



# A revision of sea anemones of the genus *Cribrinopsis* Carlgren, 1921 (Actiniaria: Actiniidae) from British Columbia with the description of a new species

Nadya P. Sanamyan<sup>1</sup> · K. E. Sanamyan<sup>1</sup> · N. McDaniel<sup>2</sup> · A. V. Martynov<sup>3</sup> · T. A. Korshunova<sup>3,4</sup> · E. S. Bocharova<sup>4,5</sup>

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## Abstract

Sea anemones of the genus *Cribrinopsis* Carlgren, 1921 from the NE Pacific are revised. Specimens traditionally identified from this region as *Cribrinopsis fernaldi* Siebert & Spaulding, 1976, actually represent two distinct species with different morphology and habitat. One species has long tentacles, a ring of distinct rounded marginal projections, a white column and usually inhabits muddy bottom. The other has short tentacles, no special marginal projections, a bright coloration (usually variations of red and occasionally white), and inhabits rocky substrates. Additionally, the distinction of two sympatric species is supported by molecular analysis. The holotype of *C. fernaldi* was re-examined, which revealed that in its original description several key features of this species were incorrectly described. The holotype is conspecific with the first (white) species, which therefore should retain the name *C. fernaldi*. The other species is described as *Cribrinopsis rubens* sp. nov. Study of the holotype of another *Cribrinopsis* species previously reported from the NE Pacific, *Cribrinopsis williamsi* Carlgren, 1940, showed that this species does not resemble any known actiniid species from the NE Pacific. It probably does not belong to *Cribrinopsis* and its taxonomic position needs further investigation.

**Keywords** Cnidaria · Anthozoa · NE Pacific · Taxonomy · Sister species · Sympatric speciation

## Introduction

The sessile shallow-water bottom fauna of British Columbia (Canada) and adjacent regions is now well documented by divers, as demonstrated by the availability of numerous underwater pictures of sea anemones. Photographs taken by one of the authors (N. McDaniel) helped to record species

previously unknown from this region (Sanamyan et al. 2018) and to describe new species (Sanamyan et al. 2013), whereas other photographs show sea anemones that cannot be assigned to any known species. Our previous studies in the NW Pacific have revealed considerable hidden species diversity (e.g., Sanamyan and Sanamyan 2006), and we therefore expect that the fauna of sea anemones in British Columbia is

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✉ Nadya P. Sanamyan  
actiniaria@sanamyan.com

<sup>1</sup> Kamchatka Branch of Pacific Geographical Institute of Far-Eastern Branch of the Russian Academy of Sciences, Partizanskaya Str. 6, Petropavlovsk-Kamchatsky 683000, Russia

<sup>2</sup> McDaniel Marine Surveys, Vancouver, British Columbia V6N 2N5, Canada

<sup>3</sup> Zoological Museum of the Moscow State University, Bolshaya Nikitskaya Str. 6, Moscow 125009, Russia

<sup>4</sup> Koltzov Institute of Developmental Biology of Russian Academy of Sciences, Vavilova Str. 26, Moscow 119334, Russia

<sup>5</sup> Russian Federal Research Institute of Fisheries and Oceanography (VNIRO), V. Krasnoselskaya Str. 17, Moscow 107140, Russia

more diverse and the taxonomic assessment of several common species not as obvious as currently accepted. In this study, we focus on the representatives of the genus *Cribrinopsis* Carlgren, 1921 in the NE Pacific. Sea anemones of this genus comprise several conspicuous species which are common in all northern waters including North Pacific, North Atlantic, European, and Arctic seas. Their photographs often appear in underwater guides (e.g., Kukliński and Bałazy 2017) and on internet sources. Traditionally all anemones of this genus from the Pacific NW coast of North America, particularly those from British Columbia and adjacent waters, were identified as *Cribrinopsis fernaldi* Siebert & Spaulding, 1976, a species originally described from the geographically nearby San Juan Island, off Washington State (USA). These identifications were apparently based on the external features only and in fact uncritically referred to as *C. fernaldi* because this was the only species of the genus recorded from this region.

In 2016, N. McDaniel took photographs of *Cribrinopsis* species inhabiting a muddy bottom in Howe Sound, British Columbia. This species showed a white column and long tentacles and appeared distinct from the species traditionally identified as *C. fernaldi* in British Columbia. Several specimens of this species were collected. Putative *C. fernaldi* specimens from rocky substrates were also collected. The results of morphological and molecular investigations showed that these specimens indeed represent two different species. However, none of them fit perfectly the original morphological description of *C. fernaldi* published by Siebert and Spaulding (1976). To reveal the status of these species, the holotypes of *C. fernaldi* and *Cribrinopsis williamsi* Carlgren, 1940, the only other *Cribrinopsis* species reported from the NE Pacific, were re-examined. It was found that the white specimens with longer tentacles were most similar to the holotype of *C. fernaldi* and should be assigned to this species, while the other, usually red species with shorter tentacles, traditionally identified as *C. fernaldi* in this region, required a new name and is described here as *Cribrinopsis rubens* sp. nov. The holotype of *C. williamsi* is distinct from specimens of both species.

## Material and methods

Numerous specimens of *C. fernaldi* and *C. rubens* sp. nov. were observed and photographed in situ underwater. All specimens were photographed prior to collection. The samples were preserved in 4% seawater formalin and then transferred to 70% EtOH for long-term storage. A small piece of each specimen was fixed in 95% EtOH for molecular study. For the preparation of histological sections, the isopropanol-mineral oil method was used (Sanamyan and Sanamyan 2012). This is easier and produces better results, especially

for larger samples, than the traditional method which uses xylene. The samples were dehydrated in five changes of 100% isopropanol, then in two changes of isopropanol mixed with mineral oil (5:1 and 2:1 at 50 °C), then placed into pure mineral oil at 55 °C, and finally soaked in three changes of paraffin at 56–58 °C. Each stage normally took about 2 h but for larger pieces ( $\geq 2$  cm) better results were obtained when the preparation time was prolonged and the samples left in the last isopropanol bath and in the last paraffin overnight. Unlike traditional dehydration in ethanol, the prolonged dehydration in isopropanol does not excessively harden the samples and has no negative effect on sectioning. For general histology, 7  $\mu$ m sections were stained by a variant of Masson trichrome in the following sequence: acid fuchsin—5 min, phosphomolybdic acid—5 min, aniline blue—5 min (Llewellyn 2018). For study of the distribution of nematocysts, 10  $\mu$ m sections stained by toluidine blue or safranin were used.

Cnidae terminology and the usage of some terms (e.g., “gonad”) are the same as in our previous papers (see Sanamyan et al. 2012 for details). Cnidae were measured according the method of Hand (1954) for reasons explained by Sanamyan and Sanamyan (2013). Size ranges of cnidae in each body region are given, but statistical data (mean and standard deviation) are not calculated for several reasons. First, calculating the mean values assumes that the measurements were made haphazardly, but this is not applicable if the method of Hand (1954) is used. Second, the average size of the nematocysts varies along each body region, e.g., size of the basitrichs is gradually changes along the length of each tentacle and depends on the cycle number to which the tentacle belongs (Sanamyan et al. 2009, 2015). And third, according to our experience, such data are not useful in taxonomic practice to differentiate actiniarian species.

Total DNA was extracted using Wizard SV Genomic DNA Purification System (Promega) following the manufacturer’s protocol. The mitochondrial gene fragments (12S rRNA, 16S rRNA, and COIII) and the nuclear gene fragments (18S rRNA and 28S rRNA) were amplified using published primers and protocols (Geller and Walton 2001; Bocharova 2015; Sanamyan et al. 2018). Sequence reaction was run using the BigDye v3.1 reagent kit (Applied Biosystems©). Purified and denatured reaction products were analyzed in the capillary molecular analyzer ABI PRISM 3500 (Applied Biosystems©) using POP7 gel polymer. In order to treat the chromatograms, Sequencing Analysis 3.7 (Applied Biosystems©) and Geneious 8.1.5 were applied. Forward and reverse sequences were assembled by Geneious alignment with default parameters and compared (via BLAST) against the nucleotide database of GenBank to determine whether the target locus and organism were sequenced rather than a symbiont or other contaminant.

Sequences were manually edited and aligned using the Muscle algorithm with default parameters in Geneious 8.1.5.

Complete and reduced alignments for each marker were analyzed separately and as a concatenated dataset in MEGA 6.0 (Tamura et al. 2013). Reduced alignments were obtained manually by trimming of sequence ends on the base of reference sequences. Several analytical methods of phylogenetic reconstruction were used (Nei and Kumar 2000).

Phylogenetic analysis was performed using the Hasegawa-Kishino-Yano model (Hasegawa et al. 1985) with gamma distribution in MEGA 6.0. The bootstrap values were calculated for maximum likelihood phylogram: rates among sites—gamma distributed (G), No. of discrete gamma categories—2, Nearest-Neighbor-Interchange heuristic method, NJ initial tree, very strong branch swap filter, 1000 replicates.

Evolutionary analyses (pairwise distances between species and standard errors) were conducted using the Hasegawa-Kishino-Yano model (Hasegawa et al. 1985) in MEGA 6.0. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). Codon positions included were 1st + 2nd + 3rd + noncoding. All positions containing gaps and missing data were eliminated.

The specimens are deposited in the Zoological Institute of the Russian Academy of Sciences, St. Petersburg (ZIN) and Kamchatka Branch of the Pacific Geographical Institute, Petropavlovsk-Kamchatsky (KBPGI). Type specimens of *C. fernaldi* and *C. williamsi* were acquired on loan from the Smithsonian Institution, National Museum of Natural History (USNM).

## Results

Order Actiniaria Hertwig, 1882

Family Actiniaria Rafinesque, 1815

***Cribrinopsis fernaldi* Siebert & Spaulding, 1976**

(Figs. 1, 2, 3, 4, 5a, b, and 6)

*Cribrinopsis fernaldi* Siebert and Spaulding 1976: 129 (part). ? Stevens and Anderson 2000: 77. Not Uchida and Soyama 2001: 83. Not Lamb and Hanby 2005. Not Harbo 2011.

**Differential diagnosis:** Large species with pale column and with ring of rounded marginal projections (pseudospherules). Tentacles longer than diameter of oral disc. Arrangement of tentacles may be interpreted as decamerous or hexamerous; arrangement of mesenteries more clearly decamerous. Rare, small, and often curved basitrichs (10–15 µm) present in ectoderm of tentacles.

**Material examined:** East Defence Island, Howe Sound, British Columbia, Canada, 49° 34.645' N, 123° 16.339' W, depth ~20 m, 2 July 2016, collector N. McDaniel. Three specimens, KBPGI 495/1, 496/2, 497/3. **Additional material examined:** Holotype of *Cribrinopsis fernaldi*, USNM 54132, Friday Harbor, Washington.

**Specimens from British Columbia:** A large, conspicuous anemone, reaching 15 cm in column diameter. The tentacles are up to 20 cm long; inner tentacles distinctly longer than the outer ones and longer than the diameter of the oral disc (Fig. 1a, b). The tentacles are conical, rather thick at bases, with a veined surface (Fig. 1a–d). The cylindrical column is usually attached to small rocks and cobble imbedded in silty or mud substrates so that only upper and middle parts of column is visible on the surface, or it may be attached directly to bedrock or boulders. The surface of the column is clear; a thin cuticle is present only at pedal disc. The column has numerous verrucae (sensu Den Hartog 1987) which are present over all endo- and exocoels, better developed on the upper half of column. The verrucae are not prominent, become inflated and clearly visible on the partially contracted specimens (Fig. 1c, d), are not adhesive, are marked by white pigment, and have a thin mesogloea at their summit, which looks like a transparent spot or depression (Fig. 1d). On histological sections, verrucae are cup-shaped, have a thin mesogloea at the center without muscle processes on the endodermal side, and with somewhat modified ectoderm (Fig. 3d). There is a ring of prominent rounded marginal projections (pseudospherules sensu Carlgren 1949), one projection on each endo- and exocoel (Fig. 1c–e). They also have a spot of thin mesogloea (Fig. 1d).

In living specimens the circular oral disc and the tentacles are raised rather high above the bottom; the tentacles may occasionally touch the surface of the sediment but do not lie on the bottom (Fig. 1a).

The color is fairly consistent among all studied specimens. The column is always uniformly white with slightly raised white spots marking verrucae. The oral disc is pinkish, with reddish radial lines marking insertions of the mesenteries (Fig. 1b). The actinopharynx is white. The tentacles have distinct rose-colored vein-like or a zig-zag pattern (moiré) on a pale-rose semitransparent background (Fig. 1c, d). In some specimens, the tentacles have vaguely yellowish tips.

All tentacles of all three preserved specimens are exposed, not hidden by the upper part of the column (Fig. 1e–g). Their surface is raised into numerous crowded small elevations (Figs. 1e and 3a), longitudinally sulcated. The column of preserved specimens is wider than high, 57–67 mm in diameter and 40–51 mm high.

The number and the arrangement of the tentacles and mesenteries differ somewhat in three examined specimens. These differences appear only in two adjacent primary exocoels on each side of one directive pair (labeled “dd” in Fig. 2). The specimens are symmetric in relation to directive plane.

The specimen KBPGI 495/1 has 92 tentacles. As it appears on the photograph of the live specimen (Fig. 1b), the arrangement of the tentacles may be considered either hexamerous in five cycles (6 + 8 + 14 + 19 + 45) or decamerous in four cycles (10 + 12 + 25 + 45) with the duplications in the second and



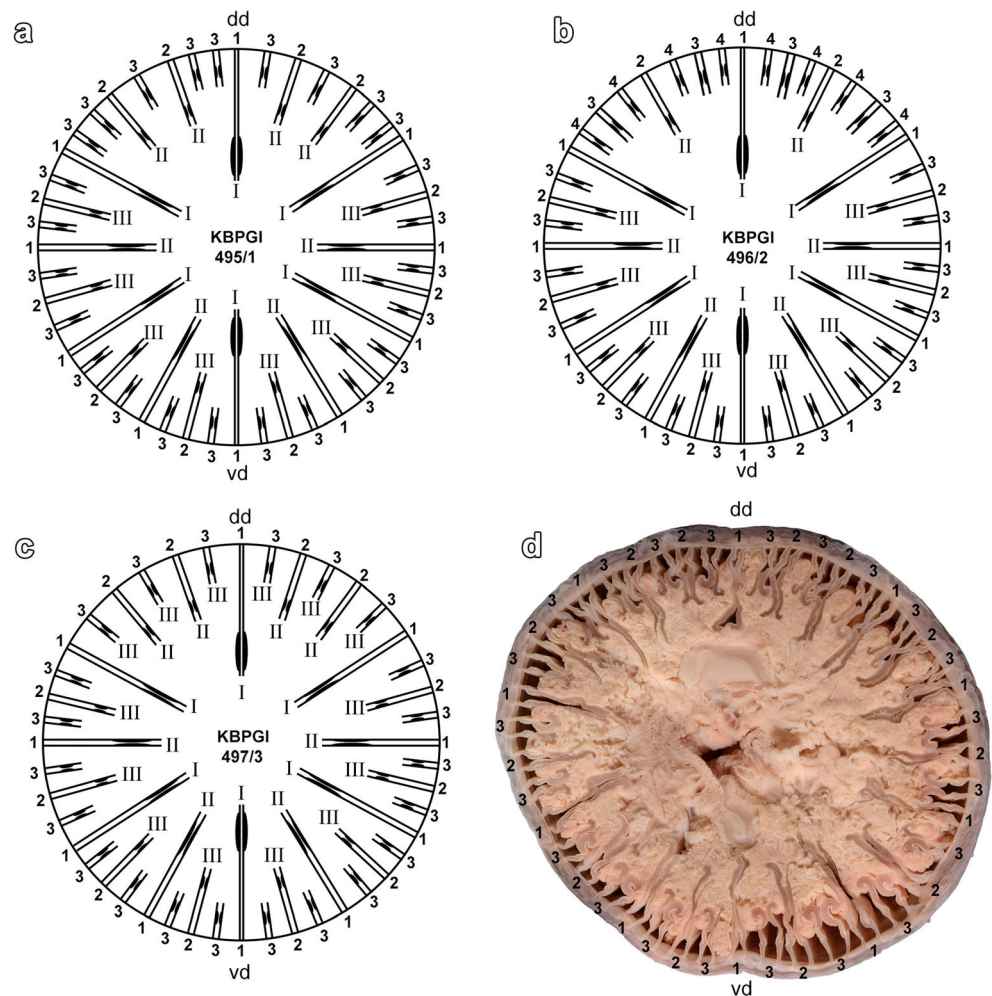
**Fig. 1** *Cribrinopsis fernaldi*: **a** Specimen KBPGI 495/1 from the side to show typical position on the bottom with partially buried column; **b** The same specimen from the top to show arrangement of the tentacles (tentacle cycles indicated by numbers, fourth cycle not labeled); **c** Partially

contracted specimen to show appearance of marginal projections and verrucae on column; **d** Margin of the partially contracted specimen; **e** Specimen KBPGI 495/1, preserved; **f** Specimen KBPGI 496/2, preserved; **g** Specimen KBPGI 497/3, preserved

third cycles in two adjacent primary exocoels. The arrangement of the mesenteries is more clearly decamerous. The mesenteries are arranged in three cycles ( $10 + 12 + 25 = 47$  pairs)

with duplications in the second and third cycles in two adjacent primary exocoels which lie symmetrically on two sides of a directive pair (Fig. 2a).

**Fig. 2** *Cribrinopsis fernaldi*, arrangement of mesenteries: **a** KBPGI 495/1; **b** KBPGI 496/2; **c**, **d** KBPGI 497/3. Numbers (1, 2, 3, 4) indicate cycle number of the mesenterial pairs; roman numbers (I, II, III) indicate primary, secondary, and tertiary mesenterial pairs; dd, “dorsal” directives; vd, “ventral” directives



Specimen KBPGI 496/2 has 96 tentacles, of which 48 are exocoelic (outer ring), arranged hexamerously in four cycles ( $12 + 12 + 24 + 48 = 96$ ). The mesenteries in this specimen are arranged decamerously because two secondary pairs of the mesenteries are shorter (on transverse section) than the mesenteries of the first cycle and their length is about the same as the length of the mesenteries of the second cycle (Fig. 2b). In two adjacent primary exocoels, lying symmetrically on the sides of one directive pair, the mesenteries of the fourth cycle are developed (Fig. 2b), while in the rest of column only three cycles of mesenteries are present ( $10 + 10 + 20 + 8 = 48$  pairs).

Specimen KBPGI 497/3 has 84 tentacles arranged in four cycles ( $10 + 12 + 22 + 40 = 84$ ). The mesenteries here are arranged decamerously in three cycles ( $10 + 12 + 22 = 44$  pairs), with the duplications of the mesenteries of the second cycle (and, correspondingly, an addition of two pairs of mesenteries of the third cycle) in two adjacent primary exocoels lying on two sides of a directive pair (Fig. 2c, d).

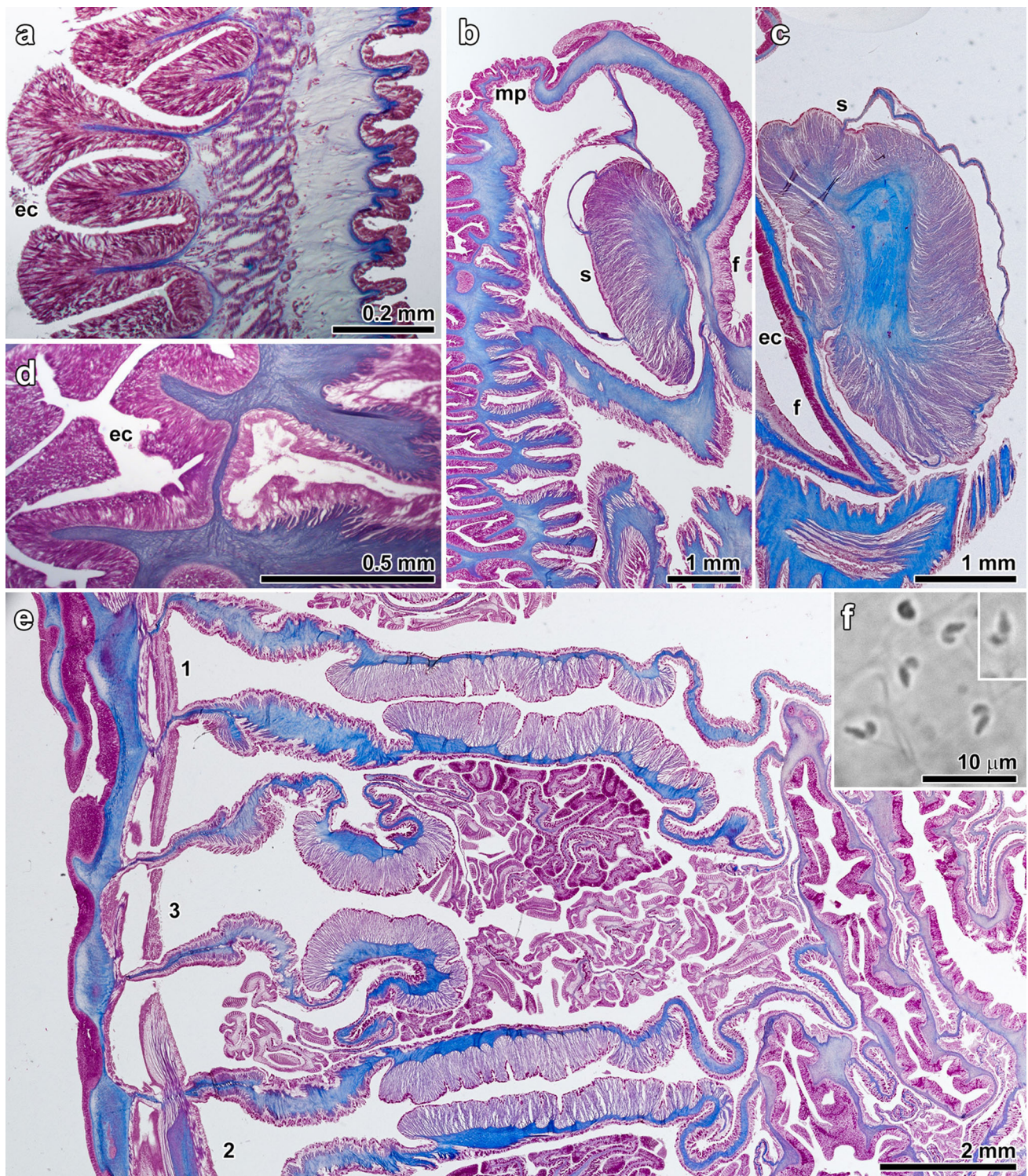
All three cycles of the mesenteries are perfect but mesenteries of the third cycle are attached to the actinopharynx only on its distal (upper) part. The retractors are restricted, rather strong (Fig. 3e). A small pennon is usually present on the

parietal side of the retractors (closer to the column wall), especially on the mesenteries of the first and the second cycles. The parietobasilar muscles have a small distinct flap.

The marginal endodermal sphincter muscle is strong, circumscribed, and with thick central mesogloal part attached to a wall of fossa by a short lamella (Fig. 3b, c). The long and very numerous mesogloal processes are branched and occasionally anastomose. The longitudinal muscles of the tentacles are mesogloal (Fig. 3a) and radial muscles of the oral disc are ectomesogloal to mesoectodermal. The actinopharynx has two siphonoglyphs supported by two pairs of directives.

Gonads are present on all mesenteries except directives. The sexes are separate. One specimen (KBPGI 495/1) has ova up to  $800 \mu\text{m}$  in diameter; the other two specimens are males. Spermatozoa  $3\text{--}3.5 \times 1\text{--}1.5 \mu\text{m}$  with mitochondrial complex about  $1 \mu\text{m}$  in diameter (Fig. 3f). We found no juveniles in the coelenteron of examined specimens.

Cnidom: spirocysts, basitrichs, p-mastigophores A, and p-mastigophores B1 (Table 1, Fig. 4). The columnar spirocysts (Fig. 4c) are rare at limbus, few at the middle of the column and common at the margin. The nematocysts of marginal projections (pseudospherules) are the same as in the upper part of

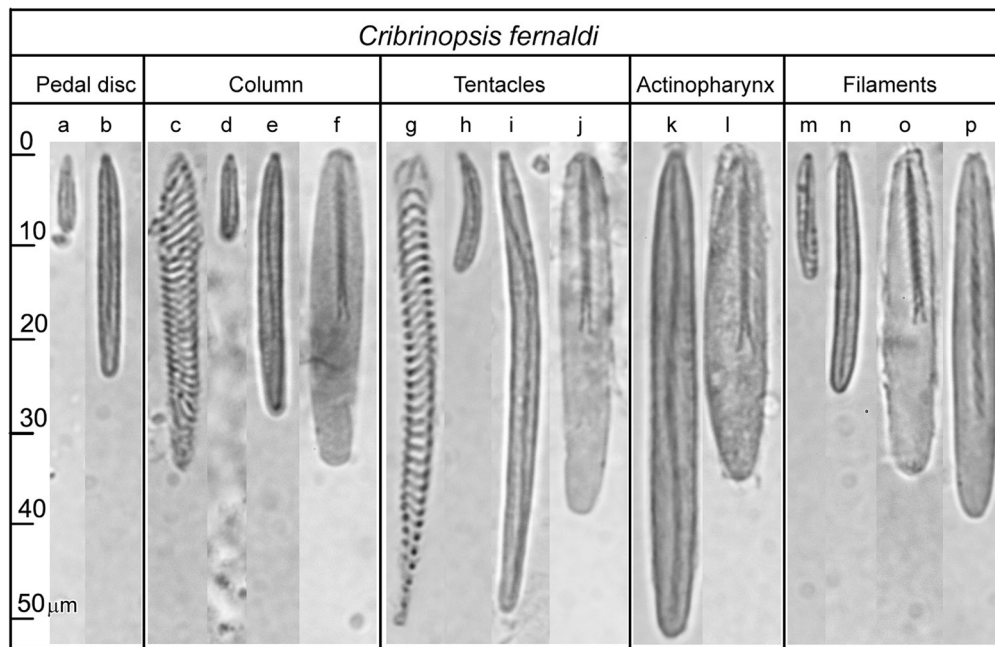


**Fig. 3** *Cribrinopsis fernaldi*: **a** Transverse section of the tentacle, specimen KBPGI 497/3; **b** Longitudinal section through the margin, specimen KBPGI 496/2; **c** Marginal sphincter, specimen KBPGI 497/3; **d** Longitudinal section of the column through verruca; **e** Transverse

section of column, specimen KBPGI 497/3, cycles of the mesenteries indicated by numbers; **f** Spermatozoa of specimen from British Columbia and holotype of *C. fernaldi* (on insertion). ec, ectoderm; f, fosse; mp, marginal projection; s, sphincter muscle

the column. The nematocysts in the fossa are the same as in the column but are more sparse. Large basitrichs in the tentacles (Fig. 4i) are common at the bases of the tentacles and

numerous at tips where they may be as numerous as spirocysts. The ectoderm of the oral disc contains sparse spirocysts and small, often curved basitrichs similar to those



**Fig. 4** *Cribrinopsis fernaldi*, cnidom of the specimens from British Columbia

in the tentacles (Fig. 4h). Cnidoglandular tracts of trilobate filaments contain sparse basitrichs  $22\text{--}28 \times 2\text{--}2.5 \mu\text{m}$  (resembling those pictured on Fig. 4n). P-mastigophores B1 (Fig. 4p) in cnidoglandular tracts of unilobate filaments are heavily stained by basic stains (safranin or toluidine blue) as all basitrichs.

**Habitat:** The species lives on muddy or rocky substrates along the BC coast, especially in coastal fjords such as Howe Sound, Knight Inlet, and Sechelt Inlet where there are negligible tidal currents. It is also found in Lynn Canal, Alaska, and Puget Sound, Washington. All specimens were observed deeper than 20 m and in low density. This sea anemone may host commensal shrimps, including *Lebbeus groenlandicus* (Fabricius, 1775), *Spirontocaris snyderi* Rathbun, 1902, *Spirontocaris lamellicornis* (Dana, 1852), *Heptacarpus kincaidi* (Rathbun, 1902), *Lebbeus grandimanus* (Bražnikov, 1907), and *Eualus suckleyi* (Stimpson, 1864) (see Jensen 2014; Bražnikov 1907; Dana 1852; Fabricius 1775; Rathbun 1902; Stimpson 1864).

**Redescription of the holotype of *Cribrinopsis fernaldi*:** The holotype (USNM 54132) is intact (Fig. 5a). The column is wider than high, 28 mm high and 60 mm in diameter. The pedal disc is flat, slightly drawn below the limbus, with remnants of cuticle in some places. The column is slightly wrinkled, with longitudinal rows of inconspicuous verrucae present as small elevations about 1 mm diameter with a depression in the center. There is a distinct ring of prominent rounded marginal projections (pseudospherules according to Siebert and Spaulding 1976) about 1 mm diameter (Fig. 5b). The actinopharynx is protruded outside and hides the oral disc. Two siphonoglyphs are visible. The tentacles are not covered

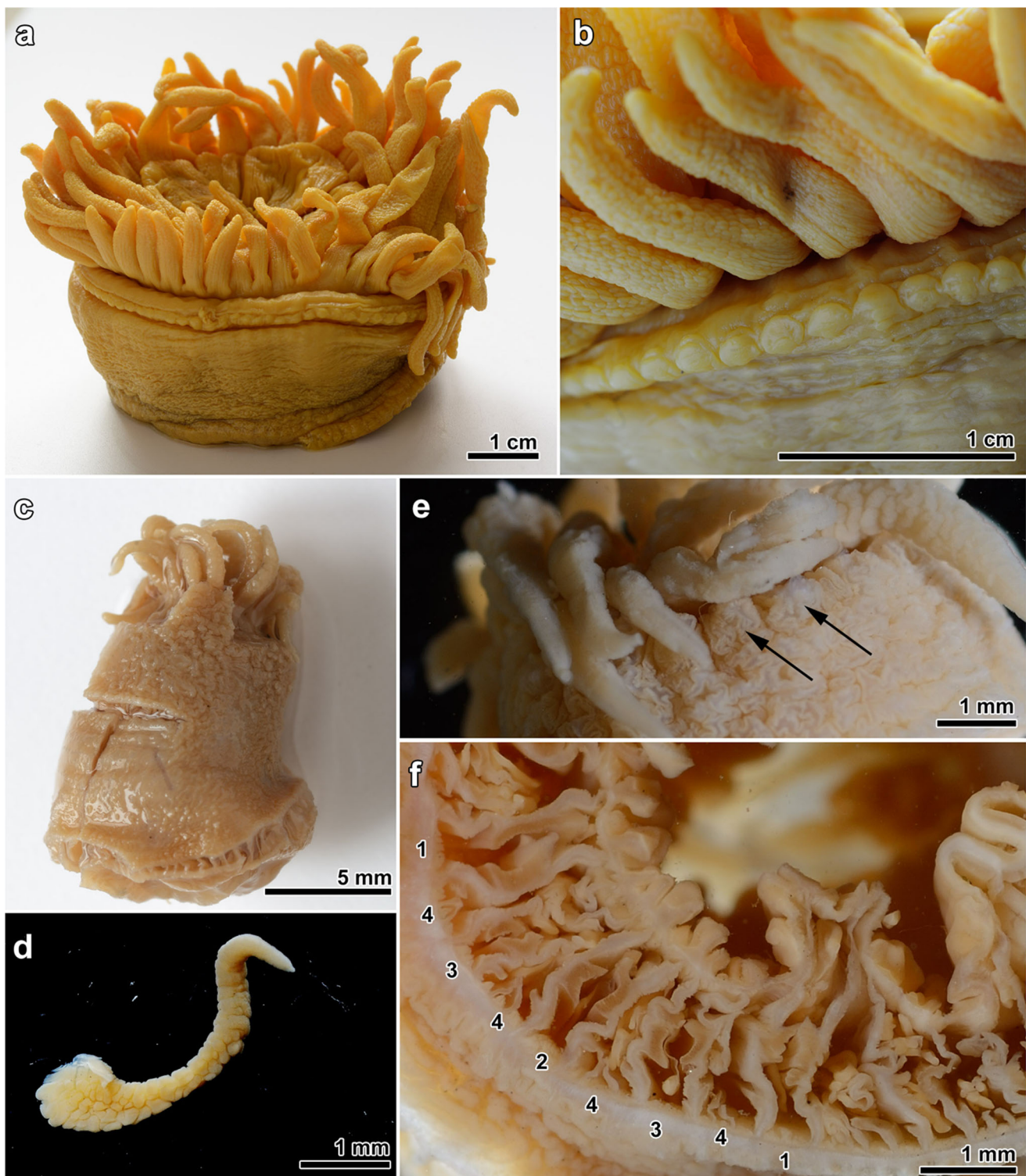
by the upper part of the column, all are visible, up to 25 mm long, and the inner ones are longer. The surface of the tentacles is raised into numerous small, more or less regularly placed, crowded elevations. Weak longitudinal furrows are visible on the proximal part of the aboral side of the tentacles (Fig. 5b). The tentacles are arranged decamerously in four cycles, with seven duplications in the third cycle:  $10 + 10 + 27 + 47 = 94$  tentacles. The decamerous arrangement is not perfect and the arrangement of the tentacles may be also interpreted as hexamerous (as in specimens described above).

Without permission, dissection of this specimen was not possible, but based on the arrangement of the tentacles, we can extrapolate that the mesenteries are probably arranged decamerously in three cycles with seven duplications in the third cycle:  $10 + 10 + 27 = 47$  pairs.

**Cnidom:** spirocysts, basitrichs, p-mastigophores A, and p-mastigophores B1 (Table 2, Fig. 6). All spirocysts are gracile, although in some the tubule was not evenly packed. Large basitrichs (Fig. 6i) are numerous in the tips of the tentacles. On the tips of the inner tentacles they are larger ( $40\text{--}60 \mu\text{m}$ ) than in ectoderm of the outer tentacles ( $20\text{--}56 \mu\text{m}$ ). The ectoderm of tips of the outer tentacles contains numerous inclusions, possibly sporozoans, which are not present in the inner tentacles. The nematocysts of marginal projections (pseudospherules) are the same as in the upper part of the column. The thin-walled p-mastigophores A (Fig. 6l) are not present in the distal (upper) part of the actinopharynx.

Spermatozoa  $3 \times 1 \mu\text{m}$ , mitochondrial complex about  $1 \mu\text{m}$  (insertion on Fig. 3f).

**Remarks:** The original description of *Cribrinopsis fernaldi* was based on sea anemones collected at San



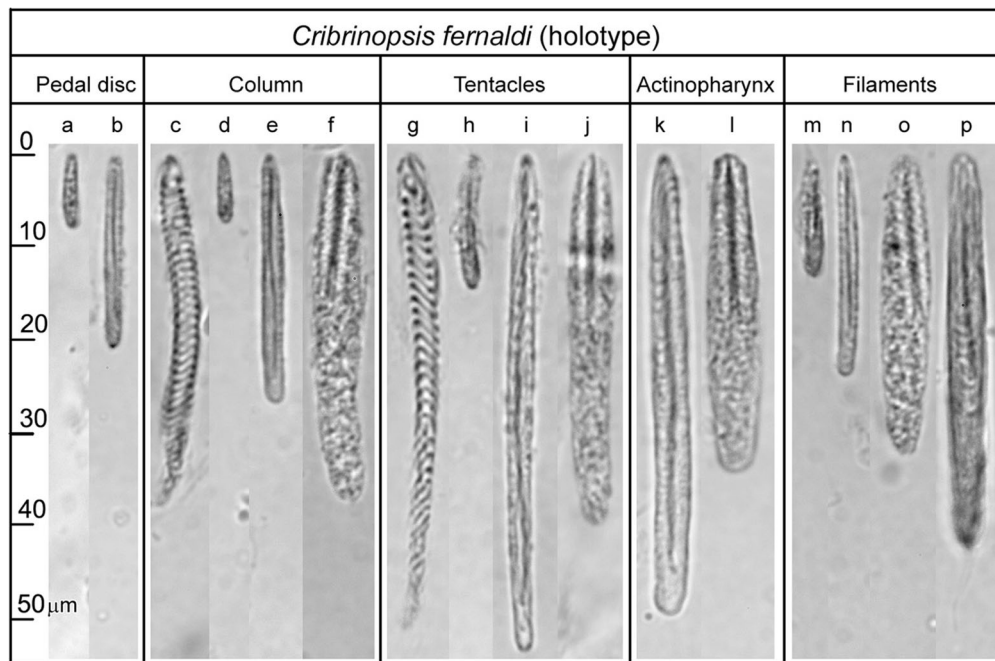
**Fig. 5** **a** *Cribrinopsis fernaldi*, holotype, external appearance; **b** Margin of the holotype of *C. fernaldi*; **c** *Cribrinopsis williamsi*, holotype, external appearance; **d** Separated tentacle of *C. williamsi*; **e** Margin of

*C. williamsi*, arrows point to marginal projections; **f** Transverse section of *C. williamsi*, numbers indicate mesenterial cycles

Juan Island, Washington. The examination of the holotype of *C. fernaldi* and additional specimens identified as *C. fernaldi* revealed that Siebert and Spaulding (1976) misinterpreted or incorrectly reported several key features

of this species. The statement that fully developed specimens have 96 pairs of mesenteries arranged hexamerously is obviously incorrect. The specimens of this species may have 96 individual mesenteries (but not pairs) and their





**Fig. 6** *Cribrinopsis fernaldi*, cnidom of the holotype

arrangement is probably decamerous, as in all other *Cribrinopsis* species in which the arrangement of the mesenteries has been studied in detail. In *C. fernaldi*, the first cycle of the mesenteries consists of six primary pairs and four secondary pairs (marked by roman numerals “I” and “II” in Fig. 2a–c). The latter, situated symmetrically in lateral exocoels and on two sides of one directive pair, conditionally called “ventral” (“vd” in Fig. 2a–c), reach the same size as the mesenteries of the primary pairs. Thus, decamerous symmetry is formed. In two primary “dorsal” exocoels, the secondary mesenterial pairs are shorter and form the second cycle of the mesenteries together with tertiary mesenterial pairs (marked by “III” in Fig. 2a–c) in other exocoels. Thus, the second cycle of mesenteries (marked by “2” in Fig. 2) is composed of eight pairs of tertiary mesenteries (“III” in Fig. 2a–c) and two–four pairs of secondary mesenteries (“II” in Fig. 2a–c). Correspondingly, the third cycle of the mesenteries may be composed of 16 pairs of quaternary mesenteries (in primary “lateral” and “ventral” exocoels) and 6–12 pairs of tertiary mesenteries (in primary “dorsal” exocoels), or it is possible to distinguish eight pairs of mesenteries of fourth cycle (as in Fig. 2b, the specimen KBPGI 496/2). The arrangement of the tentacles on the photographs of live specimens may be interpreted as hexamerous with the duplications in the second cycle in two adjacent primary (“dorsal”) exocoels, or it may be interpreted as decamerous, because six tentacles of the first cycle are placed slightly closer to the mouth than four other tentacles of this cycle (Fig. 1b). The arrangement of the mesenteries is more obviously decamerous. The

number of the tentacles and mesenteries varies only in two adjacent (“dorsal”) primary exocoels and all three studied specimens were bilaterally symmetrical with respect to a directive plane.

Siebert and Spaulding (1976) found rare atrichs in the tentacles of *C. fernaldi*, a category of nematocysts not

**Table 1** Size ranges (length × width, in microns) and distribution of cnidae of *Cribrinopsis fernaldi*, specimens from British Columbia (measured in three specimens)

Body region	Cnidae	Size ranges (μm)
Pedal disc	(a) basitrichs (very rare)	8–9 × 1.5
	(b) basitrichs (few)	18–27 × 2–2.5
Column	(c) spirocysts (common)	25–50 × 2.5–4.5
	(d) basitrichs (rare)	7–9 × 1.5–2
	(e) basitrichs (common)	18–30 × 2–3
	(f) p-mastigophores A (rare)	27–42 × 5–7
Tentacles	(g) spirocysts (numerous)	20–60 × 2–4
	(h) basitrichs (very rare)	10–15 × 1.5–2
	(i) basitrichs (numerous)	29–55 × 2–3.5
	(j) p-mastigophores A (rare)	30–43 × 5–6
Actinopharynx	(k) basitrichs (numerous)	38–58 × 3–5
	(l) p-mastigophores A (few)	30–43 × 5–7
Unilobate filaments	(m) basitrichs (few)	11–15 × 1.5–2.5
	(n) basitrichs (numerous)	19–31 × 2–2.5
	(o) p-mastigophores A (common)	29–37 × 5–6.5
	(p) p-mastigophores B1 (rare)	35–45 × 5–6

Letters in brackets correspond to letters in Fig. 4

**Table 2** Size ranges (length × width, in microns) and distribution of cnidae of holotype of *Cribrinopsis fernaldi*

Body region	Cnidae	Size ranges (μm)	<i>n</i>
Pedal disc	(a) basitrichs (very rare)	7–9 × 1.5–2	3
	(b) basitrichs (few)	20–25 × 2–2.5	9
Column	(c) spirocysts (common)	25–40 × 2–3.5	28
	(d) basitrichs (very rare)	6–9 × 1.5–2	10
	(e) basitrichs (common)	(16) 19–29 × (1.5) 2–3	71
	(f) p-mastigophores A (very rare)	23–38 × 5.5–6	2
Tentacles	(g) spirocysts (numerous)	16–63 × 2–4	90
	(h) basitrichs (very rare)	12–14 × 1.5–2	2
	(i) basitrichs (numerous)	20–60 × 2–3.5	245
	(j) p-mastigophores A (very rare)	41 × 5	1
Actinopharynx	(k) basitrichs (common)	(28) 33–58 × 3–5	63
	(l) p-mastigophores A (few)	33–38 × 4.5–6	7
Unilobate filaments	(m) basitrichs (few)	11–13 × 1.5–2	18
	(n) basitrichs (numerous)	15–25 × 1.5–2	44
	(o) p-mastigophores A (common)	30–35 × 4–6	40
	(p) p-mastigophores B1 (rare)	38–43 × 4–4.5	5

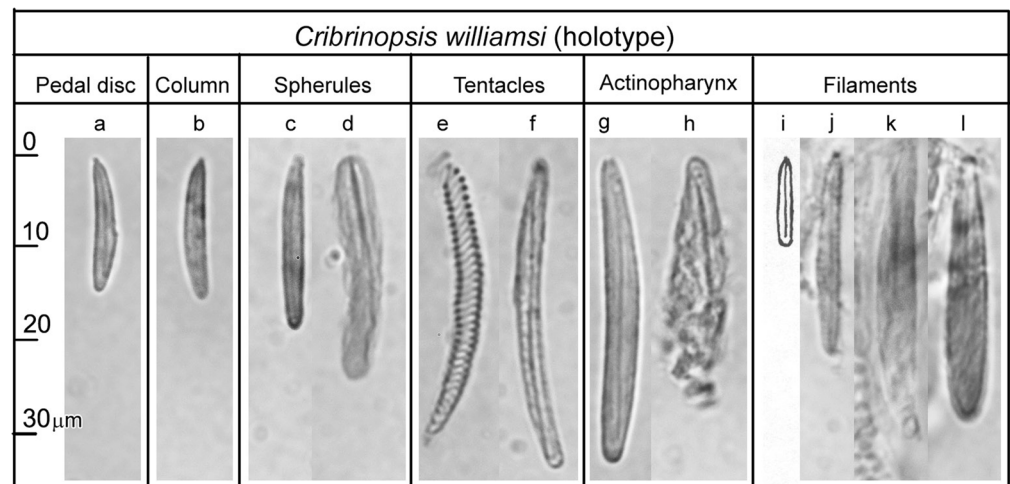
Letters in brackets correspond to letters in Fig. 6

*n* number of measured capsules

known in other species of the genus. We took seven samples from various parts of the tentacles of the holotype: from the tips of the outer, middle, and inner tentacles, the oral and aboral sides of the bases of the tentacles, but failed to find holotrichs (“atrichs”) reported in the original description. These capsules are not present in this species. Sometimes immature spirocysts may resemble holotrichs. Immature spirocysts have wider capsules (this is true for other cnidae too, see Sanamyan et al. 2015) and coils of the tubule are not as easily discernible as in mature ones.

The application of the term “verrucae” for non-adhesive columnar structures in *C. fernaldi* needs some clarification. The distinction between verrucae and vesicles was discussed by Daly (2004: 380), who, with a reference to Stephenson (1928), Den Hartog (1987) and Riemann-Zürneck and

Gallardo (1990), defined the term “verrucae” as “an adhesive, hollow evagination of all three layers of the column wall [...]”. However, Den Hartog (1987) explicitly wrote in his definition that verrucae may be non-adherent, and Riemann-Zürneck and Gallardo (1990: 554) defined them as verrucae “irrespective of whether they are adhesive or not.” Most other authors who worked with specimens of the genera *Urticina* and *Cribrinopsis* (e.g., Stephenson 1935; Carlgren 1921, 1940, 1949; Manuel 1988), where these structures show a wide range of variations, always used the term “verruca” not only for adhesive but also for weakly adhesive or non-adhesive columnar evaginations. We prefer to follow this practice rather than try to find an obscured boundary and separate (by using different terms) adhesive and non-adhesive variants of columnar structures.

**Fig. 7** *Cribrinopsis williamsi*, cnidom of the holotype

**Table 3** Size ranges (length  $\times$  width, in microns) and distribution of cnidae of holotype of *Cribrinopsis williamsi*

Body region	Cnidae	Size ranges ( $\mu\text{m}$ )	<i>n</i>
Pedal disc	(a) basitrichs (common)	12–18 $\times$ 2–2.5	17
Column	(b) basitrichs (common)	11–19 $\times$ 2–2.5	43
Marginal projections	(c) basitrichs (common)	16–19 $\times$ 2–2.5	6
	(d) p-mastigophores A (few)	23–27 $\times$ 3.5–5	6
Tentacles	(e) spirocysts (numerous)	17–38 $\times$ 2–3	32
	(f) basitrichs (numerous)	21–39 $\times$ 2–3.5	118
Actinopharynx	(g) basitrichs (numerous)	23–42 $\times$ 3–4.5	57
	(h) p-mastigophores A (?)	23 $\times$ 6	1
Unilobate filaments	(i) basitrichs (?)	11–12 $\times$ 2	2
	(j) basitrichs (?)	17–25 $\times$ 2–3	6
	(k) p-mastigophores A (?)	30 $\times$ 4	1
	(l) p-mastigophores B1 (common)	19–29 $\times$ 3.5–5	13

Letters in brackets correspond to letters in Fig. 7

*n* number of measured capsules

The most distinguishing feature of this species is the ring of prominent rounded marginal projections. Marginal projections (as perforated pseudospherules) were also reported for *C. williamsi* but this species cannot be confused with *C. fernaldi* and probably does not belong to *Cribrinopsis* (see below).

### *Cribrinopsis williamsi* Carlgren, 1940

(Figs. 5c–f and 7)

*Cribrinopsis williamsi* Carlgren, 1940: 24

**Material examined:** Holotype of *Cribrinopsis williamsi*, USNM 43441, Humpback Bay, Alaska, 19 August 1937, 56° 11' N, 131° 54' W, M.S. *Stranger*, 15 fathoms but near shore.

The specimen was sectioned by previous investigators: the column is cut transversely, the limbus is cut out (probably to count mesenteries at limbus), there are cuts on the margin (Fig. 5c), and two tentacles were separated. One of them was figured by Carlgren (1940), Fig. 4) to show its texture, another is reproduced here (Fig. 5d). The column is wider at limbus and gradually becomes narrower at the margin. The tentacles are partially contracted with only their distal parts visible. The tentacles are rather long, rugose (“papillose” according to Carlgren 1940: 24). The pedal disc wide, slightly convex, without cuticle. The column wall is finely wrinkled. The specimen probably has small projections (pseudospherules sensu Carlgren, 1940) on the margin but they are obscured by wrinkles (Fig. 5e). Twelve projections are recognizable in the present state; we estimate that the specimen had at least 20 projections. There are 84 mesenteries at limbus (only 72 reported by Carlgren 1940). The mesenteries are arranged in four cycles (Fig. 5f). The mesenterial retractors are short and restricted (Fig. 5f).

Cnidom: spirocysts, basitrichs, p-mastigophores A, and p-mastigophores B1 (Table 3, Fig. 7).

**Remarks:** The original description of *C. williamsi* is based on a small specimen (holotype) described by Carlgren (1940)

and redescribed in the present study. Its external live appearance is not known and this species has not been recorded again. The holotype was initially fixed in alcohol and the nematocysts are therefore difficult to study. Their size ranges agree with the data of Carlgren (1940), but we unite the two size ranges of basitrichs reported by Carlgren (1940) in the actinopharynx.

The generic assignment of this species is controversial. Carlgren (1940) reported 48 tentacles (which cannot be counted precisely on the dissected specimen), while the number of mesenteries at limbus is almost two times higher. Obviously the specimen has an additional cycle of the mesenteries at the base, a feature not characteristic for *Cribrinopsis* in which the number of the tentacles is about the same as the number of the mesenteries. This is true for the type species of the genus and for all other species, for which assignment to this genus is well established. The exception is *C. robertii* Parulekar, 1971, in which the tentacles are two times more numerous than the mesenteries at base, but this species, according to original description of Parulekar (1971), has “acrospheres” with “atrichs” (= acrorhagi) and is certainly wrongly assigned to *Cribrinopsis*. Also, in *Cribrinopsis fernaldi*, the marginal projections have the same types and density of the nematocysts as in the column, while the marginal projections of *C. williamsi* have an additional nematocyst type, p-mastigophore A, which is not present in the column or margin between the projections. This species does not resemble any known actiniid species from the NE Pacific. We doubt that it belongs to *Cribrinopsis* but its correct generic assignment cannot be established until additional specimens from the same locality are found.

### *Cribrinopsis rubens* sp. nov.

(Figs. 8, 9, and 10)

*Cribrinopsis fernaldi* Siebert and Spaulding, 1976: 129 (part, not holotype); Lamb and Hanby 2005; Harbo 2011.

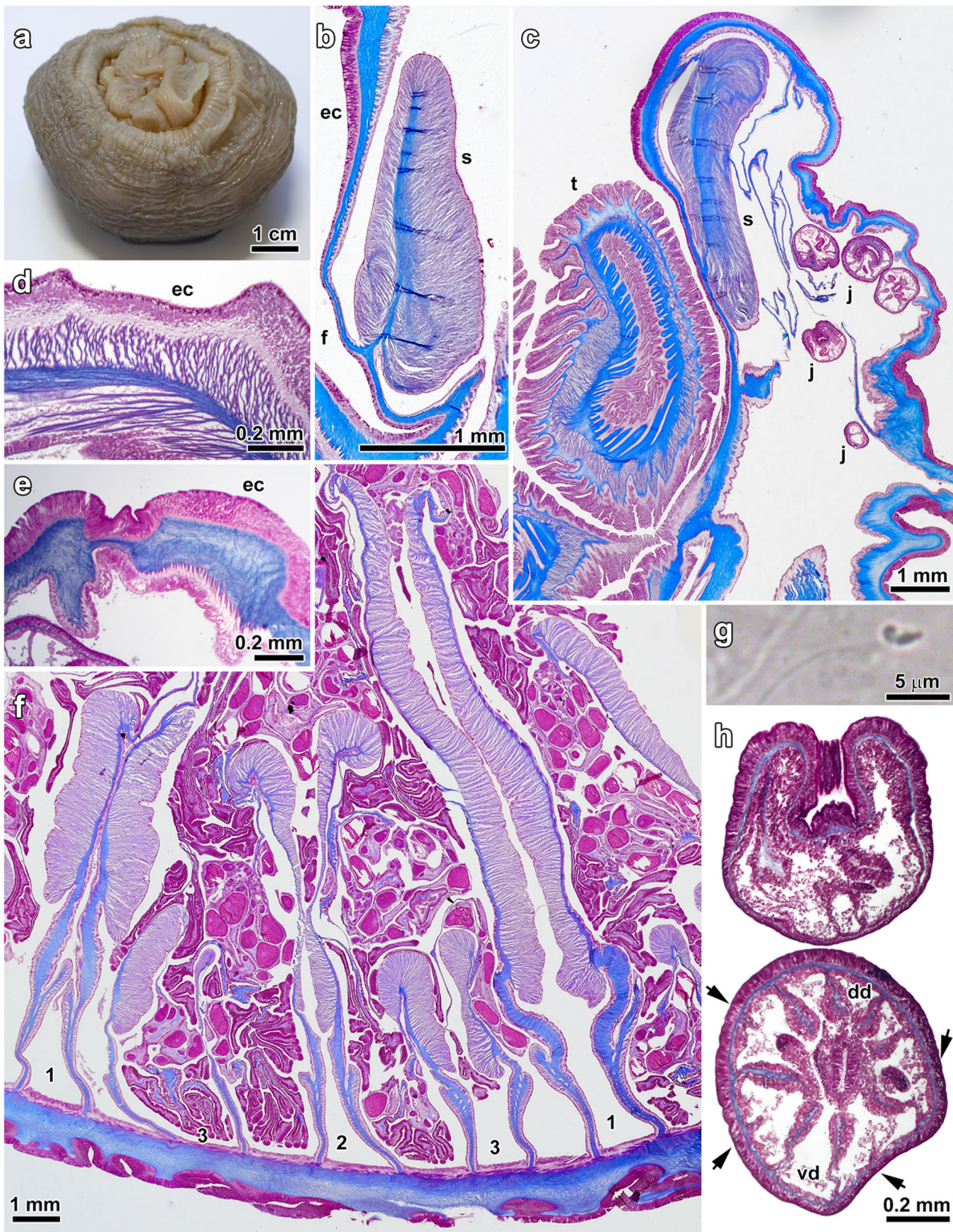


**Fig. 8** *Cribrinopsis rubens* sp. nov. **a** Holotype ZIN 11876 in natural habitat (tentacle cycles indicated by numbers, fourth cycle not labeled); **b** Paratype ZIN 11878; **c** Close up of the tentacles and symbiotic shrimp

*Lebbeus grandimanus*; **d** Specimens on bedrock habitat in Jervis Inlet, BC; **e** Specimens of variable color in current-swept rocky habitat in Weynton Passage, BC

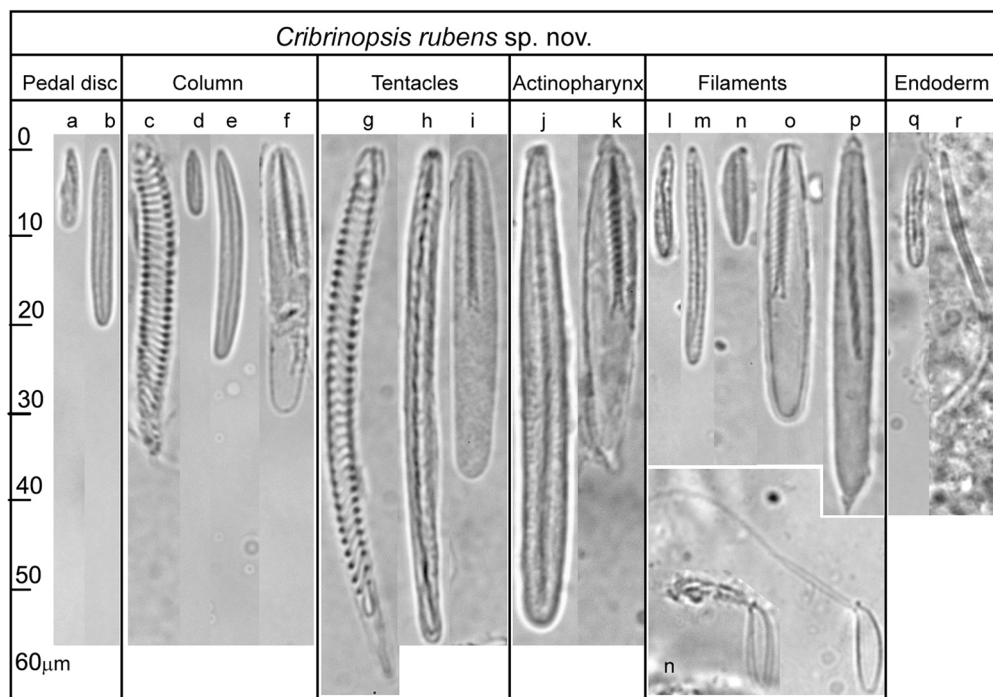
**Differential diagnosis:** Column usually pink-red, occasionally paler to white. Tentacles of same length or shorter than diameter of oral disc. No rounded marginal projections

(pseudospherules). Tentacles and mesenteries arranged decamerously. Filaments contain small (8–11  $\mu\text{m}$ ) b-mastigophores with flap displaced to one side.



**Fig. 9** *Cribrinopsis rubens* sp. nov. **a** Holotype ZIN 11876, preserved; **b** Marginal sphincter, paratype ZIN 11878; **c** Longitudinal section through the margin, holotype; **d** Radial musculature of the oral disc, holotype; **e** Longitudinal section of the column through verruca; **f** Transverse section

of column, holotype, cycles of the mesenteries indicated by numbers; **g** Spermatozoon; **h** Juveniles in the coelenteron of the holotype, arrows point to imperfect mesenteries. dd, dorsal directives; ec, ectoderm; f, fosse; j, juveniles; s, marginal sphincter; t, tentacle; vd, ventral directives



**Fig. 10** *Cribrinopsis rubens* sp. nov. cnidom

**Material examined:** Kuldekduma Island, Weynton Passage, British Columbia, Canada 50° 35.303' N, 126° 50.080' W, depth 20 m, 13 May 2016, collector N. McDaniel. Three specimens: holotype ZIN 11876, paratypes ZIN 11877 and ZIN 11878.

A large, conspicuous sea anemone, reaching 12 cm in column diameter and with the diameter of the tentacular crown up to 15 cm. The tentacles are conical, and inner ones slightly longer than the outer ones. The column is relatively short, attached by a circular base to exposed surface of bedrock or boulders. Its surface is clean, free from any attached matter. The mesenterial insertions are clearly visible on partially contracted specimens as longitudinal grooves. The verrucae are not adhesive, present over all exo- and endocoels, and most of them are of similar and very small size, slightly raised above the surface of column; those on the margin may appear larger, especially when they become inflated in contracted specimens (Fig. 8b). On histological sections, they appear as structures with thickened mesogloea on the sides and very thin mesogloea in the center with modified ectoderm and without muscle processes in the endoderm (Fig. 9e). Special marginal projections (pseudospherules) are not present.

Color and size are variable: specimens in British Columbia coastal fjords such as Jervis Inlet are uniformly pink-red, both on the column and disc, and have longer tentacles and a taller column (Fig. 8d). Specimens in high current habitats such as Weynton Passage are generally smaller (a maximum of 10 cm column diameter) with shorter tentacles and columns and show a broad range of color from pink-red to nearly white (Fig. 8e). The verrucae on the column are marked by white

spots in longitudinal rows. Some verrucae on the distal part of column and a ring of marginal verrucae have very small depressions at the summit, which look like a translucent spot. The color of the oral disc may vary from red to white. The area around the mouth on the oral disc is commonly white or pale rose (Fig. 8a). This area may be narrow, looking as a white thin ring around oral cone, or it may be wide, expanding to the whole oral disc, and in this case, the disc is white with red lines along the insertions of the mesenteries near the bases of the tentacles. The tentacles are marked by distinct reddish vein-like patterns on rose or white background (Fig. 8c), except for white specimens in which red pigment is not present.

Preserved holotype 30 mm high and 60 mm in diameter. The surface of column is wrinkled, and verrucae are not visible on preserved specimens. The actinopharynx partially protrudes and is visible from exterior (Fig. 9a). The tentacles are hidden by contraction of the upper part of column. They are decamerously arranged in four cycles:  $10 + 11 + 26 + 47 = 94$  tentacles with one duplication in the second cycle and five duplications in the third cycle (Fig. 8a). Mesenteries arranged decamerously in three cycles  $10 + 11 + 26 = 47$  pairs, with duplicated pairs in the second and third cycles.

Paratype ZIN 11877, 17 mm high, 47 mm in diameter, has 96 tentacles arranged decamerously in four cycles:  $10 + 10 + 28 + 48$  with eight duplications in the third cycle. Mesenteries are arranged decamerously in three cycles:  $10 + 10 + 28 = 48$  pairs, with eight duplicate pairs in the third cycle.

Paratype ZIN 11878 has a column that is 22 mm high and 52 mm in diameter. It has 92 tentacles arranged not symmetrically in four cycles:  $11 + 11 + 24 + 46$ .

**Table 4** Size ranges (length × width, in microns) and distribution of cnidae of *Cribrinopsis rubens* sp. nov. (measured in three specimens)

Body region	Cnidae	Size ranges (μm)
Pedal disc	(a) basitrichs (very rare)	6–9 × 1.5–2
	(b) basitrichs (few)	19–26 × 2–2.5
Column	(c) spirocysts (common)	23–43 × 2.5–4
	(d) basitrichs (rare)	6–9 × 1–2
	(e) basitrichs (common)	16–28 × 2–2.5
	(f) p-mastigophores A (rare)	28–38 × 4.5–7
Tentacles	(g) spirocysts (numerous)	20–73 × 2–4.5
	(h) basitrichs (numerous)	27–60 × 2.5–3.5
	(i) p-mastigophores A (rare)	29–41 × 5–6 (8)
Actinopharynx	(j) basitrichs (numerous)	37–58 × 4–5
	(k) p-mastigophores A (few)	29–39 × 5–7
Unilobate filaments	(l) basitrichs (few)	9–16 × 1.5–2
	(m) basitrichs (common)	17–30 × 2–2.5
	(n) b-mastigophores (common)	8–11 × 2–3
	(o) p-mastigophores A (few)	28–35 × 5–6
	(p) p-mastigophores B1 (rare)	35–42 × 4–5
Endoderm	(q) basitrichs (rare)	9–15 × 1.5–2
	(r) S-basitrichs (rare)	24–39 × 1–2

Letters in brackets correspond to letters in Fig. 10

All mesenteries are perfect. The mesenterial retractors well developed, restricted, and long (Fig. 9f). Small penons are often present on the parietal part of retractors (closer to the wall of column). The parietobasilar muscles have a well-developed flap. The mesogloea of the outer part of the mesenteries, in the region from the parietobasilar flap to the body wall, contains oval or elongate lacunae arranged into a single series. The actinopharynx has two siphonoglyphs supported by two pairs of directives. The shape of the marginal endodermal sphincter muscle is characteristic in all examined specimens. It is circumscribed, pinnate, rather long on transverse sections, with long crowded, slightly branched lamellae which are almost not anastomosing and tightly packed in the center of the sphincter, forming a long and thick central lamella. The central lamella is united with the wall of the fossa by a short stem (Fig. 9b, c). The longitudinal muscles of the tentacles are mesogloea (Fig. 9c). The radial muscles of the oral disc are mesoectodermal to ectodermal (Fig. 9d).

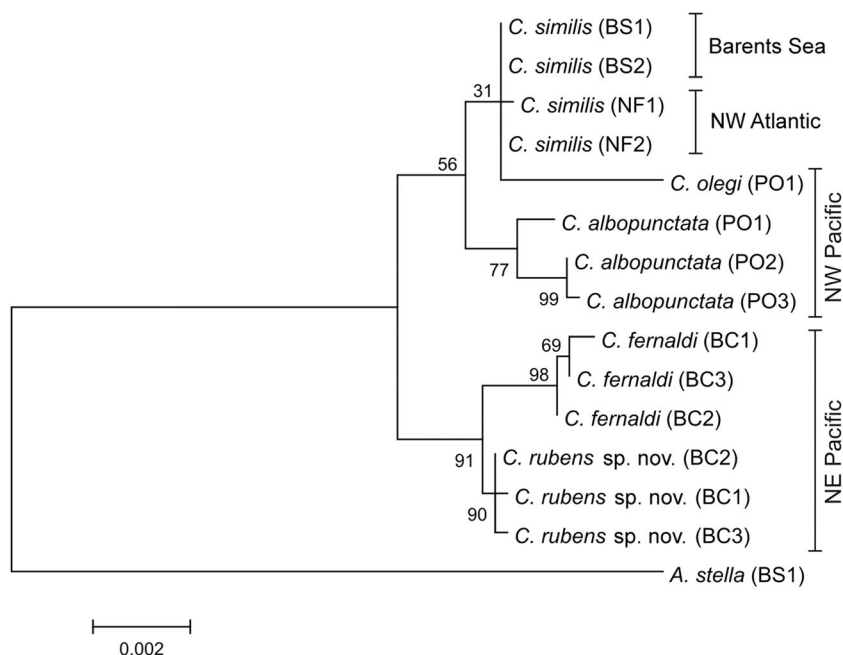
The sexes are separate. All three cycles of the mesenteries are fertile. The holotype is a female with ova up to 800 μm in diameter and with numerous small (up to 1 mm in diameter) juveniles in the coelenteron (Fig. 9c). Juveniles are in an *Edwardsia* stage. They have no tentacles. Juveniles have 12 mesenteries, eight of which are perfect and have retractors and filaments, and four smaller mesenteries without filaments and retractors (Fig. 9h). Juveniles are visible on the photographs of

live specimens in the tentacles and also in the coelenteron through the oral disc and body wall. Both paratypes are males. Spermatozoa 3–3.5 × 1–1.5 μm (Fig. 9g).

Cnidom: spirocysts, basitrichs, b-mastigophores, p-mastigophores A, and p-mastigophores B1 (Table 4, Fig. 10). Large basitrichs in the tentacles (Fig. 10h) are much shorter at tentacle bases (27–42 μm) than in the tips of the tentacles (36–60 μm). A small capsule in the filaments (Fig. 10n) is possibly a b-mastigophore. It has a flap which is somewhat displaced to one side from the usual apical position (visible as a black dot in Fig. 10n) and are fired tubule, which has differentiated shaft, is always placed at an angle to the axis of the capsule. The endoderm of column of all specimens contains rare small basitrichs (Fig. 10q) and thin long S-shaped basitrichs (Fig. 10r). The nematocysts of the juveniles are smaller than in adult specimens: in the ectoderm of column spirocysts are 13–21 × 1.5–2.5 μm, basitrichs 15–22 × 2–3 μm and 6 × 1.5 μm (very rare), p-mastigophore A 25 × 3.5 μm; in the endoderm of column S-shaped basitrichs 18–32 × 1–1.5 μm (more frequent than in adults) and small basitrichs 6–7 × 1.5–2 μm; in filaments basitrichs are 7–11 × 1.5–2 μm and 17–20 × 2.5–3 μm and p-mastigophores A 16–22 × 3–5 μm.

**Habitat:** This species is commonly found in habitats with moderate to strong tidal currents attached to bedrock or boulders. It can be found in high densities, particularly on vertical rock walls in Jervis Inlet, BC. This anemone hosts the commensal shrimps *Lebbeus grandimanus* (Bražnikov, 1907) (Fig. 8c) and *Heptacarpus kincaidi* (Rathbun, 1902) (see Jensen 2014). A single anemone may host several individuals of both species. The shrimps tend to congregate around the base of the anemone but occasionally are found among the tentacles. They appear to be immune to the sting of the nematocysts.

**Remarks:** *Cribrinopsis rubens* sp. nov. is a common species in coastal British Columbia. Photographs can be found in numerous websites and color guides where they are commonly identified as *C. fernaldi* (e.g., Lamb and Hanby 2005; Harbo 2011). However, the external appearance of *C. fernaldi* is different. Generally, it is taller than *C. rubens* sp. nov. the tentacles are longer, and the column is white. The most significant difference is the absence of marginal projections (pseudospherules) in *C. rubens* sp. nov. while those in *C. fernaldi* are prominent. The decamerous arrangement of the tentacles is more obvious in most specimens of *C. rubens* sp. nov. than in those of *C. fernaldi*. The cnidom of both species is similar. However, *C. rubens* sp. nov. lacks small curved basitrichs in the tentacles, which are present in *C. fernaldi*, and has unique small b-mastigophores which were previously not reported for other *Cribrinopsis* species. Also, these two species occupy different habitats: *C. rubens* sp. nov. occurs on current-swept rocky substrates while *C. fernaldi* inhabits muddy or rocky bottoms in areas with negligible currents. According to Siebert and Spaulding (1976: 132), *C. fernaldi* “is found on rock walls sloping to vertical”, a habitat



**Fig. 11** Phylogram resulting from maximum likelihood analysis of concatenated 12S rRNA, 16S rRNA, and COIII mitochondrial gene sequences, 18S rRNA and 28S rRNA nuclear gene sequences of *Cribrinopsis fernaldi*, *C. rubens* sp. nov. *C. similis*, *C. albopunctata*, *C. olegi*, and *Aulactinia stella* as an outgroup. The tree was

reconstructed by Hasegawa-Kishino-Yano model (MEGA 6.0). Numbers on nodes represent bootstrap values (%). BC, British Columbia (Canada); BS, the Barents Sea; NF, Newfoundland; PO, the Pacific Ocean, numbers after letters indicate genotype number

characteristic for *C. rubens* sp. nov. rather than for *C. fernaldi*. We assume that the original description *C. fernaldi* may have been based simultaneously on two species, which Siebert and Spaulding (1976) failed to distinguish.

Externally *Cribrinopsis rubens* sp. nov. most closely resembles *Cribrinopsis similis* Carlgren, 1921 from Europe and the North Atlantic. Both species usually have a red and relatively

short column, tentacles of similar length and number, and a similar color pattern composed of red lines on the oral disc. Both species may form rather dense settlements on open rocky surfaces (see color photographs in Kukliński and Bałazy 2017; Bałazy et al. 2014). However, the shape of the marginal sphincter muscle probably differs in these two species: in *C. rubens* sp. nov. the sphincter on transverse sections is

**Table 5** GenBank accession numbers of *Cribrinopsis* and *Aulactinia* species

Genotypes of <i>Cribrinopsis</i> and <i>Aulactinia</i> species	Mitochondrial gene fragments			Nuclear gene fragments	
	12S rRNA	16S rRNA	COIII	18S rRNA	28S rRNA
<i>C. olegi</i> (PO1)	MH385361	MH385366	MK304505	MH376911	MH380004
<i>C. albopunctata</i> (PO1)	MH385362	MH385367	MK304506	MH376912	MH380005
<i>C. albopunctata</i> (PO2)					MH380006
<i>C. albopunctata</i> (PO3)				MH376913	
<i>C. rubens</i> sp. nov. (BC1)	MH385363	MH385368	MK304507	MH376914	MH380007
<i>C. rubens</i> sp. nov. (BC2)				MH376915	MH380008
<i>C. rubens</i> sp. nov. (BC3)				MH376916	
<i>C. fernaldi</i> (BC1)	MH385364	MH385369	MK304508	MH376917	MH380009
<i>C. fernaldi</i> (BC2)				MH376918	MH380010
<i>C. fernaldi</i> (BC3)					MH380011
<i>C. similis</i> (BS1)	MH385365	MH385370	MK304509	MH376919	MH380012
<i>C. similis</i> (BS2)					
<i>C. similis</i> (NF1)	MK287981	MK307748	MK304514	MK307728	MK307740
<i>C. similis</i> (NF2)				MK307729	MK307741
<i>A. stella</i> (BS1)	KT310208	JQ927444	KT310207	MH376920	MH380013



elongated and has thinner central lamella, while the specimens of *C. similis* from Newfoundland examined by us (unpublished data) and the original specimens studied by Carlgren (1921) have a very thick main central lamella which is distally (on its free edge) wider than proximally. Further, we found juveniles with developed mesenteries in the coelenteron of *C. rubens* sp. nov. a feature unknown for *C. similis* (see Carlgren 1921).

*Cribrinopsis albopunctata* Sanamyan & Sanamyan, 2006 known from the NW Pacific usually also has a red column with white spots marking verrucae arranged into longitudinal rows (Sanamyan and Sanamyan 2006). However, its tentacles lack the distinct veined pattern as observed in *C. rubens* sp. nov. and the verrucae are adhesive. According to molecular data (Fig. 11), *C. rubens* sp. nov. is rather distant from both *C. similis* and *C. albopunctata*.

*Cribrinopsis japonica* Tsutsui et al., 2014 from Japan differs from *C. rubens* sp. nov. in habitat (soft bottom), absence of white marking on verrucae, the shape of the sphincter with a very thin central lamella (see Tsutsui et al. 2014, Fig. 3a), and details of cnidom. In particular it has no p-mastigophores A in the tentacles and b-mastigophores in any region. In *Cribrinopsis* species p-mastigophores A in the tentacles are known only from two British Columbia species (*C. fernaldi* and *C. rubens* sp. nov.). According to Tsutsui et al. (2014), *C. japonica* was previously mistaken with *C. fernaldi* in Japan; however, it lacks the marginal projections characteristic of *C. fernaldi*.

Two remaining NW Pacific *Cribrinopsis* species, *C. olegi* Sanamyan & Sanamyan, 2006 and *C. asiatica* (Averincev, 1967), differ distinctly from *C. rubens* sp. nov.: *C. olegi* has short pear-shaped tentacles, adhesive verrucae, and column buried in sand or gravel and *C. asiatica* has strongly developed adhesive verrucae and different coloration (Sanamyan and Sanamyan 2006; Averincev 1967).

## Results of molecular analysis

Five gene fragments of mitochondrial DNA (12S rRNA, 16S rRNA, COIII) and nuclear DNA (18S rRNA, 28S rRNA) of three specimens of *Cribrinopsis fernaldi* and three specimens of *Cribrinopsis rubens* sp. nov. collected from British Columbia were obtained and compared with corresponding sequences of other *Cribrinopsis* species. After the treatment, the lengths of the fragments were as follows: 12S rRNA—781 nucleotides, 16S rRNA—506 nucleotides, COIII—485 nucleotides, 18S rRNA—1497–1499 nucleotides, 28S rRNA—820–834 nucleotides. The sequences of *C. similis*, *C. albopunctata*, *C. olegi*, and *Aulactinia stella* (Verrill, 1864) (as an outgroup) obtained in our previous investigation were added to the phylogenetic reconstruction and were deposited in GenBank (Table 5). 12S rRNA and 16S rRNA sequences of all studied *Cribrinopsis* species were found to be identical, respectively. The COIII gene sequences of almost all these species belong to one haplotype with the exception of *C. olegi* haplotype, which includes only

one nucleotide substitution. For phylogenetic reconstruction mitochondrial (12S rRNA, 16S rRNA, COIII) and nuclear (18S rRNA, 28S rRNA) fragments were concatenated and analyzed in MEGA 6.0 (Tamura et al. 2013). The topology of resulting maximum likelihood unrooted phylograms was identical; thus, only one of them is presented (Fig. 11). Bootstrap values for maximum likelihood (ML) were calculated and the Hasegawa-Kishino-Yano model (Gamma distributed) was found to be the best one for these phylogenetic analyses (Hasegawa et al. 1985).

Obtained ML phylogram (Fig. 11) showed that each *C. rubens* sp. nov. and *C. fernaldi* formed separate clades with 90% and 98% bootstrap support, respectively. Molecular analysis confirmed the conclusion made on the basis of the morphological data that *C. rubens* sp. nov. and *C. fernaldi* are two distinct species.

## Discussion

This study presents first integrative data on the genus *Cribrinopsis* from the Pacific NW coast of North America. Both morphological and molecular data confirm the existence of two species of *Cribrinopsis* in shallow waters of British Columbia, *C. fernaldi* and *C. rubens* sp. nov. which were previously mixed together under the name *C. fernaldi*. The two species may be distinguished from each other by gross appearance, color, and habitat: *C. fernaldi* has a larger maximum size and has a white column with prominent rounded marginal projections (pseudospherules), which in most cases could be easily recognized on the photographs of live specimens. It lives attached to cobbles or small boulders imbedded in soft sediments or attached directly to bedrock or boulders in areas of weak tidal currents. *Cribrinopsis rubens* sp. nov. is generally smaller, is usually has a red column, and has no marginal projections (pseudospherules) and it lives on rocky substrates swept by tidal currents. The original description of *C. fernaldi* was based on both species; however, its holotype corresponds to the larger species with the white column and it retains the original name *C. fernaldi*, while the species from current-swept rocky substrates is described as *C. rubens* sp. nov. It is interesting that according to molecular data *C. rubens* sp. nov. is closer to *C. fernaldi* than to the morphologically more similar *C. similis* from Atlantic and European waters. However, *C. rubens* sp. nov. is distinguished from the North Atlantic *C. similis* by the presence of internal brooding up to the polypoid stage. It is important to note that Siebert and Spaulding (1976): Fig. 3c) in the specimen bearing pseudospherule (which represents true *C. fernaldi*) have recorded eggs which were visible through them, whereas observations of the brooding by Siebert and Spaulding (1976) probably should be applied to *C. rubens* sp. nov. In the present study, only a single female of *C. fernaldi* was available, which shows no juveniles inside. Therefore, until more specimens of *C. fernaldi* become available, we conclude

that most likely *C. fernaldi* is a non-brooding species, compared to its sympatric congener *C. rubens* sp. nov.

Sympatric speciation currently is considered one of major forms of speciation, though its importance was previously considerably underestimated (e.g., Dieckmann and Doebeli 1999; Bolnick and Fitzpatrick 2007; Foote 2018). According to our integrative, morphological, molecular, and biogeographical data, we suggest that the major driving force for the sympatric speciation in the NE Pacific species of the genus *Cribrinopsis* was ecological differentiation into the low current, soft, and rocky habitats in *C. fernaldi* and rocky habitats with stronger currents in *C. rubens* sp. nov. respectively. These habitat differences might further lead to the development of different reproductive strategies: broadcast release in *C. fernaldi* (likely an ancestral trait) and brooding of the juveniles in the coelenterone of the females in *C. rubens* sp. nov. The acquisition of the brooding behavior in the new species might reduce the juvenile mortality under the strong current conditions and therefore facilitate survival in this complex habitat. The present case of possible sympatric speciation in sea anemones is in agreement with notable examples of the reproductive isolation firstly triggered by the ecological shifts in various metazoan lineages, for instance in such different groups as insects and fishes (e.g., Forbes et al. 2017; van Rijssel et al. 2018; Whitney et al. 2018). A comparable case of habitat-related sympatric speciation, which is supported by both morphological and molecular data, was recently presented for two species of the nudibranch molluscs of the genus *Zelentia* Korshunova, Martynov & Picton, 2017 from the Barents Sea, one of which inhabits the intertidal zone and has direct development, whereas the other inhabits the subtidal (usually deeper than 10 m) in the same geographic location as its sister species, but possessing planctotrophic larva (Korshunova et al. 2017).

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

**Sampling and field studies** All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements, if applicable.

**Data availability** The datasets generated during and/or analyzed during the current study are available in the GenBank repository, <https://www.ncbi.nlm.nih.gov/nucleotide>. GenBank accession numbers are listed in Table 5.

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