

Austropallene halanychi sp. nov., a new species of sea spider (Pycnogonida, Callipallenidae) from the Ross Sea, Antarctica

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Abstract

Here we present *Austropallene halanychi* sp. nov., a new species of pycnogonid within the family Callipallenidae (Pycnogonida), collected from the Ross Sea, Antarctica. While retaining key morphological features known for the genus *Austropallene* Hodgson, 1915a, the new species is distinguished from congeners by its much larger size, along with the combined absence of a denticle on the inner surface of the fixed finger of the chelifore claw along with the presence of small conical outgrowths where the fixed finger of the chelifore claw meets the movable finger on both the dorsal and ventral sides, and also the ability to fully close the chelifore claw. Additionally, the complete mitochondrial genome of *A. halanychi* is consistent with other members of the genus *Austropallene* in terms of gene order and directionality. A phylogenetic tree consisting of mitochondrial protein-coding gene data places *A. halanychi* as sister to *Austropallene cornigera* (Möbius, 1902). Additionally, a phylogenetic tree constructed using partial COI data from other callipallenids placed the new species in a clade containing the genus *Austropallene*. The combination of molecular data in addition to key morphological differences from similar species in the genus leaves no doubt that the new taxon is a new Antarctic species of *Austropallene*.

Key words: Biodiversity, morphology, mtDNA, phylogeny, taxonomy

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Introduction

The family Callipallenidae (Pycnogonida, Arthropoda) is represented globally by 17 genera and over 200 species and subspecies (Bamber et al. 2023). Representatives of the family are highly variable in size, color, and form but are characterized by the presence of functional chelifores and ovigers in both males and females, and with compound spines being present on the ovigers (Arango and Brenneis 2013). The genus *Austropallene* Hodgson, 1915a was created for a species that did not fit into any of the previously established genera within Callipallenidae, including *Pseudopallene* Wilson, 1878 and *Cordylochele* Sars, 1888. The original description of the genus *Austropallene* Hodgson (1915a) is listed by Fry and Stock (1978) in their bibliography as “Hodgson (1914)”. Following the creation of *Austropallene*, Hodgson (1915b) more fully described the genus and several species. Hodgson’s original description of *Austropallene* is as follows:

"A genus established to include those forms which Möbius, Prof. Bouvier, and the present writer have included in different genera. The presence of cephalic spurs is a most noticeable feature and is confined to all these southern species. Body robust or slender, segmentation distinct, lateral processes close together or widely separated. Large and stout cephalic spurs. Eyes well developed. Proboscis tapering, with or without a setose wreath. Cheliferi stout, chelae short and powerful. Palps no trace. Ovigers 10-jointed, without a terminal claw. In the male a distal swelling on the fifth joint. No auxiliary claws." (Hodgson 1915a: 161)

Subsequently three species, originally placed within *Pseudopallene*, were included in *Austropallene*: *A. cornigera* (Möbius, 1902), *A. cristata* (Bouvier, 1911), and *A. spicata* Hodgson, 1915a. Despite Hodgson citing *Cordylochele* Sars, 1888, no *Cordylochele* species were moved to *Austropallene* (Hodgson 1915a, 1915b). Subsequently, *A. spicata* was determined by Calman (1915) to be synonymous with Bouvier's previously described species, *A. brachyura* Bouvier, 1911 and, in addition, he described *A. tibicina* Calman, 1915 and clarified that "[...] in *Austropallene* there is usually, perhaps always, a minute terminal spine, if not a 'claw', in the ovigers" (Calman 1915: 38). *A. calmani* Gordon, 1944 and *A. gracilipes* Gordon, 1944 and the smallest member of the genus, *A. tcherniai* Fage, 1952, were described later and then the genus was re-described by Pushkin in 1993, with the addition of three new species: *A. tenuicornis* Pushkin, 1993, *A. spinicornis* Pushkin, 1993, and *A. bucera* Pushkin, 1993. Apart from *A. lukini* Turpaeva, 2002, found in the northern Sea of Okhotsk, the ten other species within *Austropallene* described to date are restricted to Antarctic waters.

Within Pycnogonida, 26 complete or nearly complete mitochondrial genomes have been reported (Hassanin et al. 2005; Podsiadlowski and Braband 2006; Park et al. 2007; Masta et al. 2010; Dietz et al. 2011; Carapelli et al. 2013; Zehnpfennig et al. 2022), including two belonging to the genus *Austropallene*: *A. cornigera* and *A. bucera* (Suppl. material 1). Mitochondrial genomes contain a moderate amount of gene order conservation among related taxa and arrangements in gene order are often used for phylogenetic inferences (Boore and Staton 2002; Varney et al. 2021; Zehnpfennig et al. 2022). Published pycnogonid mitochondrial genomes have a conserved protein-coding gene order that contains the standard complement of bilaterian mtDNA genes (i.e. 13 protein-coding genes, 2 ribosomal RNA genes, and 22 tRNAs (Boore 1999; Vallès et al. 2008). Furthermore, Zehnpfennig et al. (2022) found that the order and directionality of protein-coding genes found in pycnogonids are identical in each family as well as in other chelicerates (e.g. *Limulus polyphemus*; Lavrov et al. 2000). The order of tRNA genes, however, differ by families, with representatives of Nymphonidae and Callipallenidae having distinct tRNA arrangements, exclusive to members within those families.

In this study, we describe *Austropallene halanychi* sp. nov., a new species from the Ross Sea, Antarctica (Southern Ocean). The description of this novel species is supported by morphological characteristics as well as phylogenetic analyses conducted using data from its complete mitochondrial genome. The combination of morphology and molecular data support the distinction of this new species from other described species within the genus.

Materials and methods

Study site and sample collection

A single specimen of *Austropallene halanychi* sp. nov. was collected via Blake trawl while aboard the RVIB *Nathaniel B. Palmer* (NBP12-10) on 31 January 2013 as part of a multi-institutional research expedition involving Auburn University and Central Michigan University to investigate the genetic connectivity and biogeographic patterns of Antarctic benthic invertebrates. The specimen was collected in Antarctica from the Ross Shelf (Ross Sea) at 560 m depth (75°19'46.7"S, 176°59'06.3"W, Fig. 1). Upon collection, the specimen was identified to genus and preserved in ~95% ethanol until it could be returned to the laboratory for further investigation. A map of the study site (Fig. 1) was created using the maps package in R (R Core Team 2022). The specimen was donated to the Smithsonian National Museum of Natural History (NMNH) invertebrate zoology collection under accession number 1548440.

Morphological identification

The specimen was identified to the genus level by consulting the original description of *Austropallene* by Hodgson (1915a, 1915b) as well as the description by Pushkin (1993), in the most recent review of *Austropallene*. Species-level identification was attempted using the available morphological keys as well as the original descriptions of closely related species (Hodgson 1915a, 1915b; Pushkin 1993; Child 1995). This revealed that the specimen does not belong to any described

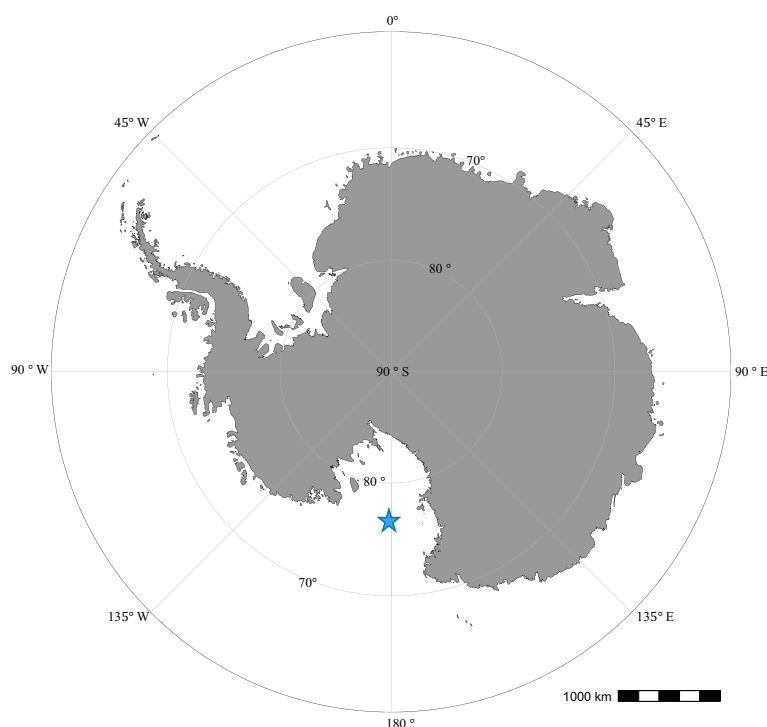


Figure 1. *Austropallene halanychi* sp. nov., collection location (blue star) within the Ross Sea, Antarctica.

species of *Austropallene*. Stereomicroscopic images of the specimen were taken with a Leica M165C, with LAS v. 4.3 software. Measurements of structures were determined using the optical micrometer provided with the LAS software.

Molecular methods

Genomic DNA was extracted from the specimen's leg muscle tissue with the Qiagen DNEasy Blood and Tissue Kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's recommendations. Extracted DNA was submitted to the RTSF Genomics Core at Michigan State University for library preparation and sequencing. The sample (in addition to other samples) was sequenced with a SP 300 cycle flow cell and Illumina NovaSeq with PE150 v2 chemistry.

Data analyses

Paired-end sequences were quality checked using FASTQC (Andrews et al. 2010) and assembled with NOVOPlasty v. 4.3.1 (Dierckxsens et al. 2016) using the previously published whole mitochondrial genome of *Achelia bituberculata* Hedgepeth, 1949 (Ammonoidea) as a reference sequence (Park et al. 2007). The assembly was screened with BLAST (Altschul et al. 1990) to determine if the resulting contig hit (with the highest percent query cover) to a previously published pycnogonid mitochondrial genome. This was used as a baseline for mitochondrial DNA identification. The contig containing the mitochondrial genome was annotated with the MITOS web server (Bernt et al. 2013) and gene boundaries were checked manually in Artemis v. 17.0 (Rutherford et al. 2000). Total G-C content (as percent total DNA) was determined using Quast v. 5.0 (Gurevich et al. 2013) on the assembled mitochondrial genome. Gene length, directionality, and order for the assembled mitochondrial genome were also assessed and summarized (Suppl. material 2).

Protein-coding gene sequences from the resulting mitochondrial genome, as well as complete or nearly complete mitochondrial genomes belonging to pycnogonids available from publicly available repositories (e.g. GenBank), partial mitochondrial gene data for individuals belonging to the family Ascorhynchidae, and mitochondrial protein-coding gene data published for *Limulus polyphemus* (as an outgroup) were compiled for phylogenetic reconstruction. To create a phylogeny containing representatives from all known sea spider families, partial mitochondrial gene data were used to represent Ascorhynchidae as there are no currently available complete mitochondrial genomes belonging to this family. Nucleotide sequences from protein-coding genes were aligned using MAFFT (Kato et al. 2002). TrimAL was used to trim protein-coding genes for each family (Capella-Gutiérrez et al. 2009). A concatenated matrix of aligned and trimmed protein-coding genes was created using FASconCAT v.1.0 (Kück and Meusemann 2010). Phylogenetic analyses were conducted using IQtree2 (Minh et al. 2020) with a partitioned maximum likelihood analysis using the best-fitting model for each gene resulting in a phylogeny of sea spider families. The resulting topology was evaluated based on 1000 rapid bootstrap replicates. To further investigate the placement of *A. halanychi* sp. nov., an additional phylogenetic analysis utilizing partial COI sequences available for members of the family Callipallendidae was conducted with COI data from *Nymphon australe* Hodgson,

1902 as an outgroup using the aforementioned methods. (Suppl. material 1). To visualize our resulting phylogenetic trees, we used FigTree v. 1.4.4 (Rambaut and Drummond 2012). MEGA v. 11 (Tamura et al. 2021) was used to assess genetic distance (using uncorrected *p*-distance) between the protein-coding genes and ribosomal RNA genes from *A. halanychi* sp. nov. and the already published genomes of *A. cornigera* and *A. buccera* (Zehnpfennig et al. 2022). The protocol for mitochondrial genome assembly and annotation is available at: <https://www.protocols.io/view/mitochondrial-genome-assembly-and-annotation-cvfgw3jw>.

Results

Systematics

Class Pycnogonida Latreille, 1810

Order Pantopoda Gerstäcker, 1863

Family Callipallenidae Hilton, 1942

Genus *Austropallene* Hodgson, 1915

***Austropallene halanychi* sp. nov.**

<https://zoobank.org/C15B8CD5-0E11-47C4-8626-81D33D718055>

Figs 2–4

Type locality. Antarctica, Ross Sea, Ross Shelf, 570 m depth, 75°19'46.7"S, 176°59'06.3"W, collected via benthic trawl, 31 January 2013, RVIB *Nathaniel B. Palmer* (NBP12-10), A. Mahon leg.

Type specimen. Holotype male preserved in ethanol, original label "Antarctica, Ross Sea, Ross Shelf, 570 m depth, 75°19'46.7"S, 176°59'06.3"W, 31 January 2013, J. Zehnpfennig and A. Mahon", NMNH 1548440"; handwritten label "Ch 226.1E".

Diagnosis. The new species can be differentiated from all other described *Austropallene* species based on its much larger size, as well as its unique and distinctive chelifores. The chelifore claw of *A. halanychi* sp. nov. closes completely when the fingers converge, with no visible space remaining between the fixed and movable fingers. The movable finger of the chelifore claw is distinctly shorter than the fixed finger, and the chelifore fingers do not contain any denticles or notches on their inner surfaces.

Description of holotype (male). Slender appearance, fully segmented trunk; neck distinct, large oblong ovigers attaching laterally; cephalic spurs present (Figs 2A–C, 3A, B). Ocular tubercle (Figs 2A–C, 3B, C) low, rounded, slightly inclined backwards, prominent distal papillae; four darkly pigmented eyes, anterior pair larger than posterior pair (Fig. 2A, I). Lateral processes long, smooth, without spines. Abdomen short, conical, swollen distally, cleft anal opening. Proboscis (Figs 2A–C, 3B, D, E) directed ventrally, broad at base, conical, slight mid-point constriction, distally tapering; mouth surrounded by setose wreath (Figs 2G, H, 3E).

Chelifore (Figs 2D–F, 3B, D, F, G), scape large and oblong, shorter than proboscis, directed slightly anteroventrally, slight tapering towards base; chelae long, 77.5% of scape length; tips of chela fingers blackened; fingers longer than one-half of palm length, fixed finger of chelifore concave with pointed tip. Movable finger 0.73 mm shorter than fixed finger, convex, with rounded tip.

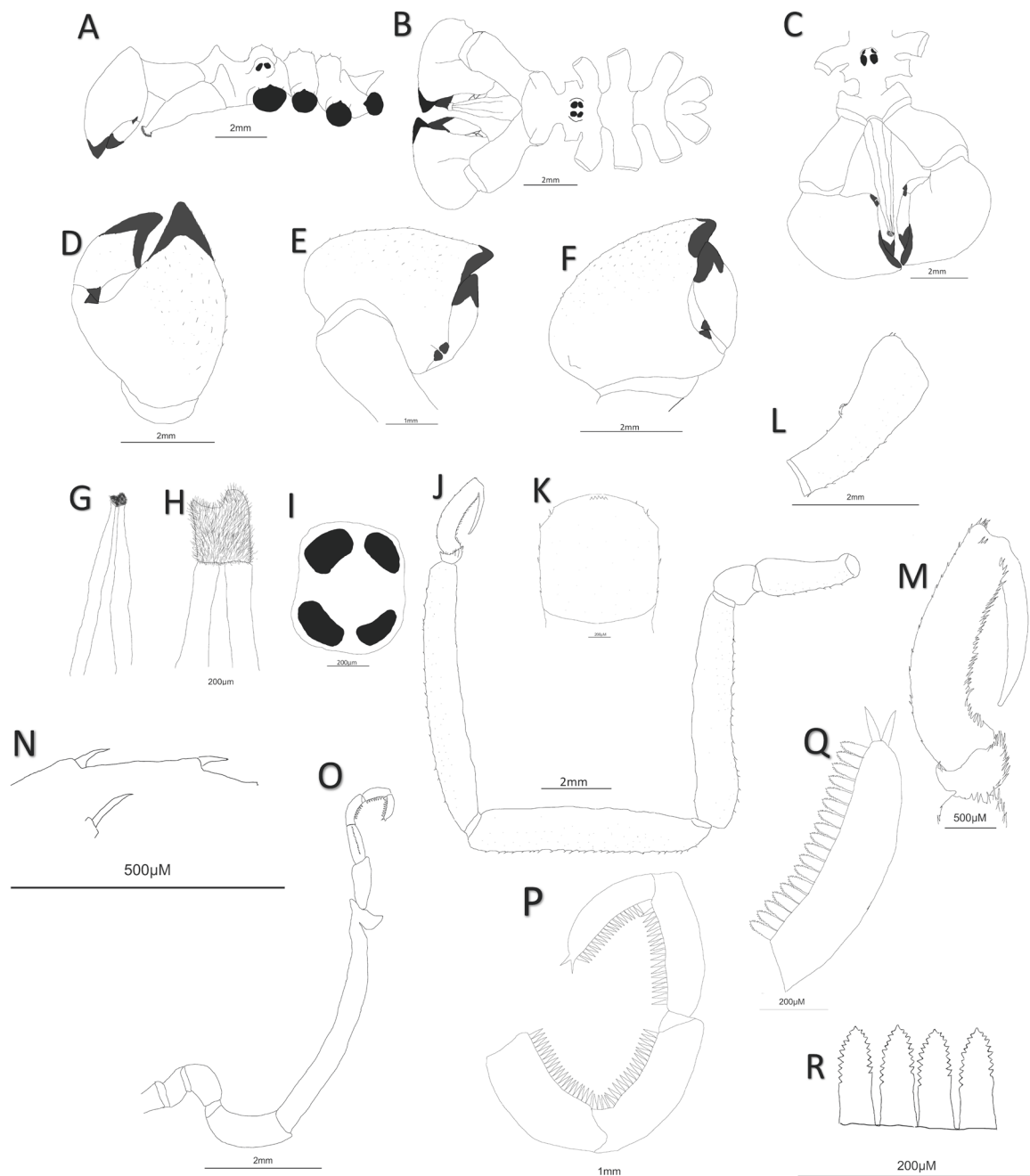


Figure 2. *Austropallene halanychi* sp. nov., male holotype **A** side view (scale) **B** dorsal view **C** front view, note cephalic spurs **D** dorsal view of opened chelifore claw; note absence of denticle and conical outgrowth at base of fixed and movable fingers **E** dorsal view of chelifore claw **F** ventral view of chelifore claw; note conical outgrowth **G** dorsal view of proboscis with setose wreath **H** setose wreath around proboscis **I** dorsal view of eyespot **J** third walking leg **K** first coxa of third walking leg **L** side view of second coxa of third walking leg **M** propodus of walking leg **N** tubercles and spines on walking legs **O** ovigerous leg of male **P** strigilis of ovigerous leg **Q** 10th segment of oviger; note terminal spines **R** serrated ovigerous leg spines (compound spines) on strigilis of ovigerous leg.

Chelifore fingers converge completely, no space present between fingers, denticles not present on inner surface of either finger. Two small conical outgrowths present where fingers of chelifore claw meet (Fig. 3B, arrow). Chelifore scape and claw contain small setae along dorsal and ventral sides.

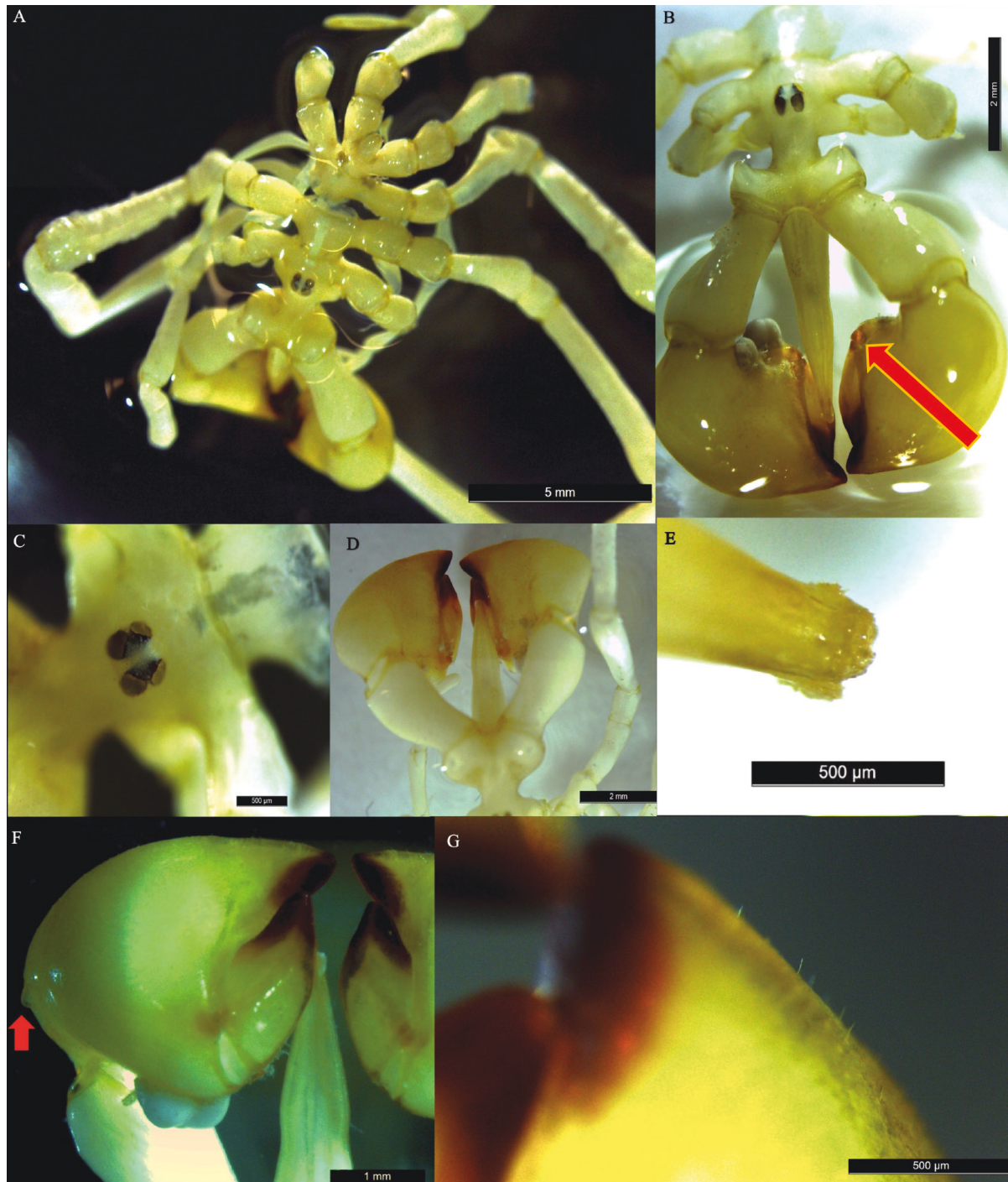


Figure 3. *Austropallene halanychi* sp. nov., male holotype **A** dorsal view **B** dorsal-frontal view; note shape, relative size, and black tips of chela fingers, cephalic spurs, and eye tubercle and eyes; note sharp conical outgrowths at base of fixed and movable fingers of chelifores (red arrow) **C** eye tubercle, top view **D** dorsal view of cephalic spurs, chelifore claws, and proboscis; note setose wreath on proboscis **E** setose wreath of proboscis **F** dorsal view of chelifore claws; note lack of space between chelifore fingers and small outgrowth (red arrow) **G** dorsal view of tip of chelifore claw; note presence of bristles along top of immovable finger.

Oviger 10-segmented (Figs 20–R, 4A–G), fifth segment longest, slightly curved, with prominent apophysis distally; oviger compound spines serrated, present on terminal four segments of the strigillis, strigilis formula: 20:20:18:18, with 2 prominent terminal spines. Some of the spines are broken off (Fig. 4E, F).

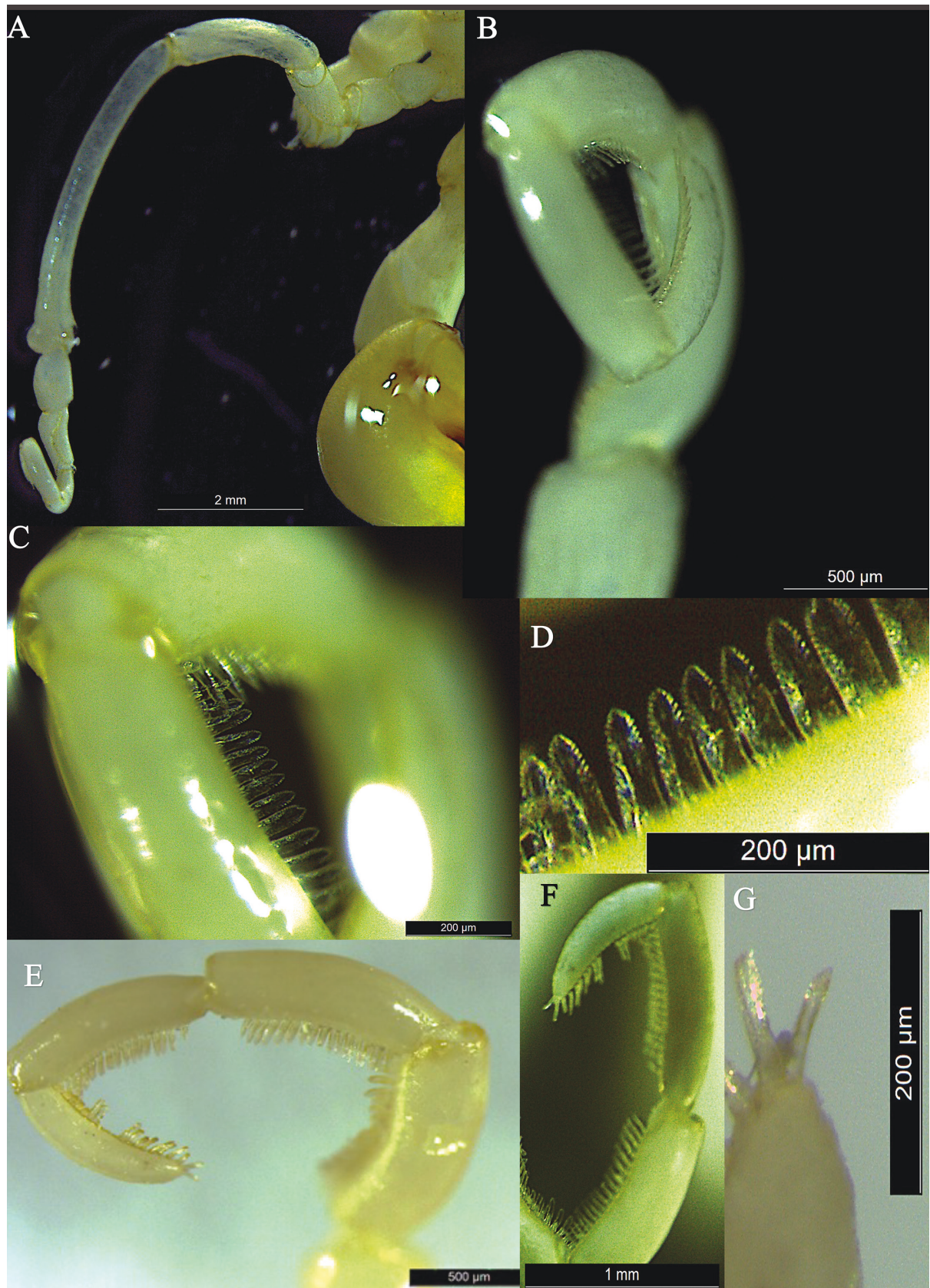


Figure 4. *Austropallene halanychi* sp. nov., male holotype **A** oviger, entire **B** strigilis, close-up view showing compound spines on segments 7–10 **C** compound spines on segment 9 **D** compound spines on segment 9 **E** strigilis, side view, showing compound spines on segments 7–10 (some spines on segment 10 are damaged or missing) **F** segments 8–10 with compound spines, and segment 10 with two terminal spines **G** segment 10, top view, close-up, with two terminal compound spines.

Walking legs (Fig. 2J–N), long, slender, sparse short spinules dorsally and ventrally on major segments; small conical tubercles and setae on first coxa. Femur shortest leg segment; tibia 2 longest segment; tarsus short, curved, small distal spine in line with heel spines; propodus curved, distinct propodal heel; five heel spines, relatively similar in size; many sole spines present; auxiliary claws absent, main claw extends nearly to heel. Gonopores and cement glands not observed.

Measurements (male holotype-mm). Body length (anterior end of cephalon to posterior end of trunk) = 10.50; body width (right lateral process of leg 1 + width of trunk + left lateral process of leg 1) = 4.02; trunk width = 1.24; abdomen length = 6.97; ocular tubercle height = 0.39; proboscis length = 2.73; chela fingers = 2.61; main claw length = 3.37; scape = 2.39; oviger 5th segment = 4.18; 10th segment = 0.74; terminal oviger spines = 0.10; leg span 63.84mm (distance between terminal claws second pair of walking legs); walking legs 29.78 mm in length (from where first coxa meets lateral process to terminal claw on second pair of walking legs); 3rd walking leg coxa 1 = 1.23, coxa 2 = 2.60, coxa 3 = 1.17, femur = 6.69, tibia 1 = 6.85, tibia 2 = 7.89, tarsus = 0.32, propodus = 1.95, terminal claw = 1.39.

Etymology. The species (*halanychi*, male genitive) is dedicated to Dr Kenneth M. Halanych, a mentor, colleague, and prolific marine invertebrate scientist whose commitment and dedication to the benthic marine systems in the Southern Ocean has provided a wealth of information related to biodiversity in the Antarctic system.

Discussion

Molecular evidence

The complete mitochondrial genome of *Austropallene halanychi* sp. nov. is 15,022 bp in length, and has a G-C content of 21.10% (Table 1) and contains 13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes, along with an AT-rich control region, consistent with the other complete published pycnogonid mitochondrial genomes (Hassanin et al. 2005; Podsiadlowski and Braband 2006; Park et al. 2007; Masta et al. 2010; Carapelli et al. 2013; Zehnpfennig et al. 2022). The order and directionality (strand placement) of all the genes in the mitogenome of *A. halanychi* were found to be consistent with the two previously published mitogenomes belonging to *A. buccera* and *A. cornigera* (Suppl. material 2) (Zehnpfennig et al. 2022). The observed gene pattern follows the same gene pattern seen in representatives of Callipallenidae (*A. cornigera* and *A. buccera*) and Nymphonidae, which shows rearrangements in trnA (Alanine), trnE (Glutamic acid), trnY (Tyrosine), trnP (Proline), trnS1 (Serine), trnV (Valine), trnQ (Glutamine), trnM (Methionine), and trnI (Isoleucine) when compared to species belonging to other families in Pycnogonida (Suppl. material 2). The phylogenetic tree constructed with protein-coding gene data recovered a monophyletic clade containing *A. halanychi* as sister to *A. cornigera* (BS = 100), with this grouping placed as sister to *A. buccera* (BS = 100) (Fig. 5). Genetic distances calculated using uncorrected *p*-distances supported the findings in the phylogenetic tree, with all the protein-coding genes (excluding ND3) and ribosomal RNA genes belonging to *A. halanychi* showing closer genetic distances to *A. cornigera* than *A. buccera* (Table 2).

Table 1. Mitochondrial genome information for *Austropallene* species used in this study.

Species	Mitogeneome length (bp)	G-C content (%)	mtDNA genome
<i>Austropallene cornigera</i>	14,650 bp	21.60	OK623743
<i>Austropallene buccera</i>	15,177 bp	21.62	OK412987
<i>Austropallene halanychi</i> sp. nov.	15,022 bp	21.10	OP781307

The mitochondrial genome information includes GenBank accession numbers, mitochondrial genome lengths, and G-C content for *Austropallene halanychi*, *A. cornigera* and *A. buccera*.

Table 2. Genetic distances (calculated as uncorrected *p*-distances (%)) for mitochondrial protein-coding and ribosomal RNA genes between *Austropallene halanychi* sp. nov., *A. cornigera*, and *A. buccera*.

Genetic distance (calculated as uncorrected <i>p</i> -distance (%))			
Gene	<i>A. halanychi</i> and <i>A. cornigera</i>	<i>A. halanychi</i> and <i>A. buccera</i>	<i>A. cornigera</i> and <i>A. buccera</i>
ATP6	2.56	2.94	3.10
ATP8	2.86	5.52	2.76
COI	2.19	4.48	2.96
CO2	2.94	3.8	3.36
CO3	2.56	5.13	4.49
ND1	2.19	3.01	3.85
ND2	2.29	4.17	3.85
ND3	2.59	2.03	3.48
ND4	1.23	2.47	2.40
ND4L	1.81	3.26	2.90
ND5	1.85	3.05	2.93
ND6	1.69	2.53	2.95
CYTB	1.73	4.83	4.28
12S	1.32	2.12	1.171
16S	0.591	1.184	0.672

The phylogenetic tree constructed using partial COI data available for members within Callipallenidae (Fig. 6) recovered a clade containing all *Austropallene* species placed as sister to a clade containing representatives of the genus *Pallenella* Schimkewitsch, 1909 (BS = 77). *Austropallene halanychi* was recovered as sister to *A. buccera* (BS = 68), with this grouping placed as sister to *A. cornigera* (BS = 100). The clade containing *A. halanychi*, *A. buccera*, and *A. cornigera* was recovered as sister to *A. cristata* with strong support (BS = 95). The *Austropallene* + *Pallenella* clade was grouped as sister (BS = 43) to a clade containing representatives belonging to the genus *Callipallene* Flynn, 1929 in a monophyletic clade (BS = 97) recovered as sister to *Oropallene* Schimkewitsch, 1930 (BS = 60). Representatives belonging to the genus *Stylopallene* Clark, 1963 were recovered in a monophyletic clade (BS = 99) and placed as sister to *Propallene* Schimkewitsch, 1909 (Fig. 6). Representatives from the genera *Parapallene* Carpenter, 1892 and *Cheilopallene* Stock, 1955 were recovered as a monophyletic clade (BS = 76) placed as sister to the recovered *Stylopallene* + *Propallene* clade (BS = 62) (Fig. 6).

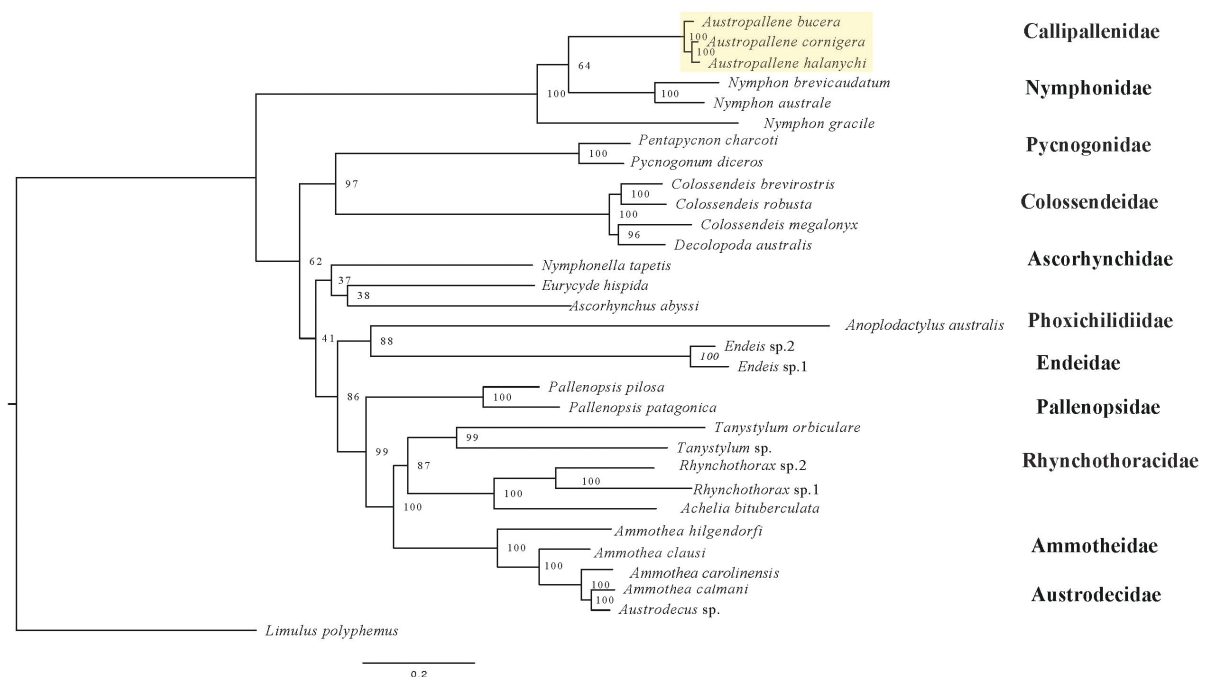


Figure 5. Phylogenetic tree constructed with protein-coding gene data. The phylogenetic tree shows placement of *Austropallene halanychi* sp. nov. and was constructed using protein-coding gene data with IQ-TREE2; it uses a maximum likelihood approach and best-fit model (GTR+F+R5). The clade containing *A. halanychi* sp. nov. is highlighted, and bootstrap support (based on 1000 rapid bootstrap replicates) is included.

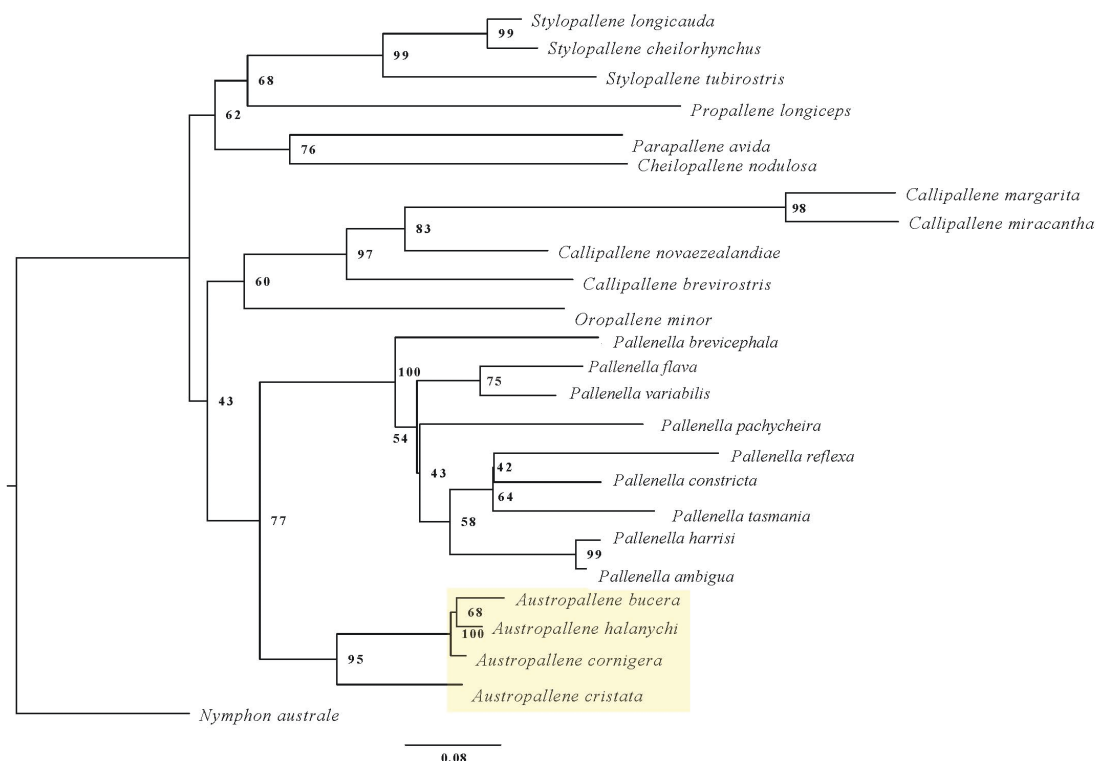


Figure 6. Phylogenetic tree constructed with partial COI data from members of the family Callipallenidae. The phylogenetic tree was constructed with IQ-TREE2 using a maximum likelihood approach and the best-fit model (GTR+F+I+G4). The clade containing *Austropallene halanychi* sp. nov. is highlighted, and bootstrap support (based on 1000 rapid bootstrap replicates) is included.

***Austropallene halanychi* sp. nov. in respect to closely related Antarctic *Austropallene* species**

Austropallene halanychi sp. nov. presents an arrangement and combination of morphological characters that have not been observed in any previously described *Austropallene* species (Table 3). We compared *A. halanychi* to three closely related and morphologically similar species of *Austropallene*: *A. cornigera* (Möbius, 1902), *A. bucera* Pushkin, 1993, and *A. tenuicornis* Pushkin, 1993. To begin, *A. halanychi* is larger and longer than the other closely related species, measuring 10.50 mm in body length, longer than *A. cornigera* (4.8 mm), *A. bucera*, (4.8 mm in male), and *A. tenuicornis* (4.5 mm) (Pushkin 1993). The length of the third walking legs in *A. halanychi* is 30.9 mm in length, longer than *A. cornigera* (22.1 mm) and *A. tenuicornis* (24.1 mm) but shorter than *A. bucera* (40.2 mm) (Pushkin 1993). One of the most distinctive characters that distinguish *A. halanychi* from these species are the powerful, relatively large, oblong chelifores. The chelifores in *A. halanychi* are longer and larger than in these species, with the scape measuring 2.40 mm and the claw measuring 3.37 mm in length, and differing from the more oval-shaped chelifores in *A. cornigera* (scape 2.2 mm, claw 2.6 mm), *A. bucera* (scape 2.0 mm, claw 2.4 mm), and *A. tenuicornis* (scape 2.30 mm, claw 2.3 mm) (Pushkin 1993). Furthermore, the fixed chelifore fingers of *A. halanychi* do not contain any denticles or notches on the inner surface, differing from *A. cornigera*, *A. bucera*, and *A. tenuicornis* all of which have denticles on the fixed finger (Table 3; Pushkin 1993). Additionally, the movable finger of the chelifore claw is distinctly shorter than the fixed finger of chelifore claw in *A. halanychi*, whereas the movable finger in *A. cornigera*, *A. bucera*, and *A. tenuicornis* is almost as long as the fixed finger (Table 3). The chelifore claw in *A. halanychi* closes completely when the fingers converge with no visible space remaining between the fixed and movable fingers; this differs from the new species' congeners. The chelifore claw has two small, conical outgrowths on each side at the base where the chelifore fingers meet, a characteristic that is shared with *A. cornigera* but not with *A. bucera* or *A. tenuicornis* (Table 3). Moreover, the first coxa of the walking legs of *A. halanychi* contains small spines and setae, similar to *A. cornigera* and *A. tenuicornis*, but differing from *A. bucera* where the first coxa is smooth (Pushkin 1993). The listed similarities and differences distinguish *A. halanychi* from other similar species in the genus.

Conclusion

Morphologically, the comparably larger, more robust, and oblong chelifores and the absence of space between chelifore fingers, unequal length of chelifore fingers, absence of a denticle on either the movable or fixed chelifore claws, and ability to fully close chelifore fingers together all serve to distinguish this large individual from previously described species of *Austropallene*. Furthermore, the complete mitochondrial genome of *A. halanychi* sp. nov. follows the same distinctive gene order and directionality found in other members of the genus. The phylogenetic tree resulting from protein-coding gene data placed *A. halanychi* as sister to *A. cornigera* with the grouping in a clade with *A. bucera*. Furthermore,

Table 3. Diagnostic characteristics for the genus *Austropallene*. The diagnostic characteristics belonging to the genus *Austropallene*, as well as known distribution locations for every Antarctic *Austropallene* species as described by Munilla and Soler Membrives (2008) with the addition of *Austropallene halanychi* sp. nov. *Austropallene lukini* was not included here since it is not Antarctic in distribution.

Species	Trunk segments on dorsal side smooth and without spines	Cephalic spurs present	Presence of terminal spines on 10 th segment of ovigerous leg	Well pronounced expansion (heel) on the propodus of all walking legs	Tubercles with a bristle on the apex on the first coxa of walking legs	Cement glands present on femur in male	Denticle present in the inner surface of the fixed finger on the chelifore claw	Fixed finger of the chelifore claw (oval or pointed)	Conical outgrowths where fixed finger meets movable finger on dorsal and ventral sides	Movable finger of chelifore claw shorter than fixed finger	Tips of fingers converge when closed	No proximal gap observed when chelifore fingers close	Tubercles and setae present on chelifore scapes and claws	Known distribution locations
<i>Austropallene brachyura</i>	X	X	X			X		Oval			X			Circumpolar
<i>Austropallene bucera</i>	X	X	X	X		X	X	Pointed		X				Scotia Sea–Subantarctic waters
<i>Austropallene calmani</i>	X	X	X			X		Pointed		X			X	Circumpolar
<i>Austropallene cornigera</i>	X	X	X	X	X	X	X	Pointed			X			Circumpolar
<i>Austropallene cristata</i>		X	X	X		X		Pointed			X			Circumpolar
<i>Austropallene gracilipes</i>	X	X	X			X		Pointed			X			Scotia Sea–Subantarctic waters
<i>Austropallene halanychi</i> sp.nov.	X	X	X	X	X	X		Pointed	X	X	X	X	X	Ross Sea
<i>Austropallene spinicornis</i>	X	X	X	X		X	X	Pointed			X			Scotia Sea–Subantarctic waters
<i>Austropallene tcherniai</i>	X	X	X			X		Pointed			X			Circumpolar
<i>Austropallene tibicina</i>	X	X	X			X		Pointed		X				Ross Sea and Scotia Sea
<i>Austropallene tenuicornis</i>	X	X	X	X			X	Pointed			X		X	Subantarctic Waters

the phylogenetic tree resulting from partial COI data for members belonging to the Callipallenidae placed *A. halanychi* in a monophyletic clade with *A. cornigera*, *A. bucera*, and *A. cristata*, with this clade recovered as sister to a clade comprised of representatives from the genus *Pallenella*; this supports the placement of the new species in *Austropallene*. *Austropallene halanychi* shares many morphological characteristics with *A. cornigera* (Table 3), indicating that these two species are closely related to one another. Overall, the combination of morphological characteristics and molecular evidence leaves no doubt that this is a new Antarctic species of *Austropallene*.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

JRZ contributed to the Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing the original draft as well as a review and editing. ARM contributed to Funding acquisition, Project Administration, Sample Collection, Resources, Supervision, Investigation, Validation as well as review and editing of the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information. The mitochondrial genome of *Austropallene halanychi* sp. nov. is deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>; accession number [OP781307](#)).

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *Journal of Molecular Biology* 215(3): 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)

- Andrews S, Krueger F, Segonds-Pichon A, Biggins L, Krueger C, Wingett S (2010) FastQC. A quality control tool for high throughput sequence data, 370 pp.
- Arango CP, Brenneis G (2013) New species of Australian *Pseudopallene* (Pycnogonida: Callipallenidae) based on live colouration, morphology and DNA. *Zootaxa* 3616(5): 401–436. <https://doi.org/10.11646/zootaxa.3616.5.1>
- Bamber RN, El Nagar A, Arango CP (2023) Pycnobase: World Pycnogonida Database. <https://doi.org/10.14284/360> [Accessed on: 2023-08-06]
- Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF (2013) MITOS: Improved *de novo* metazoan mitochondrial genome annotation. *Molecular Phylogenetics and Evolution* 69(2): 313–319. <https://doi.org/10.1016/j.ympev.2012.08.023>
- Boore JL (1999) Animal mitochondrial genomes. *Nucleic Acids Research* 27(8): 1767–1780. <https://doi.org/10.1093/nar/27.8.1767>
- Boore JL, Staton JL (2002) The mitochondrial genome of the sipunculid *Phascolopsis gouldii* supports its association with Annelida rather than Mollusca. *Molecular Biology and Evolution* 19(2): 127–137. <https://doi.org/10.1093/oxfordjournals.molbev.a004065>
- Bouvier EL (1911) Les pycnogonides du “*Pourquoi Pas*”? *Comptes Rendus des Seances Hebdomadaires de l'Academie des Sciences, Paris* 152: 1136–1141.
- Calman WT (1915) Pycnogonida. British Antarctic (Terra Nova) Expedition, 1910. *Zoology* (Jena, Germany) 3(1): 1–74.
- Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* (Oxford, England) 25(15): 1972–1973. <https://doi.org/10.1093/bioinformatics/btp348>
- Carapelli A, Torricelli G, Nardi F, Frati F (2013) The complete mitochondrial genome of the Antarctic sea spider *Ammothea carolinensis* (Chelicerata; Pycnogonida). *Polar Biology* 36(4): 593–602. <https://doi.org/10.1007/s00300-013-1288-6>
- Carpenter GH (1892) Reports on the zoological collections made in Torres Straits by Professor A.C. Haddon, 188–1889. XL. Pycnogonida. *The Scientific Proceedings of the Royal Dublin Society. Royal Dublin Society* 7: 552–558.
- Child CA (1995) Antarctic and Subantarctic Pycnogonida III. The family Callipallenidae. In: *Antarctic and Subantarctic Pycnogonida: Nymphonidae, Colossendeidae, Rhynchothoracidae, Pycnogonidae, Endeididae, and Callipallenidae*. American Geophysical Union (AGU): 1–68. <https://doi.org/10.1029/AR069p0001>
- Clark WC (1963) Australian Pycnogonida. *Records of the Australian Museum* 26(1): 1–81. <https://doi.org/10.3853/j.0067-1975.26.1963.669>
- Dierckxsens N, Mardulyn P, Smits G (2016) NOVOPlasty: *De novo* assembly of organelle genomes from whole genome data. *Nucleic Acids Research* 45(4): e18. <https://doi.org/10.1093/nar/gkw955>
- Dietz L, Mayer C, Arango CP, Leese F (2011) The mitochondrial genome of *Colossendeis megalonyx* supports a basal position of Colossendeidae within the Pycnogonida. *Molecular Phylogenetics and Evolution* 58(3): 553–558. <https://doi.org/10.1016/j.ympev.2010.12.016>
- Fage L (1952) 2. Pycnogonides. *Missions du batiment polaire Commandant Charcot, recoltes faites en Terre Adelie* (1950). *Bulletin du Museum National d'Histoire Naturelle, Paris* (2) 24(2): 180–186.
- Flynn TT (1929) Pycnogonida from the Queensland coast. *Memoirs of the Queensland Museum* 9(3): 252–260. <https://www.biodiversitylibrary.org/page/52430210>
- Fry WG, Stock JH (1978) A pycnogonid bibliography. *Zoological Journal of the Linnean Society* 63(1–2): 197–238. <https://doi.org/10.1111/j.1096-3642.1978.tb02098.x>

- Gordon I (1944) Pycnogonida. Reports of the British. Australian and New Zealand Antarctic Research Expedition (B) 5(1): 1–72.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G (2013) QUASt: Quality assessment tool for genome assemblies. *Bioinformatics* (Oxford, England) 29(8): 1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>
- Hassanin A, Léger N, Deutsch J (2005) Evidence for multiple reversals of asymmetric mutational constraints during the evolution of the mitochondrial genome of Metazoa, and consequences for phylogenetic inferences. *Systematic Biology* 54(2): 277–298. <https://doi.org/10.1080/10635150590947843>
- Hedgepeth JW (1949) Report on the Pycnogonida collected by the *Albatross* in Japanese waters 1900 and 1906. *Proceedings of the United States National Museum* 98(3231): 233–321. <https://doi.org/10.5479/si.00963801.98-3231.233>
- Hodgson TV (1902) Crustacea (Pycnogonida). Report on the collections of natural history made in the Antarctic Regions during the voyage of the “*Southern Cross*.” British Museum, London, 343 pp. [53 pls] <https://doi.org/10.5962/bhl.title.12292>
- Hodgson TV (1915a) Preliminary report on the Pycnogonida of the German Southpolar Expedition, 1901–1903. *Zoologischer Anzeiger* 45(4): 158–165.
- Hodgson TV (1915b) The Pycnogonida collected by the ‘*Gauss*’ in the Antarctic regions, 1901–3.—Preliminary report. *Annals & Magazine of Natural History* 15(8): 141–149. <https://doi.org/10.1080/00222931508693621>
- Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30(14): 3059–3066. <https://doi.org/10.1093/nar/gkf436>
- Kück P, Meusemann K (2010) FASconCAT: Convenient handling of data matrices. *Molecular Phylogenetics and Evolution* 56(3): 1115–1118. <https://doi.org/10.1016/j.ympev.2010.04.024>
- Lavrov DV, Boore JL, Brown WM (2000) The complete mitochondrial DNA sequence of the horseshoe crab *Limulus polyphemus*. *Molecular Biology and Evolution* 17(5): 813–824. <https://doi.org/10.1093/oxfordjournals.molbev.a026360>
- Masta SE, McCall A, Longhorn SJ (2010) Rare genomic changes and mitochondrial sequences provide independent support for congruent relationships among the sea spiders (Arthropoda, Pycnogonida). *Molecular Phylogenetics and Evolution* 57(1): 59–70. <https://doi.org/10.1016/j.ympev.2010.06.020>
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 37(5): 1530–1534. <https://doi.org/10.1093/molbev/msaa015>
- Möbius KA (1902) Die Pantopoden der deutschen Tiefsee-Expedition, 1898–99. *Wissenschaftliche Ergebnisse der deutschen Tiefsee-Expedition auf dem Dampfer “Valdivia”, 1898–1899* 3: 177–196.
- Munilla T, Soler Membrives A (2008) Check-list of the pycnogonids from Antarctic and sub-Antarctic waters: Zoogeographic implications. *Antarctic Science* 21(2): 99–111. <https://doi.org/10.1017/S095410200800151X>
- Park SJ, Lee YS, Hwang U (2007) The complete mitochondrial genome of the sea spider *Achelia bituberculata* (Pycnogonida, Ammotheidae): Arthropod ground pattern of gene arrangement. *BMC Genomics* 8(1): 343. <https://doi.org/10.1186/1471-2164-8-343>
- Podsiadlowski L, Braband A (2006) The complete mitochondrial genome of the sea spider *Nymphon gracile* (Arthropoda: Pycnogonida). *BMC Genomics* 7(1): 284. <https://doi.org/10.1186/1471-2164-7-284>

- Pushkin AF (1993) The Pycnogonida fauna of the South Ocean. Biological Results of the Soviet Antarctic Expeditions. Explorations of the Fauna of the Seas 20: 1–397.
- R Core Team (2022) R: a language and environment for statistical computing.
- Rambaut A, Drummond AJ (2012) FigTree version 1.4. 0.
- Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, Rajandream MA, Barrell B (2000) Artemis: Sequence visualization and annotation. Bioinformatics (Oxford, England) 16(10): 944–945. <https://doi.org/10.1093/bioinformatics/16.10.944>
- Sars GO (1888) Pycnogonida borealia et arctica enumerat (Prodromus descriptionis). Archiv for Mathematik og Naturvidenskab 12: 339–356.
- Schimkewitsch W (1909) Nochmals über die Periodicität in dem System der Pantopoden. Zoologischer Anzeiger 34(1): 1–13.
- Schimkewitsch W (1930) [Pycnogonida (Pantopoda)] Fauna SSSR. Izdatel'stvo Akademii Nauk SSSR (Part 2): 225–555. [In Russian]
- Stock JH (1955) Pycnogonida from the West Indies, Central America, and the Pacific coast of North America. Papers from Dr. Th. Mortensen's Pacific Expedition 1914–1916. LXXVIII. Videnskabelige Meddelelser fra Dansk Naturhistorisk Foreningen i Kjøbenhavn 117: 209–266.
- Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis version 11. Molecular Biology and Evolution 38(7): 3022–3027. <https://doi.org/10.1093/molbev/msab120>
- Turpaeva EP (2002) Shallow-water sea spiders (Pycnogonida) from the Kurils, Komandor, and Yamskie Islands: 1. Families Colossendeidae and Callipallenidae. Entomological Review 82(1): S83.
- Vallès Y, Halanych KM, Boore JL (2008) Group II introns break new boundaries: Presence in a bilaterian's genome. PLoS ONE 3(1): e1488. <https://doi.org/10.1371/journal.pone.0001488>
- Varney RM, Speiser DI, McDougall C, Degnan BM, Kocot KM (2021) The iron-responsive genome of the chiton *Acanthopleura granulata*. Genome Biology and Evolution 13: evaa263. <https://doi.org/10.1093/gbe/evaa263>
- Wilson EB (1878) Descriptions of two new genera of Pycnogonida. American Journal of Science 15(87): 200–203. <https://doi.org/10.2475/ajs.s3-15.87.200>
- Zehnpfennig JR, Varney RM, Halanych KM, Mahon AR (2022) Mitochondrial genomes provide insight into interfamilial relationships within Pycnogonida. Polar Biology 45(9): 1513–1522. <https://doi.org/10.1007/s00300-022-03085-6>

Supplementary material 1

All species used in phylogenetic analyses for this study, which phylogenetic analysis the data was used for, and the correlated GenBank accession numbers

Authors: Jessica R. Zehnpfennig, Andrew R. Mahon

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Supplementary material 2

Mitochondrial gene order of *Austropallene halanychi* sp. nov.

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Data type: pdf

Explanation note: The mitochondrial gene order, excluding introns, and the control region for *A. halanychi* and other members within Callipallenidae and Nymphonidae, as well as other families within Pycnogonida. The underlined segments represent genes found on the reverse strand of the mtDNA.

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