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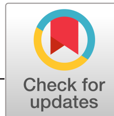

ORIGINAL ARTICLE

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Unrecognized diversity of a scale worm, *Polyeunoa laevis* (Annelida: Polynoidae), that feeds on soft coral

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Abstract

A goal of taxonomy is to employ a method of classification based on phylogeny that captures the morphological and genetic diversity of organismal lineages. However, morphological and genetic diversity may not always be concordant, leading to challenges in systematics. The scale worm *Polyeunoa laevis* has been hypothesized to represent a species complex based on morphology, although there is little knowledge of its genetic diversity. Commonly found in Antarctic waters and usually associated with gorgonian corals (especially *Thouarella*), this taxon is also reported from the south-west Atlantic, Magellanic and sub-Antarctic regions. We employ an integrative taxonomic approach to examine the traditional morphological characters used for scale worm identification in combination with COI mitochondrial gene data and whole mtDNA genomes. Moreover, we consider *P. laevis*'s association with *Thouarella* by examining data from the mMutS gene, a soft-coral phylogenetic marker. Analyses for *P. laevis* recovered 3 clades, two in Antarctic waters and one from the Argentina-Indian Ocean. Interestingly, genetic and morphological results show differences between specimens from South Argentina and the Antarctic region, suggesting that open ocean barriers might have limited gene flow from these regions. Bayesian phylogenetic analyses for *Thouarella* resulted in at least 12 lineages, although some of the lineages consist of only a single individual. Our results show different evolutionary histories for both species, confirming that association between these scale worms and their hosts is not restrictive. For both taxonomic groups, biodiversity in the Southern Ocean appears to be underestimated.

KEYWORDS

COI, mtMuts, Southern Ocean, unrecognized diversity

1 | INTRODUCTION

The Southern Ocean consists of approximately eight percent of global ocean surface area, and waters surrounding Antarctica are estimated to sustain around five percent of all marine diversity (Barnes & Peck, 2008; Poulin, González-wevar, Díaz, Gérard, & Hüne, 2014). Several studies on a wide variety of taxa including isopods, molluscs, pycnogonids and echinoderms (Brandt et al., 2007; Galaska, Sands, Santos, Mahon, & Halanych, 2017a; Held & Leese, 2007; Hunter & Halanych, 2008; Mahon, Thornhill, Norenburg, & Halanych, 2010; Munilla & Soler Membrives, 2009) have shown high levels of endemism and species radiations, which may have been driven by the glacial and oceanographic history

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of the Southern Ocean (e.g. Antarctic Circumpolar Current and Antarctic Polar Front) (Baird, Miller, & Stark, 2011; Halanych & Mahon, 2018; Thatje, Hillenbrand, & Larter, 2005). Additionally, molecular studies have revealed the presence of previously unrecognized diversity in numerous Antarctic taxonomic groups (Allcock et al., 2011; Brokeland & Rapauch, 2008; Hunter & Halanych, 2008; Janosik & Halanych, 2010; Linse, Cope, Lörz, & Sands, 2007; Mahon, Thornhill, Norenburg, & Halanych, 2010). More recently, sampling of deep-sea Antarctic polychaetes recovered cryptic diversity in 50% of species sampled based on comparison of mitochondrial DNA (Brasier et al., 2016), suggesting that diversity for this group is highly underestimated. Systematic studies on annelids from Antarctica have been mostly dependent on morphology (Blake, 2015; Parapar & Moreira, 2008), but including molecular data will improve understanding of species boundaries.

The scale worm *Polyeunoa laevis* McIntosh, 1885 (Polynoidae), offers an example of a species whose taxonomy may not match recognized morphological variation and genetic diversity. This species was first described from Prince Edward Island in the Indian Ocean but was also reported during the same expedition in the Strait of Magellan (McIntosh, 1885). Records of *P. laevis* also include the Southern Ocean, sub-Antarctic waters and southwest Atlantic (Barnich, Gambi, & Fiege, 2012; Pettibone, 1969; Stiller, 1996). In addition to their wide distribution, previous studies (Barnich et al., 2012; McIntosh, 1885) have shown that morphological characters used to identify *P. laevis* show considerable variation. The presence of cephalic peaks, coloration (Figure 1a–c) and number and arrangements of pairs of elytra have created confusion when identifying closely related species of scale worms with similar morphology. Further complicating identification, the original descriptions of *Polyeunoa* species were ambiguous to the point that past workers have stated that *P. laevis* could not be identified based on information in the literature (see Barnich et al., 2012). To help with this situation, Barnich et al. (2012) proposed a redescription for *P. laevis*, identifying this species based on the tip of the neuropodial acicular lobe not being extended to supra-acicular process and most of the neurochaetae being unidentate (Figure 1d–f). Their description facilitated the identification of *P. laevis*, although they hypothesized that this taxon might represent a species complex (i.e. two or more species previously classified as one species) (Barnich et al., 2012) of *P. laevis* and undescribed species.

Polyeunoa laevis is considered a specialist, feeding primarily on gorgonian cnidarians (Stiller, 1996) including *Thouarella*, a widespread soft-coral species commonly called the bottlebrush corals. Even though this genus can be very common, especially in Antarctic and sub-Antarctic waters, species identification and delimitation is problematic (Taylor, Cairns, Agnew, & Rogers, 2013) as morphological features are variable. The association between *P. laevis* and *Thouarella* species has been documented in several studies (Barnich et al., 2012; Pettibone, 1969; Stiller, 1996). Hartmann-Schröder (1989) reported finding a worm on every *Thouarella* collected in her study, and they proposed that this association afforded the worms protection from predators. However, no study has examined whether species-level diversity of *Polyeunoa* corresponds to species-level diversity of *Thouarella*.

Given the distribution of *P. laevis*, we examined the following questions: (a) Does current taxonomy accurately reflect morphological diversity? (b) Are the morphological features described in the literature useful for characterizing diversity? (c) Lastly, given the feeding association, do *P. laevis* and *Thouarella* have similar biogeographic patterns in Antarctica? To this end, we employ an integrative taxonomic approach that examines the traditional morphological characters used for scale worm identification in combination with molecular markers. Moreover, we consider *P. laevis*'s relationship to *Thouarella* species in the Southern Ocean.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Specimens were collected during four expeditions (i.e. *RVIB Nathaniel B. Palmer* NBP12-10, *R/V Laurence M. Gould* LMG13-12, LMG04-14 and LMG06-05) to South America and Antarctica (Figure 2a). *Polyeunoa laevis* was collected from 1 station in South America and 31 stations from the Antarctic waters including the Antarctic Peninsula and Weddell, Bellingshausen, Amundsen and Ross Seas (Figure 2a; Table 1; Table S1). *Thouarella* specimens were collected from 32 stations in Antarctic waters (Figure 2a; Table 1; Table S2). Samples were obtained with a Blake trawl. Samples were frozen after collection at −80°C, preserved in ~95% ethanol, or 4% formalin.

2.2 | Morphological examination

A total of 111 *Polyeunoa* worms were initially examined individually under a Leica MDG41 dissecting scope. Identifications were based on the morphological characters outlined in Barnich et al. (2012), including number of elytral insertions, presence of cephalic peaks, subarticular process and type of chaetae (i.e. unidentate or slightly bidentate). Most worms were disassociated from their soft-coral host because *Polyeunoa* tended to fall off of soft coral when collected by dredging and because of the specimen preservation workflow used on the ship. However, 16 of the 111 sampled worms were retrieved directly from known *Thouarella* specimens sampled for this study.

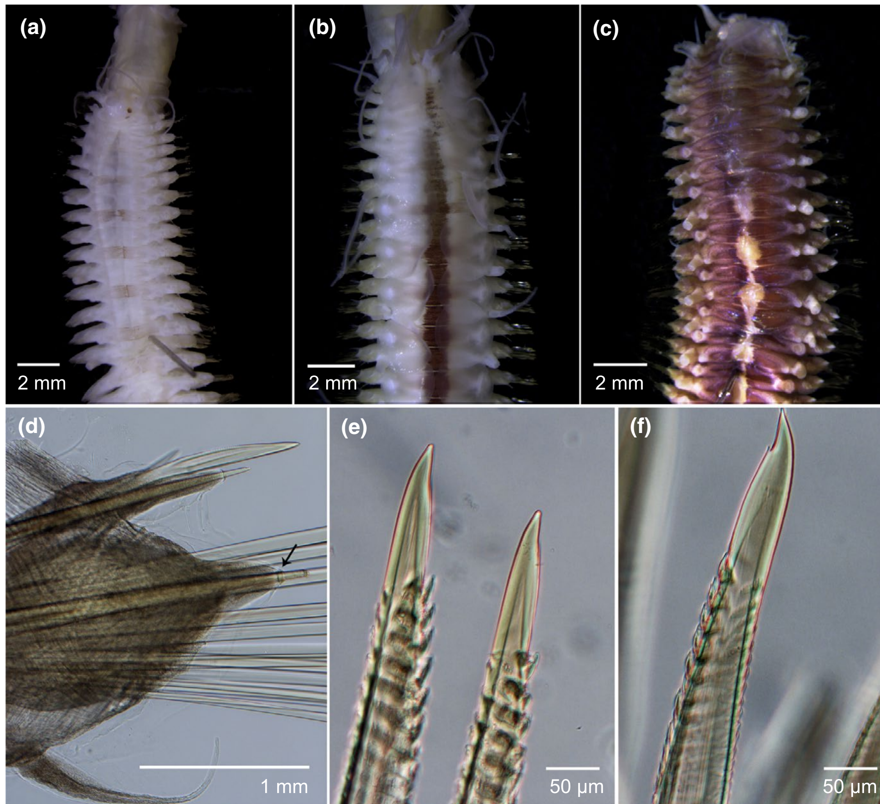

FIGURE 1 *Polyeunoa laevis*: (a-c) coloration patterns observed in the AP-Ross clade; (d) anterior parapodia, arrow points to the end of the neuropodial acicular lobe; (e) unidentate chaeta; (f) slightly bidentate chaeta

A total of 82 *Thouarella* individuals were identified based on descriptions from Taylor et al. (2013). We documented sclerite and polyp morphology of a representative subset of species using scanning electron microscopy (*SEM*). Individuals examined on *SEM* were chosen based on preservation quality of morphological features while also trying to analyse at least one individual per clade (see below). Selected specimens were washed in 30% ethanol and then transferred to successively increasing dilutions up to 100% ethanol before being air-dried. Dry specimens were mounted and sputter-coated using gold prior to analysis on a Zeiss EVO 50 *SEM*. After placing morphological examinations in the context of molecular phylogenetic results (see below), we determined that morphological characters alone were not sufficient for species-level identification of *Thouarella*. Thus, species examined here are identified by genetic/phylogenetic similarity to specimens identified and sequenced in other studies, or in the absence of genetically similar individuals from past studies, as "*Thouarella* sp.".

Polyeunoa laevis and *Thouarella* specimens are deposited at Auburn University Museum of Natural History (AUMNH; Tables 1 and 2).

2.3 | Molecular data

One millimetre tissue clips were taken from individual *Polyeunoa* specimens for DNA extraction. Similarly, 3-mm clips were taken from *Thouarella* stalks. For both taxa, whole genomic DNA was obtained using the Qiagen DNeasy® Blood and Tissue Kit. The standard barcoding region of COI was amplified and sequenced for all *Polyeunoa* using primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994). Polymerase chain reaction (PCR) mix for 25 µl reaction consisted of 1 µl of DNA template, 2.5 µl $Mg(OAc)_2$ (25 mM), 2.5 µl Taq buffer (10X), 2.5 µl dNTPs (10 mM), 1 µl of each primer (10 µM), 0.3 µl Taq DNA polymerase (25mM) and 14.2 µl water (ddH_2O). PCR cycling protocol consisted of denaturation at 96°C for 4 min followed by 35 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 1 min, followed by a final elongation at 72°C for 8 min.

Octocorals have a lower rate of mitochondrial substitution compared with other metazoans (France & Hoover, 2002; McFadden et al., 2011; Shearer, Oppen, Romano, & Worheide, 2002), and thus, we used a portion of mtMutS gene for *Thouarella* samples. The mtMutS gene fragment has been proposed to have an appropriate rate of substitution for octocoral species delimitation (McFadden et al., 2011; McFadden, Ofwegen, Beckman, Benayahu, & Alderslade, 2009). PCR used the primers ND42599F (5′-GCCATTATGGTTAACTATTAC-3′) (France & Hoover, 2002) and MUT-2458R (5′-TSGAG CAAAAGCCACTCC-3′) (McFadden, Alderslade, Ofwegen, Johnsen, & Rusmevichientong, 2006) and a cycling protocol consisted of initial denaturation at 94°C, 35 cycles of 94°C

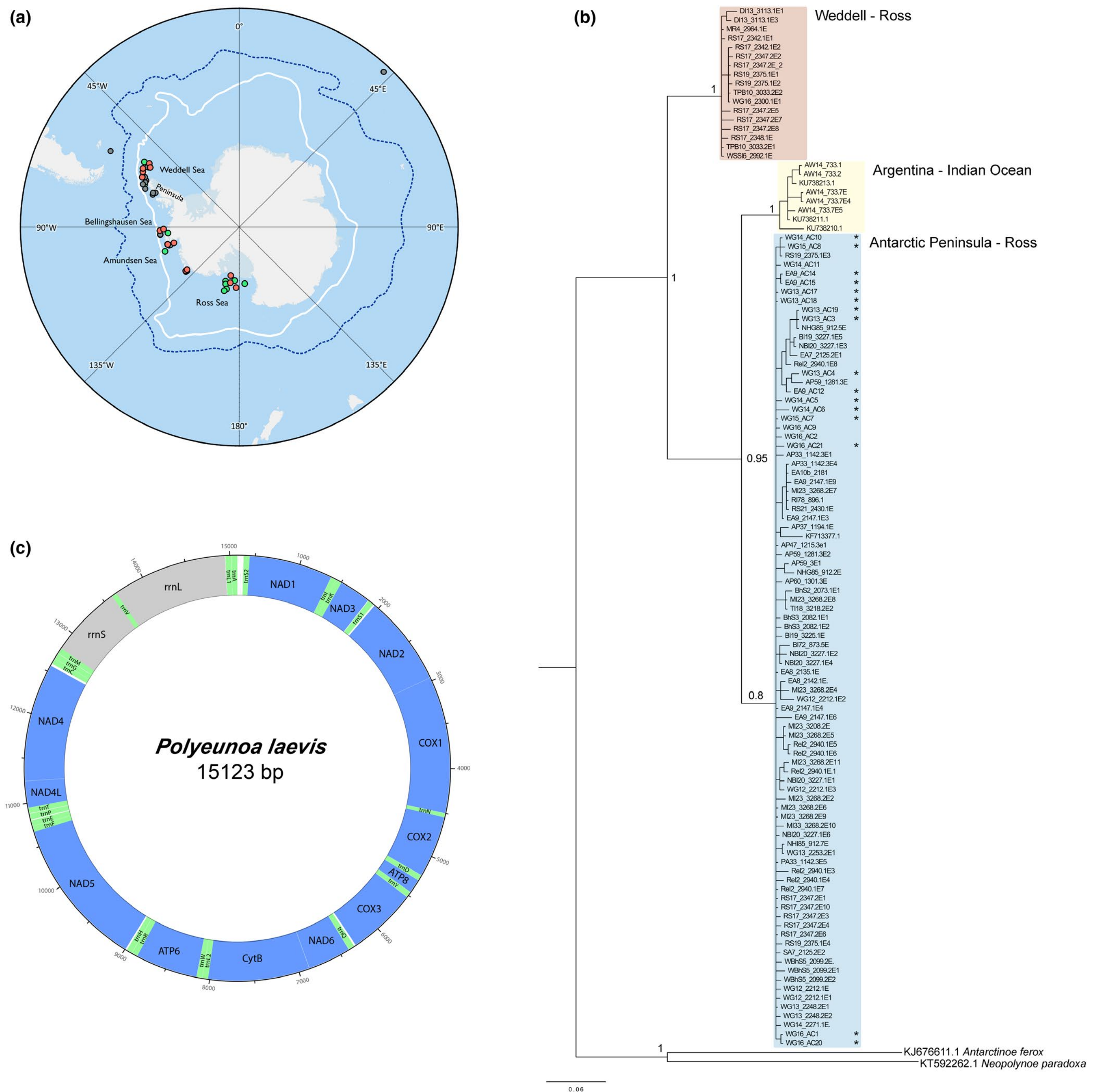

FIGURE 2 (a) Sampling localities for *Polyeunoa laevis* and *Thouarella*. Gray circles represent sampling localities for *P. laevis* only, green circles are sampling localities for *Thouarella*, and orange circles represent localities were both organisms were collected. White line represents the Southern boundary of the Antarctic Circumpolar Current, while dotted blue line represents the Antarctic Polar Front. Both of these features vary in position with season and climate. Map created with Quantarctica package (Matsuoka, Skoglund, & Roth, 2018). (b) Bayesian phylogenetic analysis for *Polyeunoa* laevis based on COI data (for specimens information see Table S1). Asterisks indicate the organisms retrieved directly from *Thouarella*. Topology is consistent with ABGD and PTP species delimitation results (see Data S2). (c) Gene order of the mitochondrial genome of *Polyeunoa laevis*. White spaces correspond to unannotated base pairs

for 1 min, 57°C for 1 min and 72°C for 1 min followed by a final extension step of 72° for 5 min. For all PCR samples, products were purified using the Qiagen QIAquick PCR Purification Kit. Purified PCR products were bidirectionally Sanger sequenced by GENEWIZ Inc. Sequences were assembled and proofread by eye with Geneious R6.

Partial COI sequences for *Polyeunoa* were aligned using default parameters of MUSCLE (Edgar, 2004) and visually inspected (GenBank accession numbers MK593024-MK593134; Table S1). Partial mtMutS sequences for *Thouarella* were aligned with MACSE (Ranwez, Harispe, Delsuc, & Douzery, 2011) using the alignSequences

TABLE 1 Summary of sampling stations and organisms collected in each location for *Polyeunoa laevis* and *Thouarella*

Station/ Locality	*Polyeunoa*	*Thouarella*	Latitude	Longitude	Depth
Station 33 Antarctic Peninsula	✓		−67.740	−69.290	122
Station 37 Antarctic Peninsula	✓		−68.186	−67.595	232
Station 47 Antarctic Peninsula	✓		−67.663	−68.245	170
Station 59 Antarctic Peninsula	✓		−64.925	−63.534	360
Station 60 Antarctic Peninsula	✓		−65.026	−63.304	400
Station 14 Argentinean Waters	✓		−54.690	−59.392	207
Station 7 Eastern Amundsen	✓		−72.483	−104.563	591
Station 8 Eastern Amundsen	✓		−72.781	−104.554	496
Station 23 Myriad Islands	✓		−65.021	−64.425	312
Station 85 Near Hugo Island	✓		−64.688	−65.927	368
Station 2 Racovitza Island	✓		−64.411	−61.963	664
Station 78 Renaud Island	✓		−65.624	−67.785	217
Station 10 Tabarin Peninsula	✓		−63.686	−56.859	400
Station 5 Western Bellingshausen	✓		−70.842	−95.411	472
Station 12 Wrights Gulf	✓		−73.159	−129.895	440
Station 16 Wrights Gulf	✓	✓	−73.247	−129.503	478
Station 14 Wrights Gulf	✓	✓	−73.499	−129.919	516
Station 9 Eastern Amundsen	✓	✓	−73.722	−103.617	699
Station 13 Wrights Gulf	✓	✓	−73.297	−129.192	506
Station 15 Wrights Gulf	✓	✓	−73.710	−129.056	655
Station 2 Bellingshausen	✓	✓	−70.812	−92.522	430
Station 3 Bellingshausen	✓	✓	−71.702	−91.504	430
Station 19 Brabant Island	✓	✓	−63.845	−62.624	248
Station 20 North Brabant Island	✓	✓	−63.834	−62.664	256
Station 13 Danvers Island	✓	✓	−63.576	−54.629	227
Station 10b Eastern Amundsen	✓	✓	−72.204	−103.596	612
Station 18 Tower Island	✓	✓	−63.389	−60.120	310
Station 4 Montravel Rock	✓	✓	−62.996	−58.599	320
Station 17 Ross Shelf	✓	✓	−75.330	−176.985	570
Station 19 Ross Shelf	✓	✓	−76.341	−170.850	531
Station 21 Near Ross Shelf	✓	✓	−78.063	−169.991	549
Station 6 Weddell Sea	✓	✓	−64.302	−56.136	290
Station 4 Bellingshausen		✓	−72.699	−94.694	670
Station 5 Western Bellingshausen		✓	−70.842	−95.411	472
Station 10 Eastern Amundsen		✓	−72.177	−103.514	341
Station 11 Eastern Amundsen		✓	−71.147	−108.005	627
Station 20 Ross Shelf		✓	−76.479	−165.738	457
Station 22 Near Ross Shelf		✓	−76.998	−175.093	541
Station 23 Mid-Ross Sea		✓	−76.245	174.504	604
Station 25 North of Ross Island		✓	−75.833	−166.505	552
Station 26 Northwest Ross Island		✓	−74.708	−168.408	489
Station 27 Northwest Ross		✓	−74.182	−166.661	390
Station 5 Anderson Island		✓	−63.701	−56.079	293
Station 11 Erebus Terror Bay		✓	−63.935	−56.571	394

(Continues)

TABLE 1 (Continued)

Station/ Locality	*Polyeunoa*	*Thouarella*	Latitude	Longitude	Depth
Station 12 Dundee Island		✓	−63.754	−55.684	334
Station 14 NE D'Urville Island		✓	−62.442	−55.459	245
Station 25 Hugo Island		✓	−65.087	−65.809	202
Indian Ocean*	✓		−41.346	42.922	1,361
Indian Ocean*	✓		−41.346	42.922	1,358
Indian Ocean*	✓		−41.380	42.854	1,017
Indian Ocean*	✓		−41.357	42.918	917

Note: Asterisks indicate samples from Serpetti et al. (2016).

command. Aligned COI and mtMutS sequences were translated using the invertebrate mitochondrial code 5 and coelenterate mitochondrial code 4, respectively, to assure that stop codons or frameshift mutations were not present in alignments (GenBank accession numbers MN121855–MN121936; Table S2). Available sequences from GenBank for *P. laevis* and *Thouarella* were retrieved and included in the analyses (Tables S1 and S2).

2.4 | Mitochondrial genome of *Polyeunoa laevis*

To further assess genetic variation within *Polyeunoa* individuals, the whole mitochondrial genome was sequenced for three specimens (AW14_733.7E, RS19_2375.1E2, NBI20_3227.1E3; Table S1). DNA extractions were performed as described above. Sequencing of total genomic DNA was performed by Novogene Inc. on an Illumina HiSeq 2500 platform, using 2 x 150 pair-end v4 chemistry. Paired-end reads were assembled de novo with Ray 2.2.0 (k-mer = 31; Boisvert, Raymond, Godzaridis, Laviolette, & Corbeil, 2012). Potential mitochondrial genomes were identified using BLASTn (Altschul et al., 1997) and previously published COI sequences of *P. laevis* (GenBank accession numbers KU738210–KU738214, KF713377) were used as bait against the assembled data (GenBank accession numbers MN057924–MN057926). Annotation was conducted using MITOS2 web server (Bernt et al., 2013), and gene boundaries were manually verified. Uncorrected pairwise genetic distances (*p*) were estimated with MEGA7 (Kumar, Stecher, & Tamura, 2016).

2.5 | Molecular analyses

Bayesian inference of nucleotide data was used to reconstructed trees for *Polyeunoa* and *Thouarella* using MrBayes 3.2.6 (Huelsenbeck & Ronquist, 2001) using best-fit partitions as indicated by PartitionFinder (Lanfear, Calcott, Ho, & Guindon, 2012) (i.e. *Polyeunoa* COI: split by codon position; *Thouarella* mtMutS: single partition). Additionally, sequences of *Antarctinoe ferox*, *Neopolynoe paradoxa* (GenBank accession numbers KJ676611, KT592262; Polynoidae, Annelida) and *Calyptrophora* (GenBank accession numbers DQ297427, DQ234756, JX561183; Primnoidae, Cnidaria) were chosen to serve as outgroups for *P. laevis* and *Thouarella,* respectively, based on current understanding of phylogeny (Cairns & Wirshing, 2018; Zhang et al., 2018).

For each partition, a reversible jump Markov chain Monte Carlo (MCMC) was used for model averaging across the GTR models and rate heterogeneity for each partition was modelled using four discrete categories of a gamma distribution as indicated by PartitionFinder. Four independent runs with four Metropolis-coupled chains each were run for 10,000,000 MCMC generations and sampled every 1,000 generations. For each data set, commands used in MrBayes analyses can be found in Data S1. Stationarity of each run was checked with Tracer 1.7.1 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018), and 25% burn-in was determined to be appropriate for each analysis. Both analyses appeared to reach convergences as all parameters had a potential scale reduction factor of 1.0. A 50% majority rule consensus tree was calculated from each analysis using the "sumt" command in MrBayes.

2.6 | Genetic distance, species delimitation and haplotypes

Uncorrected pairwise genetic distances (*p*) were obtained with MEGA7. Arlequin 3.5.2.2 (Excoffier & Lischer, 2010) was used to conduct a Tajima's D (Tajima, 1989) test to determine whether sequence variation fit the neutral mutation model. Neutrality tests such as Tajima's D can provide some insights about demographic forces affecting a population. Analyses of molecular (AMOVA) variance were also conducted with Arlequin 3.5.2.2 to test for genetic differentiation; regions were defined by sampling locality and then grouped by geographic regions (e.g. Amundsen, Antarctic

TABLE 2 Nucleotide and haplotype diversity for the 3 lineages of *Polyeunoa laevis* and Tajima's *D*

Clade	ns	vs	nh	π	*h*	Tajima's *D*
AP-Ross	90	56	59	0.00995 ± 0.0005	0.980 ± 0.004	-1.89399 *
Argentina-Indian Ocean	8	27	6	0.0016 ± 0.004	0.944 ± 0.070	-0.83614
Weddell-Ross	17	22	13	0.0056 ± 0.0008	0.956 ± 0.037	-1.72597

Note: Asterisk indicates significant values ($p < .005$).

Abbreviations: *h*, haplotype diversity; nh, number of haplotypes; ns, number of samples; vs., number of variable sites; π, nucleotide diversity.

Peninsula, Bellingshausen, Ross; Table S1). Haplotype diversity and nucleotide diversity were also estimated for each clade.

We used two methods to assess species boundaries for *Polyeunoa* and *Thouarella*. First, barcode gap discovery (ABGD; Puillandre, Lambert, Brouillet, & Achaz, 2012) was used to determine whether a barcode gap in percent nucleotide difference existed with the data sets that could be used for species delimitation. ABGD default values were used for *P. laevis*, while 0.3% intraspecific cutoff value was used for *Thouarella* as suggested by Quattrini et al. (2019). However, the lack of a barcode gap in mtMutS (Wirshing & Baker, 2015) might limit resolution of ABGD for *Thouarella* species delimitation. We also used Poisson Tree Processes (PTP; Zhang, Kapli, Pavlidis, & Stamatakis, 2013) which uses non-ultrametric trees with a scale of expected substitutions/site. For this, 10,000 trees from the posterior distribution of MrBayes analyses (i.e. 2,500 from each of the four independent runs) were used as input.

A TCS network was constructed for the largest of three clades containing *Polyeunoa laevis* individuals (see below) was generated using PopART (http://popart.otago.ac.nz), which utilizes statistical parsimony (Clement, Posada, & Crandall, 2000).

3 | RESULTS

Given the focus of this study, results of both the morphological and molecular analyses for *Polyeunoa* will be described first, followed by results for *Thouarella*.

3.1 | Morphological comparisons

Morphological characters showed different patterns between the *Polyeunoa laevis* from South Argentina and Antarctica. Individuals from South Argentina present between 12 and 18 alternate pairs of elytra after setiger 32 (determined by the presence of elytra insertions), whereas specimens from the Southern Ocean only have 0, 1 or 2 pairs of elytra after setiger 32, except for one specimen that showed 4 (Table S3). These comparisons were made for individuals with 60–80 segments. Specimens from Antarctica showed morphological variation, but consistent differences were not observed between individuals from different localities. Dorsal coloration, for example varies from red horizontal lines, to one red longitudinal line (anterior to posterior region), or most of the dorsal region covered with a red-purple pigment (Figure 1a–c); cephalic peaks were present in some specimen and absent in others (Table S3). Neurochaetae were also examined for each specimen and were found to be consistent with previous descriptions, with most of the worms having unidentate neurochaetae and some with bidentate tips (Figure 1d–f). The chaetae, parapodia from the anterior, middle and posterior region of 9 specimens (3 from each clade, see below), were more closely examined with scanning microscopy (*SEM*), although no clear differences were observed (Figure S1).

Syntypes deposited at the Natural History Museum of London (NHM) were also examined for this study as a holotype is not available. Given the proximity with samples used in the COI analysis from Serpetti et al. (2016; Figure 2a), we expected the distribution of the elytra after setiger 32 to follow a similar pattern to specimens from southern Argentina and the Indian Ocean. However, the syntypes are morphologically more similar to the specimens from the Southern Ocean and only have a few (0–3) elytra scars after setiger 32.

3.2 | Molecular analyses

The molecular data set for *Polyeunoa laevis* consisted of a 657 bp fragment of the mitochondrial COI for 115 *Polyeunoa* individuals (including four sequences from GenBank that included 1 Southern Ocean sequence and 3 Indian Ocean sequences (Gallego, Lavery, & Sewell, 2014; Serpetti et al., 2016; Table S1). The data set included 116 (18%) variable sites and 82 (12.5%) parsimony informative sites. Out of the 115 sequences, 78 haplotypes were recovered, and only 17 haplotypes were sampled more than once. Bayesian inference recovered 3 main clades (Figure 2b). One clade included most of the samples from the East Antarctic Peninsula, Weddell and Ross Seas (Weddell-Ross), a second clade corresponds to the samples from Argentina and the Indian Ocean, and the third clade includes specimens from the West Antarctic Peninsula, Bellingshausen Sea, Amundsen Sea and Ross Sea

(AP-Ross). The same analysis was conducted using midpoint rooting, and the recovered topology was the same. Individuals from the two Antarctic clades lacked morphological differentiation. In contrast, the clade from Argentina showed genetic and morphological distinction (see above morphological comparisons) when compared to the Antarctic clades.

3.3 | Mitochondrial genome

A single contig representing the mitochondrial genome was recovered for all three samples of *Polyeunoa* sequenced in this study. These contigs ranged in length from 15,118 to 15,123 bp and had an average GC content of 33.4% (Tables S4). MITOS2 annotation recovered 13 protein coding, 22 tRNA and 2 rRNA genes. Composition and gene order (Figure 2c) is consistent with previously published mitochondrial genomes of annelids and specifically polynoids (Boore, Boore, & Brown, 2000; Jennings & Halanych, 2005; Vallès & Boore, 2006; Weigert et al., 2016; Zhang et al., 2018). Uncorrected pairwise genetic distance (*p*) for complete mitochondrial genomes ranged around 4% among the genomes of representatives from AP-Ross and Argentina-Indian Ocean clades, and 7%, when comparing Weddell-Ross against the other clades (Tables S4).

3.4 | Genetic distances, species delimitation and haplotype network

For *Polyeunoa*, the average of uncorrected pairwise genetic distances (*p*) within groups was <1% (Tables S4). Between clades, genetic distances ranged from 4% to 8%. The greater distances were found when comparing the Weddell-Ross clade with the two other clades. Tajima's *D* test was negative and significant for the AP-Ross clade (Tables S4). Tajima's *D* values were not significant for the other clades. Significant negative values of Tajima's *D* are the result of rare polymorphism in a population, which could be an indication of purifying selection or a recent population size expansion (Tajima, 1989). Both of the species delimitation methods, ABGD and PTP, suggest three distinct species that are congruent with the clades recovered with Bayesian analysis (Data S2).

Haplotype diversity (*h*) values were similar for each clade (Table 2). Nucleotide diversity (π) was higher in the AP-Ross clade (0.009), while Argentina-Indian Ocean clade showed the lowest value (0.001; Table 2). A haplotype network was constructed for the AP-Ross clade, and 59 haplotypes out of 90 individuals were identified. This group included most of the specimens from the Western Antarctic Peninsula, Bellingshausen and Amundsen Sea (Figure 3a), although no obvious population structure is observed. This is supported by the results from the AMOVA showing that 98.35% of the variation was found within regions (Tables S4). Due to limited numbers of samples, haplotypes network and AMOVA analysis were not constructed for the other clades.

3.5 | Biogeography of *Polyeunoa laevis* and *Thouarella*

Morphology of 26 *Thouarella* individuals was documented with *SEM* images (Figure S2), although reliable morphological identification of species was not feasible (McFadden, Alderslade, et al., 2006; McFadden, France, Sánchez, & Alderslade, 2006) as individuals with identical morphologies seen through *SEM* were sometimes recovered in different clades and individuals with disparate morphologies were recovered in the same clade. Thus, individuals were identified using a barcode approach. The molecular data set for *Thouarella* consisted of 756 bp fragment of the mtMutS for 109 specimens, including 27 from GenBank (McFadden, Alderslade, et al., 2006; McFadden et al., 2011; Taylor & Rogers, 2015). The data set included 116 (15%) sites that were variable and 103 (13.6%) that were parsimony informative. Out of the 109 sequences, 35 haplotypes were recovered.

Species delimitation methods for *Thouarella* resulted in at least 12 lineages (Figure 3b), although some of the lineages consist of a single individual. ABGD partitioned the data into 12 different groups, while 27 were found with PTP (Figure 3b), although some partitions from the PTP have low support (Data S3). Additionally, various lineages that included previously identified *Thouarella* species clustered different species together (e.g. *T. variabilis* and *T. chilensis*), while other lineages remain as *Thouarella* sp. since they did not correspond to any previous *Thouarella* barcodes for the Southern Ocean. Given the small sample number of individual groups, we limit our interpretation of these results as resolving phylogenetic relationships among *Thouarella* species is beyond the scope of this study and will likely require several genomic markers (e.g. Quattrini et al., 2019).

Taken as a whole, results show different phylogeographic patterns for *P. laevis* and *Thouarella*. With respect to the worms retrieved directly from *Thouarella*, they were all found to be in the AP-Ross clade (Figure 2b); however, they inhabit different *Thouarella* lineages (Figure 3b). That is, *P. laevis* does not appear to be a species-level specialist in regard to *Thouarella* host/habitat preference although whether their preferences are limited remain unknown.

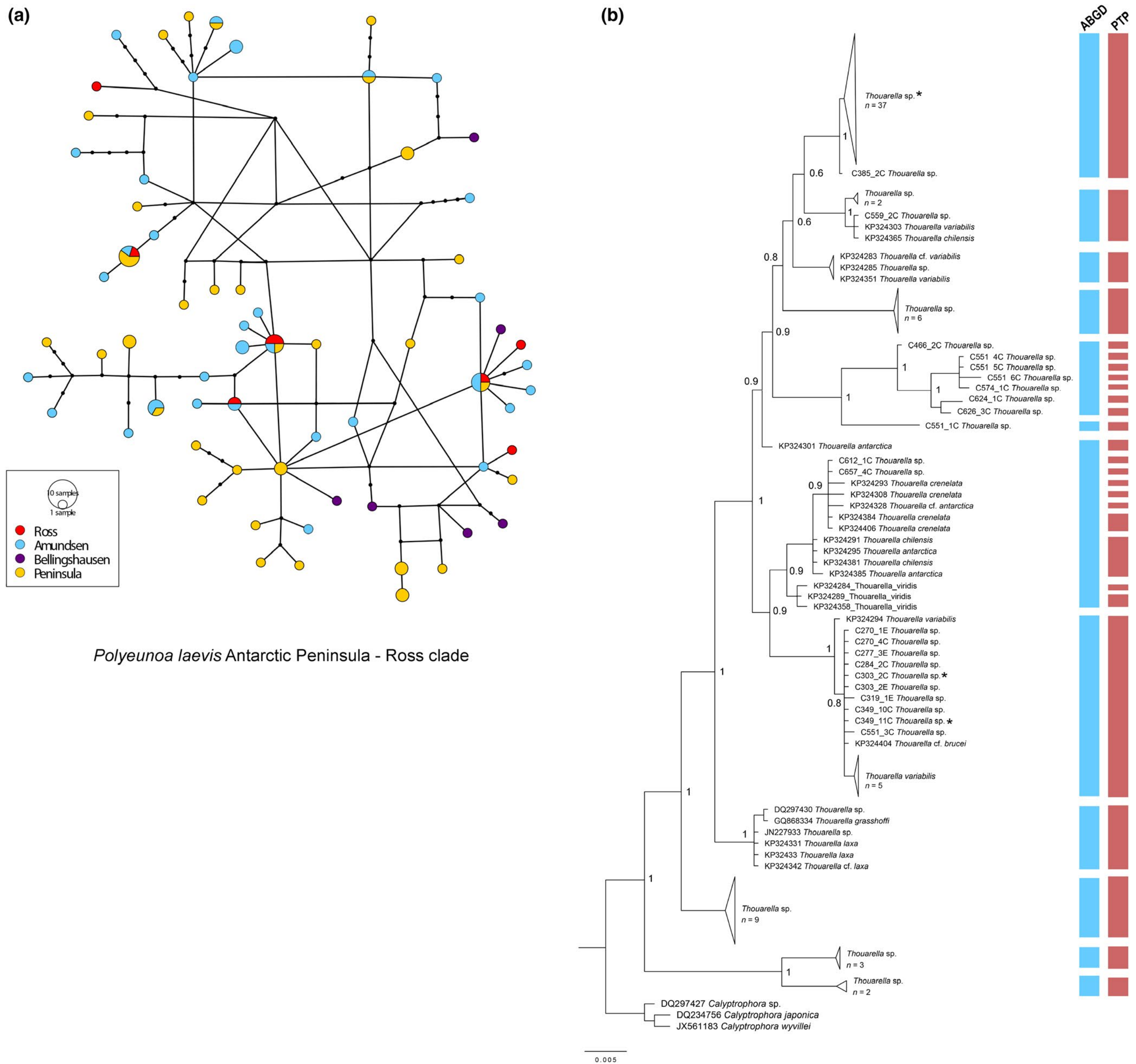

FIGURE 3 (a) COI haplotype network for AP-Ross clade of *Polyeunoa laevis*. The size of the circles represents the frequency of the haplotype, connecting lines represent one mutation step between haplotypes. Black dots indicate missing haplotypes. (b) Bayesian phylogenetic analysis for *Thouarella* based on mtMutS. Asterisks indicate the corals from which *Polyeunoa* worms were directly retrieved. Vertical bars show species delimitation results from ABDG (red line) and PTP (blue line). GenBank accession numbers are shown with the taxon labels

4 | DISCUSSION

Polyeunoa laevis represents at least 3 genetic lineages with two lineages from the Southern Ocean and a third from South America and the Indian Ocean. Distinct morphologies were found when comparing specimens from Argentina and the Southern Ocean, but the two genetic lineages from the Southern Ocean show no morphological differences. These results confirm the possibility that *P. laevis* represents a species complex (Barnich et al., 2012) and that current taxonomic delineation does not reflect the actual diversity within the group.

Although most polynoids are considered to have planktotrophic larvae with potential to disperse long distances (Giangrande, 1997; Wilson, 1991), there are no studies on the development of *P. laevis* and its larvae type is unknown. However, independent of the larval type, we found genetic connectivity over 7,000 km range (Weddell-Ross lineage) suggesting the capacity of *P. laevis* to disperse long distances, even though barriers have limited dispersion of these organisms in and out of the Southern Ocean. Genetic breaks between South America and Southern Ocean fauna have been reported in similar studies. Thornhill, Mahon, Norenburg,

and Halanych (2008) showed genetic differentiation of populations of the nemertean *Parborlasia corrugatus* in South America, Antarctic and sub-Antarctic waters, by analysing mitochondrial 16s rRNA and COI sequence data, and found two lineages to be geographically separated possibly by the Antarctic Polar Front (APF). One lineage included all organisms from Antarctic and sub-Antarctic region, and a second lineage including individuals from South Argentina only. Similarly, Shaw, Arkhipkin, and Al-Khairulla (2004) examined genetic structure of toothfish around the Southern Ocean and South Argentina and found that, despite the potential for high dispersal, there is genetic differentiation between Patagonian and Southern Ocean toothfish. These studies and others (e.g. Hunter & Halanych, 2008) suggested the APF as the main barrier restricting larval dispersion, and consequently limiting gene flow between the adults. Although, the APF is not completely impermeable, and exchange of organisms from Antarctic to South American waters have occurred promoting radiation of the group. Sands, O'Hara, Barnes, and Martín-Ledo (2015) for example, found molecular evidence of migration of the ophiuroid *Ophiura lymanii* moving from the Southern Ocean to South America (also see Galaska, Sands, Santos, Mahon, & Halanych, 2017b). As previously mentioned, *P. laevis* individuals from Argentinean waters do show morphological differences compared with Southern Ocean individuals. Unfortunately, specimens from the Indian Ocean (Serpetti et al., 2016) could not be examined morphologically, and elytra pattern of these organisms remains unclear. Given this situation, we were not able to determine whether the differences found in the distribution of the elytra can be used as a diagnostic character since organisms from Argentina and the Indian Ocean are clustered in the same genetic lineage. Additionally, syntypes from Prince Edward Island are morphologically consistent with Antarctic specimens, but whether these organisms belong to the Argentina-Indian Ocean clade or one of the Southern Ocean clades is unknown. Prince Edward Island is a sub-Antarctic Island located in the Indian region of the Southern Ocean (Ansorge, Froneman, & Durgadoo, 2012). We restrain from describing a new species until a more extensive morphological examination can be completed, especially from organisms from sub-Antarctic regions.

Interestingly, we found genetic connectivity between samples from Ross and Weddell Seas, two regions not currently connected. Previous studies have shown an affinity between the fauna of these regions, including gastropods, bivalves (Linse, Griffiths, Barnes, & Clarke, 2006) bryozoans (Barnes & Claus-Dieter, 2010), and more recently the ophiuroid *Ophionotus victoriae* (Galaska et al., 2017a). Barnes and Claus-Dieter (2010) suggested that similarity between these regions is to some extent the result of a past connectivity between Ross and Weddell Seas, as a consequence of the collapse of the West Antarctic Ice Sheet (Scherer, Aldahan, Tulaczyk, Engelhardt, & Kamb, 2009), opening a direct trans-Antarctic passage that allowed the exchange of organisms between the West Antarctic waters. Evidence from sediment cores suggests a near complete collapse occurred ~1.1 MYA and modelling suggests a collapse as recent at 125 KYA (Feldmann & Levermann, 2015; Naish et al., 2009; Pollard & DeConto, 2009). This past connectivity would help to explain the occurrence of a geographically discontinuous Antarctic lineage for the *Polyeunoa* worms.

Current morphological examination of *P. laevis* cannot resolve whether the range of variation observed in morphological characters across Antarctic and sub-Antarctic specimens represent one or more species. For several taxa in the Southern Ocean, molecular results yield lineages often unrecognized by morphological characters (e.g. annelid *Glycera kerguelensis*, Schüller, 2011; notothenioid fish Matschiner, Hanel, & Salzburger, 2009; octopuses, Allcock et al., 2011; crinoids, Hemery et al., 2012; pycnogonids, Harder, Halanych, & Mahon, 2016). Several hypotheses have been proposed to account for this discrepancy between morphology and molecular data (Allcock & Strugnell, 2012; Halanych & Mahon, 2018; Thatje et al., 2005). Among the most commonly accepted is the idea that during glaciation events benthic taxa were forced into refugia to survive. Such refugia may have been in deeper water along the continental slope or in polynyas. The resulting isolation (Thatje et al., 2005) would have facilitated establishment of genetically isolated lineages by genetic drift, even though morphologies could remain similar (Janosik & Halanych, 2010). In the case of *Polyeunoa* lineages from the Southern Ocean, the morphology is similar despite genetic differences resulting in cryptic lineages.

Given the association between *P. laevis* and *Thouarella*, we questioned if these taxa would have similar phylogeographic patterns, but that is not the case based on the data collected for this investigation. *P. laevis* is considered a poly-xenous polynoid as it has been found on other corals including *Dasytenella* and *Primnoisis* (Barnich et al., 2012; Serpetti et al., 2016; Stiller, 1996) or can be free-living even though the association with *Thouarella* might be more common (Hartmann-Schröder, 1989). However, the specificity of *P. laevis* for *Thouarella* remains unclear. Importantly, examined specimens from South Argentina showed differences in the number of elytra by having more elytra covering the posterior region. Pettibone "1991" reported that polynoids living in association with soft corals tend to have elytra present only in their anterior region, while worms showing a more free-living lifestyle have elytra in the anterior and posterior region of the body. *Thouarella* is better represented around the Southern Ocean than South Argentina in terms of number of species (Taylor & Rogers, 2015; Zapata-Guardiola & López-González, 2010) and abundance (K.M. Halanych pers. observation) Thus, the lack of elytra in the posterior

region of *Polyeunoa* lineages in the Southern Ocean possibly represents a morphological adaptation to availability of *Thouarella* hosts.

On the other hand, *Thouarella* species delimitation has been considered problematic and many species are in need of revision (Zapata-Guardiola & López-González, 2010). Furthermore, a recent study recovered *Thouarella* as a polyphyletic group (Taylor & Rogers, 2015). Molecular data generated here, for example recovered specimens previously identified as *T. variabilis*, *T. crenelata* and *T. antarctica* in multiple regions on the tree (Figure 3b), suggesting that current understanding of morphology does not align with genetics. Results from species delineation analyses (i.e. ABGD and PTP) disagree in the species boundaries for some clades (e.g. *T. crenelata*, *T. antarctica* and *T. chilensis*). These species are placed within the "Antarctica gruppe" which as described by Taylor et al. (2013) represents the *Thouarella* group with the smallest morphological variations between the species. Whether this group represents many or one single variable species has been questioned.

Scale worms are often the most common organisms found in association with soft corals, and when present can change coral growth, and alter their morphology. Molodtsova and Budaeva (2007) examined over 300 specimens of black corals and found that taxonomic characters important for species identification were modified by the scale worms, in some cases identification of the corals was not possible due to the changes caused by the worms. Morphological modification by *Polyeunoa* in *Thouarella* should be evaluated, as it could represent a source of morphological variation in an already challenging group.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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