5016.

Boyer SL, Baker CM, Popkin-Hall ZR, Laukó DI, Wiesner HW, Quay RM. 2015. Phylogeny and biogeography of the mite harvestmen (Arachnida, Opiliones, Cyphophthalmi) of Queensland, Australia with a description of six new species from the rainforests of the Wet Tropics. *Invertebrate Systematics* 29:37–70.

Boyer SL, Markle TM, Baker CM, Luxbacher AM, Kozak KH. 2016. Historical refugia have shaped biogeographical patterns of species richness and phylogenetic diversity in mite harvestmen (Arachnida, Opiliones, Cyphophthalmi) endemic to the Australian Wet Tropics. *Journal of Biogeography* 43:1400–1411.

Buckley TR, Krosch M, Leschen RA. 2015. Evolution of New Zealand insects: Summary and prospectus for future research. *Austral Entomology* 54:1–27.

Buckley TR, Marske K, Attanayake D. 2009a. Identifying glacial refugia in a geographic parthenogen using palaeoclimate modelling and phylogeography: The New Zealand stick insect *Argosarchus horridus* (White). *Molecular Ecology* 18:4650–4663.

Buckley TR, Marske K, Attanayake D. 2009b. Phylogeography and ecological niche modelling of the New Zealand stick insect *Clitarchus hookeri* (White) support survival in multiple coastal refugia. *Journal of Biogeography* 37:682–695.

Burge PI, Shulmeister J. 2007. Re-envisioning the structure of last glacial vegetation in New Zealand using beetle fossils. *Quaternary Research* 68:121–132.

Buzzato BA, Machado G. 2014. Male dimorphism and alternative reproductive tactics in harvestmen (Arachnida: Opiliones). *Behavioural Processes* 109:2–13.

Buzzato BA, Tomkins JL, Simmons LW, Machado G. 2014. Correlated evolution of sexual dimorphism and male dimorphism in a clade of neotropical harvestmen. *Evolution* 68:1671–1686.

Chousou-Polydouri N, Carmichael A, Szűts T, Saucedo A, Gillespie R, Griswold C, et al. 2019. Giant goblins above the waves at the southern end of the world: the biogeography of the spider family Orsolobidae (Araneae, Dysderoidea). *Journal of Biogeography* 46:332–342.

Clouse RM, Wheeler WC. 2014. Descriptions of two new, cryptic species of *Metasiro* (Arachnida: Opiliones: Cyphophthalmi: Neogoveidae) from South Carolina, USA, including a discussion of mitochondrial mutation rates. *Zootaxa* 3814:177–201.

Cook LG, Crisp MD. 2005. Not so ancient: the extant crown group of *Nothofagus* represents a post-Gondwanan radiation. *Proceedings of the Royal Society B* 272:2535–2544.

Cook LG, Edwards RD, Crisp MD, Hardy NB. 2010. Need morphology always be required for new species descriptions? *Invertebrate Systematics* 24:322–326.

Cooper A, Cooper RA. 1995. The Oligocene bottleneck and New Zealand biota: genetic record of a past environmental crisis. *Proceedings of the Royal Society B: Biological Sciences* 261:293–302.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10:564–567.

Fernandez R, Giribet G. 2014. Phylogeography and species delimitation in the New Zealand endemic, genetically hypervariable harvestman species, *Aoraki denticulata* (Arachnida, Opiliones, Cyphophthalmi). *Invertebrate Systematics* 28:401–414.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplifications of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3:294–299.

Forster RR. 1948. The Sub-order Cyphophthalmi Simon in New Zealand. *Dominion Museum Records in Entomology* 1:79–119.

Gellatly AF, Chinn TJH, Röthlisberger F. 1988. Holocene glacier variations in New Zealand: a review. *Quaternary Science Reviews* 7:227–242.

Giribet F, Boyer SL. 2010. 'Moa's Ark' or 'Goodbye Gondwana': is the origin of New Zealand's terrestrial invertebrate fauna ancient, recent, or both? *Invertebrate Systematics* 24:1–8.

Giribet G, Boyer SL, Baker CM, Fernández R, Sharma PP, de Bivort BL, et al. 2016. A molecular phylogeny of the temperate Gondwanan family Pettalidae (Arachnida, Opiliones, Cyphophthalmi) and the limits of taxonomic sampling. *Zoological Journal of the Linnean Society* 178:523–545.

Giribet G, Buckman-Young RS, Costa CD, Baker CM, Benavides LR, Branstetter MG, et al. 2018. The 'Peripatos' in Eurogondwana? – Lack of evidence that southeast Asian onychophorans walked through Europe. *Invertebrate Systematics* 32:842–865.

Goldberg J, Trewick SA, Paterson AM. 2008. Evolution of New Zealand's terrestrial fauna: a review of molecular evidence. *Philosophical Transactions of the Royal Society B* 363:3319–3334.

Graham CH, Moritz C, Williams SE. 2006. Habitat history improves prediction of biodiversity in rainforest fauna. *Proceedings of the National Academy of Sciences of the United States of America* 103:632–636.

Haase M, Marshall B, Hogg I. 2007. Disentangling causes of disjunction on the South Island of New Zealand: the Alpine Fault hypothesis of vicariance revisited. *Biological Journal of the Linnean Society* 91:361–374.

Harms D. 2018. The origins of diversity in ancient landscapes: Deep phylogeographic structuring in a pseudoscorpion (Pseudotyrannochthoniidae: *Pseudotyrannochthonius*) reflects Plio-Pleistocene climate fluctuations. *Zoologischer Anzeiger* 273:112–113.

Hoang DT, Chernomor O, von HaeselerA, Minh BQ, Vinh LS. 2018. UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 35:518–522.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14:587–589.

Landis CA, Campbell HJ, Begg JG, Mildenhall DC, Paterson AM, Trewick SA. 2008. The Waipounamu Erosion Surface: questioning the antiquity of the New Zealand land surface and terrestrial fauna and flora. *Geological Magazine* 145:173–197.

Machado G, Macías-Ordóñez R. 2007. Reproduction. Pp. 414–454.

In Harvestmen: The Biology of Opiliones (Pinto-da-Rocha R, Machado G, Giribet G, eds.). Harvard University Press, Cambridge MA.

Marra M. 2008. Quaternary fossil beetles from New Zealand. *New Zealand Entomologist* 31:5–16.

Marra MJ, Shulmeister J, Smith EGC. 2006. Reconstructing temperature during the Last Glacial Maximum from Lyndon Stream, South Island, New Zealand using beetle fossils and maximum likelihood envelopes. *Quaternary Science Reviews* 25:1841–1849.

Marske KA, Leschen RAB, Barker GM, Buckley TR. 2009. Phylogeography and ecological niche modelling implicate coastal refugia and trans-alpine dispersal of a New Zealand fungus beetle. *Molecular Ecology* 18:5126–5142.

Marske KA, Leschen RA, Buckley TR. 2012. Concerted versus independent evolution and the search for multiple refugia: Comparative phylogeography of four forest beetles. *Evolution* 66:1862–1877.

McGlone MS, Newnham RM, Moar NT. 2010. The vegetation cover of New Zealand during the Last Glacial Maximum: Do pollen records under-represent woody vegetation? Pp. 202–214. *In* Altered Ecologies: Fire, climate and human influence on terrestrial landscapes (Haberle SG, Stevenson J, Prebble M, eds.). ANU Press, Canberra, Australia.

McGlone MS, Salinger MJ, Moar NT. 1993. Paleovegetation studies of New Zealand's climate since the Last Glacial Maximum. Pp. 294–315. *In* Global Climates since the Last Glacial Maximum (Wright H, Kutzbach J, Webb T, Ruddiman W, Alayne S, Bartlein P, eds.). University of Minnesota Press, Minneapolis MN.

McLoughlin S. 2001. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany* 49:271–300.

Mitchell KJ, Llamas B, Soubrier J, Rawlence NJ, Worthy TH, Wood J, et al. 2014. Ancient DNA reveals elephant birds and kiwi are sister taxa and clarifies ratite bird evolution. *Science* 344:898–900.

Newnham, R., McGlone M, Moar N, Wilmshurst J. 2013. The vegetation cover of New Zealand at the Last Glacial Maximum. *Quaternary Science Reviews* 74:202–214.

Oberski JT, Sharma PP, Jay KR, Coblens MJ, Lemon KA, Johnson JE, et al. 2018. A dated molecular phylogeny of mite harvestmen (Arachnida: Opiliones: Cyphophthalmi) elucidates ancient diversification dynamics in the Australian Wet Tropics. Molecular *Phylogenetics and Evolution* 127:813–822.

Painting, CJ, Probert AF, Townsend DJ, Holwell GI. 2015. Multiple exagerated weapon morphs: a novel form of male polymorphism in harvestmen. *Scientific Reports* 5:16368.

Papadopoulou A, Anastasiou I, Vogler AP. 2010. Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. *Molecular Biology and Evolution* 27:1659–1672.

Pons J, Fujisawa T, Claridge EM, Savil RA, Barraclough TG, Vogler AP. 2011. Deep mtDNA subdivision within Linnean species in an endemic radiation of tiger beetles from New Zealand (genus *Neocicindela*). *Molecular Phylogenetics and Evolution* 59:251–262.

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67:901–904

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, et al. 2017. DnaSP v6: DNA Sequence Polymorphism analysis of large datasets. *Molecular Biology and Evolution* 34:3299–3302.

Sanmartín I. 2002. A paleogeographic history of the Southern Hemisphere. Uppsala University. Online at http://www.ebc.uu.se/systzoo/staff/sanmartin/ PaleoHistSH.pdf

Sanmartín I, Ronquist F. 2004. Southern hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic Biology* 53:216–243.

Schwendinger PJ, Giribet G. 2005. The systematics of the South-East Asian genus *Fangensis* Rambla (Opiliones: Cyphophthalmi: Stylocellidae). *Invertebrate Systematics* 19:297–323.

Schwentner M, Giribet G. 2018. Phylogeography, species delimitation and population structure of a Western Australian short-range endemic mite harvestman (Arachnida: Opiliones: Pettalidae: *Karripurcellia*). *Evolutionary Systematics* 2:81–87.

Trewick SA, Wallis GP. 2007. Bridging the 'beech-gap': New Zealand invertebrate phylogeography implicates Pleistocene glaciation and Pliocene isolation. *Evolution* 55:2170–2180.

Trifinopoulos J, Nguyen L-T, von Haeseler A, Minh BQ. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44:W232–W235.

Wallis GP, Jorge F. 2018. Going under down under? Lineage ages argue for extensive survival of the Oligocene marine transgression on Zealandia. *Molecular Ecology* 27:4368–4396.

Wallis GP, Waters JM, Upton P, Craw D. 2016. Transverse alpine speciation driven by glaciation. *Trends in Ecology and Evolution* 31:916–926.

Waters JM, Craw D. 2006. Goodbye Gondwana? New Zealand biogeography, geology, and the problem of circularity. *Systematic Biology* 55:351–356.

Willemart RH, Giribet G. 2010. Scanning electron microscope survey of the cuticle in Cyphophthalmi (Arachnida, Opiliones) with the descripton of novel sensory and glandular structures. *Zoomorphology* 129:175–183.

Xia X. 2018. DAMBE7: new and improved tools for data analysis in molecular biology and evolution. *Molecular Biology and Evolution* 35:1550–1552.

Xie W, Lewis PO, Fan Y, Kuo L, Chen MH. 2011. Improving marginal likelihood estimation for Bayesian phylogenetic model selection. *Systematic Biology* 60:150–160.

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