Phylogeny of hydrothermal vent Iphionidae, with the description of a new species (Aphroditiformia, Annelida)

Marina F. McCowin¹, Greg W. Rouse¹

¹ Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093-0202, USA

Corresponding authors: Marina F. McCowin (marruda@ucsd.edu); Greg W. Rouse (grouse@ucsd.edu)

Abstract
The scale-worm family Iphionidae consists of four genera. Of these, Thermiphione has two accepted species, both native to hydrothermal vents in the Pacific Ocean; T. fijiensis Miura, 1994 (West Pacific) and T. tufari Hartmann-Schröder, 1992 (East Pacific Rise). Iphionella is also known from the Pacific, and has two recognized species; Iphionella risensis Pettibone, 1986 (East Pacific Rise, hydrothermal vents) and I. philippinensis Pettibone, 1986 (West Pacific, deep sea). In this study, phylogenetic analyses of Iphionidae from various hydrothermal vent systems of the Pacific Ocean were conducted utilizing morphology and mitochondrial (COI and 16S rRNA) and nuclear (18S and 28S rRNA) genes. The results revealed a new iphionid species, described here as Thermiphione rapanui sp. n. The analyses also demonstrated the paraphyly of Thermiphione, requiring Iphionella risensis to be referred to the genus, as Thermiphione risensis (Pettibone, 1986).

Keywords
East Pacific Rise, Pacific Ocean, polychaete, systematics, scale-worm

Introduction
Annelid scale-worms (Aphroditiformia) are a particularly common and diverse group at hydrothermal vents (Desbruyères et al. 2006). Most of this diversity is within Polynoidae Kinberg, 1856, but there have been several records of another aphroditiform family, Iphionidae Kinberg, 1856, which currently includes four genera and 13 accepted species.

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(Read and Fauchald 2018). Iphionidae had been regarded as a subfamily of Polynoidae, until Norlinder et al. (2014) gave it family rank, as it appears it is actually most closely related to Acoetidae (Gonzalez et al. 2018). In addition to DNA sequence data, the monophyly of Iphionidae is supported by the presence of feathered notochaetae, areolae on elytra, and the absence of a median antenna (Gonzalez et al. 2018). The majority of the known diversity of iphionids are within *Iphione* Kinberg, 1856, and these are mostly shallow-water taxa. However, three genera of deep-sea hydrothermal vent iphionids have been described: *Iphionella* McIntosh, 1885 and *Thermiphione* Hartmann-Schröder, 1992, each with two species, and *Iphionides* Hartmann-Schröder, 1977, containing only *I. glabra* Hartmann-Schröder, 1977.

With regards to the hydrothermal vent-associated iphionids, *Iphionella risensis* Pettibone, 1986 was erected for specimens collected from the East Pacific Rise at 20°50’N. Similar to *I. philippinensis*, this species has 13 pairs of elytra. *Thermiphione tufari* Hartmann-Schröder, 1992, was described for specimens also collected from the East Pacific Rise at 21°30’S, well to the south of the type locality of *I. risensis*. A new genus, *Thermiphione* Hartmann-Schröder, 1992, was erected for this species. *Thermiphione* was distinguished from *Iphionella* by the presence of 14 pairs of elytra instead of 13, as well as by having a greater number of segments (Hartmann-Schröder 1992). *Thermiphione fijiensis* Miura, 1994 was subsequently described from hydrothermal vents from the western Pacific (North Fiji Basin), also with 14 pairs of elytra (Miura 1994).

This paper focuses on new deep-sea collections of Iphionidae from Pacific Ocean hydrothermal vents. DNA data was previously published for *Thermiphione fijiensis* (as *Thermiphione* sp.) in Norlinder et al. (2012); herein we add additional DNA data for this species and for the other two known hydrothermal vent Iphionidae. Furthermore, we describe a new vent-associated iphionid species from the East Pacific Rise and assess some morphological and taxonomic issues for Iphionidae.

**Materials and methods**

**Sample collection**

Sampling was conducted over several years and at multiple localities (Figure 1, Tables 1, 2). *Thermiphione rapanui* sp. n. and *T. tufari* were collected on several dives by the manned submersible *Alvin* in 2005 at hydrothermal vents of the southern East Pacific Rise (Table 2). *Thermiphione fijiensis* was collected from the Lau Back-arc Basin in 2005 utilizing the ROV *Jason II* (Table 2). *Iphionella risensis* was collected in 2012 using the ROV *Doc Ricketts* from the Alarcon Rise in the Gulf of California, just north of its type locality (Table 2). All specimens are deposited in the Scripps Institution of Oceanography Benthic Invertebrate Collection (SIO-BIC), La Jolla, California, USA. Whole specimens were photographed prior to preservation using Leica MZ8 or MZ9.5 stereomicroscopes. Post-preservation, specimens were examined and photographed using Leica S8 APO and DMR HC microscopes.
DNA extraction and amplification

DNA extraction of specimens from the aforementioned collection sites was conducted with the Zymo Research DNA-Tissue Miniprep kit, following the protocol supplied by the manufacturer. Up to 645 bp of mitochondrial cytochrome subunit I (COI) were amplified using the primer set HCO2198 and LCO1490 (Folmer et al. 1994) for multiple specimens in Table 2 and 16S rRNA, 18S rRNA, and 28S rRNA were amplified for a subset of these specimens. Up to 527 bp of 16S rRNA (16S) were amplified using the primer set 16SbrH and 16SarL (Palumbi 1996). 18S rRNA was amplified in three fragments using 18S1F, 18S3F, 18S9R, 18S5R, 18Sbi, and 18Sa2.0 (Giribet et al., 1996; Whiting et al. 1997), resulting in sequence lengths up to 1927 bp. Up to 973 bp of 28S rRNA were amplified using Po28F1 and Po28R4 (Struck et al. 2006). Amplification was carried out with 12.5µl Apex 2.0x Taq RED DNA Polymerase Master Mix (Genesee Scientific), 1µl each of the appropriate forward and reverse primers (10µM), 8.5µl of ddH2O, and 2µl eluted DNA. The PCR reactions were carried out in a thermal cycler.
### Table 1. Origin of sequenced terminals, vouchers, and GenBank accession numbers. New sequences in bold. Family assignments follow Zhang et al. (2018).

| Scientific name | Origin | Voucher | 18S  | 28S  | 16S  | COI    |
|-----------------|--------|---------|------|------|------|--------|
| *Panthalis oerstedi* | Sweden | SMNH118954 | AY839572 | JN852845 | JN852881 | AY839584 |
| *Iphione cf. treadwelli* | Eilat, Israel | – | KY823447 | – | KY823478 | KY823494 |
| *Iphione sp. 1* | Hong Kong | – | KY753852 | KY753852 | KY753835 | KY753835 |
| *Iphione sp. 2* | Papua New Guinea | SMNH118972 | JN852819 | – | JN852886 | JN852921 |
| *Iphione sp. 3* | Lord Howe Island, Australia | SIO-BIC A8708 | – | – | – | MH389786 |
| *Thermiphione risensis* (was *Iphionella risensis*) | Gulf of California | SIO-BIC A6326 | MG994954 | MH000396 | MG994947 | MG981037 |
| *Thermiphione tufari* | East Pacific Rise | SIO-BIC A7973 | MG994958 | MH000401 | MG994951 | MG981042 |
| *Thermiphione fijiensis* (Fijiensis) | Fiji, Lau Basin | SMNH118982 | JN852820 | JN852849 | JN852887 | JN852922 |
| *Thermiphione fijiensis* | Lau back-arc Basin | SIO-BIC A7975 | MG994960 | MH000402 | MG994953 | MG981044 |
| *Thermiphione rapanui sp. n.* | East Pacific Rise | SIO-BIC A7969 | MG994955 | MH000397 | MG994948 | MG981038 |

### Table 2. Sampling localities and GenBank COI accession numbers for all specimens collected and sequenced for this study.

| Specimen | Voucher | Locality | Latitude | Longitude | Depth (m) | COI Accession No. |
|----------|---------|----------|----------|-----------|-----------|-------------------|
| *Iphionella risensis* | SIO-BIC A6326 | Alarcon Rise, Gulf of California | 23°22'37"N | 108°31'52"W | 2,309 | MG981037 |
| *Thermiphione rapanui sp. n.* | SIO-BIC A7969 | Pacific Antarctic Ridge | 37°47'60"S | 110°55'0"W | 2,216 | MG981038 |
| *Thermiphione rapanui sp. n.* | SIO-BIC A7970 | Pacific Antarctic Ridge | 37°47'60"S | 110°55'0"W | 2,216 | MG981039 |
| *Thermiphione rapanui sp. n.* | SIO-BIC A8557 | Pacific Antarctic Ridge | 37°47'60"S | 110°55'0"W | 2,216 | – |
| *Thermiphione rapanui sp. n.* | SIO-BIC A7971 | East Pacific Rise | 23°32'47"S | 115°34’11"W | 2,595 | MG981040 |
| *Thermiphione rapanui sp. n.* | SIO-BIC A7972 | East Pacific Rise | 23°32'47"S | 115°34’11"W | 2,595 | MG981041 |
| *Thermiphione tufari* | SIO-BIC A7973 | East Pacific Rise | 23°32'47"S | 115°34’11"W | 2,595 | MG981042 |
| *Thermiphione tufari* | SIO-BIC A7974 | East Pacific Rise | 23°32'47"S | 115°34’11"W | 2,595 | MG981043 |
| *Thermiphione fijiensis* | SIO-BIC A7975 | Lau Back-Arc Basin | 20°19'0"S | 176°9’0”W | 2,719 | MG981044 |
| *Thermiphione fijiensis* | SIO-BIC A8510 | Kilo Moana, Lau Back-Arc Basin | 20°3’0”S | 176°9’0”W | 2,657 | MG981045 |
| *Iphione sp. 3* | SIO-BIC A8708 | Lord Howe Island, Australia | 31°31.603'S | 159°4.518'E | 5 | MH389786 |
Phylogenetic analyses

Alignments of the newly generated sequences, along with sequence data from GenBank for the four genes presented in Table 1 and published in the most recent aphroditiform phylogeny (Zhang et al. 2018) were performed using MAFFT (Katoh and Standley 2013). Poorly-aligned regions of the three rDNA genes were removed using Gblocks v.0.91b (Catresana 2000), with least stringent settings. This resulted in two concatenated alignments, referred to here as complete and Gblocked. Maximum likelihood (ML) analyses were conducted on the two datasets using RaXML v.8.2.10 (Stamatakis 2014) with each partition assigned the GTR+G model. Node support was assessed via thorough bootstrapping (1000 replicates). Bayesian Inference (BI) analyses were also conducted using MrBayes v.3.2.6 (Rohnquist et al. 2012). Best-fit models for these partitions were selected using the Akaike information criterion (AIC) in jModelTest 2 (Darriba et al. 2012; Guindon and Gascuel 2003). Maximum parsimony (MP) analyses were conducted using PAUP* v.4.0a161 (Swofford 2002), using heuristic searches with the tree-bisection-reconnection branch-swapping algorithm and 100 random addition replicates. Support values were determined using 100 bootstrap replicates. The acoetid Panthalis oerstedi Kinberg, 1856, was selected as the outgroup based on recent phylogenomic analyses that place Acoetidae as the sister clade to Iphionidae (Zhang et al., 2018). Uncorrected pairwise distances were calculated for the COI dataset with PAUP* v.4.0a161 (Swofford 2002). Median-joining haplotype networks (Bandelt et al. 1999) for Thermiphione napanui sp. n. and T. fijiensis were created with PopART v.1.7 (Leigh and Bryant 2015).

Morphology

Most parsimonious reconstructions for a few relevant characters were mapped onto the molecular phylogeny of Iphionidae using Mesquite v.3.4 (Maddison and Maddison 2018). No DNA data is presently available for Iphionella philippinensis, or Iphionides glabra, and they are not included in this study. Their eventual phylogenetic placement in Iphionidae will influence the inferred transformations found in this study. Morphological characters used were:
1. Elytra. Thirteen pairs of elytra are found in Iphionella (Pettibone, 1986), while Thermiphione has 14 pairs (Hartmann-Schröder 1992). Members of Iphione have 13 pairs of elytra (Pettibone 1986). The monotypic Iphionides has up to 20 pairs (Hartmann-Schröder 1977). Other Aphroditiformia, including the outgroup Acoetidae, normally have many elytral pairs. States, 0. Many pairs; 1. 13 pairs; 2. 14 pairs.

2. Palps. Within Iphionidae, Iphione have papillate palps, while all other Iphionidae and the outgroup have smooth palps (Pettibone 1986, Gonzalez et al. 2018). States, 0. Smooth; 1. Papillate.

3. Eyes. Within Iphionidae, Thermiphione and Iphionella risensis lack obvious eyes, while all other Iphionidae and the outgroup have them (Pettibone 1986, Gonzalez et al. 2018). States, 0. Present; 1. Absent.

4. Antennae. In general, Aphroditiformia have a median antenna, while most have lateral antennae (Gonzalez et al. 2018). Acoetidae have lateral and median antennae. A median antenna is absent in all Iphionidae, while the presence of lateral antennae varies. In Iphione, lateral antennae are present, while they are absent in Iphionella, Iphionides and Thermiphione (Pettibone 1986, Hartmann-Schröder 1992, Miura 1994). States, 0. Present; 1. Absent.

**Taxonomic note**

Iphionella was erected by McIntosh (1885) as a new genus of Polynoidae for a specimen collected from ~900 meters depth from off Philippines, identified as Iphione cimex Quatrefages, 1866. This species was therefore the type species for Iphionella by monotypy. Pettibone (1986) determined that this identification by McIntosh as Iphione cimex was incorrect as the type of Iphione cimex, described from the Malacca Strait, actually belonged to Polynoidae and should be placed in a new genus, Gaudichaudius Pettibone, 1986, and so it was referred to as G. cimex (Quatrefages, 1866). Pettibone (1986) then redescribed the specimen McIntosh (1885) had used to erect Iphionella as a new species, Iphionella philippinensis Pettibone, 1986. This was not in accordance with the International Code on Zoological Nomenclature at the time (see Art. 70.3; ICZN, 1999). According to 70.3.1, the correct type species name for Iphionella was Iphione cimex Quatrefages, which should have become Iphionella cimex (Quatrefages, 1866). Furthermore, since Iphione cimex is the type species of Gaudichaudia, then Gaudichaudia should become a junior synonym of Iphionella. As a result of this, Iphionella should be referred to Polynoidae, and the two currently accepted species of Iphionella, I. philippinensis and I. risensis Pettibone, 1986 are in the incorrect genus and require new names. While technically correct, we regard this as not being in accordance of a goal of taxonomic nomenclature to provide stability of names. We therefore endorse Pettibone’s (1986) non-ICZN-compliant actions. In order to preserve stability, the type species of Iphionella is now fixed here (under Art. 70.3.2 of the ICZN) as Iphionella philippinensis Pettibone, 1986, misidentified as Iphione cimex in the original designation by McIntosh (1885).
Results

The complete and G-blocked ML, BI and MP analyses (Figure 2) were congruent, showing the same topology for relationships and generally similar high support values within Iphionidae (Figure 2), except for relationships within Iphione. The Iphione terminals formed a sister clade to a well-supported clade comprised of all the iphionids from hydrothermal vents.

The two known Thermiphione species, T. fijiensis and T. tufari, formed a grade with respect to Iphionella risensis (Figure 2). The new species, Thermiphione rapanui sp. n., was the well-supported sister group to the sympatric T. tufari. The three East Pacific Rise taxa, I. risensis, T. tufari and T. rapanui sp. n. were recovered as the sister group to the western Pacific T. fijiensis. The taxonomic implications of the paraphyly of Thermiphione and our rationale for the generic placement of the new species are discussed below. The analysis of uncorrected pairwise COI distances (Table 3) showed that T. rapanui sp. n. was 10.5% divergent from its sister taxon, T. tufari, and 13–15% divergent from I. risensis and T. fijiensis (Table 3). For the four specimens of T. rapanui sp. n. that we obtained COI sequences for there were three haplotypes that varied from each other by only two base pairs (Figure 4B).

The parsimony reconstruction of ancestral states revealed an unambiguous convergent appearance of 14 pairs of elytra in Thermiphione fijiensis and Thermiphione tufari and that a elytral number of 13 represents the plesiomorphic state for Iphionidae. The absences of eyes and lateral antennae may be apomorphies for Thermiphione (but see below) (Figs 2, 3). The presence of papillate palps was apomorphic for Iphione (Figure 3).

Taxonomy

Iphionidae Kinberg, 1856

Thermiphione Hartmann-Schröder, 1992, emended
http://zoobank.org/7BC3CE3F-4C9B-476A-A263-B8B77B961467

Type species. Thermiphione tufari Hartmann-Schröder, 1992

Diagnosis (emended). Ventrally flattened, short, oval-shaped body. Between 28 and 32 segments in adults, with 13 or 14 pairs of elytra on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 26 (and 27, if 14 pairs) that cover dorsal side. Elytra rounded, covered with polygonal and/or hexagonal areas with lattice-like areolae; may exhibit papillae along elytral margins and on elytral surface near margins. Bilobed prostomium square to oval, merged with segment 1, with short, smooth, bulbous palps. Lateral and median antennae absent. Eyes absent. Segment 1 with paired enlarged anterior cirri (sensu Rouse and Pleijel 2001; = tentacular cirri), bearing each pair on a tentaculophore with an acicula and capillary chaetae. Mouth anterior, not ventral. Eversible pharynx with papillae and two pairs of jaws. Segment 2 bears first pair of elytra and parapodia,
spherical papillae. Segment 3 barely visible dorsally, with parapodia wedged between segments 2 and 4. Segments 4 and 7 bear spherical ventral papillae. All parapodia biramous: notopodia rounded and much smaller than neuropodia, with bundles of thin, feathered notochaetae; neuropodia large with thicker, single-tipped neurochaetae. Dorsal cirri with short papillae and cylindrical cirrophores. Ventral cirri much smaller than dorsal cirri, short and cirriform. Pygidium inconspicuous, lacking anal cirri.

**Figure 2.** Maximum likelihood tree of the combined analysis from four genes (28S, 18S, 16S, COI) aligned with MAFFT and then concatenated (No Gblocks). Numbers above nodes are bootstrap support percentages from RAxML and Maximum Parsimony analyses (separated by slashes), followed by Bayesian posterior probabilities from the complete dataset alignment (no Gblocks) and below nodes from Gblocks. Support values of 95% or greater for all analyses are indicated by stars.
Figure 3. Most parsimonious reconstructions of four traits mapped onto the molecular phylogeny (complete dataset). **A** Elytral pairs **B** Eyes **C** Palps **D** Lateral antennae.

Figure 4. Haplotype networks from COI data: **A** *Thermiphione fijiensis* network includes two sequences from specimens from the Lau Back-Arc Basin (black), and one from the type locality in Fiji (grey) **B** *Thermiphione ratanui* sp. n. network includes two sequences from 23°S (black) and two from 37°S (grey).
Remarks. Hartmann-Schröder's (1992) diagnosis of *Thermiphione* has been amended to accommodate the inclusion of *Iphionella risensis* and *Thermiphione rapanui* sp. n. The genus now comprises *Thermiphione fijiensis* (Figure 5A, D), *T. risensis* (Figure 5B, E), *T. tufari* (Figure 5C), and *T. rapanui* sp. n (Figs 6–9). The morphology of these taxa and phylogenetic evidence suggests that segment and elytral numbers are more variable than in the previous diagnosis. *Thermiphione* all have smooth palps, but this is plesiomorphic for Iphionidae. The absence of eyes may be an apomorphic state, depending on the eventual placement of *Iphionella philippinensis*, which was not included here owing to the lack of material for DNA sequencing. Similarly, the loss of lateral antennae may also be an apomorphy for *Thermiphione* once the position of *Iphionella philippinensis* and *Iphionides glabra*, which also lack them, is resolved.

*Thermiphione rapanui* sp. n.
http://zoobank.org/D201192A-0569-4C3E-8B22-4C3C3C6A27D7
Figures 6–9

Type-locality. German Flats, hydrothermal vents of Pacific Antarctic Ridge, 110°55′W, 37°48′S.

Material Examined. Type specimens. Holotype (SIO-BIC A8557) from German Flats, hydrothermal vents of Pacific Antarctic Ridge, (type locality above), HOV *Alvin* Dive 4088, 2216m depth, 22 March 2005; fixed in 10% SW formalin, preserved in 50% ethanol. The holotype was not sequenced directly to avoid damage but was morphologically identical to sequenced specimens from the same locality. Post-preservation, holotype 10 mm long, 8.5 mm wide including parapodia, 31 segments.

### Table 3. Uncorrected pairwise distances for COI data, generated with PAUP*.

|                     | *Thermiphione rapanui* sp. n. | *Thermiphione tufari* | *Thermiphione fijiensis* | *Thermiphione (Iphionella) risensis* | Iphione cf. treadwelli | Iphione sp. 1 | Iphione sp. 2 |
|---------------------|-------------------------------|-----------------------|--------------------------|--------------------------------------|------------------------|--------------|--------------|
| *Thermiphione tufari* | 10.48%                        | −                     | −                        | −                                    | −                      | −            | −            |
| *Thermiphione fijiensis* | 15.39%                       | 16.67%                | −                        | −                                    | −                      | −            | −            |
| *Thermiphione (Iphionella) risensis* | 13.39%                       | 14.25%                | 14.79%                   | −                                    | −                      | −            | −            |
| Iphione cf. treadwelli | 18.14%                        | 19.88%                | 17.27%                   | 19.23%                               | −                      | −            | −            |
| Iphione sp. 1        | 21.75%                        | 19.73%                | 20.39%                   | 21.52%                               | 18.78%                 | −            | −            |
| Iphione sp. 2        | 23.81%                        | 24.01%                | 21.66%                   | 24.00%                               | 23.35%                 | 24.73%       | −            |
| Iphione sp. 3        | 18.49%                        | 19.92%                | 17.42%                   | 19.06%                               | 0.76%                  | 19.75%       | 23.14%       |

Table 3. Uncorrected pairwise distances for COI data, generated with PAUP*.
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Figure 5. Dorsal and ventral micrographs of species in *Thermiphione*. Scale bars represent 5 mm. **A** *Thermiphione fijiensis* (SIO-BIC A7975), dorsal **B** *Thermiphione risensis* (SIO-BIC A6326, was *Iphionella risensis*), dorsal **C** *Thermiphione tufari* (SIO-BIC A7973), dorsal **D** *Thermiphione fijiensis* (SIO-BIC A7975), ventral **E** *Thermiphione risensis* (SIO-BIC A6326), ventral.

Paratypes: 1 specimen (SIO-BIC A7969) fixed and preserved in 95% ethanol, same location as holotype, post-preservation 9 mm long, 7 mm wide, 29 segments; 1 specimen (SIO-BIC A7970) from same location as holotype: anterior of specimen (approximately 14 segments) fixed in 10% SW formalin and preserved in 50% ethanol and posterior (approximately 14 segments) fixed and preserved in 95% ethanol; 2 specimens (SIO-BIC A7971, juvenile; SIO-BIC A7972) from the western flank of the Easter Microplate, East Pacific Rise, 115°34’W, 23°32’S, HOV *Alvin* Dive 4096, 2595m depth, 6 April 2005. SIO-BIC A7971 fixed and preserved in 95% ethanol, post-preservation 7 mm long, 4 mm wide, 19 segments; SIO-BIC A7972: anterior of specimen (approximately 20 segments) fixed in 10% SW formalin and preserved in 50% ethanol and posterior (approximately 9 segments) fixed and preserved in 95% ethanol.

**Diagnosis.** Ventrally flattened, oval-shaped body. Between 29 and 31 segments, with 13 pairs of elytra on segments covering dorsum. Elytra covered completely by polygonal areas enclosing areolae, with marginal papillae covering edges. Prostomium bilobed and slightly rounded. Eyes absent. Lateral and median antennae absent. Segment 1 with
Figure 6. Micrographs of live *Thermiphione rapanui*, sp. n., holotype (SIO-BIC A8557) and paratype (SIO-BIC A7969). Scale bars in A–E represent 1 mm, and scale bars in F–H represent 0.5 mm. A Dorsal view of whole body, holotype B Ventral view of whole body with pharynx everted, holotype C Dorsal view of whole body, paratype D Ventral view of whole body, paratype E Dorsal view of anterior region with scales, holotype F Dorsal view of anterior region with 2 pairs of scales removed, holotype. Abbreviations as follows: *tp*, terminal papilla; *p*, palp; *t*, tentaculophore; *e*, elytrophore G Ventral view of anterior region with pharynx and jaws everted/visible, holotype. Abbreviations: *dj*, dorsal jaw; *tp*, terminal papilla; *vj*, ventral jaw H Dorsal view of anterior region, paratype. *e*, elytrophore; *bp*, prostomium (bilobed); *mn*, medial nodule.
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Figure 7. Micrographs of Thermiphione rapanui sp. n. holotype (SIO-BIC A8557) and paratype (SIO-BIC A7971), stained with Shirlastain-A. Scale bars in A–C represent 1 mm, and scale bars in D–E represent 0.25 mm. A Dorsal view of anterior with 2 pairs of scales removed, holotype B Ventral view of anterior showing palps tentaculophore and cirri, paratype. C Ventral view of anterior with pharynx everted and jaws visible, holotype D Magnified dorsal view of anterior right side, holotype. Abbreviations as follows: e, elytrophore; p, palps; t, tentaculophore; eac, enlarged anterior cirrus E Magnified ventral view of left anterior parapodia and ventral cirri on segments 2 and 3, holotype. Abbreviations: bc, buccal cirrus; vc, ventral cirrus.

Description. In life, elytra pale brown with yellow tinge, becoming slightly paler after preservation. Body ventrally flattened, slightly tapered at anterior and posterior ends (Figure 6A–D). Holotype with 31 segments, 13 pairs of elytra, bacterial filaments on elytra (Figure 6A, B). One mature paratype SIO-BIC A7969, 29 segments, 13 pairs of elytra (Figure 6C, D). One juvenile paratype (SIO-BIC A7971), 19 segments, eight pairs of elytra (identified by scars; elytra lost in sampling).

Pharynx everted anteriorly in holotype, with 9 pairs terminal papillae, and dorsal and ventral pairs of hook-shaped jaws (Figs 6E–G, 7A–C). Prostomium bilobed, slightly rounded; eyes lacking (Figure 6H). Dorsal small circular medial nodules on segments 4 (1), and 5–8 (2 per segment) (Figure 6H). Lateral and median antennae lacking (Figs 6F–H, 7A–C). Pair of smooth palps, longer than pair of tentaculophores plus enlarged anterior cirri (tentacular cirri) (Figs 6F, 7A–B, D). Tentaculophores extending laterally to prostomium (Figs 6F, 7A–B, D), each with single acicula and very thin, short capillary chaetae on inner side. Enlarged anterior cirri, dorsal cirri, and ventral cirri with papillae (Figure 7). Buccal cirri on segment 2, also papillate, appearing larger than
remaining ventral cirri (Figure 7C, E). Thirteen pairs of elytra covering dorsum and oval in shape, on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 26 (Figure 8). First pair of elytra slightly compressed (Figure 8A); last pair much smaller in size and tapered at one end compared to other elytra (Figure 8B–C). Elytra covered completely by polygonal (generally hexagonal) areas enclosing areolae (Figure 8D–F). Thin, rounded marginal
Papillae covering lateral edges of elytra, sometimes sparsely extending towards posterior edges of elytra (Figure 8D-F). Remaining segments cirrigerous. Dorsal tubercles and dorsal cirri on segment 3, alternating on segments 6–29, with short, clavate papillae; anal cirri on segments 30, 31 (Figure 6B, D). Dorsal cirri long with short styles, usually extending to near tips of neurochaetae. Ventral cirri much shorter and smaller than dorsal cirri, present on segments 2–29 (Figure 7B–C, E). Anus dorsal; short ventral anal cirri similar to posterior dorsal cirri. Parapodia biramous (Figure 9), with short, subconical notopodia anterodorsal to larger neuropodia (Figure 9). Dense bundles of slender feathered notochaetae, shorter than neurochaetae (Figure 9F, H, J, L). Longer, simple, or slightly hooked neurochaetae, less dense but more numerous than notochaetae (Figure 9G, I, K). Upper neurochaetae generally longer than lower neurochaetae, with length of neurochaetae gradually decreasing towards dorsal and ventral edges (Figure 9).

**Variation.** Paratypes vary in segment number from holotype and were observed with fewer bacterial filaments on elytra.

**Genetic distance.** Paratype specimens from the 23°S sampling locality varied by two nucleotide bases from the holotype specimen, 37°S (Figure 4B). This genetic distance is so small that they are certainly all the same species. Unfortunately, our sampling was too limited for any analyses of connectivity.

**Etymology.** Thermiphione rapanui sp. n. is named after the traditional Polynesian name for Easter Island (Rapa Nui), which lies near one of the paratype localities. Neither of the specimens from near Easter Island were chosen as the holotype as they were in poor condition.

**Remarks.** Thermiphione rapanui sp. n. was collected from hydrothermal vents across 15 degrees of latitude, with the northernmost samples collected from the western flank of the Easter Microplate region at 23°S latitude, and the samples from further south collected on the East Pacific Rise at 37°S. The northernmost samples of Thermiphione rapanui sp. n. were collected from the same locality as samples of its sister taxon, *T. tufari*, which previously has only been recorded from slightly further north at 21°30’S (Hartmann-Schröder 1992).

Thermiphione rapanui sp. n. differs from its sister taxon *T. tufari* in that it has 13 pairs of elytra instead of 14 pairs of elytra and the last pair of elytra are on segment 26 instead of segment 27 (compare dorsal photos of each in Figs 6A and 5C, respectively). Like *T. tufari*, the new species also has up to 31 segments (Hartmann-Schröder 1992). Both *T. tufari* and *T. fijiensis* (Figure 5A) have 14 pairs of elytra and 30–31 segments (Pettibone, 1986), so elytral number may be convergent (Figure 3). Thermiphione was erected by Hartmann-Schröder (1992) and distinguished from other Iphionidae largely based on the presence of 14 pairs of elytra and 30–31 segments, but Iphionella risensis (Figure 5B), which nests within the Thermiphione (Figure 2), and Thermiphione rapanui sp. n. have 13 elytral pairs (Pettibone 1986). However, the two latter species differ in that *I. risensis* has 28–29 segments (Pettibone 1986) and *T. rapanui* sp. n. has 29–31 segments. *T. rapanui* sp. n. also differs from *I. risensis* in the presence of medial nodules on segments 6–8 in *T. rapanui* sp. n., which are absent on these segments in *I. risensis* (Pettibone 1986).
Figure 9. Interference contrast micrographs of *Thermiphione rapanui* sp. n. parapodia, (paratype SIO-BIC A7969). Scale bars in A–D represent 0.5 mm, and scale bars in E–L represent 0.1 mm. A Right parapodium 1 B Right parapodium 2 C Right parapodium 13 D Right parapodium 25 E Enlarged view of ventral cirrus (parapodium 2) F Feathered notochaetae (parapodium 2) G Chaetae of parapodium 25 H Notochaetae of right parapodium 2. I Slightly hooked neurochaetae (right parapodium 25) J Feathered notochaetae of parapodium 25 K Simple neurochaetae (some slightly hooked) from right parapodium 13. L Feathered notochaetae from right parapodium 13.
**Discussion**

The topologies of the likelihood and parsimony phylogenies are similar to those recovered in the recent analyses of Norlinder et al. (2012), Gonzalez et al. (2018), and Zhang et al. (2018) and support the maintenance of Iphionidae as a family distinct from Polynoidae.

The phylogeny demonstrates that our newly generated sequences for *Thermiphione fijiensis* represent the same species as the *Thermiphione* sp. published in Norlinder et al. (2012). These specimens were collected on the same cruise as the Norlinder et al. (2012) specimen. The *Thermiphione* sp. (Norlinder) specimen was collected at the White Lady hydrothermal vent, near the type locality for *Thermiphione fijiensis*. It is therefore identified here as *T. fijiensis*. The two specimens of *Thermiphione fijiensis* collected from the Lau Back-Arc basin, varied at most by a single base pair from the Norlinder et al. (2012) sequences (Figure 4A).

The distribution of the three East Pacific Rise iphionids sampled in this study (Table 2) and the phylogenetic results (Figure 2) indicate that *Iphionella risensis* forms a northern sister clade to the more southern *Thermiphione rapanui* sp. n. and *T. tufari* clades. This combined eastern Pacific clade is then sister group to *Thermiphione fijiensis* (Figure 2). The placement of *Iphionella risensis* makes *Thermiphione*, as currently formulated, paraphyletic. To resolve the paraphyly of *Thermiphione*, *Iphionella risensis* should be placed within *Thermiphione* and we do so here by amending the diagnosis for *Thermiphione* to allow for the presence of 13 or 14 pairs of elytra and 28–31 segments (see below). No DNA data currently exists for the type species of *Iphionella, I. philippinensis*.

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**References**

Bandelt H, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16(1): 37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036
Catresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17: 540–522. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8): 772. https://doi.org/10.1038/nmeth.2109

Desbruyères D, Segonzac M, Bright M (2006) Handbook of Deep-Sea Hydrothermal Vent Fauna. Biologiezentrum der Oberösterreichische Landesmuseen, Linz, Austria, 565 pp.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294–299. https://www.mbari.org/wp-content/uploads/2016/01/Folmer_94MxBB.pdf

Giribet G, Carranza S, Baguna J, Riutort M, Ribera C (1996) First molecular evidence for the existence of a Tardigrada plus arthropoda clade. Molecular Biology and Evolution 13: 76–84. https://doi.org/10.1093/oxfordjournals.molbev.a025573

Gonzalez BC, Martinez A, Borda E, Iliffe TM, Eibye-Jacobsen D, Worsaae K (2018) Phylogeny and systematics of Aphroditiformia. Cladistics 34: 225–259. https://doi.org/10.1111/cla.12202

Guindon S, Gascuel O (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Systematic Biology 52: 696–704. https://doi.org/10.1080/10635150390235520

Hartmann-Schröder G (1977) Die Polychaeten der Kubanisch-Rumänischen Biospeologischen Expedition nach Kuba 1973. Résultats des expéditions biopéologiques cubano-roumaines à Cuba 2: 51–63.

Hartmann-Schröder G (1992) Zur Polychaetenfauna in rezenten hydrothermalen Komplexmassivsulfiderzen (”Schwarze Raucher”) am Ostpazifischen Rucken bei 21°30’S. Helgoländer Meeresuntersuchungen 46: 389–403. https://doi.org/10.1007/BF02367206

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. https://doi.org/10.1093/molbev/mst010

Kinberg J (1856) Nya slägten och arter af Annelider, Öfversigt af Kongl. Vetenskaps-Akademins Förhållningar Stockholm 12: 381–388.

Maddison WP, Maddison DR (2018). Mesquite: a modular system for evolutionary analysis. Version 3.40. http://mesquiteproject.org

McIntosh WC (1885) Report on the Annelida Polychaeta collected by H.M.S. Challenger during the years 1873–1876. Ser. Zoology 12: 1–554.

Miura T (1994) Two new scale-Worms (Polynoidae: Polychaeta) from the Lau Back-Arc and North Fiji Basins, South Pacific Ocean. Proceedings of the Biological Society of Washington 107: 532–543.

ICZN (1999) International Code of Zoological Nomenclature. Fourth Edition. The International Trust for Zoological Nomenclature, London, UK. http://www.iczn.org/iczn/index.jsp

Norlinder E, Nygren A, Wiklund H, Pleijel F (2012) Phylogeny of scale-worms (Aphroditiformia, Annelida), assessed from 18SrRNA, 28SrRNA, 16SrRNA, mitochondrial cytochrome c
oxidase subunit I (COI), and morphology. Molecular Phylogenetics and Evolution 65: 490–500. https://doi.org/10.1016/j.ympev.2012.07.002

Palumbi SR (1996) Nucleic acid II: the polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK (Eds) Molecular Systematics. 2nd ed, Sinauer Associates, Inc, Sunderland, MA, 205–247.

Pettibone MH (1986) Review of the Iphioninae (Polychaeta: Polynoidae) and revision of *Iphione cimex* Quatrefages, *Gattiana deludens* Fauvel, and *Harmothoe iphionelloides* Johnson (Harmothoinae). Smithsonian Contribution to Zoology 428: 1–43. https://doi.org/10.5479/si.00810282.428

Quatrefages A de (1866) Histoire naturelle des Annelés marins et d’eau douce. Annélides et Géphyriens. Librarie Encyclopédique de Roret, Paris, France, 588 pp.

Read G, Fauchald K (Eds) (2018) World Polychaeta database. Iphionidae Kinberg, 1856. http://www.marinespecies.org/polychaeta/aphia.php?p=taxdetails&id=155222 [on 2018-05-23]

Rouse GW, Pleijel F (2001) Polychaetes. Oxford University Press, London, 354 pp.

Ronquist F, Teslenko M, Mark P van der, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Struck T, Purschke G, Halanych K (2006) Phylogeny of Eunicida (Annelida) and Exploring Data Congruence Using a Partition Addition Bootstrap Alteration (PABA) Approach. Systematic Biology 55: 1–20. https://doi.org/10.1080/10635150500354910

Swofford DL (2002) Phylogenetic analysis using parsimony (*and other methods) v.4.0a161. Sinauer Associates, Sunderland, Massachusetts.

Whiting MF, Carpenter JC, Wheeler QD, Wheeler WC (1997) The Strepsiptera Problem: Phylogeny of the Holometabolous Insect Orders Inferred from 18S and 28S Ribosomal DNA Sequences and Morphology. Systematic Biology 46: 1–68. https://doi.org/10.2307/2413635

Zhang Y, Sun J, Rouse GW, Wiklund H, Pleijel F, Watanabe HK, Chen C, Qian P, Qiu J (2018) Phylogeny, evolution and mitochondrial gene order rearrangement in scale worms (Aphroditiformia, Annelida). Molecular Phylogenetics and Evolution 125: 220–231. https://doi.org/10.1016/j.ympev.2018.04.002