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Isanthidae

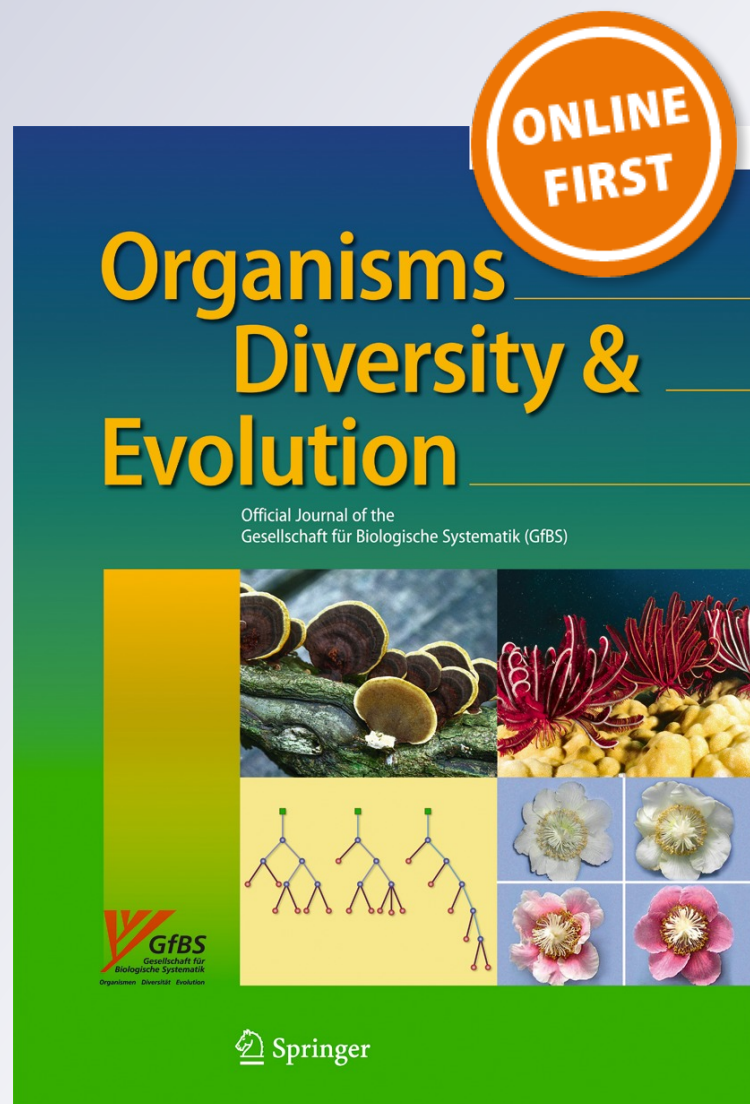
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Isoparactis fionae sp. nov. (Cnidaria: Anthozoa: Actiniaria) from Southern Patagonia with a discussion of the family Isanthidae

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Abstract A new species from Southern Patagonia, *Isoparactis fionae* sp. nov., is described. *Isoparactis fionae* sp. nov. differs from other species in the genus by having longitudinal rows of adherent rugae in the column, number of tentacles, cnidae and geographic distribution. We amend the generic position of the previously termed *Paraisanthus fabiani* (now *Isoparactis fabiani* comb. nov.), transferring it to *Isoparactis* as we discovered acontia in the type material of this species. We amend the binomen of *Acraspedanthus ferax* and use the correct binomen for the species *Isoparactis ferax*. In addition, we transfer the genus *Isoparactis* (previously within Bathypheilliidae as *Acraspedanthus*) to Isanthidae based on molecular evidence and because its diagnosis corresponds better with this family; we amend the diagnosis of Isanthidae accordingly. Finally, we discuss phylogenetic relationships within Isanthidae and among members of the superfamily Metridioidea. Our results show that Isanthidae is composed of derived acontiate sea anemones that have diversified in shallow waters of the Southern Hemisphere

and that the family is putatively the sister group of a deep-sea lineage.

Keywords Acontia · Argentina · Beagle Channel · Chile · Sea anemone · Straits of Magellan

Introduction

Carlgren (1924) erected the genus *Acraspedanthus* Carlgren, 1924, to accommodate *A. elongatus* Carlgren, 1924 –the type species of the genus by monotypy – from New Zealand. *Acraspedanthus* was initially placed within the recently resurrected family Phelliidae Verrill, 1868 (see Rodríguez et al. 2012); later it was transferred to Bathypheilliidae Carlgren, 1932, a family defined by having acontia with only basitrichs and mesenteries divisible into macro- and micro-cnemes. The diagnosis of *Acraspedanthus* also agrees well with that of Isanthidae Carlgren, 1938 (mesogleal marginal sphincter, macro- and micro-cnemes, basilar muscles and very strong retractor muscles), but members of the five currently recognized genera lack acontia (Carlgren 1949; Häussermann and Försterra 2008). Rodríguez et al. (2012) resurrected the superfamily Metridioidea Carlgren, 1893 [formerly Subtribe Acontiaria (sic)], a group including all sea anemones having acontia, but also some families lacking these structures. Based on molecular evidence and morphological data – in recognition of acontia having been lost in several instances – a few families (such as Isanthidae) have been transferred to Metridioidea (Rodríguez et al. 2012).

Parry (1951, 1952) synonymized *Isoparactis ferax* (Stuckey, 1909) with *Acraspedanthus elongatus*. Because the genera *Isoparactis* Stephenson, 1920, and *Acraspedanthus* only had one species each at the time both genera are synonyms, with *Isoparactis* having priority over *Acraspedanthus* (International Code of Zoological Nomenclature 1999). However, Parry (1952) used the incorrect binomen for the species and listed it

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as *Acraspedanthus ferax*. Here, we amend Parry's mistake and use the correct binomen, *Isoparactis ferax*.

We describe *Isoparactis fionae* sp. nov. – from shallow waters of Southern Patagonia – based on 18 specimens collected from the northern shore of the Straits of Magellan in 1998 and in the Beagle Channel in 2010 and 2012. One of the two only other representatives of Isanthidae in South America is the recently described *Paraisanthus fabiani* Häussermann and Försterra, 2008. However, the initial assessment of *P. fabiani* lacking acontia (Häussermann and Försterra 2008) is equivocal: although scarce and rather small, we found acontia in the type material of *P. fabiani*. Because the type species of *Paraisanthus* Sanamyan and Sanamyan, 1998, lacks acontia (Sanamyan and Sanamyan 1998; Sanamyan personal communication), we amend the generic position of the previously termed *P. fabiani* as *Isoparactis fabiani* comb. nov. We transfer the genus *Isoparactis* (previously within Bathypheiliidae as *Acraspedanthus*) to Isanthidae and amend the diagnosis of this family accordingly. Finally, we discuss phylogenetic relationships within Isanthidae and among members of the superfamily Metridioidea.

Materials and methods

Taxonomic data collection

The 18 examined specimens were collected via SCUBA diving in Southern Patagonia (Fig. 1). Specimens were relaxed with menthol crystals prior to fixation in 10 % seawater-buffered formalin and after a few months were transferred to 70 % ethanol for long-term storage. Tissue subsamples from several specimens were fixed in 96 % ethanol and RNA_{later} (Life technologies) for molecular analysis.

Specimens were examined whole and some were dissected. Histological sections 5 to 10 μ m thick were made from different parts of seven specimens; they were stained with Azocarmin Triple Stain (Humason 1967) or Ramón y Cajal's Triple Stain (Gabe 1968).

The distribution of cnidae in the tissues was analyzed from nine specimens of *Isoparactis fionae* sp. nov., two paratypes of *I. fabiani* comb. nov., and two syntypes and two additional specimens of *I. ferax* using light microscopy (1,000 \times magnification, oil immersion). Forty non-fired capsules of each cnida type (whenever possible) were haphazardly measured and photographed. Mean and standard deviation is provided to give an estimate of size distribution; these are not statistically significant (see Williams 1998, 2000 for minimal requirements for statistical significance in cnida sizes) but provide some qualitative information about variability in capsule size for each type of nematocyst. Cnida nomenclature follows Mariscal (1974).

The studied material has been deposited in the Museo Argentino de Ciencias Naturales (MACN) in Argentina, the

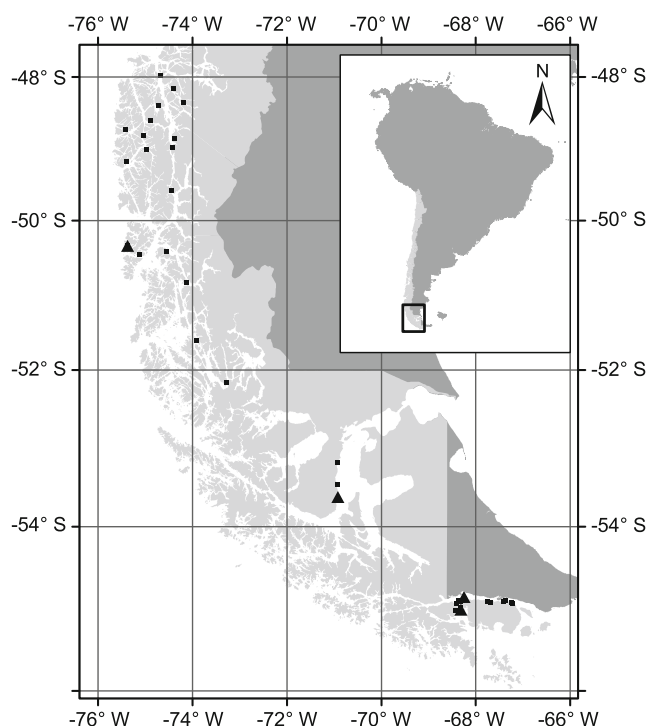


Fig. 1 Geographic distribution of *Isoparactis fionae* sp. nov. Triangles indicate localities where *I. fionae* sp. nov. was found; squares indicate those localities where *I. fionae* sp. nov. was not found. Light shading corresponds to Chile

American Museum of Natural History (AMNH) in the USA, the Zoologische Staatssammlung München (ZSM) in Germany and the Museo de Zoología de la Universidad de Concepción (MZUC-UCCC) in Chile.

Molecular data collection and analysis

Total genomic DNA was extracted from tissue using a DNeasy Blood & Tissue Kit (Qiagen), with slight modifications (following overnight digestion with Proteinase K, we added another 20 μ l Pro K and incubated for 3 h; 5 min AE buffer incubation at room temperature; eluted AE buffer twice, 50 μ l each time). We targeted three mitochondrial (12S and 16S rDNA, and *cox3*) and two nuclear (18S and 28S rDNA) gene regions using primers previously published by Apakupakul et al. (1999; 18S), Medina et al. (2001; 28S), Geller and Walton (2001; 16S and *cox3*) and Chen et al. (2002; 12S). PCR was performed using Fisher BioReagents *Taq* DNA polymerase [final concentrations in a 25 μ l reaction: 1X Buffer A (includes $MgCl_2$); 0.8 mM dNTP mixture (total); 0.4 μ M of each primer; 0.03 U/ μ l *Taq* polymerase; 0.1 mg/ml acetylated bovine serum albumin (Promega); 40–45 ng template; brought to final volume with dH_2O]. PCR products were cleaned using a Thermo Scientific Fermentas clean-up protocol utilizing Exonuclease I and FastAPTM thermosensitive alkaline phosphatase (per manufacturer's specifications, except that shrimp alkaline phosphatase

was replaced with FastAP™). Purified PCR product was cycle sequenced directly in an ABI BigDye® Terminator v3.1 (Applied Biosystems) cycle sequencing reaction following the manufacturer's protocols (except for one-fourth of the recommended 'Ready Reaction Premix' in 10- μ l total volume reactions), and cleaned using Centri-Sep columns (Princeton Separations; following the manufacturer's protocols) containing DNA-grade Sephadex (G-50 Fine; GE Healthcare). Cycle sequencing products were electrophoresed on an ABI PRISM® 3730xl Genetic Analyzer. All sequence traces were edited using Sequencher™ version 5.0 (Gene Codes Corp.), gene identity was verified with BLAST (Altschul et al. 1990), and sequences were subsequently transferred to Se-AL v2.0a11 Carbon. The multiple sequence alignment was determined with MAFFT (Multiple Alignment using Fast Fourier Transform) version 7 (online at <http://mafft.cbrc.jp/alignment/server/>) using the following parameters: strategy: L-INS-i, scoring matrix: 200PAM/ $k=2$, gap open: 1.53, gap offset: 0.05 (Katoh et al. 2002, 2005; alignment available upon request). The Akaike information criterion (AIC) was implemented within jModelTest v2.1.2 (Darriba et al. 2012) to determine the appropriate evolutionary model (GTR+I+G) and corresponding parameters [p-inv: 0.3230, gamma shape: 0.4530, freqA: 0.2425, freqC: 0.2311, freqG: 0.2740, freqT: 0.2524, (AC): 0.9162, (AG): 2.8799, (AT): 0.9435, (CG): 1.2928, (CT): 6.0144, (GT): 1.0000] for the concatenated data set (number of candidate models: 88; number of substitution schemes: 11; base tree for likelihood calculations: ML optimized using PhyML v3.0 (Guindon et al. 2010)). The concatenated data set consisted of 87 taxa and 5,504 sites. Herein we provide new sequences for *I. fionae* sp. nov. and *I. ferax*, which we added to the data matrix presented in Rodríguez et al. (2012). For a complete account of taxa included in this study, we refer readers to Rodríguez et al. (2012). New sequences have been deposited in GenBank (Table 1). We searched for optimal trees using maximum likelihood within RAxML-HPC BlackBox (v7.3.1, released by Alexandros Stamatakis) on the CIPRES Science Gateway web portal (Miller et al. 2010). We conducted rapid bootstrapping (RB) with a subsequent ML search and let RAxML halt

bootstrapping automatically (RAxML executed 350 RB replicates with MRE-based bootstopping criterion). We also conducted tree searches under maximum parsimony (results not shown) with TNT v1.1 (random and consensus sectorial searches, tree drifting and 100 rounds of tree fusing; Goloboff et al. 2008). In all analyses, gaps were treated as missing data (-). Trees of minimum length were found at least five times. The concatenated data set was subjected to 1,000 rounds of bootstrap resampling to assess support for clades. In addition, we searched for optimal trees using Bayesian criterion within MrBayes v3.1.2 (data set not partitioned, number of runs: 2, number of chains: 4, number of generations: 5 million, chain sample frequency: 1,000, sump and sumt burnin: 1,250; Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Priors were fixed for substitution rates (revmat), stationary nucleotide frequencies (statefreq), proportion of invariable sites (pinvar) and gamma, as output by jModelTest v2.1.2.

Systematics

Suborder NYNANTHEAE Carlgren, 1899

Superfamily METRIDIOIDEA Carlgren, 1893

Family ISANTHIDAE Carlgren, 1938

DIAGNOSIS (after Carlgren 1949 and Rodríguez et al. 2012; modifications in bold)

Metridioidea with basilar muscles and mesogleal **marginal** sphincter. Mesenteries divisible into macro- and microcnemes. Retractor muscles strongly restricted (reniform) to almost circumscribed. **Acontia if present with basitrichs and microbasic p-amastigophores. Cnidom: spirocysts, basitrichs, holotrichs and microbasic p-amastigophores.**

Included genera. *Isoparactis*; *Isanthus* Carlgren, 1938; *Paraisanthus*; *Eltaninactis* Dunn, 1983; *Zaolutus* Hand, 1955; *Austroneophellia* Zamponi, 1978.

REMARKS. The diagnosis now includes the possibility of acontia being present and details of the cnidom. We have referred to the cnida that Sanamyan and Sanamyan (1998) called a heterotrich in the diagnosis of *Paraisanthus* as a

Table 1 GenBank accession numbers of new sequences provided in this study. See Rodríguez et al. (2012) for a complete list of taxa and data included in the analysis. Voucher specimens are located in the American Museum of Natural History

Species	<i>cox3</i>	12S	16S	18S	28S
<i>Isoparactis fionae</i> sp. nov.	KC700007	KC700001	KC700003	KC700004	No data
<i>Isoparactis ferax</i> (Stuckey, 1909)	KC700008	KC700002	No data	KC700005	KC700006

cox3: Reference=*Metridium senile cox3* (GenBank accession no. NC_000933; 789 bp); alignment begins 88 bp into *cox3* (alignment length: 603 bp, shortest sequence: 280 bp, longest sequence: 603 bp). 12S: Reference=*M. senile* 12S (NC_000933; 1,082 bp); alignment begins 219 bp into 12S (alignment length: 982 bp, shortest seq: 619 bp, longest seq: 859 bp). 16S: Reference=*M. senile* 16S (NC_000933; 2,189 bp); alignment begins 1,352 bp into 16S (alignment length: 535 bp, shortest seq: 262 bp, longest seq: 495 bp). 18S: Reference=*Nematostella vectensis* 18S (AF254382; 1,723 bp); alignment begins on first base of 18S and extends 20 bp into ITS1 (alignment length: 2,203 bp, shortest seq: 844 bp, longest seq: 1,929 bp). 28S: Reference=*Tethya* sp. 28S [AY626300 (poriferan); 3,565 bp]; alignment begins 284 bp into 28S (alignment length: 1,181 bp, shortest seq: 55 bp, longest seq: 961 bp)

holotrich. As defined by Mariscal (1974) and England (1991), a heterotrich has a tubule that is dilated and bears longer spines at the base. These attributes are specific to discharged capsules; Sanamyan and Sanamyan (1998) only mention undischarged capsules. In the undischarged capsules we examined and in the illustrations provided by Sanamyan and Sanamyan (1998), we do not find any evidence of differentiation along the tubule, and thus prefer to be more conservative and use the term holotrich.

Isoparactis Stephenson, 1920

DIAGNOSIS (after Carlgren 1924; modifications in bold)

Isanthidae with definite pedal disc. Column smooth **or with adherent rugae**; sometimes clinclides proximally. Margin rather distinct, without fosse. Tentacles short. Longitudinal muscles of tentacles and radial muscles of oral disc ectodermal. Two well-developed siphonoglyphs. More mesenteries distally than proximally. Six perfect pairs of mesenteries, fertile, **with strong, circumscribed retractor muscles**. Basilar muscles well developed. Acontia present with basitrichs **and in some specimens microbasic p-amastigophores**. Cnidom: spirocysts, basitrichs and microbasic p-amastigophores.

TYPE SPECIES. *Paractis ferax* Stuckey, 1909.

INCLUDED SPECIES. *Isoparactis ferax*; *I. fabiani* comb. nov.; *I. fionae* sp. nov.

REMARKS. The diagnosis now includes adherent rugae (*sensu* Stephenson 1928) in the column because they are present in *Isoparactis fionae* sp. nov. Also included is the presence of microbasic p-amastigophores in the acontia as they are found in all three species, although not in every specimen.

Isoparactis fionae sp. nov.

(Figs. 1, 2, 3, 4 and 5, Tables 1, 2 and 3)

Type material

Holotype (MACN-IN 39151); Beagle Channel, Tierra del Fuego, Argentina (54°51'S, 68°14'W), December 2010, between 7–15 m. Paratypes (AMNH 6041); 3 specimens, Beagle Channel, Tierra del Fuego, Islas Bridges, Argentina, (54°51'53.13"S, 68°14'55.98"W), December 2010, 6 m. (ZSM 20051668); 4 specimens, Fuerte Bulnes, Chile, (53°50'S, 70°56'W), March 1998, between 4–7 m.

Additional material

(MACN-IN 38991); 2 specimens, Beagle Channel, Tierra del Fuego, Argentina (54°52'41.46"S, 68°14'53.70"W), March 2012, between 7–15 m. (MZUC-UCCC); 2 specimens, Fuerte Bulnes, Chile, (53°37'S, 70°55'W), December 2010, between 5–17 m. During the course of this study we utilized all remaining tissue for an additional 6 specimens: 1 specimen, Fuerte Bulnes, Chile, (53°37'S, 70°55'W), December 2010,

between 5–17 m; 5 specimens, Beagle Channel, Tierra del Fuego, Islas Bridges, Argentina, (54°52'41.46"S, 68°14'53.70"W), December 2010, 5 m.

Comparative material examined

Acraspedanthus elongatus Carlgren, 1924: (SMNH-Type 4046) (2 syntypes). New Zealand, North Cape; Mortensens Exp. 1914-15.

Isoparactis ferax (Stuckey 1909): (AMNH 6039), 3 specimens, Island Bay, New Zealand (41°20'5"S, 174°45'5"E), November 2011, 1 m.

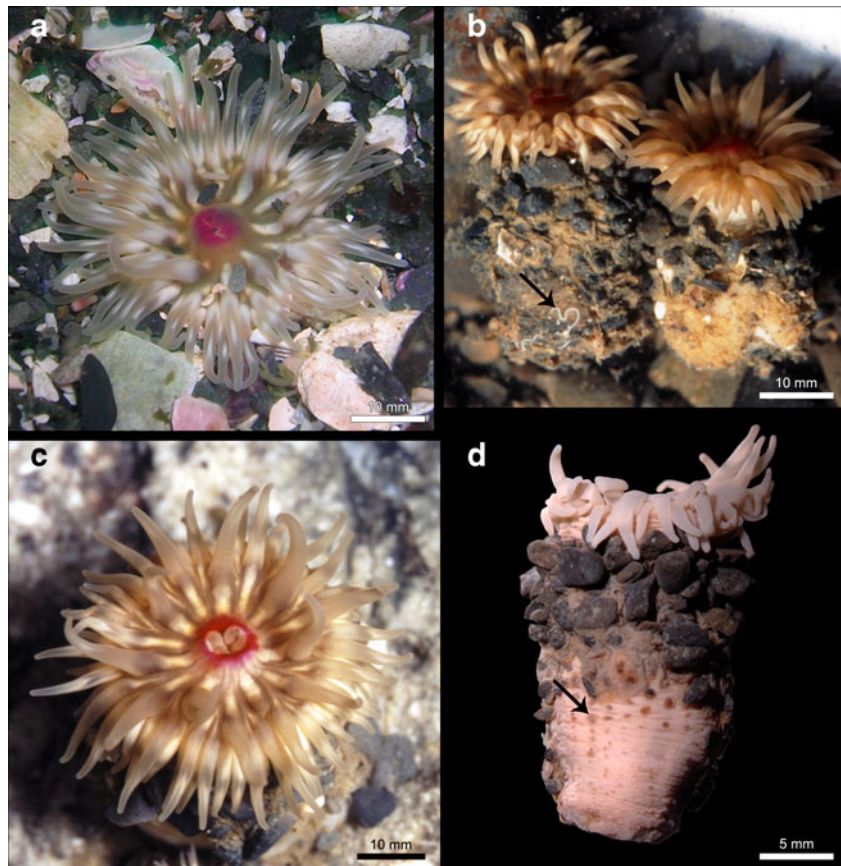
Paraisanthus fabiani Häussermann and Försterra, 2008: (ZSM 20070250) (1 paratype). (ZSM 20051705) (half paratype). (ZSM 20070251) (1 paratype).

Description

External anatomy. Pedal disc well developed, flat, wider than column; most examined specimens not attached to the substratum. Column elongated (Fig. 2b, d), 15.8–20.0 mm in height and 6.1–14.0 mm in diameter in preserved specimens and to 50 mm in height and 15 mm in diameter in live specimens. Column in live specimens light pink or brownish; in preserved specimens column darker pink or light brown with slight pink tone. Column with 24 endocoelic longitudinal rows of adherent rugae more evident distally, with small rocks and debris firmly attached (Figs. 2d and 3d). Some preserved specimens with smooth column proximally. Oral disc to 8.1–10.8 mm in diameter in preserved specimens (to 30.0 mm in live specimens). Tentacles hexamerously arranged, to 68 (Fig. 2a, c), with perforated tip, to 25.0 mm in live specimens (to 1.6 mm in preserved ones); inner ones longer than outer ones, on outer third of oral disc; innermost 12 tentacles (two cycles) often directed upward (Fig. 2a, c). Exocoels on oral disc ochre to brown; endocoels corresponding to inner 12 tentacles somewhat translucent brown to whitish. Tentacles translucent brown with more or less broad whitish line on oral side, which might be interrupted by one to two darker arrows. Bright pink circle around mouth opening (Fig. 2a, c); actinopharynx translucent brown.

Internal anatomy. More mesenteries distally than proximally. Forty-eight mesenteries (24 pairs) at actinopharynx level hexamerously arranged in three cycles (6+6+12=24) (Fig. 3b). Only first cycle including directives perfect and fertile (macrocnemes). Second and third cycle with poorly developed longitudinal muscles, without mesenterial filaments or gametogenic tissue (microcnemes). Two siphonoglyphs, each with one pair of directives attached. Gonochoric; specimens collected in March and December with gametogenic tissue well developed (oocytes to 0.425 mm in diameter in December). Retractor muscles strong, circumscribed (Fig. 3e). Parietobasilar muscles

Fig. 2 External anatomy of *Isoparactis fionae* sp. nov. **a, c** Oral view of living specimen *in situ*. **b** Lateral view of living specimens; notice acontium (arrow). **d** Lateral view of a preserved specimen; notice longitudinal rows of adherent rugae (arrow)



strong, with thick mesogleal pennon (Fig. 3e). Marginal sphincter muscle mesogloea, strong, reticulated, occupying almost entire width of mesogloea distally (Fig. 3a). Longitudinal muscles of tentacles and radial muscles of oral disc ectodermal (Fig. 3f, c). Acontia only in macro-cnemes, coiled and relatively short. Basilar muscles well developed (Fig. 3g). Epidermis thicker than mesoglea and gastrodermis at actinopharynx level, to 0.50 mm thick; mesogloea to 0.32 mm thick, gastrodermis to 0.22 mm thick.

Cnidom. Spirocysts (in tentacles and pedal disc), basitrichs (in all tissues) and microbasic *p*-amastigophores (in all tissues) (Fig. 4). See Table 2 for size and distribution.

Common name. Pink mouth anemone.

ETYMOLOGY. The species is named after Fiona Häussermann, who in 2010 at the age of 1 year accompanied us on the expedition during which the species was collected. She is the sister of Fabian Häussermann after whom the sister species of *I. fionae* sp. nov. is named (*I. fabiani* comb. nov.).

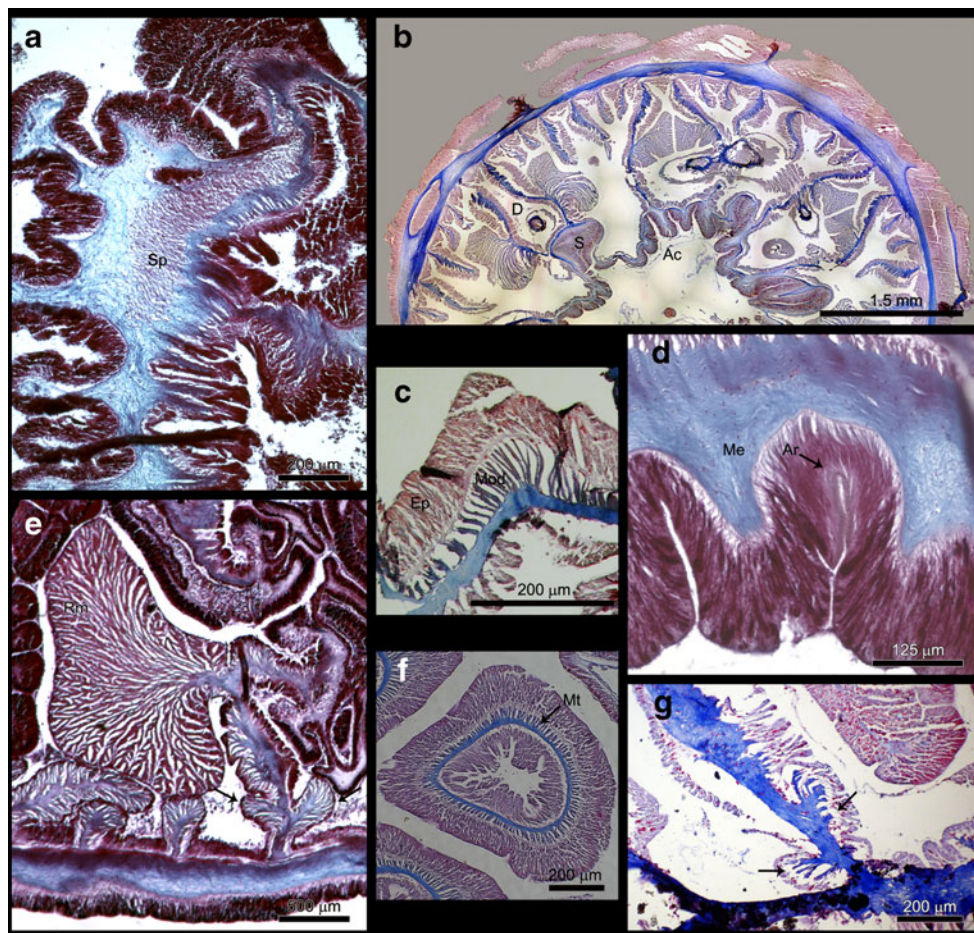
DISTRIBUTION AND NATURAL HISTORY. *Isoparactis fionae* sp. nov. inhabits the Southern Patagonian coast. It was observed on the northern shore of the Straits of Magellan and on both shores of the Beagle Channel (Tierra del Fuego and Navarino Island) but not along the Argentinean Atlantic coast; additionally, we recently observed it as far north as the Archipelago Madre de Dios (Fig. 1). The

specimens were found between 3–17 m, but not in the intertidal zone. In the Beagle Channel, the specimens were burrowed into coarse soft bottoms – composed mainly of sand, small rocks and shell fragments – in exposed areas, but strongly attached to rocks or stones when in sand-filled crevices or fixed on smaller loose rocks on a pebble substrate. However, specimens in the Straits of Magellan were found in hard substrate, hidden in small crevices; only the oral disc was visible in both areas. The species was present only at 5 of more than a 100 examined sites along the Chilean coast.

Phylogenetic analysis

All phylogenetic reconstructions (parsimony, maximum likelihood and Bayesian criterion) using the concatenated data set (*cox3-12S-16S-18S-28S*) agreed in basic topology: *Isoparactis fionae* sp. nov. is sister to *I. fabiani* comb. nov., and both are sister to *I. ferax* within Metridioidea (Fig. 5). *Isoparactis ferax* does not group with *Bathypheilia*, the latter being the only member of Bathypheiliidae included in our analysis. Although only including two of the six currently recognized genera, members of Isanthidae are resolved together with high support [100 % bootstrap support (BS), 100 % Bayesian posterior probability (BPP)]. While the Isanthidae

Fig. 3 Internal anatomy of *Isoparactis fionae*, sp. nov. **a** Longitudinal section of distal part of the column showing the mesogleal marginal sphincter muscle. **b** Cross section at the actinopharynx level showing cycles of mesenteries. **c** Section of the oral disc showing the ectodermal longitudinal muscles. **d** Detail of an adherent rugae. **e** Detail of retractor and parietobasilar muscles (arrows). **f** Section of a tentacle showing the ectodermal longitudinal muscles. **g** Longitudinal section of the pedal disc showing the basilar muscles (arrows). *Ac*, actinopharynx; *Ar*, adherent rugae; *D*, directives; *Ep*, epidermis; *Me*, mesogloea; *Mod*, longitudinal muscles of the oral disc; *Mt*, longitudinal muscles of the tentacle; *Rm*, retractor muscle; *S*, siphonoglyph; *Sp*, sphincter



groups as sister to Deepsina — a clade including sea anemones from deep-sea and chemosynthetic environments in which some members have also lost the acontia (Rodríguez and Daly 2010; Rodríguez et al. 2012) — there is little to no support (21 % BS, 54 % BPP). A Isanthidae+Deepsina relationship was also recovered using PhyML v3.0, but once again lacked support (28 % BS; phylogeny not shown).

For the MrBayes analysis, the average standard deviation of split frequencies at 5 million generations was 0.164792, suggesting that the chains did not converge (a subsequent run using 10 million generations increased the average SD to 0.194383). Thus, Bayesian posterior probabilities should be interpreted with caution.

Discussion

On *Isoparactis ferax*

Parry (1951, 1952) synonymized *Acraspedanthus elongatus* with *Isoparactis ferax* based on the live aspect of the species. By synonymizing both species, Parry implied that Stuckey's specimens had acontia and cinclides, but failed to see them

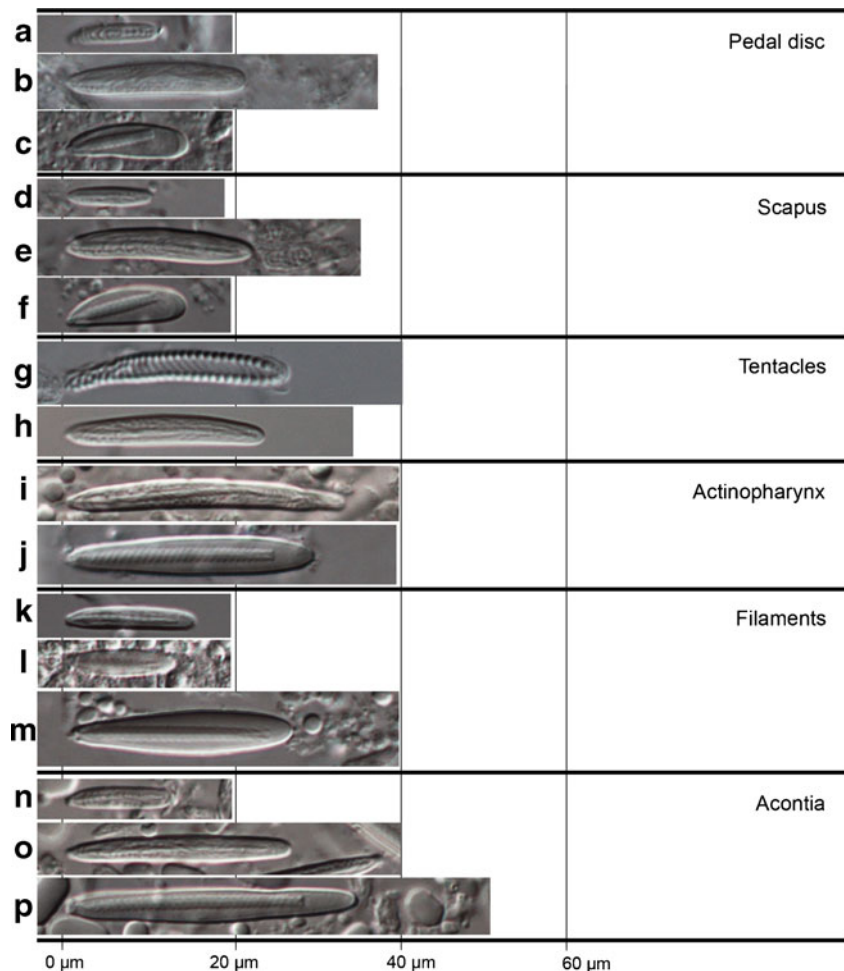
because he did not study Stuckey's specimens. Regrettably, the type material of *I. ferax* is not locatable and cannot be examined to check what constitutes *Isoparactis*. However, the study of specimens that could be identified using Stuckey's description of living material as *P. ferax* (M. Daly personal communication) has led us to the conclusion that this species is identical with the type material of *A. elongatus*. Acontia and cinclides are often easily overlooked in some taxa, and both species were collected from relatively close localities (south and north of the North Island of New Zealand, respectively). Thus, we render *A. elongatus* as a junior synonymy of *I. ferax*.

Familial placement of *Isoparactis*

Although the diagnosis of *Isoparactis* (as *Acraspedanthus*) agrees well with that of Isanthidae provided by Carlgren (1938, 1949), the presence of acontia in *Isoparactis* obscures the similarity between *Isoparactis* and other isanthid genera. However, the morphology, anatomy and cnidae of the genus together with molecular evidence support the placement of the genus within the family (Rodríguez et al. 2012).

Isoparactis differs from other genera in the family in the number of perfect mesenteries, number of mesenteries

Fig. 4 Cnidae of *Isoparactis fionae* sp. nov. **a-b, d, e, h, i, k, n, o** Basitrichs. **c, f, j, l, m, p** Microbasic *p*-amastigophores. **g** Spirocyst



proximally and distally, presence of adherent rugae, cinclides and acontia, and cnidae (see Table 3).

Generic placement

Isoparactis fionae sp. nov. agrees with most of the diagnostic characters of *Isoparactis*: it has a well-developed mesogloea marginal sphincter, two well-developed siphonoglyphs, more mesenteries distally than proximally, six pairs of perfect and fertile mesenteries, and the acontia and the longitudinal muscles of the tentacles and radial muscles of the oral disc are ectodermal (Carlgren 1924, 1949). Although *Isoparactis* (as *Acraspedanthus*) was defined as having only basitrichs in the acontia (Carlgren 1945, 1949), we found microbasic *p*-amastigophores in the acontia of at least one of the two syntypes of *I. ferax*; capsules of microbasic *p*-amastigophores were relatively common but easily overlooked because of their similar shape and size ranges to the very abundant basitrichs. These probably also correspond with the wider cnida capsules that Carlgren (1924) reported in the acontia of only one specimen of *I. ferax* but interpreted at the time as developmental stages of the basitrichs. Microbasic *p*-amastigophores

have also been reported to be scarce or only present in the acontia of some specimens in taxa such as *Bathypheilia* (see Riemann-Zürneck 1997; Sanamyan et al. 2009). Similarly, we found microbasic *p*-amastigophores in only one specimen of the newly collected material of *I. ferax* and in four of the six specimens of *I. fionae* sp. nov. that had acontia (see Table 2). Carlgren (1924) describes cinclides proximally in the column of *I. ferax*. We did not detect cinclides in the type material of *I. ferax*; however, we did not prepare histological sections of the type material. Similarly, we did not detect cinclides in *I. fionae* sp. nov. or *I. fabiani* comb. nov.

The presence of adherent rugae in *I. fionae* sp. nov. and the lack of cinclides in *I. fionae* sp. nov. and *I. fabiani* comb. nov. might be considered generic-level characters and warrant erecting a new genus for the South American species of the genus. Furthermore, our molecular phylogenetic analysis resolved *I. fionae* sp. nov. and *I. fabiani* comb. nov. as sister to *I. ferax*, supporting a closer relationship between *I. fionae* sp. nov. and *I. fabiani* comb. nov. than to *I. ferax*. It should be noted that the current molecular toolkit for sea anemones and closely related taxa (e.g., antipatharians and scleractinians) includes markers (primarily mitochondrial DNA) that cannot

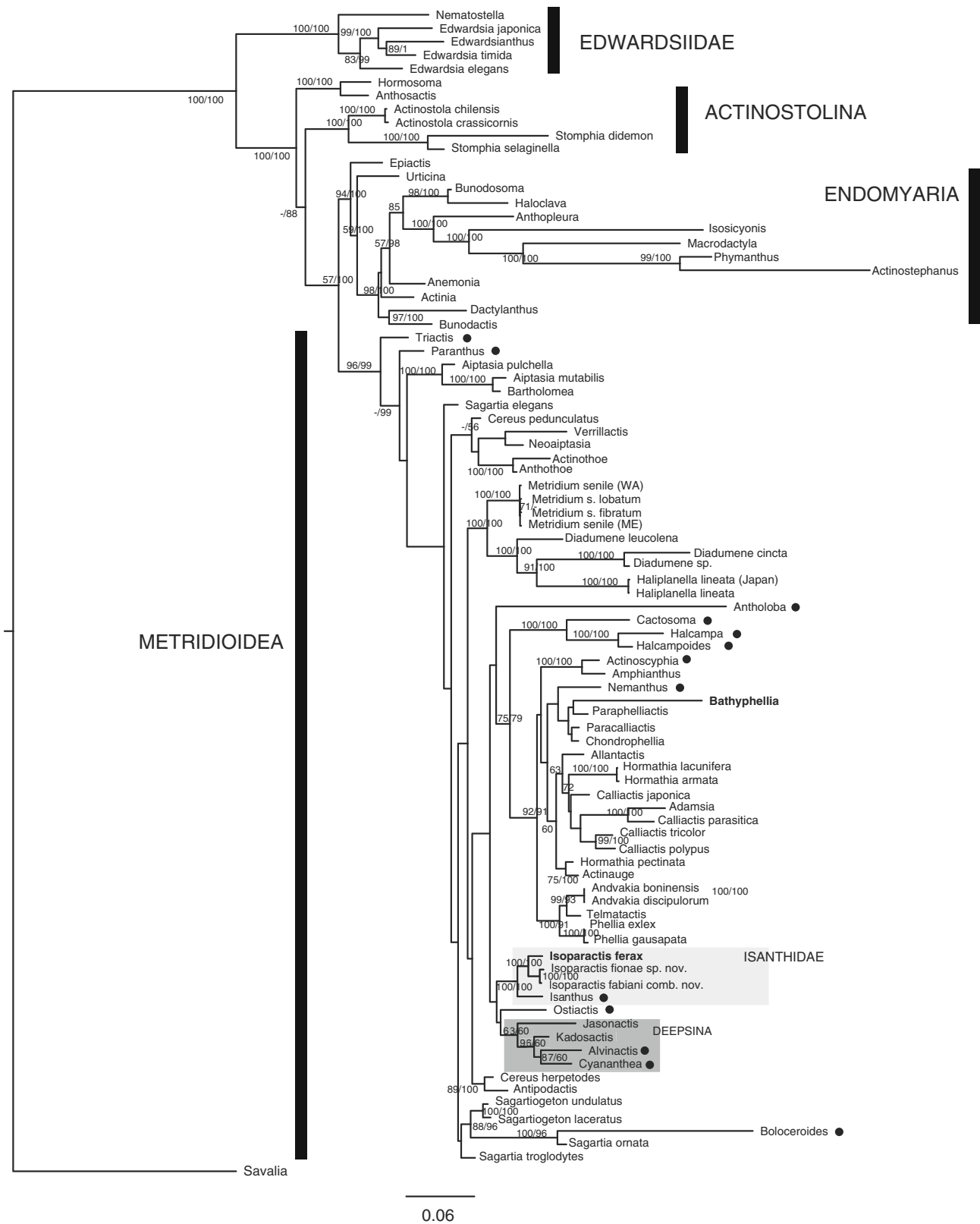


Fig. 5 Phylogenetic reconstruction of the concatenated data set (87 taxa, 5,504 sites) comprised of three mitochondrial genes (*cox3*, 12S, 16S) and two nuclear genes (18S, 28S). While topology and distance estimates are ML based, node support includes both bootstrap support

values and Bayesian posterior probabilities (BS/BPP). Only support values >50 % are shown. *Circles* indicate taxa within Metridioidea that lack acontia. Taxa in **bold** formerly classified together within the Bathypheiliidae

Table 2 Size ranges of the cnidae of *Isoparactis fionae* sp. nov., *I. fabiani* comb. nov. and *I. ferax*

Categories	Range of length and width of capsules (µm) <i>I. fionae</i> sp. nov.	$\bar{X} \pm SD$	S	N	F	Range of length and width of capsules (µm) <i>I. fabiani</i> comb. nov.	S	N	Range of length and width of capsules (µm) <i>I. ferax</i>
Pedal disc									
Spirocyst (-)	11.0–27.1 x 3.4–4.0	18.5 ± 5.0 x 3.9 ± 0.2	3/4	11	—	—	—	—	No data/[33.0] x [8.0]
Basitrichs 1 (a)	8.0–11.6 x 1.7–2.3	10.3 ± 0.9 x 2.0 ± 0.1	3/4	14	+	(12.0–14.5 x 2.0–3.0)	2/2	39	No data/[10.0–11.0] x [2.0]
Basitrichs 2 (b)	18.0–27.0 x 2.8–4.4	22.9 ± 1.6 x 3.6 ± 0.5	4/4	85	+/+/+	(23.0–29.0 x 3.0–4.5)	2/2	11	No data/[23.0–24.0] x [3.0–4.0]
M <i>p</i> -amastigophores (c)	12.0–17.0 x 3.0–4.4	14.7 ± 1.0 x 3.9 ± 0.4	4/4	24	++	(12.0–20.0 x 3.0–4.0)	2/2	9	No data/[-]
Scapus									
Basitrichs 1 (d)	9.0–13.0 x 1.5–2.0	11.3 ± 1.3 x 1.9 ± 0.2	2/4	14	+/+	(9.0–12.0 x 2.0–3.5)	5/5	13	(10.0–12.7) x (1.5)/[10.0–13.0] x [2.0]
Basitrichs 2 (e)	19.0–26.5 x 2.8–4.6	22.9 ± 1.6 x 3.6 ± 0.5	4/4	88	+++	(25.0–29.0 x 3.0–4.0)	5/5	39	(15.5–20.4) x (2.8) / [17.0–24.0] x [3.0–4.0]
M <i>p</i> -amastigophores (f)	13.0–20.0 x 3.0–5.0	15.6 ± 1.6 x 3.9 ± 0.5	4/4	43	++	(16.0–22.5 x 3.0–4.5)	5/5	13	(-)/[19.0] x [2.0]
Tentacles									
Spirocysts (g)	14.0–33.5 x 2.0–6.0	24.8 ± 4.5 x 3.2 ± 0.7	4/4	84	++	(15.0–32.0 x 2.0–4.5)	5/5	40	(30.0) x (2.8)/[17.0–26.0] x [3.0–4.0]
Basitrichs (h)	9.0–28.0 x 1.5–4.0	22.6 ± 2.7 x 2.8 ± 0.5	4/4	79	+/+/+	(17.0–30.5 x 2.0–4.0)	5/5	40	(16.9–22.6) x (2.5–2.8)/[18.0–24.0] x [3.0]
Actinopharynx									
Basitrichs 1 (-)	10.0–16.3 x 1.5–2.7	13.3 ± 2.4 x 2.2 ± 0.5	3/4	5	+	(7.0–11.4 x 1.0–1.9)	1/8	7	(-)/[-]
Basitrichs 2 (i)	22.0–36.0 x 2.7–4.0	33.0 ± 2.7 x 3.1 ± 0.3	4/4	38	++	(34.0–43.0 x 3.5–5.0)	8/8	28	(25.4–29.0) x (2.1–2.8)/[19.0–30.0] x [2.0–3.0]
M <i>p</i> -amastigophores 1 (-)	—	—	—	—	—	(15.2–19.1 x 3.2–3.8)	2/8	5	(-)/[-]
M <i>p</i> -amastigophores 2 (i)	24.0–40.0 x 3.0–6.0	31.0 ± 4.2 x 4.6 ± 0.6	4/4	84	+++	(32.0–41.5 x 2.5–4.0)	8/8	39	(21.0–31.0) x (4.2)/[21.0–33.0] x [4.0–5.0]
Filaments									
Basitrichs 1 (k)	12.0–32.0 x 1.8–5.0	19.0 ± 7.2 x 3.0 ± 0.7	4/4	56	++	(8.0–38.0 x 2.0–4.5)	5/5	30	(12.7–16.9) x (2.1–2.8)/[14.0–16.0] x [2.0–3.0]
M <i>p</i> -amastigophores 1 (l)	15.0 x 3.0	—	1/4	1	—	(12.7–14.3 x 2.2–2.5)	3/5	7	(10.0–13.4) x (2.8)/[13.0–14.0] x [3.0–4.0]
M <i>p</i> -amastigophores 2 (m)	23.4–36.9 x 3.0–6.0	28.0 ± 2.7 x 4.6 ± 0.6	4/4	81	+++	(27.0–36.0 x 3.5–5.0)	5/5	36	(21.1–25.4) x (4.2–5.0)/[20.0–27.0] x [4.0–5.0]
Acontia*									
Basitrichs 1 (n)	9.3–18.3 x 1.0–3.0	14.3 ± 1.9 x 2.8 ± 0.5	6/6	19	+	[11.0–15.0] x [2.0]	1/1	5	(-)/[16.0–18.0] x [2.0–3.0]
Basitrichs 2 (o)	26.4–39.3 x 2.0–4.0	30.3 ± 2.1 x 2.9 ± 0.4	3/6	99	+++	[31.0–36.0] x [3.0–4.0]	1/1	7	(25.4–31.0) x (2.8)/[26.0–32.0] x [3.0]
M <i>p</i> -amastigophores (p)	32.0–43.0 x 3.0–4.0	37.9 ± 3.1 x 3.9 ± 0.2	4/6	32	+/+	[27.0–41.0] x [2.5–3.0]	1/1	6	(-)/[28.0–31.0] x [4.0]

\bar{X} : mean length by mean width of capsules. SD: standard deviation. S: ratio of number of specimens in which each cnidae was found to number of specimens examined. N: total number of capsules measured. F: Frequency; +++, very common; ++, common; +, somewhat common; —, sporadic. M, Microbasal. Values from pooled samples. *In *I. fionae* sp. nov. acontia were only found in six of nine specimens examined. Measurements in parentheses for *I. fabiani* comb. nov. and *I. ferax* from Häussermann and Försterra (2008) and Carlgrén (1945), respectively. New data from *I. fabiani* comb. nov. and *I. ferax* (SMNH-type 4046) in brackets. Letters next to cnidae categories correspond to images in Fig. 4

Table 3 Comparison of generic-level characters within the family Isanthidae

Genus	M/MP	Fertile M	More mesenteries	Siphonoglyph	Mesogaeal sphincter	Retractor muscles	Tentacles	Cinclides	Column surface	Acontia
<i>Isoparactis</i> Stephenson, 1920	24++incomplete cycle / 6	1st cycle (6)	Distally	2	Well developed	Restricted to circumscribed	Up to 110	Proximally or absent	Adherent rugae/smooth	Yes
<i>Isanthus</i> Carlgren, 1938	Not numerous/6	1st cycle (6)	=	2	Not strong	Reniform	Not numerous	No	Smooth	No
<i>Zoalattus</i> Hand, 1955	Usually 48 in mid-column/≤ 12	Up to 2nd cycle (12)	Distally	2	Two sphincters	Restricted, those of directives sometimes reniform	Numerous (up to 6 cycles)	No	Smooth or with microscopic papillae	No
<i>Austroneophellia</i> Zamponi, 1978	24/12	?	?	2	Palmate circumscribed	Circumscribed	>50	No	Smooth	No
<i>Eltaninactis</i> Dunn, 1983	≤12/6	1st cycle (6)	Equal or proximally	Absent or indistinct	Weak	Strong, circumscribed	24, 16 or 17	No	Tenaculi and cuticle	No
<i>Paraisanthus</i> Sanamyan & Sanamyan, 1998	24–48/6	1st cycle (6)	Distally	2	Well developed	Strongly restricted, reniform or almost circumscribed	48–74	In longitudinal rows	Smooth	No

M, number of pairs of mesenteries; MP, number of pairs of perfect mesenteries; = same number of mesenteries proximally and distally. Updated from Häussermann and Försterra (2008)

delineate species-level relationships in all instances (Shearer et al. 2002, Forsman et al. 2009, Brugler and Rodríguez unpublished data); there is also an example of morphologically defined genera that cannot be distinguished (Brugler 2011). Thus, we prefer to be conservative and tentatively place *I. fionae* sp. nov. and *I. fabiani* comb. nov. within *Isoparactis* until further revision is done.

Differential diagnosis of *Isoparactis fionae* sp. nov.

Isoparactis fionae sp. nov. differs from *I. fabiani* comb. nov. and *I. ferax* in the column, color pattern of the oral disc, number of tentacles, cnidae and geographical distribution. The column of *I. fionae* sp. nov. has longitudinal rows of adherent rugae, whereas the column of *I. fabiani* comb. nov. and *I. ferax* is smooth. Although the general color pattern of the oral disc might vary within specimens of the three species, all specimens of *I. fionae* sp. nov. have a distinct pink ring around the mouth that is absent in *I. fabiani* comb. nov. and *I. ferax*; this pink ring is only discernible in living specimens. *Isoparactis ferax* has cinclides proximally (Carlgren 1924), whereas cinclides have not been detected in the other two species. *Isoparactis fionae* sp. nov. and *I. ferax* have four cycles of tentacles (6+6+12+24) plus an incomplete fifth cycle (Carlgren 1924), whereas *I. fabiani* comb. nov. has up to 110 tentacles (corresponding to an additional sixth cycle of tentacles) (Häussermann and Försterra 2008). The cnidae also differ slightly among the species: *I. fionae* sp. nov. has larger microbasic *p*-amastigophores and shorter basitrichs 2 in the actinopharynx, and slightly shorter basitrichs 1 in the pedal disc than *I. fabiani* comb. nov. *Isoparactis fabiani* comb. nov. lacks spirocysts in the pedal disc, but these are present (although not very commonly) in *I. fionae* sp. nov. and *I. ferax*. Although the ranges overlap, *I. ferax* has shorter basitrichs in the actinopharynx, filaments and acontia as compared to *I. fionae* sp. nov. and *I. fabiani* comb. nov.; in addition, microbasic *p*-amastigophores in the pedal disc of *I. fionae* sp. nov. and *I. fabiani* comb. nov. are absent in *I. ferax*. Furthermore, Häussermann and Försterra (2008) reported an additional category of small microbasic *p*-amastigophores in the actinopharynx of two specimens of *I. fabiani* comb. nov. that is absent in *I. fionae* sp. nov. and *I. ferax*; based on the number of capsules that they found, this category of cnidae seems to be rare.

Finally, the species differ in their geographical distribution: *Isoparactis ferax* is recorded from the North Island in New Zealand; *I. fionae* sp. nov. inhabits the southernmost part of Patagonia (Straits of Magellan and Beagle Channel) although it reaches as far north as the Archipelago Madre de Dios (50°20'23.1"S 75°22'39.2"W); however, *I. fabiani* comb. nov. is found along the Chilean coast between Faro Corona, Chiloé Island (41°47'02.0"S 73°52'58.8"W) and Archipelago Madre de Dios.

Systematics of *Isoparactis* and the family Isanthidae

Molecular and morphological data support the placement of *Isoparactis* within Isanthidae rather than within Bathyphelellidae. Bathyphelellids usually have a regionated column with tenaculi and are usually from the deep-sea (Carlgrén 1949, Riemann-Zürneck 1997), whereas in isanthids the column is usually smooth and not regionated — except in *Eltaninactis* (Table 3) — and it comprises mostly shallow-water taxa. Nevertheless, the relationship of *Eltaninactis* and the family Isanthidae has been questioned (Sanamyan 2001, Häussermann and Försterra 2008). The finding of acontia in *I. fabiani* comb. nov. and the discovery of *I. fionae* sp. nov. reinforces the recent placement of Isanthidae within acontiate actinarians (i.e., Metriodidae, Rodríguez et al. 2012). Furthermore, confirmation of the presence of microbasic p-amastigophores in the acontia of the type material of *I. ferax* further supports the homogeneity of the group and its placement within Isanthidae. Although support values are low, our results suggest that Isanthidae is the sister group of Deepsina (a clade including members of the family Kadosactinidae Riemann-Zürneck, 1991). Similarly to Isanthidae, Kadosactinidae includes taxa with and without acontia and cinclides, but with no other morphological disagreement among members. However, it is remarkable that the putative sister group of Isanthidae (mostly shallow-water taxa) is the Deepsina, a deep-sea clade. If this relationship is confirmed using more informative nuclear DNA markers, we can begin to test whether shallow-water sea anemones from the Southern Hemisphere were seeded by ancestors living in the deep sea (the cold, wind-blown, turbid waters of the Southern Hemisphere may mimic conditions in the deep sea); additionally, deep water renewal events may be responsible for seeding deep-water species over sills and shallow plains (which limit water exchange) and into semi-isolated basins, such as the Straits of Magellan (Antezana 1999) — isolation leading to speciation. Isanthidae appears to be a family that diversified in the Southern Hemisphere, with only a few representatives in the northern hemisphere (e.g., *Paraisanthus*, *Zaolatus*) and some other representatives whose affiliation with the family or identity is uncertain (e.g., *Isanthus homolophilus* Chintiroglou and Doumenc, 1998, or *Austroneophellia*; see Häussermann and Försterra 2008). Similarly, the majority of members from the Kadosactinidae are from the Southern Hemisphere.

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