First Molecular Evidence for Underestimated Biodiversity of *Rhachotropis* (Crustacea, Amphipoda), with Description of a New Species

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Abstract

The crustacean genus *Rhachotropis* has a worldwide distribution and amongst the largest bathymetric range known from any amphipod genus. DNA barcoding of new material from around New Zealand and the Ross Sea indicated depth-related biogeographic patterns. New Zealand *Rhachotropis* do not form a monophyletic clade. Species from bathyal depths on the Chatham Rise, east of New Zealand, show lower sequence divergence to bathyal species from California and the Arctic than to abyssal New Zealand species. Species sampled in the Kermadec Trench, north of New Zealand below 5000 m, seem to be more closely related to Ross Sea abyssal species than to the New Zealand shelf species. The worldwide geographic and bathymetric distribution for all *Rhachotropis* species is presented here. Depth may have a greater influence on phylogeny than geographic distance. Molecular and morphological investigations of *Rhachotropis* specimens from the Chatham Rise, New Zealand revealed a species new to science which is described in detail, including scanning electron microscopy. This increases the number of described species of *Rhachotropis* to 60 worldwide.

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Introduction

The amphipod genus *Rhachotropis* (Eusiridae) contains 59 known species with a worldwide distribution (Fig. 1), [1]. *Rhachotropis* species appear to have a patchy distribution although some species are locally very abundant [1,2], especially in benthic slope communities [3]. Species in general have a relatively high swimming capacity, indicative of a partial pelagic lifestyle [3]. Phylogenetic analyses based on morphological characters have been unsatisfying or not possible. The numerical analysis of 20 morphological characters and corresponding character states by Bousefield & Hendrycks [4] focused on gross external morphology rather than mouthparts and reproductive features that may actually prove more significant phylogenetically as the authors suggested. Even though *Rhachotropis* show an impressive horizontal and vertical distribution, the genus has not been studied with molecular phylogenetic tools. This is a first preliminary analysis of the mitochondrial **cytochrome oxidase** c subunit **1** (COI) sequences of *Rhachotropis* specimens collected from bathyal and abyssal depths around New Zealand and in the Ross Sea.

This paper describes one new species collected on the Chatham Rise, east of New Zealand, and increases the number of known and described *Rhachotropis* species to 60, and the New Zealand *Rhachotropis* to four species. At least two further species from New Zealand waters appear to be new, but in too poor condition as to be formally described.

Results

COI

Relationships for nine *Rhachotropis* specimens from New Zealand and the Ross Sea are shown in Fig. 2 and represent the topology inferred by both analyses. The trees were rooted with the Antarctic outgroup *Eusirus* sp., and include additional close matches for northern hemisphere *Rhachotropis* COI sequences held in Genbank: *R. inflata*, *R. aculeata*, *R. inflata*, *R. helleri, and a putative new species from California R.* sp 28 (Table 1). The DNA barcodes revealed six well supported clades of *Rhachotropis* specimens from New Zealand and the Ross Sea with a further four clades for the northern hemisphere species (Fig. 2). Three specimens from the Chatham

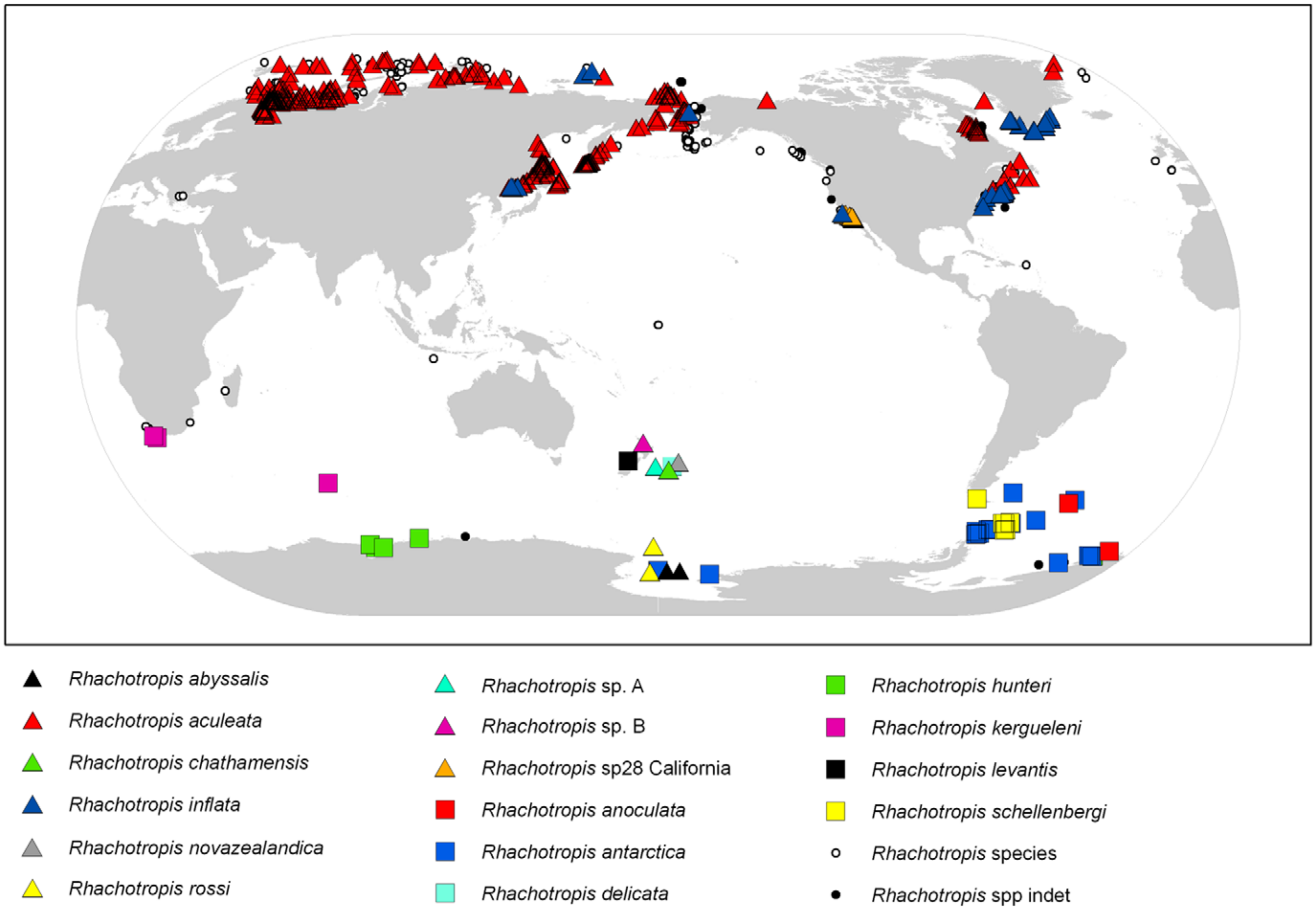

Figure 1. Global distribution map of the genus *Rhachotropis* with the species represented in the molecular part of this paper in triangles, the Southern Ocean species in squares and the remaining species (~40), including unidentified ones in small circles.
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Rise, New Zealand, had identical COI sequences and were described as *R. chathamensis* Lörz, 2010. Two specimens from the Ross Sea had identical COI sequences and belong to *R. abyssalis* Lörz, 2010. A further three specimens from New Zealand had three unique COI sequences; one specimen which is described in this paper as *R. novazealandica*, Lörz, 2012 (Fig. 2), while the other two specimens remain undescribed: R. sp. A and R. sp. B (Fig. 2). A fourth single specimen from the Ross Sea with a unique sequence was described as *R. rossi* Lörz, 2010 (Fig. 2).

Sequence divergence was zero within the *R. chathamensis* and *R. abyssalis* clades, and low within the 24 specimens of *R. aculeata* (0.0089), 9 specimens of *R. helleri* (0.0003), and 13 specimen of *R. inflata* (0.037). A single specimen identified as *Rhachotropis inflata* (Cornwallis Island) is separated distinctly from the remaining clade (separated by 3% sequence divergence). And one tentative species, *R.* sp 28 from California, is represented by one sequence retrieved from GenBank. Inter-clade sequence divergences ranged from 0.143–0.370 with an overall average divergence 0.284. The lowest divergence (0.143, Table 2) was between *R. novazealandica* spec. nov. from New Zealand and R. sp. 28 from California, while the greatest divergence was between the two putative species *R.* sp. A and *R. sp. B* (0.370, Table 2) from New Zealand.

Morphological investigation revealed a species new to science which is described herein. Even though only a single damaged specimen exists, the COI sequence and detailed morphological descriptions will allow corroboration by future collections.

Systematics

Order AMPHIPODA Latreille, 1816
Suborder GAMMARIDEA Latreille, 1802
Family EUSIRIDAE Stebbing, 1888
Genus *Rhachotropis* S.I. Smith, 1883
Rhachotropis novazealandica spec. nov.
Lörz, 2012
(Figs. 3, 4, 5, 6, 7)

Material examined. Holotype: NIWA 42864, female, 17 mm TAN0705/12, 13 Apr 2007, Box corer at 520 m, 44* 7.57 S, 174* 50.74E, R.V. Tangaroa, Collector: Ocean Survey 20/20 Chatham Rise, New Zealand.

Etymology. *Rhachotropis novazealandica* spec. nov. is named after New Zealand where the species was collected.

Diagnosis. Body delicate. Rostrum longer than head. Eyes absent. Head twice as long as pereonite 1, lateral lobes produced. Pereonites smooth. All pleonites bearing dorsal processes, pleonite 1 also bearing dorsolateral processes.

Description. Antenna 1 second article of peduncle with several plumose setae, article 2 slightly shorter than article 1, twice as long as article 3; flagellum broken after 10th-articulate. Antenna 2 peduncle article 3 longer than article 4, several plumose setae on third article; flagellum broken after 6th article.

Mandible with smooth incisor process well developed, lacinia mobilis denticulate, molar process conical. Left and right molars have several pores in the middle. The tip of the left mandibular palp

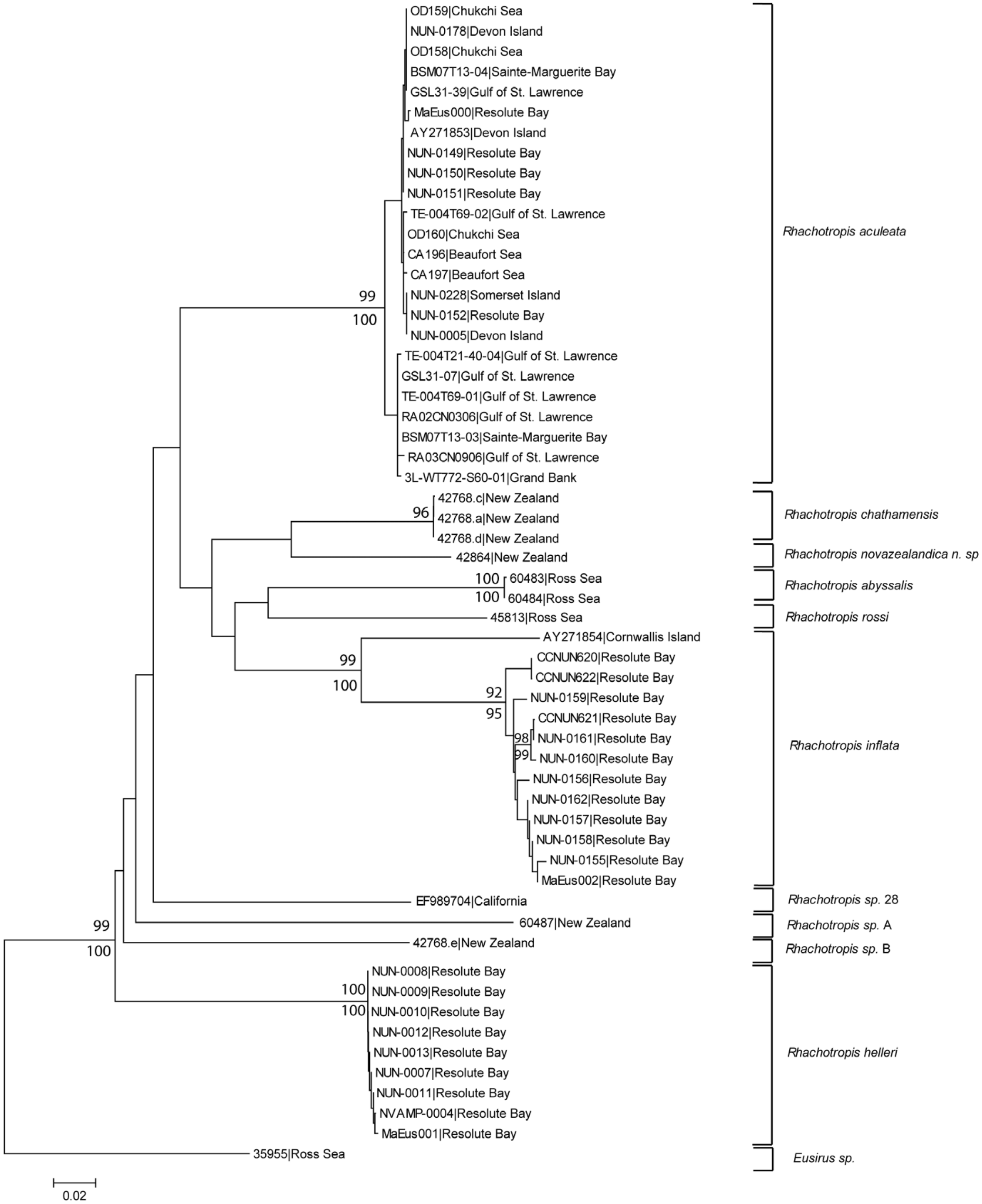

Figure 2. Relationships of COI sequences from *Rhachotropis* specimens. BOLD Accession Numbers are given for each specimen along with locations. Numbers at nodes are ML bootstrap percentages (>80%) after 1000 replications, and Bayesian inference posterior probability values (>0.90); scale bar represents an interval of the TIM+I+G model. The tree topology represents the 50% majority rule consensus of all Bayesian trees and has been rooted with the Antarctic *Eusirus*.
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Table 1. *Rhachotropis* and outgroup accession numbers in BOLD, GenBank and station data.

Species	BOLD	Sample ID	GenBank Acc#	Area	Lat	Lon	Depth
Rhachotropis abyssalis	AMPNZ095-09	60483	GU804296	Ross Sea	−76.19	176.30	447
Rhachotropis abyssalis	AMPNZ094-09	60484	GU80484	Ross Sea	−76.19	176.30	447
Rhachotropis aculeata	WWGSL070-08	GSL31-39	FJ581879	St. Lawrence Gulf	48.15	−63.54	
Rhachotropis aculeata	WW865-08	GSL31-07	FJ581880	St. Lawrence Gulf	47.90	−65.35	
Rhachotropis aculeata	WWGSL098-08	TE-004T21-40-04	FJ581881	St. Lawrence Gulf	48.39	−59.55	150
Rhachotropis aculeata	WW851-08	TE-004T69-02	FJ581882	St. Lawrence Gulf	50.82	−58.59	233
Rhachotropis aculeata	WW850-08	TE-004T69-01	FJ581883	St. Lawrence Gulf	50.82	−58.59	233
Rhachotropis aculeata	WW105-07	RA03CN0906	FJ581884	St. Lawrence Gulf	49.92	−64.62	
Rhachotropis aculeata	WW129-07	RA02CN0306	FJ581885	St. Lawrence Gulf	51.14	−58.05	
Rhachotropis aculeata	WW459-08	BSM07T13-04	FJ581886	Cote-Nord	50.25	−66.70	
Rhachotropis aculeata	WW458-08	BSM07T13-03	FJ581887	Cote-Nord	50.25	−66.70	
Rhachotropis aculeata	BENTH312-08	OD158	JQ412470	Chukchi Sea	70.00	−168.40	45
Rhachotropis aculeata	BENTH313-08	OD159	JQ412471	Chukchi Sea	70.00	−168.40	45
Rhachotropis aculeata	BENTH314-08	OD160	JQ412469	Chukchi Sea	70.00	−168.40	45
Rhachotropis aculeata	WW402-08	3L-WT772-S60-01	JQ412480	Grand Bank	46.61	−49.24	74
Rhachotropis aculeata	CCNUN228-07	NUN-0228	JQ412476	Somerset Island	72.77	−93.36	
Rhachotropis aculeata	CCNUN149-07	NUN-0149	JQ412465	Resolute	74.68	−94.86	
Rhachotropis aculeata	CCNUN150-07	NUN-0150	JQ412468	Resolute	74.68	−94.86	
Rhachotropis aculeata	CCNUN151-07	NUN-0151	JQ412467	Resolute	74.68	−94.86	
Rhachotropis aculeata	CCNUN152-07	NUN-0152	JQ412466	Resolute	74.68	−94.86	
Rhachotropis aculeata	CCNUN178-07	NUN-0178	JQ412475	Devon Island	74.67	−91.70	
Rhachotropis aculeata	CCNUN005-07	NUN-0005	JQ412473	Devon Island	75.76	−88.12	
Rhachotropis aculeata	RBGC036-03	MaEus000	DQ889127	Resolute			
Rhachotropis aculeata	WW023-07	CA196	JQ412474	Beaufort Sea	70.90	−128.90	
Rhachotropis aculeata	WW024-07	CA197	JQ412472	Beaufort Sea	70.90	−128.90	
Rhachotropis aculeata	GBCMA0080-06	AY271853	AY271853	Resolute			
Rhachotropis chathamensis	AMPNZ101-09	42768.d	GU804298	New Zealand	−43.80	175.32	418
Rhachotropis chathamensis	AMPNZ100-09	42768.c	GU804299	New Zealand	−43.80	175.32	418
Rhachotropis chathamensis	AMPNZ098-09	42768.a	GU804300	New Zealand	−43.80	175.32	418
Rhachotropis helleri	CCNUN449-08	NVAMP-0004	JQ412483	Resolute	75.08	−94.86	
Rhachotropis helleri	CCNUN007-07	NUN-0007	JQ412484	Resolute	74.68	−94.86	
Rhachotropis helleri	CCNUN008-07	NUN-0008	JQ412482	Resolute	74.68	−94.86	
Rhachotropis helleri	CCNUN009-07	NUN-0009	JQ412481	Resolute	74.68	−94.86	
Rhachotropis helleri	CCNUN010-07	NUN-0010	JQ412477	Resolute	74.68	−94.86	
Rhachotropis helleri	CCNUN011-07	NUN-0011	JQ412480	Resolute	74.68	−94.86	
Rhachotropis helleri	CCNUN012-07	NUN-0012	JQ412485	Resolute	74.68	−94.86	
Rhachotropis helleri	CCNUN013-07	NUN-0013	JQ412479	Resolute	74.68	−94.86	
Rhachotropis helleri	RBGC037-03	MaEus001	JQ412478	Resolute			
Rhachotropis inflata	CCNUN620-08	CCNUN620	JQ412491	Resolute	75.08	−94.86	
Rhachotropis inflata	CCNUN621-08	CCNUN621	JQ412492	Resolute	75.08	−94.86	
Rhachotropis inflata	CCNUN622-08	CCNUN622	JQ412493	Resolute	75.08	−94.86	
Rhachotropis inflata	CCNUN334-07	NUN-0334	JQ412487	Igloolik	69.37	−81.79	
Rhachotropis inflata	CCNUN154-07	NUN-0154	JQ412489	Resolute	74.68	−94.86	
Rhachotropis inflata	CCNUN155-07	NUN-0155	JQ412498	Resolute	74.68	−94.86	
Rhachotropis inflata	CCNUN156-07	NUN-0156	JQ412488	Resolute	74.68	−94.86	
Rhachotropis inflata	CCNUN157-07	NUN-0157	JQ412497	Resolute	74.68	−94.86	
Rhachotropis inflata	CCNUN158-07	NUN-0158	JQ412499	Resolute	74.68	−94.86	
Rhachotropis inflata	CCNUN159-07	NUN-0159	JQ412495	Resolute	74.68	−94.86	
Rhachotropis inflata	CCNUN160-07	NUN-0160	JQ412494	Resolute	74.68	−94.86	
Rhachotropis inflata	CCNUN161-07	NUN-0161	JQ412490	Resolute	74.68	−94.86	

Table 1. Cont.

Species	BOLD	Sample ID	GenBank Acc#	Area	Lat	Lon	Depth
Rhachotropis inflata	CCNUN162-07	NUN-0162	JQ412496	Resolute	74.68	−94.86	
Rhachotropis inflata	RBGC038-03	MaEus002	JQ412486	Resolute			
Rhachotropis inflata	GBCMA0081-06	AY271854	AY271854	Resolute			
Rhachotropis novazealandica n. sp.	AMPNZ128-09	42864	GU804309	New Zealand	−44.13	174.85	520
Rhachotropis rossi	ANZR470-08	45813	JF498593	Ross Sea	−76.59	176.83	369
Rhachotropis sp. 28	GBCMA1154-08	EF989704	EF989704	California	36.33	122.90	300–700
Rhachotropis sp. A	AMPNZ184-10	60487	JF498594	New Zealand	−36.52	179.20	5173
Rhachotropis sp. B	AMPNZ102-09	42768.e	HM372956	New Zealand	−43.80	175.32	418
Eusirus sp. (outgroup)	ANZR028-08	35955	JQ412464	Ross Sea	−72.08	175.55	1620

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bears six plumose setae. Maxilla 1 inner plate bearing 1 subterminal seta, outer plate with 9 denticulate spines. Maxilliped outer plate 2.5 times as long as inner plate, reaching half of article 2 of maxillipedal palp; inner margins of palp, outer plate and terminal end of inner plate setose. Labrum entire, smooth and broadly rounded. Hypopharynx setose, outer lobes with broad gap.

Gnathopod 1 coxa 1 produced, reaching to end of head, coxa 2, 3 and 4 subquadrate. Gnathopods similar in shape, subchelate. Gnathopod 1 slightly smaller than gnathopod 2, basis bearing several small spines at anterior side; merus with long setae at posteroventral corner; carpus lobe extending width of propodus, spines at terminal end of lobe; propodus widened, oval; dactylus slender, reaching end of palm.

Pereopod 5 basis small, narrow; merus longer than carpus. Pereopod 6 basis larger than of pereopod 5, posteroventral angle produced. Pereopod 7 basis widened, posterior margin serrate, posteroventral angle strongly produced; merus posteroventral angle produced.

Uropod 1 rami same length.

Remarks. *Rhachotropis novazealandica* spec. nov. differs from the other four *Rhachotropis* species from New Zealand (*R. chathamensis* Lörz 2010; *R. delicata* Lörz 2010; *R. levantis* Barnard 1961 and *R. spec* Dahl, 1959) by the combination of following characters: rounded coxa 1 (vs R. *chathamensis*), coxa 2 smaller than coxa 3 (vs *R. chathamensis*), coxa 3 and 4 ventral margin slightly bilobed (vs straight *R. chathamensis*), second segment of maxillipedal palp not broadend (vs R. spec Dahl, 1959) gnathopod 2 propodus extension exceeding article (as *R. delicata* vs *R. levantis*), uropod 1 rami same length (as *R. delicata*, vs *R. levantis*), gnathopod 1 and 2 dactylus as long as palm, basis pereopod 6 and 7 strong dorsolateral projection (vs *R. delicata*).

Distribution. New Zealand, Chatham Rise, 520 m.

Discussion

This is the first molecular study of *Rhachotropis* and has revealed a high level of diversity among specimens from the northern and southern hemispheres. The historic *Rhachotropis* collections, including the type material for most the species, were preserved in formalin or other DNA degrading media and are therefore not suitable for routine molecular investigations. Some fragile *Rhachotropis* specimens collected on recent expeditions were damaged and unsuitable for detailed morphological descriptions, but were fixed in ethanol to enable molecular studies. This study

Table 2. Nucleotide distances (TIM+I+G) within and between species/clades of *Rhachotropis*.

	N	within	*R. aculeata*	*R. inflata*	*R. helleri*	*R. abyssalis*	*R. chathamensis*	*R. sp.* **28**	*R. rossi*	*R. zealandica*	*R. sp.* **A**	*R. sp.* **B**	**Outgroup**
R. aculeata	24	0.00887											
R. inflata	13	0.03672	0.27756										
R. helleri	9	0.00035	0.26149	0.27599									
R. abyssalis	2	0	0.25909	0.26891	0.29778								
R. chathamensis	3	0	0.22547	0.25206	0.19702	0.28893							
R. sp. 28	1	n/a	0.24149	0.30575	0.27101	0.31554	0.2622						
R. rossi	1	n/a	0.2502	0.28218	0.28517	0.28076	0.2164	0.26698					
R. novazealandica	1	n/a	0.23667	0.26022	0.24123	0.27004	0.2435	0.14342	0.24142				
R. sp. A	1	n/a	0.30815	0.32293	0.31698	0.30095	0.3544	0.31781	0.31284	0.3328			
R. sp. B	1	n/a	0.26806	0.30257	0.25556	0.29027	0.3214	0.30855	0.30804	0.315	0.3699		
Outgroup	1	n/a	0.30998	0.30515	0.29003	0.30271	0.3229	0.28589	0.3078	0.3151	0.2916	0.365	

N = number of specimens.

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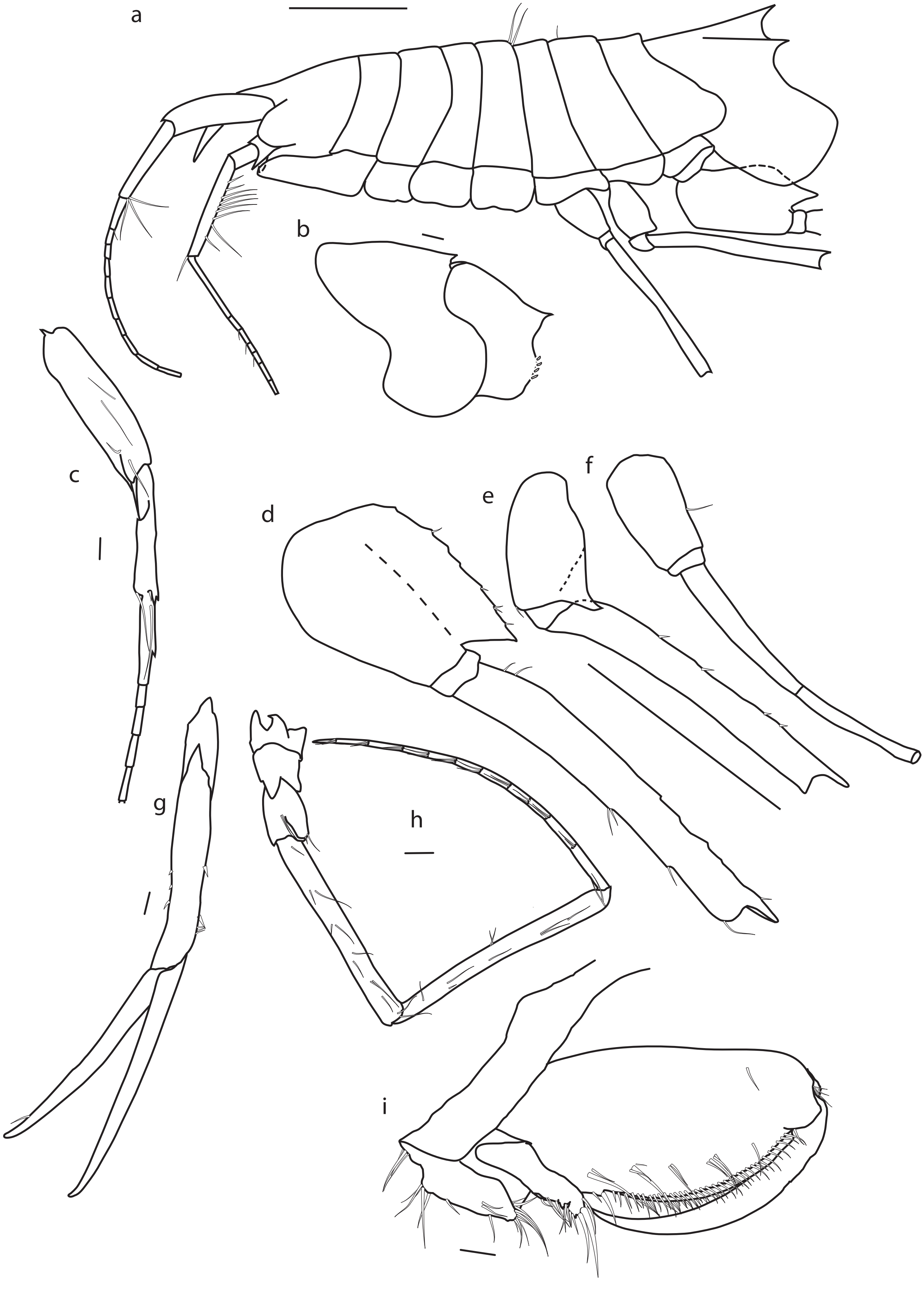
a
b
c
d
e
f
g
h
i

Figure 3. *Rhachotropis novazealandica* spec. nov., female holotype NIWA 42864. a) habitus lateral, b) epimeral plates 2 and 3, c) antenna 1, d) pereopod 7, e) pereopod 6, f) pereopod 5, g) uropod 1, h) antenna 2, i) gnathopod 1 Scalebars:a,d,e,f = 1 mm; b = 200 μm; c,g,h,i = 100 μm.
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continues the integrative approach of DNA barcoding and classic taxonomy.

Most barcode projects aim to develop open-access libraries derived from referenced (vouchered) specimens that will improve understanding of biodiversity, highlight cryptic species, and provide rapid tools for identification of a wide range of species [5,6,7]. While barcoding has its limitations, especially the discrimination of recently diverged species that underwent introgressive hybridisation, the COI barcode region has been shown to be appropriate for discrimination between closely related species across diverse animal phyla [7–10]. Barcoding can highlight potentially cryptic species that appear in discrete clades with high sequence divergences as in the *Rhachotropis* case here. High intra-specific divergences indicate that additional data are required to distinguish potential new species from known species. The barcode databases, once established can be applied to the DNA identification of specimens where traditional morphological methods are inappropriate such as stomach contents in fishes [11,12], fish fillets [13,14] and environmental barcoding for biomonitoring [15].

Although there are ongoing discussions about the level of intra- and inter-specific divergences in amphipods and the concept of species to be used [16,17] molecular species recognition is mostly based on the barcode "gap" between intra- and interspecific variations, with high inter- and low intra-clade sequence divergences indicative of cryptic species. Based on the barcode gap and consistent morphological differences, Lörz et al. [18] described and redescribed species of Antarctic Amphipoda and suggested that benthic species of Amphipoda do not occur circum Antarctic.

The inter- and intra-specific divergences of the *Rhachotropis* clades are in the same order of magnitude as for other deep sea Amphipoda (e.g. [10,18,19]). Interspecific uncorrected COI sequence distances in the Antarctic Iphimediidae varied from 7.9% (*Echiniphimedia scotti* to *E. hodgsoni*) to 29.5% (*Iphimediella cyclogena* to *I. georgei*) [18]. The deepwater Antarctic *Rhachotropis* species from the Admiralty seamount and Scott Island, to the north of the Ross Sea were in the same range, 28%.

Within the Epimeriidae sequence divergence varied from 8.5% (*E. schiaparelli* to *E. macrodonta*) to 26.15% (*E. horsti* to *E. annabellae*) [18]. Sequences of species from New Zealand's seamounts, *Epimeria horsti* and *E. bruuni* were more similar to each other than to any of the remaining Antarctic *Epimeria* species, but the distance between them was high with nearly 20%. The Antarctic *Epimera* species formed a monophyletic clade [18] while this study found the New Zealand *Rhachotrois* not to be monophyletic with the largest genetic distance of 37% between species..

Rhachotropis specimens are found in all major oceans of the world: Arctic, Atlantic Ocean, Mediterranean Sea, Carribean Sea, Indian Ocean, Pacific Ocean and the Southern Ocean (see Fig. 1 map). *Rhachotropis* specimens have been collected in all water depths (see Fig. 8a,b), from the shelf (e.g. [20]) to abyssal and hadal sampling sites (*R. rossi*, *R. abyssalis* Lörz 2010), in trenches (*R. flemmingi* Dahl 1959, Sunda Trench 7160 m; *R. spec A* Kermadec Trench, 7180, Dahl 1959), as well as around hydrothermal vents

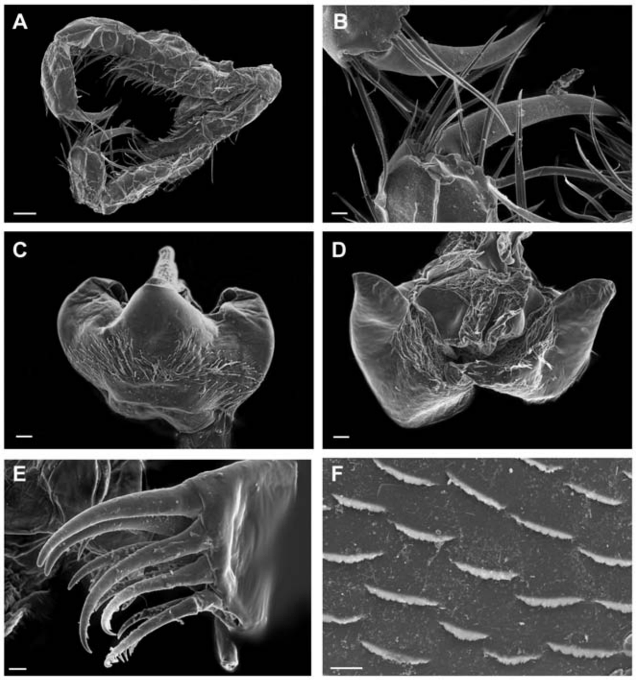

Figure 4. *Rhachotropis novazealandica* spec. nov., female holotype NIWA 42864. A) maxilliped, B) dactyli of maxillipedal palp, C) labrum, D) hypopharynx, E) maxilla 1 outer lobe, F) surface on epimal plate 2. Scalebars: A = 100 μm, B, C, D = 20 μm; E, F = 10 μm.
doi:10.1371/journal.pone.0032365.g004

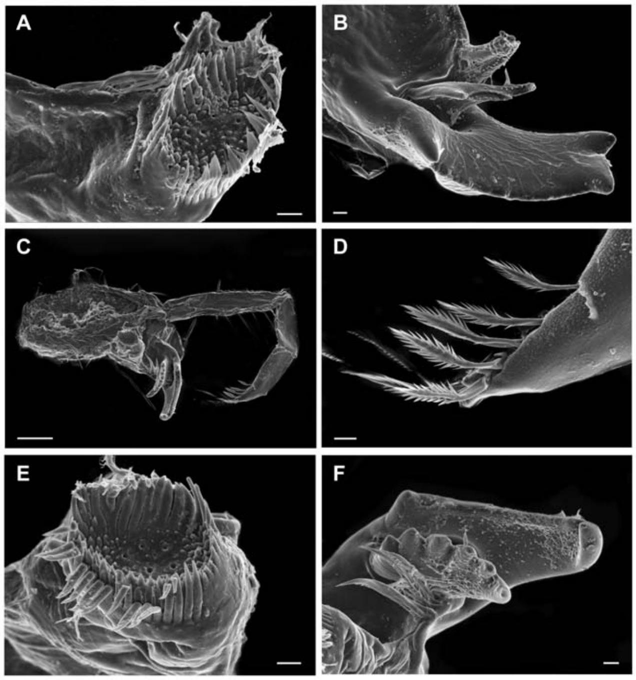

Figure 5. Mandible of *Rhachotropis novazealandica* spec. nov., female holotype NIWA 42864. A) molar, B) incisor and lacina mobilis right mandible, C) left mandible, D) mandibular palp terminal end, E) molar, F) incisor and lacina mobilis, left mandible. Scalebars: A, B, D, E, F = 10 μm, C = 100 μm.
doi:10.1371/journal.pone.0032365.g005

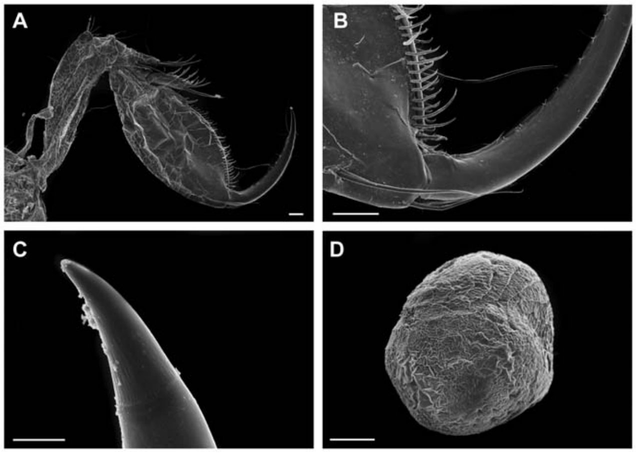

Figure 6. *Rhachotropis novazealandica* spec. nov., female holotype NIWA 42864. A) Gnathopod 1 v 2, B) palm of gnathopod 1 v 2, C) tip of dactylus, D) egg. Scalebars: A, B, D = 100 µm, C = 10 µm.
doi:10.1371/journal.pone.0032365.g006

(e.g. [21]). Specimens used in this study are from three oceans, the Arctic, Southern and Pacific Oceans. Generally more species are currently known from the shelf and upper slope area, however, the observed depth pattern is heavily sample/collection biased and areas with more stations show more species. For example, detailed sampling at one deepwater location (2700 m Iceland Basin) shows four species. Similar results are found for Southern Ocean species in general [22] and in specific groups, such as isopods and gastropods [23].

The worldwide and broad depth distribution makes *Rhachotropis* an ideal model group to test the relationship between shelf and trench faunas or biogeographic "processes" such as sub– or emergence events. Submergence describes the downwards movement/shift of taxa from the shelf/shallow water depth to deeper waters (continental slopes and abyss) while emergence represents the upward movement of taxa from deeper to shallower depth [24] Currently there is insufficient specimen or habitat coverage to provide such comparisons and present a phylogeny of the genus, but this snapshot of *Rhachotropis'* molecular biodiversity provides an indication of what could be found with integrative methods and extensive sampling.

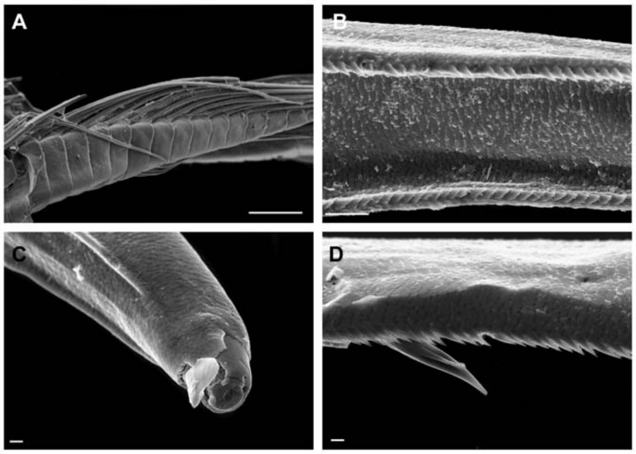

Figure 7. *Rhachotropis novazealandica* spec. nov., female holotype NIWA 42864. A) rami of pleopod 1, B) mid rami of uropod 1, C) tip of rami uropod 1, D) setation on peduncle margin of uropod 1. Scalebars: A = 100 µm, B = 10 µm, C, D = 2 µm.
doi:10.1371/journal.pone.0032365.g007

Our preliminary study suggests that the New Zealand *Rhachotropis* fauna is not monophyletic (Fig. 2), with the highest sequence divergence among all *Rhachotropis* specimens found between two species from New Zealand waters, *R.* sp. A from the Kermadec Trench (>5000 m), and *R.* sp. B, sampled from the Chatham Rise, east of New Zealand (418 m). Their position in the tree remains to be inconclusive with no node support. This suggests the use of additional molecular markers in subsequent studies. Based on COI New Zealand bathyal species seem to be closer related to Californian and Arctic shelf species than to New Zealand abyssal species. The New Zealand trench specimen shows a divergence of 30% to the Antarctic abyssal species, sampled below 3000 m at the Admiralty seamount and Scott Island. We therefore hypothesise that depth has a greater influence on the phylogeny of *Rhachotropis* than geography. The Kermadec Trench is one of the coldest trenches in the world [25]. The Deep Western Boundary Current purges Antarctic Bottom Water from the southern entrance into the Kermadec Trench [26], and it appears likely that the New Zealand trench species derive from Antarctica. However, further studies with additional molecular markers are needed to better resolve the tree and to support this hypothesis.

Further specimens from a comprehensive species set, from the shelf to abyssal and hadal depths, and additional genetic markers are required to test sub- or emergence theories. Our preliminary analyses testing DNA divergence against geography (Fig. 1) and depth (Fig. 8a, b), indicate that *Rhachotropis* could be a deep-sea taxon that has undergone several speciation events establishing it at bathyal depths (Emergence) in oceans around the world.

Materials and Methods

All necessary permits were obtained for the described field studies. Studies in the Ross Sea were undertaken under permit number AMLR07/005/Tangaroa/ZMFR, issued by the New Zealand Government by the Minister of Fisheries Jim Anderton on 19 December 2007 under New Zealand Antarctic Marine Living Resources Act 1981, for the CCAMLR statistical subareas 88.1 and 88.2. Collection of bio samples from the Kermadec Trench expedition (KAH0910) and for the Oceans Survey 2020 Chatham Challenger project (TAN0705) was undertaken under Special Permits (421 and 318) issued by the Ministry of Fisheries pursuant to section 97 (1)(i) and (ii) of the New Zealand Fisheries Act 1996.

Taxon sampling

Rhachotropis amphipods were collected during the Ocean Survey 2020 voyages of RV Tangaroa to the Chatham Rise 2007 (TAN0705) east of New Zealand and to the western Ross Sea 2008 (IPY-CAML, TAN0802), and during the RV Kaharoa voyage HADEEP #6 to the Kermadec Trench north of New Zealand 2009 (KAH0910). Specimens were immediately sorted on deck, often photographed alive on board to record live coloration, fixed in 98% ethanol and later transferred to 70% ethanol.

The amphipod specimens were identified to species level by the first author using identification keys based on morphological characters.

The amphipod specimens including the type material have been registered and curated at the National Institute for Water & Atmospheric Research (NIWA) Invertebrate Collection (NIC) in Wellington, New Zealand.

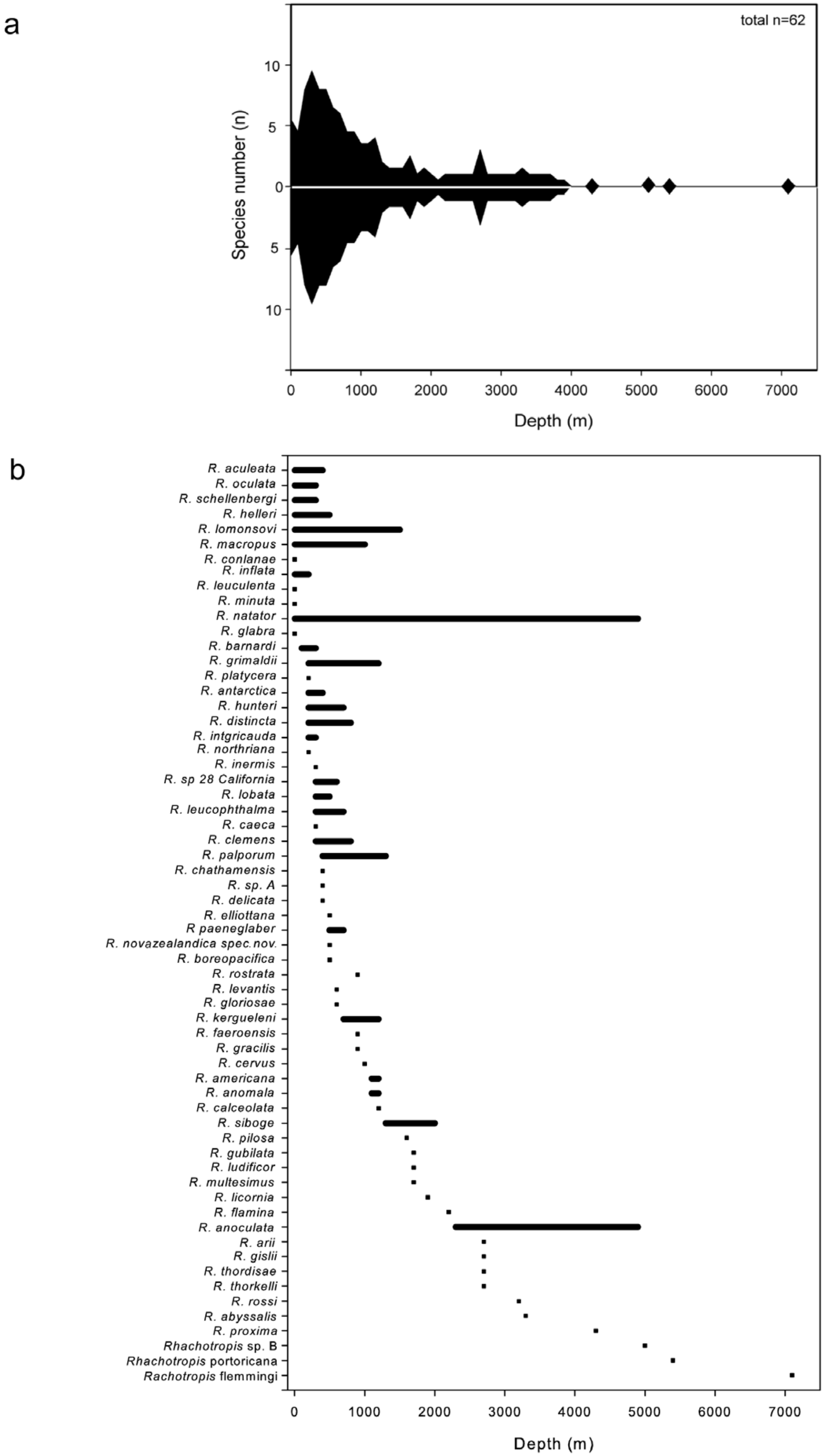
a
total n=62
Species number (n)
10
5
0
5
10
0
1000
2000
3000
4000
5000
6000
7000
Depth (m)
b
R. aculeata
R. oculata
R. schellenbergi
R. helleri
R. lomonsovi
R. macropus
R. conlanae
R. inflata
R. leuculenta
R. minuta
R. natator
R. glabra
R. barnardi
R. grimaldii
R. platycera
R. antarctica
R. hunteri
R. distincta
R. intgricauda
R. northriana
R. inermis
R. sp 28 California
R. lobata
R. leucophthalma
R. caeca
R. clemens
R. palporum
R. chathamensis
R. sp. A
R. delicata
R. elliottana
R paeneglaber
R. novazealandica spec.nov.
R. boreopacifica
R. rostrata
R. levantis
R. gloriosae
R. kergueleni
R. faeroensis
R. gracilis
R. cervus
R. americana
R. anomala
R. calceolata
R. siboge
R. pilosa
R. gubilata
R. ludificor
R. multesimus
R. licornia
R. flamina
R. anoculata
R. arii
R. gislii
R. thordisae
R. thorkelli
R. rossi
R. abyssalis
R. proxima
Rhachotropis sp. B
Rhachotropis portoricana
Rachotropis flemmingi
0
1000
2000
3000
4000
5000
6000
7000
Depth (m)

Figure 8. a) Depth distribution of *Rhachotropis* species showing that the genus spans from the shallow to the abyss; b) the depth range of the 59 named plus the 3 spp from this study (A, B and sp. 28 California) *Rhachotropis* species.
doi:10.1371/journal.pone.0032365.g008

DNA extraction and analyses

DNA was extracted from a sub-sample of muscle tissue from nine specimens using an automated Glass Fiber protocol [27]. The 650 bp barcode region of COI was amplified under the following thermal conditions: 1 min at 94°C; 5 cycles of 94°C for 40 s, 45°C for 40 s and 72°C for 1 min, followed by 35 cycles at 94°C for 40 s, 40 s at 51°C, and 1 min at 72°C; and a final step of 72°C for 1 min. The 12.5 µl PCR reaction mixes included 6.25 µl of 10% trehalose, 2.00 µl of ultrapure water, 1.25 µl 10× PCR buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl], 0.625 µl $MgCl_2$ (50 mM), 0.125 µl of each primer [0.01 mM, using LCO1490/HCO2198 [28] with M13 tails], 0.062 µl of each dNTP (10 mM), 0.060 µl of Platinum® Taq Polymerase (Invitrogen), and 2.0 µl of DNA template. PCR amplicons were visualized on a 1.2% agarose gel E-Gel® (Invitrogen) and bidirectionally sequenced using sequencing primers M13F or M13R and the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) on an ABI 3730 capillary sequencer following manufacturer's instructions.

Sequences were edited in CHROMAS 2.3 (Technelysium, Queensland, Australia), and aligned using CLUSTAL [29] in MEGA v 5.0 [30]. Net sequence divergences among taxa were estimated in MEGA v 4.1 [28]. Maximum likelihood and Bayesian analyses were performed using a nucleotide substitution model selected in Modeltest version 0.1.1 [31] using BIC and AIC criteria, and the TIM+I+G model was selected for both analyses. COI sequences in GenBank for five northern hemisphere taxa: *R. inflata*, *R.* sp 28 California, *R. aculeata*, *R. inflata*, and *R. helleri* were included in phylogenetic analyses. Maximum likelihood analysis was done using PAUP v. 4b10 [32], with support for each internode evaluated by 1000 bootstrap replications [33]. Bayesian phylogenetic analyses were estimated with MrBayes version 3.1.2 [34]. Four simultaneous Monte Carlo chains were run for 1×10^6 generations, saving the current tree every 1000 generations. Consensus trees with posterior probabilities were created with a burnin value equal to 1000 (the first 1000 trees were discarded). COI sequences for an Antarctic *Eusirus* species were used to root the trees. *Eusirus* is closely related to *Rhachotropis* and also belongs to the family Eusiridae. COI sequence data are available in BOLD and GenBank (Table 1).

Morphological description

The specimen of the new species was dissected under a Leica MZ12 stereomicroscope and drawn using a camera lucida. All illustrations were digitally 'inked' following Coleman [35,36]. Inking was done with the software Adobe Illustrator 14.0 and an A3 drawing table (Wacom Intuos 9×12).

Parts of selected specimens (mouthparts, antennae, coxal plates) were dried, coated with gold-paladium and investigated via a Scanning electron microscope LEO1525.

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The LSID for *Rhachotropis novazealandica* spec. nov. is:

urn:lsid:zoobank.org:act:F270B26E-A63D-42A2-B9F0-62A502-E2EFB4

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Author Contributions

Conceived and designed the experiments: ANL. Performed the experiments: ANL DS PS KL. Analyzed the data: ANL KL DS. Contributed reagents/materials/analysis tools: ANL KL DS PS. Wrote the paper: ANL KL DS.

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