ORIGINAL PAPER

Morphological and molecular assessment of large sea anemones (Actiniaria: Actiniidae) in Newfoundland (eastern Canada)

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Abstract

Literature records of large actiniid sea anemones along the Atlantic coast of Canada currently include three *Urticina* species: *U. felina*, *U. fecunda* and *U. crassicornis*. The findings of the present morphological and molecular study conducted in eastern Newfoundland suggest that *U. felina* is often misidentified, and that this region may only harbor two similar-looking species: *U. crassicornis* and *Cribrinopsis similis*. The latter were identified using genetic analysis and comparison of key characters with the same species collected from other regions of the North Atlantic (Barents Sea), whereas no specimen corresponding to *U. felina* was found. Mitochondrial gene sequences of *U. crassicornis*, *U. felina* and *C. similis* were identical except for a different haplotype found in several specimens of *U. crassicornis* (with one nucleotide substitution), in contrast to five nucleotide insertions in 16S rRNA fragments of *U. fecunda*. Phylogenetic analysis based on three mitochondrial and two nuclear gene fragments revealed that the most closely related species among the above-mentioned were *U. crassicornis* and *U. felina*, nevertheless *U. fecunda* groups in the same clade as the *Urticina* species.

Introduction

Despite being one of the most extensively studied areas in the world ocean, the North Atlantic remains incompletely studied and still harbors its share of unrecorded species and taxonomic debates (e.g., Haydar 2011; Saucier et al. 2017). Among the least understood and most often misidentified

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taxa occurring at scuba diving depths in the Northwest Atlantic are cnidarians belonging to the order Actiniaria (sea anemones). Species in the genera *Metridium* (family Metridiidae), *Hormathia* (family Hormathiidae), *Stomphia* (family Actinostolidae), *Aulactinia* and *Urticina* (family Actiniidae) are the most common and often the largest sea anemones found off north-eastern North America (Brunel et al. 1998). Along the coast of Canada, the large conspicuous actiniids can reach densities of 5–6 individuals m^{-2} on rocky bottoms between the lower intertidal zone and 80 m depth (J-F Hamel, personal observation). A number of catalogs, reports and scientific publications designate some of these large actiniid sea anemones by the name *Urticina felina* (Linnaeus, 1761), with no clear reference to taxonomic support (e.g., Bolton 1981; Himmelman et al. 1983; Lavergne and Himmelman 1984; Logan 1988; Brunel et al. 1998; Collie et al. 2009; Nadon and Himmelman 2010; Mercier et al. 2011; Morrison and Redden 2012; Novaczek et al. 2017; Daly and Fautin 2018). Mercier et al. (2011) studied a species in Newfoundland (as *U. felina*) that broods offspring to the planula stage before releasing the larvae in the water column; however, according to Carlgren (1921) and Manuel (1988) *U. felina* is not a brooder. Notably, Brunel et al. (1998) had already highlighted the possible confusion

plaguing this group of sea anemones in eastern Canada, where the presence of the species *Urticina fecunda* (Verrill, 1899) was only recently acknowledged (Larson et al. 2012).

In the present study, we examined a substantial sampling of similar-looking actiniid sea anemones collected along the coast of Newfoundland (eastern Canada) from the same general location and habitat. Morphological and genetic investigations showed that they belonged to two different species: *Cribrinopsis similis* Calgren 1921 and *Urticina crassicornis* (Müller 1776), which are known from European and North American coasts of the North Atlantic (Carlgren 1921; Verrill 1922; Fautin 2013; Murillo et al. 2016). However, the analyses failed to find any *U. felina* and examination of published pictures suggest that specimens previously identified as *U. felina* in this region instead belong to the species *C. similis*. The present contribution thus aims to shed some light on the characteristics of each of these actiniid species and ultimately helps untangle a long-standing confusion.

Material and methods

Collection and morphological study

Thirty large specimens of actiniid sea anemones exhibiting different color morphs were collected by the Field Services of the Department of Ocean Sciences (Memorial University) in coastal waters of Newfoundland, eastern Canada (Fig. 1): individuals U1, U2, U6, U12–U17, U21–U28 were sampled from Cape Broyle in the Admirals Cove area (around 47° 06′ N: 52° 54′ W; date of collection 1.06.2017, max depth 12 m; water temperature 1.7 °C); samples U3–U5, U7–U11 and U29 were out of Bay Bulls, Bread and Cheese Cove (around 47° 18′ N: 52° 47′ W; date of collection 22.06.2017; max depth 16 m; water temperature 2.7 °C); U18–U20, and U30 had been kept in the aquarium for a long time and were collected in Bay Bulls and Bread and Cheese Cove at a depth of 11 m (Fig. 1). A morphological study was conducted on all live specimens, which were photographed to record color and external appearance, as well as the presence of brooded juveniles or larvae. All specimens were examined alive and then five of them, which were the most variable in color, were fixed in 4% formaldehyde. Histological sections were
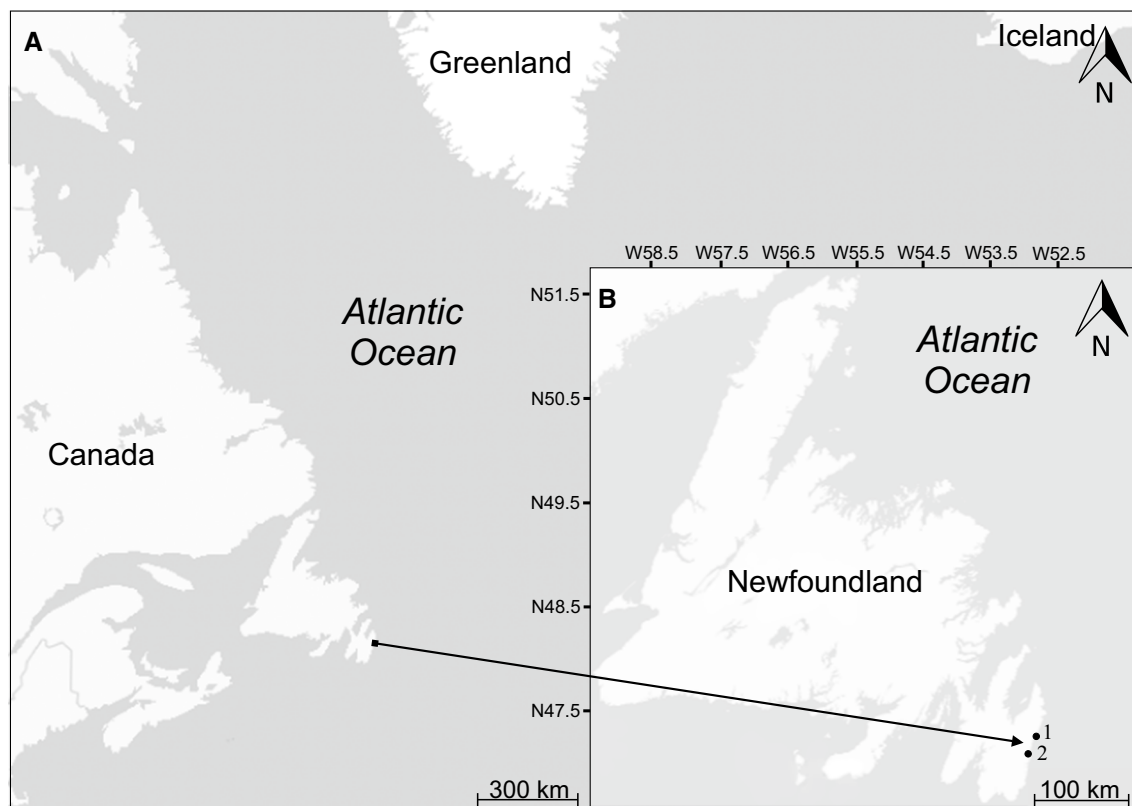
Fig. 1 Map of collection localities in Newfoundland (eastern Canada): 1—Bay Bulls, Bread and Cheese Cove (47° 18′ N: 52° 47′ W), 2—Cape Broyle in the Admirals Cove area (47° 06′ N: 52° 54′ W)

prepared using isopropanol—mineral oil method (Sanamyan and Sanamyan 2012). Size ranges of cnidae were measured on small sections of macerated tissue and details of distribution of cnidae in different tissues were assessed from histological sections stained by basic dyes, e.g., Safranin or Toluidine blue (see details in Sanamyan et al. 2013). Cnidae terminology follows Weill (1934) and Carlgren (1949), but classification of *p*-mastigophores follows Schmidt (1969, 1972, 1974) with the modification applied by den Hartog (1995) as per Sanamyan et al. (2012). The preserved specimens are stored at the Kamchatka Branch of the Pacific Geographical Institute (KBPGI 490/1, 491/2, 492/1, 493/2, 494/3).

Molecular techniques

Samples of pedal disc from all 30 specimens were preserved in 96% ethanol prior to DNA extraction. 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 were amplified using published primers and protocols (Geller and Walton 2001; Bocharova 2015).

The nuclear gene fragments (18S rRNA and 28S rRNA) were amplified with specially designed primers and the Geneious 8.1.5 software (https://www.geneious.com) (Sanamyan et al. 2018). PCR cycling conditions were as follows: initial denaturation for 2 min at 95 °C, followed by 35 cycles

of denaturation for 30 s at 95 °C, annealing for 30 s at 55 °C and extension for 1 min at 72 °C, and additional extension for 5 min at 72 °C.

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. To treat the chromatograms, Sequencing Analysis 3.7 (Applied Biosystems©) and Geneious 8.1.5 were applied. After the treatment, the lengths of the fragments were as follows: 12S rRNA—781 nucleotides, 16S rRNA—506–511 nucleotides, COIII—537 nucleotides, 18S rRNA—1498 nucleotides, 28S rRNA—820–836 nucleotides. Forward and reverse sequences were assembled in 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. All assembled sequences have been deposited in GenBank (Table 1).

Data analysis

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) and MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001). Several analytical methods of phylogenetic

Table 1 GenBank accession numbers of newly and previously obtained sequences

Species	Samples	Mitochondrial gene sequences			Nuclear gene sequences	
		12S rRNA	16S rRNA	COIII	18S rRNA	28S rRNA
C. similis (NF1)	U1, U3-U5, U9, U10, U17-U19, U21, U29	MK287981	MK307748	MK304514	MK307728	MK307740
C. similis (NF2)	U2, U6-U8, U11				MK307729	MK307741
C. similis (BS)	–	MH385365	MH385370	MK304509	MH376919	MH380012
U. crassicornis (NF1)	U12	MK287979	MK307745	MK304512	MK307725	MK307733
U. crassicornis (NF3)	U16, U30					MK307734
U. crassicornis (NF4)	U28				MK307726	
U. crassicornis (NF2)	U13, U22		MK307746			MK307733
U. crassicornis (NF5)	U23, U25-U27		MK307743			MK307734
U. crassicornis (NF6)	U14, U15, U24				MK307725	
U. crassicornis (BS1)	–			MK304511	MK307724	MK307731
U. crassicornis (BS2)	–		MK307744			MK307732
U. felina (BS1)	–	MK287980	MK307747	MK304513	MK307727	MK307735
U. felina (BS2)	–					MK307736
U. felina (BS3)	–					MK307737
U. felina (BS4)	–					MK307738
U. felina (BS5)	–					MK307739
U. fecunda (NF1)	U20	–	MK307749	–	MK307730	MK307742
A. stella (BS)	–	–	JQ927444	–	MH376920	MH380013

reconstruction were used (Nei and Kumar 2000) and were submitted to TreeBASE (submission ID 23878). In Bayesian analysis, the number of substitution types was fixed to 2, and the 4by4 model was used for substitution, while rates variation across sites was fixed to "gamma," and four Markov Chain Monte Carlo (MCMC) chains were run for 10,000 generations, sampling every 10 generations, with the first 250 sampled trees discarded as "burn-in." Finally, a 50% majority rule consensus tree was constructed.

Phylogenetic analyses [Maximum Likelihood (ML), Neighbor-Joining (NJ) and Minimum Evolution (ME)] were performed using the Kimura 2-parameter model with gamma distribution in MEGA 6.0. The bootstrap values were calculated for (1) ML: rates among sites = gamma distributed (G), No of discrete gamma categories = 2, Nearest-Neighbor-Interchange heuristic method, BioNJ initial tree, very strong branch swap filter, 1000 replicates; (2) NJ: rates among sites = gamma distributed, No of discrete gamma categories = 1, 1000 replicates; (3) ME: rates among sites = gamma distributed, No of discrete gamma categories = 1, Close-Neighbor-Interchange heuristic method, Neighbor-Joining initial tree, search level = 1, 1000 replicates. Neighbor-Joining algorithm was used to find the phylograms.

Evolutionary analyses (pairwise distances between species and standard errors) were conducted using the Kimura 2-parameter model (Kimura 1980) 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.

Taxonomy

Cribrinopsis similis Carlgren, 1921

Cribrinopsis similis Carlgren, 1921, p. 156; 1928, p. 279 (and synonymy); Zhubicas (1977, p. 108).

Not *Cribrinopsis similis*: Sanamyan and Sanamyan (2006, p. 369).

Material examined

Two specimens: U18 (KBPGI 490/1), U19 (KBPGI 491/2).

Description

The two specimens measure 36 and 34 mm in height and 22 and 18 mm in column diameter, respectively. Pedal disc has thin cuticle. Their color alive is similar: the column is reddish-pink with whitish verrucae arranged into clear longitudinal and faint transversal rows (one longitudinal row of verrucae in each endo- and exocoel). Verrucae are better

developed in the distal (upper) half of column and gradually disappeared in the proximal (lower) part of column, toward the limbus (Fig. 2a). Oral disc and tentacles are beige-pink in color. Reddish pigment is present around the base of the tentacles on the outer half of the disc. Wide short red bands run radially toward the center of the oral disc from the base of the tentacles. These bands end abruptly on different levels, depending of the cycle number of the tentacle, and clearly mark ten tentacles of the first cycle (Fig. 2b). The tentacles are of uniform color, with peculiar transverse zigzag lines or "moire" (a feature characteristic of certain *Cribrinopsis* species), and yellowish tips. Tentacles are arranged decamerously in four cycles (10 + 10 + 20 + 40) and are transversely wrinkled in the preserved specimens.

Circular muscles of column are well developed (Fig. 2c).

On histological sections, verrucae are marked by much thinner mesogloea; circular columnar muscles are not present in these places. The most distal (marginal) verrucae have especially thin mesogloea at their central part with tiny (30 μm) perforation in it (Fig. 2d). There are well-formed parapet, fossa and short capitulum.

The endodermal marginal sphincter is circumscribed, pinnate with very thick main central lamella, which is much wider distally (on its free edge) than proximally (in the place where it is attached to column wall) (Figs. 2c; 3b). Muscle processes in the sphincter muscle are very numerous, anastomosing in some places (Fig. 2e).

Longitudinal muscles of the tentacles are mesogloeal (Fig. 3a). Radial muscles of the oral disc are ectodermal to meso-ectodermal.

Mesenteries are arranged decamerously in three full cycles (10 + 10 + 20 = 40 pairs or 80 mesenteries). Two pairs of directives are attached to two siphonoglyphes. At least two first cycles of mesenteries are perfect. Both examined specimens were sterile.

Retractors are rather strong, diffuse, their parietal sides (closer to the wall of column) end abruptly and often form distinct pennon.

Parietobasilar muscles are well developed and form distinct free flap. Mesogloea of the parietal part of the mesenteries, in the region of parietobasilar muscles, contains a chain of oval or elongated lacunae running longitudinally (on transverse sections; Fig. 3c).

Cnidome contains spirocysts, basitrichs, *p*-mastigophores A, *p*-mastigophores B1 (Table 2, Fig. 4). Size ranges of cnidae are in agreement with data published by Carlgren (1921, 1945) for this species. Basitrichs in the actinopharynx are of about the same length as those in the tentacles, but thicker. Columnar spirocysts and *p*-mastigophores A are present only in the distal (upper) part of column, but absent in its proximal part. Columnar basitrichs (Fig. 4E) are numerous near the limbus. Cnidoglandular tracts of filaments contain three types of nematocysts: larger basitrichs (Fig. 4N),

Fig. 2 *Cribrinopsis similis* (specimen U18): **a, b** Live specimen (white arrow shows reddish pigment around the bases of the tentacles, black arrows points to wide short red bands); **c** longitudinal section through distal column showing sphincter muscle; **d** longitudinal section through the marginal verruca; **e** detail of the sphincter muscle, enlarged. *c* column, *cl* central lamella of the sphincter, *cm* circular columnar muscles, *ec* ectoderm, *en* endoderm, *f* fossa, *s* sphincter, *t* tentacle, *v* verruca
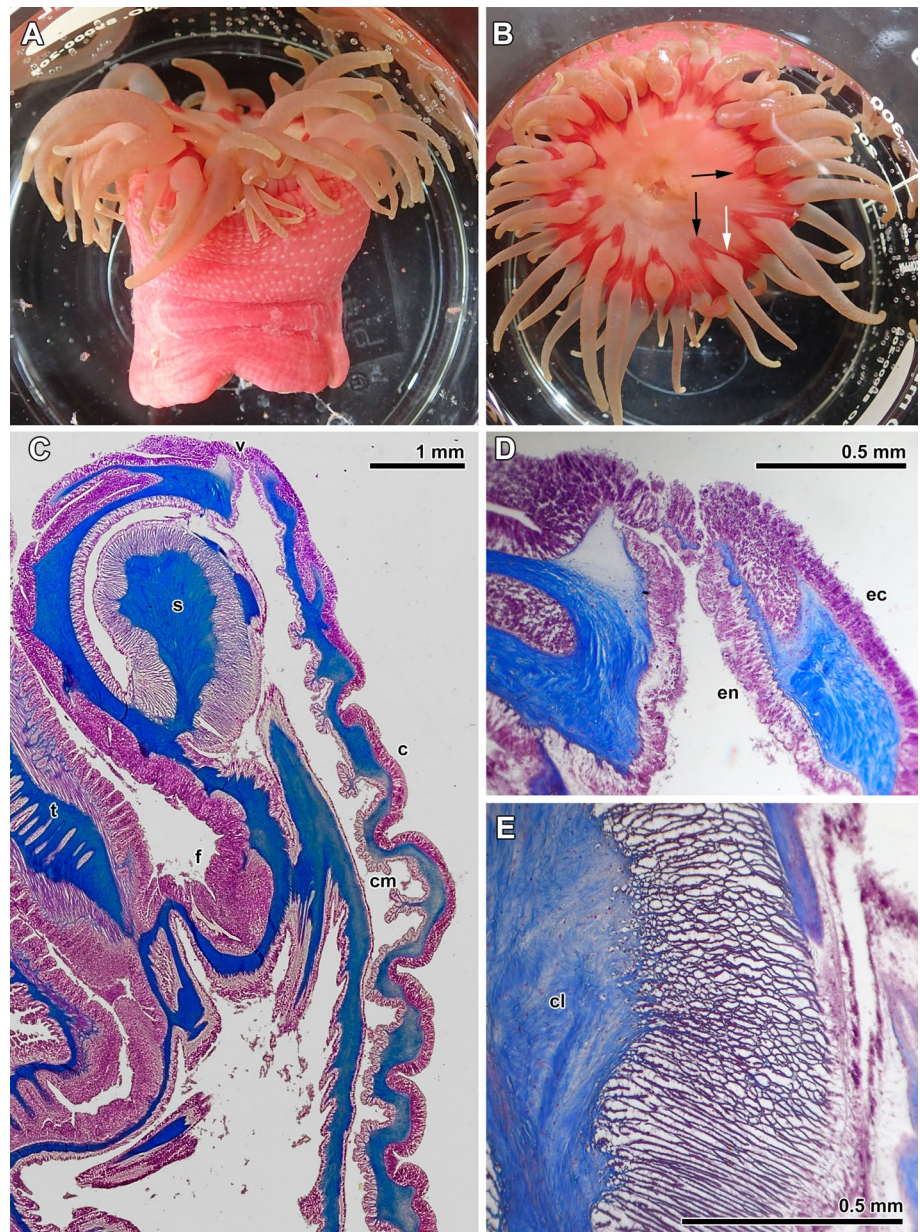
p-mastigophores A and B1. Small basitrichs (Fig. 4M) occur (commonly) in the endoderm of filaments near cnidoglandular tracts. In addition, in the endoderm of filaments near cnidoglandular tracts, two types of larger basitrichs occur: 21–24 × 2–2.5 μm (very rare) and 38 × 2.5–3 μm. Small basitrichs (Fig. 4R) and thin and long S-shaped basitrichs (Fig. 4Q) occur in whole endoderm.

Urticina crassicornis (Müller, 1776)

Actinia crassicornis Müller (1776, p. 231).
    Rhodactinia daevisii Agassiz (1847, p. 677).
    Actinia obtruncata Stimpson (1853, p. 7).
    Urticina felina: McMurrich (1911, p. 65).

    Urticina felina crassicornis: Carlgren (1921, p. 170) (and synonymy).
    Urticina crassicornis: Verrill (1922, p. 104) (part).
    Tealia felina var. *crassicornis*: Stephenson (1935, p. 150).
    Not *Tealia crassicornis*: Hand (1955, p. 72); Chia and Spaulding (1972, p. 206); Sebens and Laakso (1977, p. 165); Widersten (1976, p. 865).
   Not *Urticina crassicornis*: Sanamyan and Sanamyan (2006, p. 372).

Material examined

Three specimens: U22 (KBPGI 492/1), U24 (KBPGI 493/2), U25 (KBPGI 494/3).

Fig. 3 *Cribrinopsis similis*: **a** longitudinal muscles of the tentacle (arrow) (specimen U18); **b** marginal sphincter (specimen U19); **c** transverse section of column (specimen U18) (arrows point to pennons on retractors; numbers indicate cycle number of mesenterial pairs). *c* column, *ec* ectoderm, *en* endoderm, *f* fossa, *fl* filaments, *ph* actinopharynx, *s* sphincter, *sf* siphonoglyph
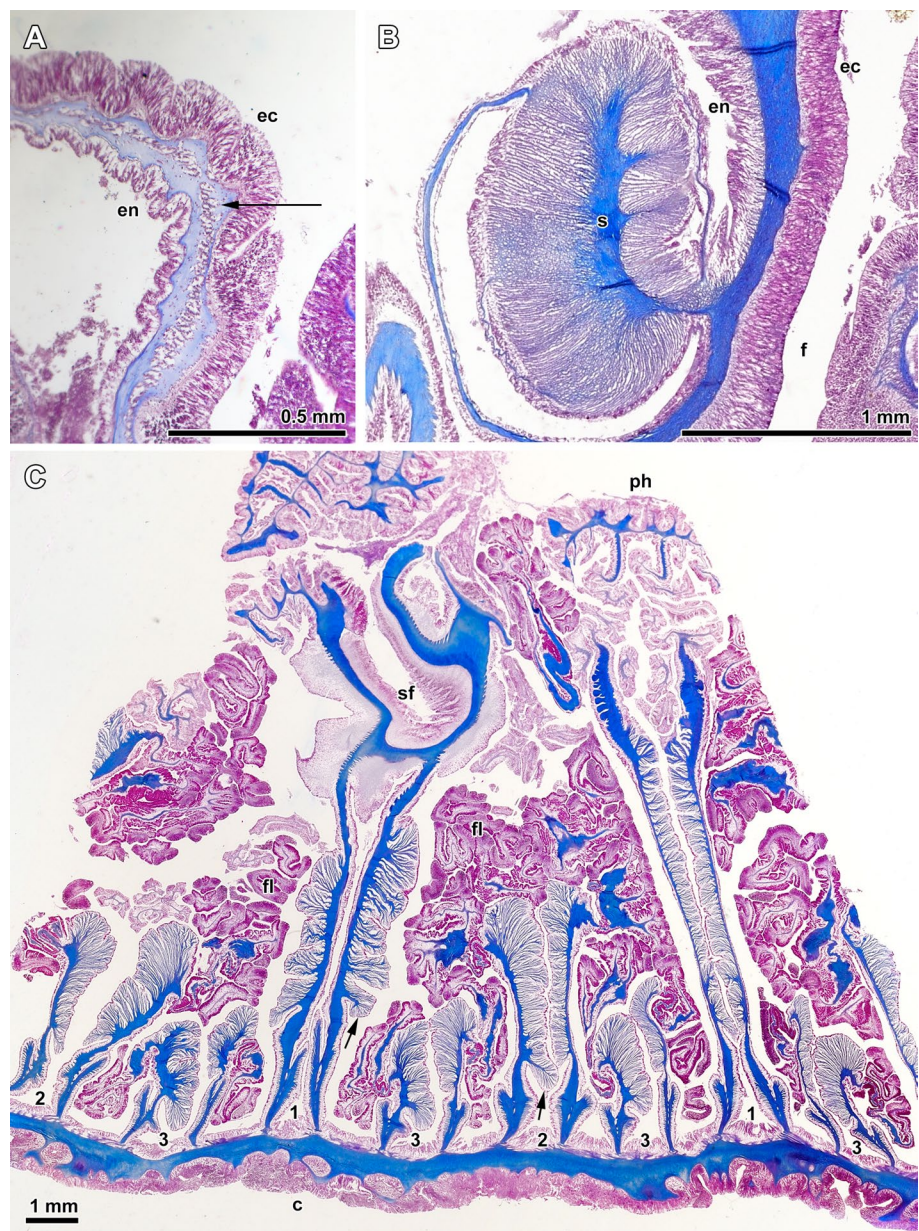
Description

The dimension of the column is 27 mm in diameter and 12 mm in height in preserved specimen (U24). Column of live specimen is bright red with small whitish spots spread over the whole column (Fig. 5a, b). Oral disc and tentacles are roughly of the same bright red color. Mesenterial insertions on the oral disc are marked by red lines (darker than background red color of the disc). Specimen U22 is about the same size as U24 and in life has a dull reddish-brown column with bluish tint and small white dots (Fig. 5c). The oral disc is bluish with red lines over mesenterial insertions. The proximal part of each tentacle is of the same color as the oral disc, the distal part is

lilac, separated from the proximal part by a light lilac band (Fig. 5d). Specimen U25 (30 mm) has marble color with red blurs on yellowish column.

In all specimens, the tentacles are arranged decamerously in four cycles (10 + 10 + 20 + about 40), and the last cycle may be incomplete. In preserved specimens, the tentacles are longitudinally sulcate (unlike *Cribrinopsis similis*).

The column wall of preserved specimens is wrinkled, but histological sections show no trace of verrucae. Circular muscles are well developed on the whole column. There are well-formed parapet, fossa and short capitulum (Fig. 6a).

The endodermal marginal sphincter is circumscribed, pinnate, about 1 mm in diameter, with very thin and short main central lamella (about one third of the diameter of the

Table 2 Size ranges (length × width, in μm) and distribution of cnidae of *Cribrinopsis similis* (measured in two specimens)

Body region	Cnidae	Size ranges (μm)
Pedal disc	(A) basitrichs (common)	6–10×1.5–2.5
	(B) basitrichs (few)	20–31×2–3
Column	(C) spirocysts (rare)	30–39×2.5–4
	(D) basitrichs (very rare)	7–13×1.5–2
	(E) basitrichs (common)	24–33×2–3.5
	(F) *p*-mastigophores A (very rare)	26–33×5–6
Tentacles	(G) spirocysts (very numerous)	18–51×2.5–4
	(H) basitrichs (very rare)	10–12×1.5–2
	(I) basitrichs (numerous)	35–55×2.5–3
Actinopharynx	(J) basitrichs (very rare)	24–37×2–3
	(K) basitrichs (common)	41–55×3–4.5
	(L) *p*-mastigophores A (few)	33–43×4.5–6.5
Filaments	(M) basitrichs (few)	10–15×1.5–2.5
	(N) basitrichs (common)	19–34×2.5–3
	(O) *p*-mastigophores A (common)	27–38×5–8
	(P) *p*-mastigophores B1 (common)	35–48×4.5–6
Endoderm of column	(Q) basitrichs (very rare)	27–42×1.5–2
	(R) basitrichs (very rare)	11–14×1.5–2.5
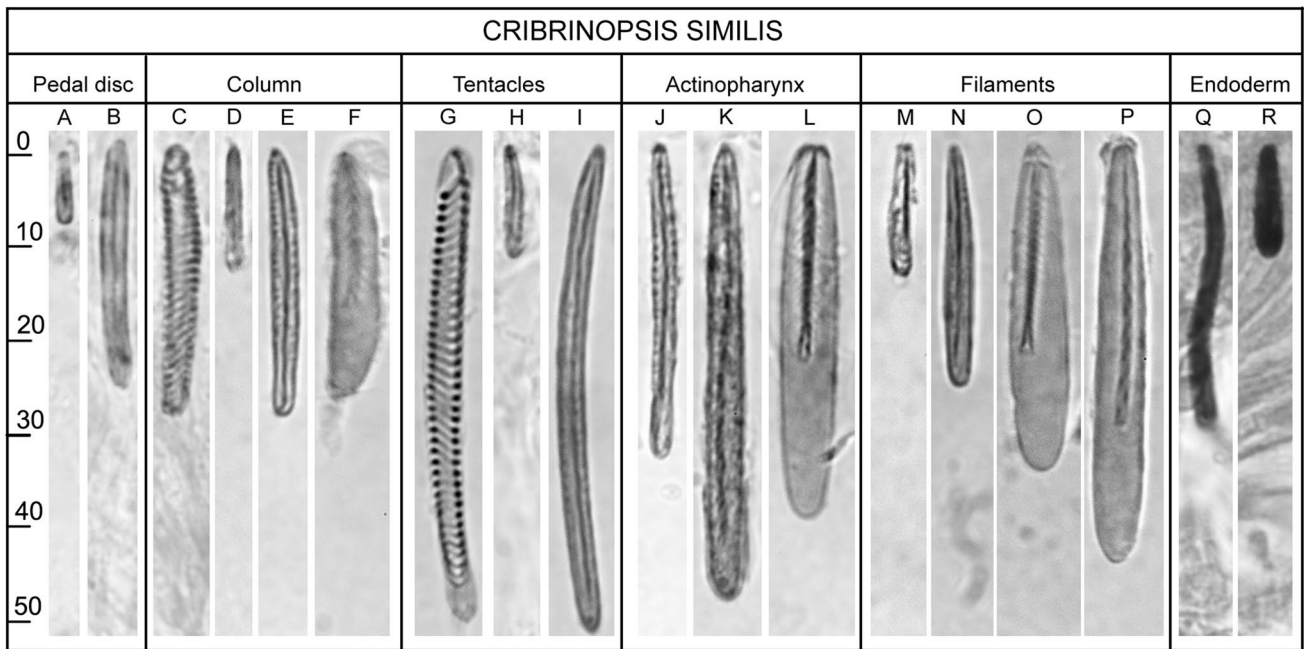
Fig. 4 *Cribrinopsis similis*, cnidom

sphincter). Muscle processes in the sphincter muscle are very numerous, sometimes anastomosing (Fig. 6a, c).

Longitudinal muscles of the tentacles and radial muscles of the oral disc are ectomesogloeal to mesogloeal (Fig. 6b).

Mesenteries are arranged decamerously in three cycles (10 + 10 + 18 = 38 pairs or 76 mesenteries in specimen U24). All three cycles are perfect. There are two pairs of directives attached to two siphonoglyphes.

Retractors are rather strong, diffuse; on transverse sections their distal ends (closer to the actinopharynx) end abruptly and often form distinct pennon. On parietal side of retractors (closer to the wall of column) muscle processes gradually become smaller (Fig. 7a).

Parietobasilar muscles are well developed and form distinct free flap. Mesogloea of the parietal part of the

Fig. 5 *Urticina crassicornis*, live specimens: **a**, **b** specimen U24; **c**, **d** specimen U22
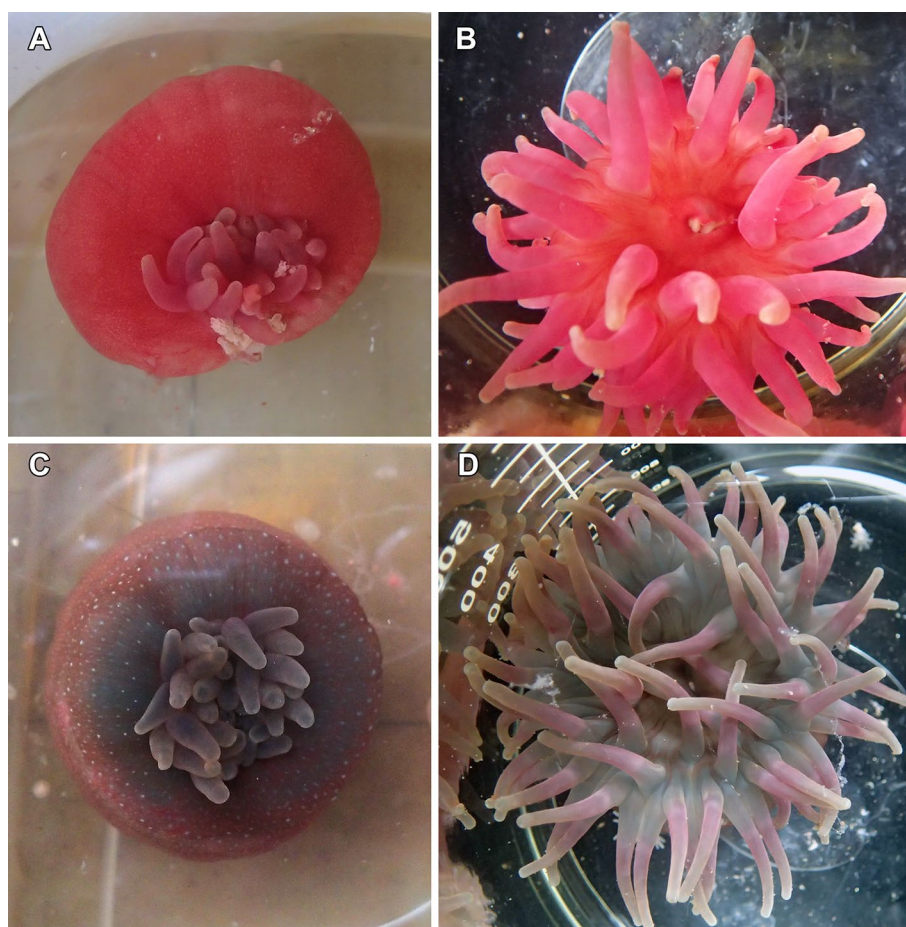
mesenteries, in the region of parietobasilar muscles, contains a chain of elongated lacunae (Fig. 7a).

The second and third cycles are fertile. Specimen U22 (female) had oocytes up to 300 μm in diameter. The other two specimens had numerous male follicles containing immature spermatozoa. All three specimens (female and males) contained juvenile sea anemones in the coelenteron, about 3 mm in diameter, bearing at least two cycles of tentacles, developed mesenteries, retractors and filaments (Fig. 7b).

Cnidome contains spirocysts, basitrichs, *p*-mastigophores A, *p*-mastigophores B1 (Table 3, Fig. 8). Basitrichs in the actinopharynx are much larger than those in the tentacles, and their size ranges do not overlap. Nematocysts of the pedal disc are the same as in the rest of column but are very sparse. Small basitrichs and thin and long S-shaped basitrichs occur in whole endoderm, both are rare. Size ranges of cnidae are in agreement with data published by Carlgren (1921) for this species.

Results of molecular analysis

Firstly, 16 specimens were morphologically identified as *Cribrinopsis similis* (U1–U11, U17–U19, U21, U29, U30), 13 specimens as *Urticina crassicornis* (U12–U16, U22–U28) and the last one as *Urticina fecunda* (U20). Five gene fragments of mitochondrial DNA (12S rRNA, 16S rRNA, COIII) and nuclear DNA (18S rRNA, 28S rRNA) of the 30 specimens collected from Newfoundland were obtained and compared with corresponding sequences deposited in GenBank (Table 1). Moreover, previously obtained gene sequences of *Urticina felina* (five specimens: BS1-BS5), *U. crassicornis* (six specimens of two genotypes: BS1, BS2), *C. similis* (two specimens of one genotype: BS1) and *Aulactinia stella* (Verrill, 1864) (this last as an outgroup) from the Barents Sea (Nemetsky Peninsula, Varanger Fjord, see Bocharova 2015) are added to the phylogenetic reconstruction and Table 1 (Sanamyan

Fig. 6 *Urticina crassicornis* (specimen U24): **a** longitudinal section through distal column; **b** longitudinal muscles of the tentacle (arrow); **c** marginal sphincter muscle. *c* column, *ec* ectoderm, *en* endoderm, *f* fossa, *s* sphincter
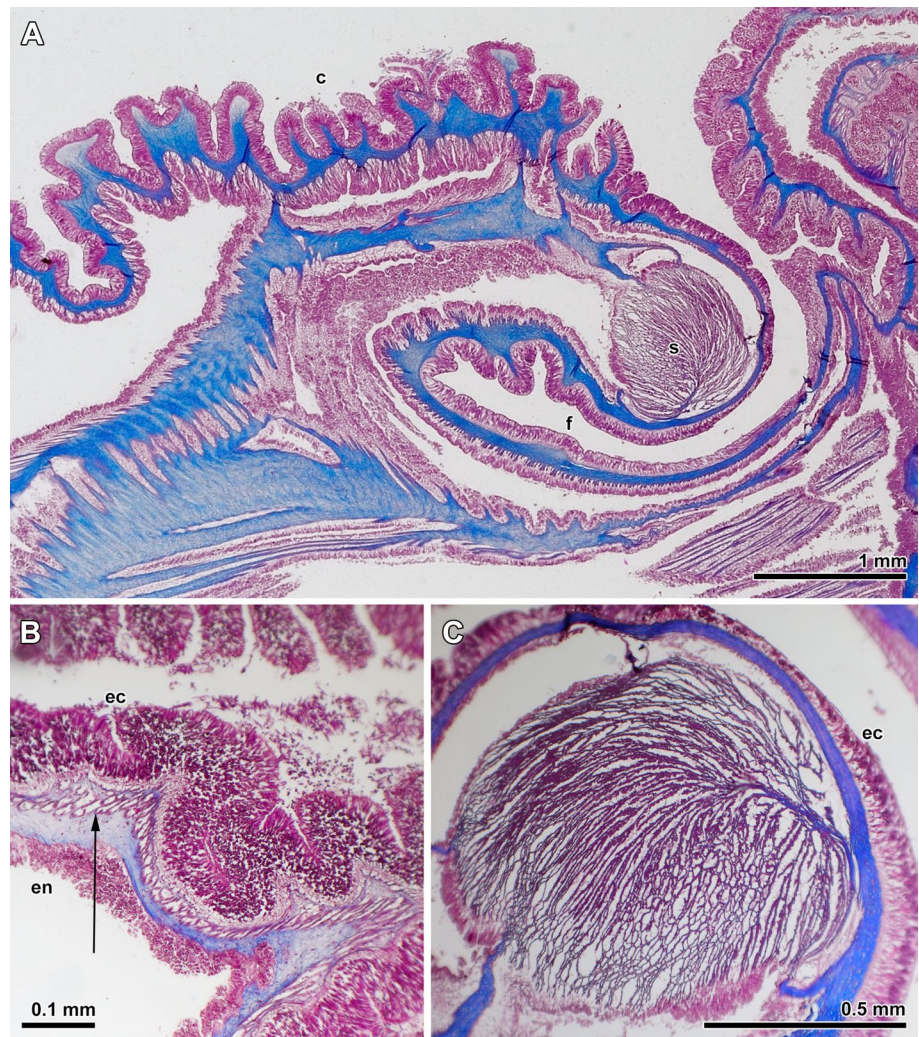
et al. 2019). All 12S rRNA sequences of the three studied species (*Urticina felina*, *U. crassicornis*, *C. similis*) were found to be identical and all COIII sequences were the same as well, so they were removed from the analysis. The 16S rRNA gene sequences of almost all these species belong to one haplotype, but some specimens of *U. crassicornis* from Newfoundland as well as from the Barents Sea have the second haplotype, which includes only one nucleotide substitution. This mitochondrial fragment (16S rRNA) in *U. fecunda* is clearly different from the others due to five nucleotide insertions. For phylogenetic reconstruction, mitochondrial (16S rRNA) and nuclear (18S rRNA, 28S rRNA) fragments were concatenated and analyzed in MEGA 6.0 (Tamura et al. 2013) and MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001). The resulting tree of Bayesian phylogenetic analysis (Fig. 9a) and the phylogram based on Neighbor-Joining algorithm (Fig. 9b) differ in the position of *U. fecunda* (separately from other

Urticina species in the Bayesian analysis or in one clade but with weak support in the NJ analysis). Different bootstrap values for Maximum Likelihood (ML), Neighbor-Joining (NJ) and Minimum Evolution (ME) algorithms were calculated (Fig. 9) and Kimura 2-parameter model (Gamma distributed) were found to be the best for these phylogenetic analyses (Kimura 1980). The estimates of evolutionary divergence show that the most closely related species among the above-mentioned are *U. crassicornis* and *U. felina* (between group mean distance = 0.002, SE = 0.001, Table 3) forming a separate clade with 71/87/87% bootstrap support, respectively, and 100% support in Bayesian analysis. Another major clade found by the analysis includes all *C. similis* specimens (from Newfoundland and the Barents Sea) with 100/100/100% bootstrap support, respectively, which are clearly distinct from the other species (between group mean distance = 0.006 to 0.009, SE = 0.002, Table 4).

Fig. 7 *Urticina crassicornis* (specimen U24): **a** transverse section of column (arrows point to pennons on retractors; numbers indicate cycle number of mesenterial pairs); **b** juvenile in the coelenteron of adult male specimen. *c* column, *fl* filaments, *g* male follicles, *ph* actinopharynx, *t* tentacle
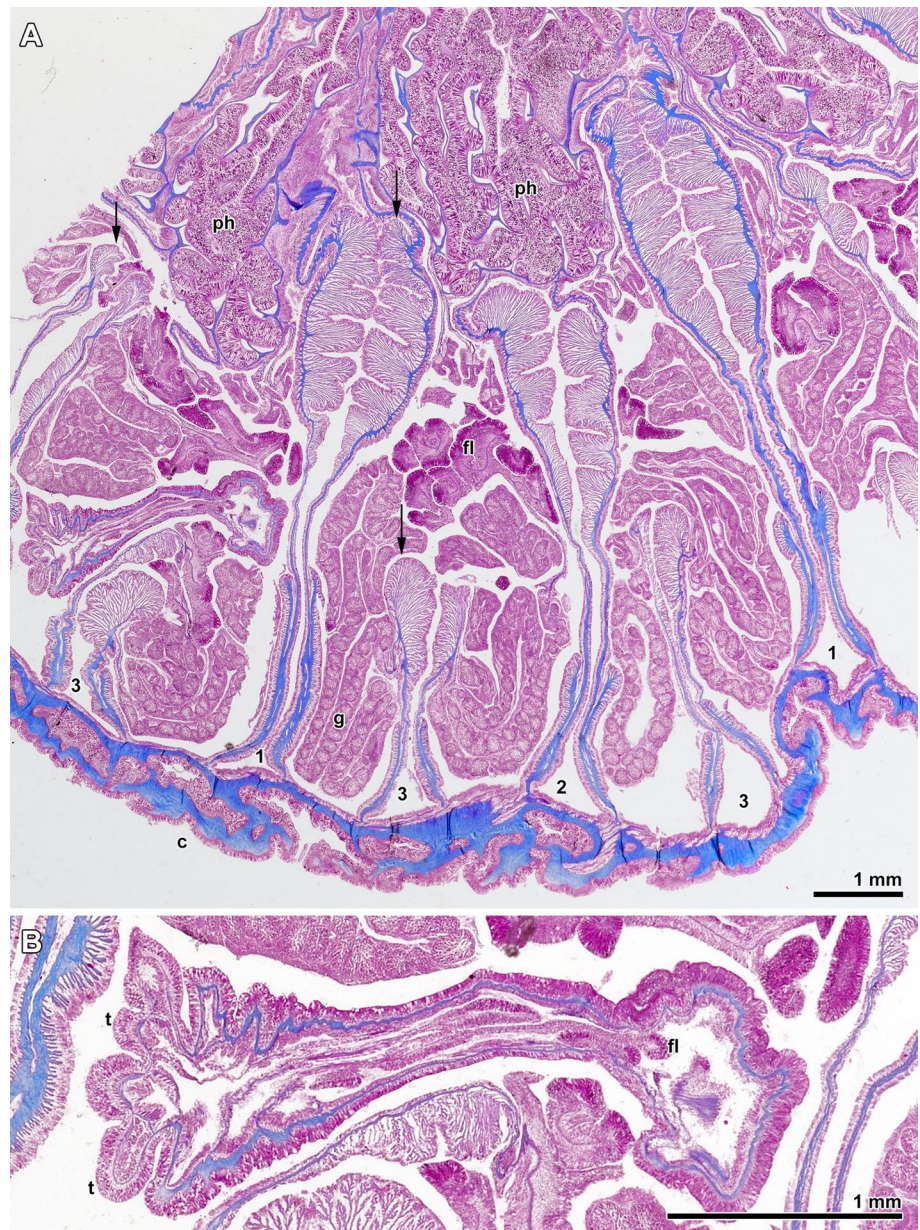

Discussion

The genera *Urticina* and *Cribrinopsis* contain several characteristically similar-looking actiniid species, which in most cases are decamerous (with few deviations). They are very common and diverse in cold and temperate waters of the Northern Hemisphere. The species belonging to these genera have often been confused in the past (see Sanamyan and Sanamyan 2006; Sanamyan et al. 2013) despite key distinguishing features outlined by Carlgren (1921) when he established the genus *Cribrinopsis*. Specifically, (1) members of the genus *Urticina* have basitrichs of the actinopharynx that are much larger than the basitrichs of the tentacles, while in *Cribrinopsis* they are of about the same length; and

(2) in *Cribrinopsis* most mesenteries are fertile, while in *Urticina* the oldest 10–20 pairs (i.e., mesenteries of the first and the second cycles) are usually sterile, though sometimes only the six oldest pairs are sterile. Sanamyan and Sanamyan (2006, p. 383, Fig. 11) provided a diagram illustrating size ranges of basitrichs from the actinopharynx and tentacles of several species of *Urticina* and *Cribrinopsis*, showing that the basitrichs in the actinopharynx of all *Urticina* species are much larger than the basitrichs in the tentacles and that their size ranges do not overlap. This character alone clearly identifies members of *Urticina*. The second feature, the distribution of gonads, is more ambiguous as a feature for delimitating these genera. Indeed, in all species of *Cribrinopsis*, mesenteries of all cycles (including the mesenteries of the

Table 3 Size ranges (length × width, in µm) and distribution of cnidae of *Urticina crassicornis* (measured in three specimens)

| Body region	Cnidae	Size ranges (µm)
Column	(A) basitrichs (common)	7–12×1.5–2
	(B) basitrichs (common)	18–28×2.5–3
Tentacles	(C) spirocysts (numerous)	25–55×2–3.5
	(D) basitrichs (common)	22–34×2–3
Actinopharynx	(E) basitrichs (numerous)	48–78×4–6
	(F) *p*-mastigophores A (very rare)	21–28×5–6
Filaments	(G) basitrichs (few)	13–16×2–3
	(H) basitrichs (few)	20–40×2–4
	(I) basitrichs (few)	50–62×4–5
	(J) *p*-mastigophores A (common)	23–32×4.5–6.5
	(K) *p*-mastigophores B1 (numerous)	24–39×4–7
Endoderm of column	Basitrichs (very rare)	29–34×1.5–2
	Basitrichs (very rare)	11–15×2–2.5

Fig. 8 *Urticina crassicornis*, cnidom
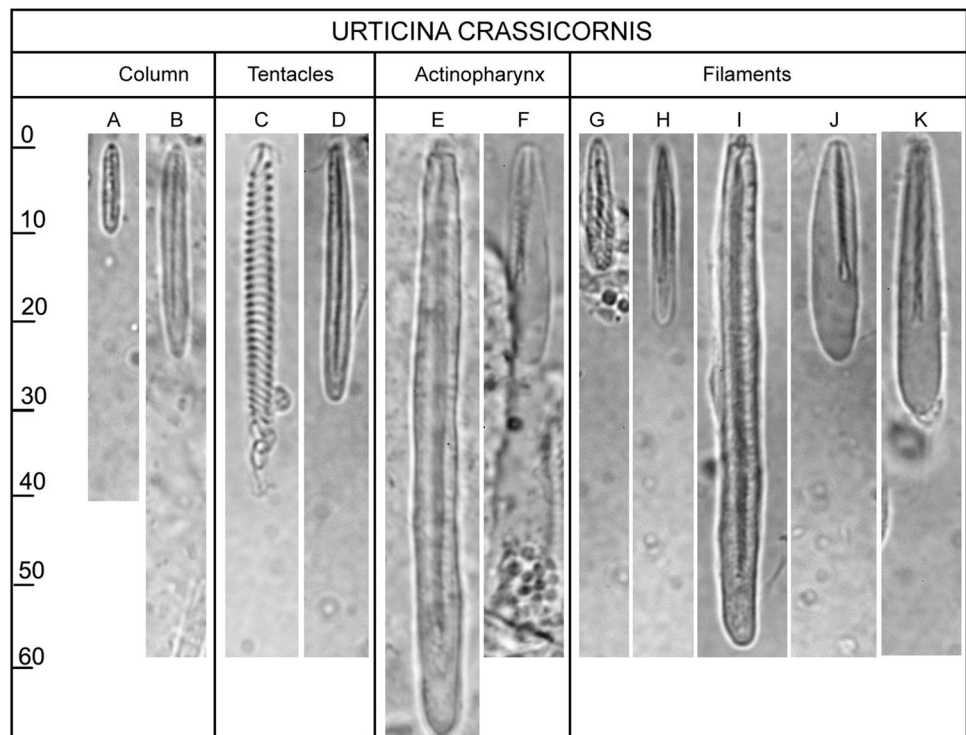
first cycle) may be fertile, while in most specimens of most *Urticina* species the mesenteries of the first cycle are sterile. However, Sanamyan and Sanamyan (2006) showed that some specimens of *Urticina* may also exhibit occasional fertile mesenteries (usually few) in the first cycle. Moreover, at least in one species (*Urticina clandenstina* Sanamyan et al. 2013) all mesenteries of the first cycle are fertile (see Sanamyan et al. 2013).

Confusion between the genera *Cribrinopsis* and *Urticina* persists to this day, especially when specimens are not dissected and examined to assess the characters mentioned above. Thus, field guides and scientific papers have

mislabeled them, further complicating species identification for non-taxonomists. The distinguishment between the species within these genera is an even more difficult task, especially if live specimens, or good photographs of them, are not available. Key features, including presence or absence of verrucae, their size and whether they are strongly, weakly or non-adhesive, and the color pattern of the disc and tentacles, are either lost completely or very difficult to study on preserved specimens.

The two species described in the present paper, *Cribrinopsis similis* and *Urticina crassicornis*, are rather common and widely distributed, and they are known from the
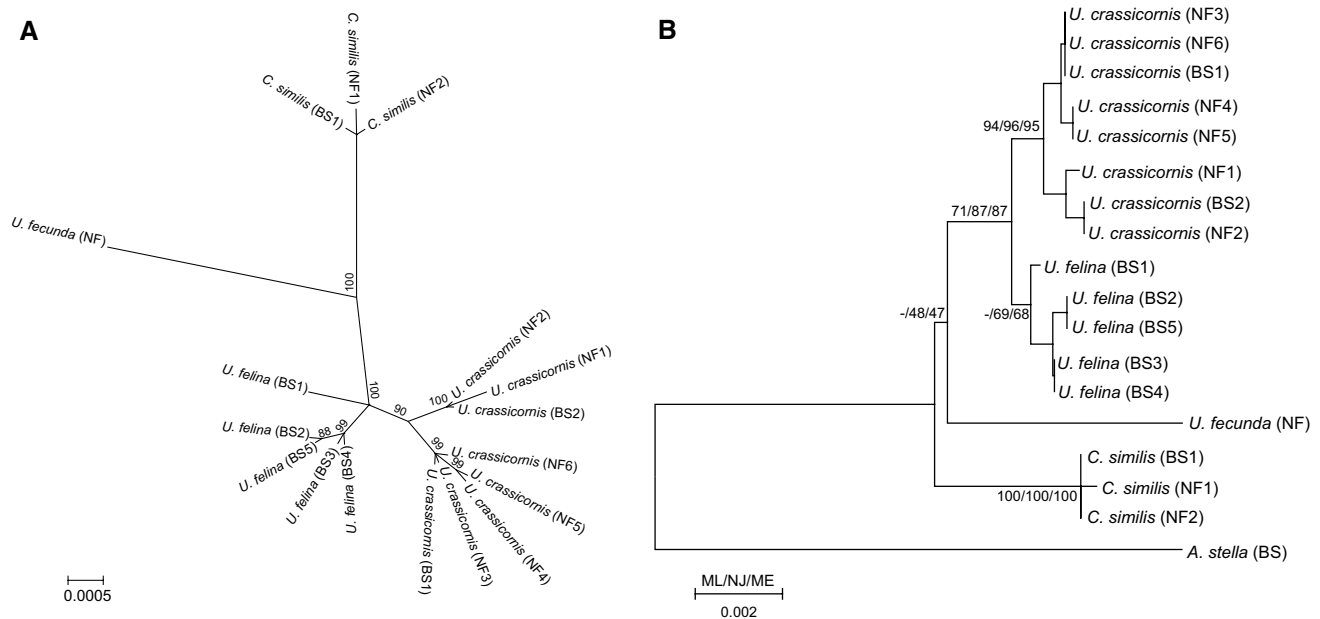
Fig. 9 Trees resulting from: **a** Bayesian phylogenetic analysis (MrBayes 3.2.6); **b** Neighbor-Joining (NJ) analysis (MEGA 6.0) of concatenated 16S rRNA mitochondrial gene sequences, 18S rRNA and 28S rRNA nuclear gene sequences of *Cribrinopsis similis*, *Urticina crassicornis*, *Urticina felina*, *Urticina fecunda*, and *Aulactinia stella* as an outgroup. The NJ phylogram was reconstructed by Kimura2-parameter model; numbers on nodes represent bootstrap values calculated by Maximum Likelihood (ML), Neighbor-Joining (NJ) and Minimum Evolution (ME) methods. Populations: *BS* Barents Sea, *NF* Newfoundland. Numbers after letters indicate genotype number

American and European coasts of the North Atlantic as well as from all northern European seas. Both species were previously reported from Newfoundland (Carlgren 1921; Murillo et al. 2016). Despite records for the two species in the World Register of Marine Species (WoRMS) showing clear geographic distributions encompassing a large part of the North Atlantic in Europe, eastern USA and Canada, the bulk of the literature on large sea anemones refers to *U. felina*. The fact that the distribution patterns of *U. felina* nearly cover the entire North Atlantic hints at the potential complexity of identification issues for those large sea anemones, especially in eastern North America. In fact, *U. felina* in its current sense (as a separate species, see Manuel 1988) has not been recorded in Canadian waters in formal taxonomic works. Carlgren (1921) used the name "*felina*" as a specific epithet

for several subspecies which are now considered separate species. Two of these subspecies (*U. felina coriacea* and *U. felina tuberculata*) are now considered as synonyms of *U. felina* in its current sense; they are known only from European waters and were never recorded in North America (with Iceland being the western most point of distribution; Daly and Fautin 2018). The name *U. felina* appeared in the Catalogue of the Marine Invertebrates of the Estuary and Gulf of Saint Lawrence (Brunel et al. 1998) without any supporting morphological data. Adding to the confusion, records such as Brunel et al. (1998) present *U. crassicornis* as a subspecies of *U. felina* [*Urticina (Tealia) felina* var. *crassicornis*]. However, these species most probably do not occur in the Pacific; the specimens recorded by Sanamyan and Sanamyan (2006) as *C. similis* from the sea of Okhotsk and as *U. crassicornis* from Pacific coast of Kamchatka belong to other species based on morphological and molecular characteristics. In particular, *Cribrinopsis similis* in Sanamyan and Sanamyan (2006) differs from true *C. similis* based on morphological features and cnidom. Specifically, the central lamella of the sphincter is poorly developed, spirocysts and small basitrichs of the column are much more abundant, spirocysts of the column and the tentacles are much larger, basitrichs of the tentacles and the actinopharynx are also larger, and there is only one type of basitrichs in the actinopharynx. *Urticina crassicornis* in Sanamyan and Sanamyan

Table 4 Estimates of evolutionary divergence over sequence pairs between species

| Species/species	C. similis	U. fecunda	U. felina	U. crassicornis
C. similis		0.002[a]	0.002	0.002
U. fecunda	0.009		0.002	0.002
U. felina	0.006	0.008		0.001
U. crassicornis	0.007	0.008	0.002	

[a]Standard error estimates are shown above the diagonal

(2006) has five cycles of tentacles and four cycles of mesenteries, the two oldest cycles are usually sterile, and this species is oviparous. In addition, it has an additional type of small curved basitrichs in the tentacles.

Apart from generic features discussed earlier (nematocysts and distribution of gonads), *Cribrinopsis similis* differs from *Urticina crassicornis* on the basis of several other characters. The "moire" (zigzag pattern) of the tentacles and the presence of white verrucae on the column distinguish *C. similis* from *U. crassicornis* externally. The tentacles in preserved specimens of *C. similis* (as in most *Cribrinopsis* species) are transversely wrinkled, while in *U. crassicornis* (and in most other *Urticina* species) they are longitudinally sulcate. These two species also have different marginal sphincters: *C. similis* has a very thick main lamella (Figs. 2c, 3b), while in *U. crassicornis* it is thin and short (Fig. 6a, c). The retractors also appear to be different: those of *C. similis* have a pennon on the side, which is closer to the column wall (Fig. 3c), while in *U. crassicornis* the pennon is on the opposite side (Fig. 7a).

For example, the photo of the species identified as *U. felina* in the paper of Mercier et al. (2011, Fig. 1a) clearly shows morphological characters of *C. similis*, including the pattern on the oral disc and small verrucae on the column that differentiate *C. similis* from *U. crassicornis* and *U. felina*. The only other species belonging to these genera, for which occurrence in this region (Newfoundland) is firmly established, is *U. fecunda*, which has been redescribed in detail by Larson et al. (2012) and possesses very distinctive characters separating it from all other species.

The occurrence of *U. felina* in the focal region (and in the NW Atlantic in general) is doubtful based on the lack of definitive taxonomic record discussed earlier. Despite the extensive distribution presented in WoRMS for this species (Daly and Fautin 2018), it was not found in the present survey or in a previous investigation that looked at material from trawl collections in Newfoundland (Murillo et al. 2016). *Urticina felina* differs from both *U. crassicornis* and *C. similis* by very well-developed and strongly adhesive verrucae on the column, which invariably have particles of gravel and other debris stuck to them, a contracted anemone having the appearance of a rounded mound of gravel (see Manuel 1988 and Fig. 10 showing specimens from Europe). In contrast, the column of *U. crassicornis* is invariably smooth, while verrucae of *C. similis* are small and weakly adherent (if adherent at all). *Urticina felina* has up to 160 short tentacles, decamerously arranged into 5 cycles. For
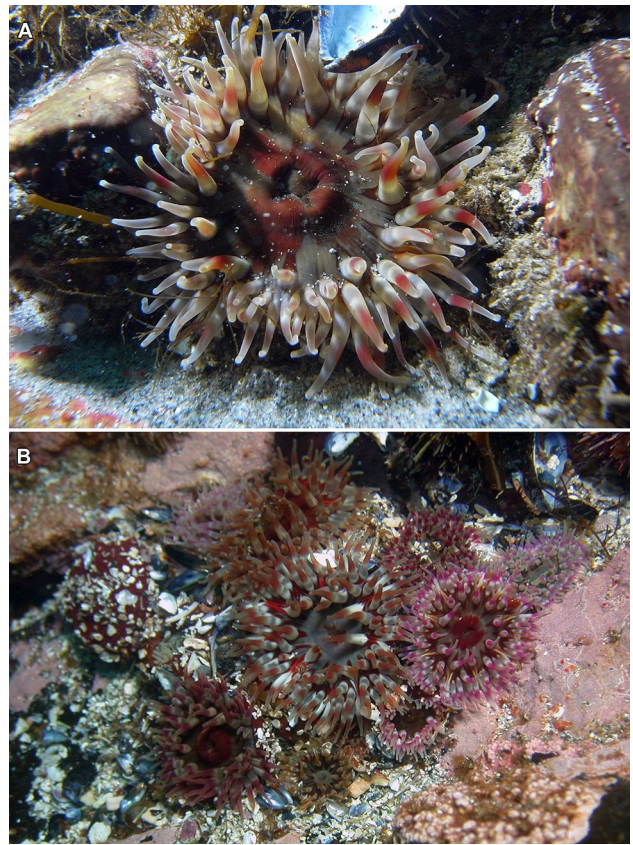
Fig. 10 *Urticina felina*, live specimens from Europe: **a** specimen BS1 from the Barents Sea (photograph by T. Anthokhina); **b** specimens from Lofoten (Norway) (photograph by D. Schories)

description of color of live specimens, see Manuel (1988). Nematocysts of this species are described by Sanamyan and Sanamyan (2006) and by den Hartog and Ates (2011). The species are compared in Table 5.

Overall, the present work provides clarification with respect to the identification of common large actiniid sea anemones in the focal region (Newfoundland) and the entire North Atlantic. It will hopefully help investigators distinguish between the genera *Cribrinopsis* and *Urticina*, and among species belonging to these genera, either while re-assessing previous records or conducting future studies.

Table 5 Distinguishing features of the discussed species

Species/characteristic	Position on substrate	Surface of column	Sphincter	Number of the mesenterial cycles	Fertile mesenteries	Brooding	Size ranges of large basitrichs in actinopharynx and tentacles
C. similis	On the surface of stones	Small non-adhesive or slightly adhesive verrucae	Pinnate with thick main central lamella	3 cycles	All cycles fertile	Juveniles not brooded in the coelenteron to polypoid stage	Significantly overlap
U. crassicornis	On the surface of stones	Smooth	Pinnate with very thin and short main central lamella	3 cycles	First cycle sterile	Juveniles brooded in the coelenteron to polypoid stage	Does not overlap
U. felina	Partly buried in gravel or in crevices between stones	Large strongly adhesive verrucae with attached gravel and other debris	Pinnate or palmate	4 cycles	First cycle sterile	Is not a brooder	Does not overlap, size ranges differ considerably

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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

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