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DNA barcoding of some Pandeidae species (Cnidaria, Hydrozoa, Anthoathecata)

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Abstract: Using 16S, COI, and ITS DNA sequences, it was possible to link a Norwegian marine hydroid formerly known as *Leuckartiara abyssi* (G.O. Sars, 1874) to a *Neoturris* medusa occurring in the same region. Although the Norwegian medusae showed some slight morphological differences to Mediterranean *Neoturris pileata*, DNA sequence comparisons show that they must be conspecific. A Mediterranean specimen of *N. pileata* showed less sequence divergences to the Norwegian *Neoturris* than was found within this latter population. The morphological differences are likely only age- and environmentally related. *Leuckartiara abyssi* is thus a subjective synonym of *Neoturris pileata* (Forsskål, 1775). The sequence analyses were embedded in a comparison with other members of the family Pandeidae. Contrary to the case for most other hydrozoans investigated, 16S sequences show very little divergences within the genus *Catablema* and it is thus not a good barcoding marker for this genus. COI sequences showed about three times greater divergence than 16S within selected pandeid species clades and are more suitable to investigate *Catablema* species, although also for COI the divergences within this group remain rather small. *Catablema nodulosum* Bigelow, 1913 was found to be most likely conspecific with *Catablema vesicarium* (A. Agassiz, 1862) and was therefore accepted only as a subspecies of the latter, thus following the opinion of most other prominent taxonomists of the last century. Medusae referred to *Catablema multicirratum* Kishinouye, 1910 originating from either the NE Pacific or the Eurasian sector of the Arctic Sea could belong to two distinct species.

The medusa *Leuckartiara longicalcar* n. spec. is described and illustrated. It occurs along the coast of North America from British Columbia to California and has been confused previously with *L. octona* (Fleming, 1823).

Keywords: *Neoturris pileata* - life cycle - barcoding - systematics - *Catablema* - *Leuckartiara* - new species - hydromedusae.

INTRODUCTION

The family Pandeidae is a group of anthoathecate hydrozoans with a cosmopolitan distribution, currently comprising 89 accepted species (Schuchert, 2017a). The family is somewhat unique among the Order Anthoathecata as many species have relatively large and conspicuous medusae (Figs 5-7, 10-16). They can occasionally be found caught in large numbers in rock-pools or protected bays and thus attract the interest of non-specialist naturalists. In contrast to the medusae, their hydroid stages are often small, rather uniform in morphology, and difficult to identify to species level. For the majority of the Pandeidae species, only the medusa phase of the life cycle is known, while their hydroids are either known as juveniles only or they remain unidentified (Bouillon *et al.*, 2006). Prudkovsky & Neretina (2016) provide list of pandeid species for which the life cycle has been elucidated, usually by rearing experiments (e.g. as described in Rees & Russell, 1937).

Because many species cannot be reared in the laboratory, DNA barcoding has recently been used successfully to link hydroid and medusae stages in different hydrozoan families (Schuchert, 2016; Schuchert *et al.*, 2017 and references therein). The present study continues these studies, focusing here mainly on the pandeid hydroid currently known as *Leuckartiara abyssi* (G.O. Sars, 1874) and its medusa. However, it was also necessary to consider also relationships to other Pandeidae, in particular *Neoturris pileata* and *N. brevicornis*.

In 1874, Georg Ossian Sars described *Perigonimus abyssi* (Fig. 1), a tiny new hydroid which he had obtained from two localities in the south-west of Norway (Island of Kvitøy near Stavanger and the Hardangerfjord near Bergen). Both samples came from relatively deep waters (146-366, resp. and 274-731 m) and they were growing on scaphopods and the bivalve *Nucula tumidula* Malm. Sars noted that the gonophores were rare, but he was quite sure that they would produce a free medusa. As he

had preserved material only, some uncertainty remained. The species was subsequently recorded from numerous localities in the North Atlantic from Greenland to the Barents Sea (listed in Edwards, 1965). However, all these records must be considered somewhat doubtful (Edwards, 1965) due to the possibility of confusions with other pandeid hydroids, e. g. *Leuckartiara octona* (Fleming, 1823). Most known pandeid hydroids are rather simple and offer few characters to differentiate them, notably in preserved material without gonophores. Rees (1938) then re-sampled living *L. abyssi* hydroids from near Bergen, where it seems to occur quite regularly. Although out of 12 colonies only one had a single gonophore, he was able to follow its development until its release as a young medusa with four tentacles lacking ocelli. This made it distinguishable from *L. octona* medusae which are released with two tentacles only (the ocelli develop later). Rees (1938) concluded that the hydroid is potentially the polyp stage of *Neoturris pileata* (Forsskål, 1775) or any other *Leuckartiara* medusae known from the region. He thus transferred it provisionally to the genus *Leuckartiara* as *L. abyssi*. Later (1956), Rees was able to re-examine the syntype material of which only the colony from Kvitsøy on the scaphopod *Antalis entalis* (Linnaeus) was left. Although with some ambiguity, Rees designated this specimen as the lectotype.

Subsequently, Naumov (1960, 1969) speculated that *L. abyssi* could be the polyp stage of *Catablema vesicarium* (A. Agassiz, 1862). The situation became much clearer when Edwards (1965) documented an identical hydroid growing on *Nucula* shells from the Firth of Clyde (Scotland). Edwards reared the medusae to a stage with well-formed gonadal pits and folds (5 mm height) and he identified it as *Neoturris pileata* based on Russell's (1953) descriptions. Edwards (1965) also found mature *N. pileata* medusae in the plankton of the region, which further supported his identification of the reared medusae. Although Edward's hydroids were indistinguishable from *L. abyssi* polyps from Norway, he refrained from synonymising *L. abyssi* with *N. pileata* because also another *Neoturris* medusa, *N. breviconis* (Murbach & Shaerer, 1902), had been reported from the Bergen region by Kramp & Damas (1925; as *Leuckartiara breviconis*). *Leuckartiara abyssi* could therefore also be the hydroid of *N. breviconis*, a medusa originally described from the NE Pacific Ocean (see Arai & Brinckmann-Voss, 1980; Schuchert, 2007, 2012). Until the re-description by Arai & Brinckmann-Voss (1980), the species was not well known, but several authors reported it also from the North Atlantic, notably the influential Russell (1953) and Kramp (1959). However, most of these records are likely incorrect identifications and at least some refer to other *Leuckartiara*, *Catablema*, and *Neoturris* species (Schuchert, 2007, 2012), notably also *Neoturris pileata*, a very conspicuous and characteristic medusa (Figs 6-7) originally described from the Mediterranean (Forsskål, 1775, 1776). Unambiguous records of *N. pileata* are

also known from the North-eastern Atlantic reaching as far north as Scotland, Norway, and Iceland (Hartlaub, 1914; Kramp & Damas, 1925; Kramp, 1926; Russell, 1953; Schuchert, 2007). However, not all individuals are as typical as shown in Fig. 7, some might be smaller or broader (Fig. 6), the apical process can be missing, and the colour of the manubrium is not always as intense as shown in Fig. 7 (Schuchert, 2007). Atlantic *Neoturris pileata* medusae are not always easy to distinguish from *N. breviconis* of the Pacific Ocean (compare Figs 3-7 and 10; see also Schuchert, 2007). The latter species is generally larger, broader, has a less developed apical process, more than 80 tentacles, the manubrium is distinctly shorter and never as red as in *N. pileata*, the gonadal folds are not clearly directed towards interradial and their arrangement resembles more a *Leuckartiara* species.

During several sampling trips in the region of Bergen (Norway), I was able to collect repeatedly *Neoturris* medusae (Figs 3-4) as well as hydroids identifiable as *L. abyssi* (Fig. 2). The *Neoturris* medusae from the Bergen area appeared mature or nearly fully grown (Fig. 4) but were differed somewhat from typical *N. pileata* as found south of the British Isles and in the Mediterranean (Figs 6-7). It was thus very interesting to use DNA barcoding (Schuchert, 2016; Schuchert *et al.*, 2017) to assess the connection of the polyps and medusae as well as relationships to other Pandeidae species.

Additional Pandeidae species, including also *N. breviconis*, could be obtained for DNA barcoding and observations related *Leuckartiara* and *Catablema* species are also reported in this context.

MATERIAL AND METHODS

Molecular biological methods as well as the sampling of the medusae have already been described in Schuchert *et al.* (2017) and Schuchert (2005, 2012, 2014, 2016). The polyp stages of *N. abyssi* (Table 1) were obtained by sampling molluscs with a modified R-P epibenthic sampler (Rothlisberg & Percy, 1976) or a triangular dredge.

The DNA samples are all stored in the DNA collection of the MHNG.

About 600 bp of the large mitochondrial ribosomal RNA (16S) was amplified using the primers SHA (ACGGAATGAACTCAAATCATGT) and SHB (TCGACTGTTTACCAAAAACATA) (Cunningham & Buss, 1993) (30 cycles, profile: 20 sec 94°C, 45 sec 50°C, and 120 sec 68°C).

Fragments of about 660 or 890 bp of the mitochondrial Cytochrome Oxidase I (COI) were amplified using the forward primer COF (TGAGTATTTTCAACTAATCAYAAAGA) combined with either the reverse primers COI3 [TAAACTTCAGGGTGACCAAAAATCA,

is HCO2198 of Folmer *et al.* (1994)] or CoR (AAGTAAGCTCTAGTATCAACRTCCAT). The PCR cycling profile for the COI fragment was: 5 cycles with 50 sec 94°C, 50 sec 45°C, and 120 sec 70°C; followed by 30 cycles with 50 sec 94°C, 50 sec 50°C, and 120 sec 68°C.

About 750 bp spanning the ITS region of the tandemly repeated ribosomal genes of *Neoturris* and *Catablema* samples was amplified using the primers IFS (GTCGCTACTACCGATTGAATGG) and IRS (CGCTTCACTCGCCGTTACTAGG) (shortened primers of Martinez *et al.*, 2010). The PCR cycling profile for the ITS fragment was: 24 cycles with 20 sec 94°C, 45 sec 51°C, and 90 sec 72°C.

All PCR reactions were done in 50 µl volume using PCR Kits of Qiagen® according to the instructions of the manufacturer. About 1-5 ng of genomic DNA were used as template. The sequencing of the products was made by Macrogen Inc.

The sample data as well as the GenBank numbers of the specimens used in this study are given in Table 1. Some additional Pandeidae 16S and COI sequences which had approximately the same lengths as the ones generated for this study could be retrieved from GenBank (16S sequences JX965913, KT809337, KT809324, KT288206, AM183136, JX965912, JQ715887, JQ715888; COI sequences KT809324, KC440110, GQ120057, KC440107, JQ716057, JX965906, Q716085).

The sequences were edited and aligned using the Bioedit Sequence Alignment Editor (Hall, 1999) and the integrated ClustalW tool with default settings (Larkin *et al.*, 2007). Regions with ambiguous alignments were not removed (removal did not change the results, not shown). Maximum likelihood analyses and substitution model selection were done as given in Schuchert (2016).

Abbreviations:

16S	16S mitochondrial ribosomal RNA gene sequence
BOLD	The Barcode of Life Data System, see Ratnasingham & Hebert (2007)
COI	Cytochrome Oxidase subunit I
DOI	Digital Object Identifier
GenBank	Genetic sequence database of the National Institute of Health, USA http://www.ncbi.nlm.nih.gov/genbank/
ITS	Internal Transcribed Spacer
MHNG	Muséum d'histoire naturelle de Genève, Switzerland

RESULTS AND DISCUSSION

Maximum likelihood analyses

16S and COI sequence data were used to obtain Maximum Likelihood trees (Figs 8-9) that graphically visualise inter- and intraspecific sequence divergences

(see also Schuchert *et al.*, 2017). The trees for ITS showed identical relationships for the examined taxa (results not shown).

Neither 16S nor COI resolves satisfactorily the phylogenetic relationships at the genus or family level. However, this was not the aim of this study and will need additional markers like 18S and 28S gene sequences. The aim of the present study was to identify the medusa of *L. abyssi* and concomitantly to evaluate the usefulness of 16S and COI for separating Pandeidae species in barcoding approaches. The 16S, COI, and ITS sequences clearly identified the *Neoturris* medusa from the same locality as the medusa stage of *L. abyssi*. More details are given below.

16S and COI intra- and interspecific variation

The mitochondrial 16S gene sequence tends to be a reliable barcode marker for Hydrozoa and largely replaces the more commonly used COI of other groups because it can be amplified with a higher success rate (Lindsay *et al.*, 2015; Zheng *et al.*, 2014; Schuchert *et al.*, 2017). For the Pandeidae analysed here, however, COI appears more suitable (Table 2, Figs 8-9). The maximal intraspecific sequence divergences range from 0.33% to 0.52% for 16S and 0.9% to 1.68% for the COI (Table 2). The latter marker has thus about three times greater divergence values. Similarly, the minimal interclade divergences (barcode gaps) tend also to be much higher for COI, resulting in trees with an apparently much better separation of the species clades through longer branches (Fig. 9). The bootstrap values, however, seem not to reflect this, the COI tree has not more supported nodes. The higher divergence of the COI is particularly useful for the *Catablema* species and will be discussed in more detail below.

Neoturris species

Five hydroid colonies of *L. abyssi* from the Bergen area could be obtained to get DNA, all growing on the same organisms as the type material (Fig. 2). Concomitantly, seven *Neoturris* medusae of different ages (sizes) were analysed from the same region (Figs 3-4). They were provisionally named *N. abyssi* after the first DNA sequence comparisons indicated their identity with the hydroid. A single adult medusa of *Neoturris pileata* suitable for DNA extraction could be obtained from the Mediterranean.

The 16S, COI, and ITS sequence data (Figs 8-9) unambiguously associated the polyps *L. abyssi* (Fig. 1-2) with the *Neoturris* medusae shown in Figures 3-4. Some polyp and medusa samples even yielded identical sequences. For both COI and 16S the intraclade divergences were similar to intraspecific variations seen in other Pandeidae (e. g. *N. brevicornis*, *L. octona*; Table 2).

Table 1. Sample data, voucher numbers, and GenBank data of specimens examined by the author. The sample data of other sequences retrieved from GenBank and shown in Figs 8-9 can be obtained by searching the accession numbers in GenBank. nd = no data.

species	WGS84 coordinates	locality	depth [m]	date collected	polyp medusa	comments, reference or DOI of photos	MHNG-INVE-voucher	DNA isolate	16S	COI	ITS
<i>Amphinema dinema</i>	48.79717, -3.96817	France, off Roscoff	0-8	30.08.2011	m	typical, mature medusa		887	KP776816	MG136807	nd
<i>Amphinema</i> spec.	41.73520, 2.95412	Spain, Catalonia, Cala Giverola	1-2	11.08.2008	p	typical hydroid for genus		688	MG250194	MG237874	nd
<i>Catabela cf. multicirratum</i>	81.36-80.97, 15.25-15.40	north of Svalbard	nd	26.08.2015	m	this study		1139	MG136740	MG136793	MG136766
<i>Catabela cf. multicirratum</i>	81.36-80.97, 15.25-15.40	north of Svalbard	nd	26.08.2015	m	this study		1140	MG136741	MG136794	MG136767
<i>Catabela multicirratum</i>	48.54514, -123.01206	USA, San Juan Island	0.5	19.05.2011	m	this study		868	MG136729	MG136781	MG136764
<i>Catabela nodulosum</i>	48.54514, -123.01206	USA, San Juan Island	0	16.05.2011	m	this study		932	MG136727	MG136779	MG136762
<i>Catabela nodulosum</i>	48.54514, -123.01206	USA, San Juan Island	0.5	20.05.2011	m	this study		957	MG136730	MG136782	MG136765
<i>Halitholus</i> spec.	48.54514, -123.01206	USA, San Juan Island	0.5	20.05.2011	m	this study		870	MG136735	MG136787	nd
<i>Hydrichthys boycei</i>	nd	South Africa, Durban	nd	July 2005	p	Schuchert (2007)	37417	383	EU448102	MG136811	nd
<i>Leuckartaria cf. nobilis</i>	56.455, -5.434	Scotland, Dunstaffnage Bay	0	07.05.2004	m	Schuchert (2007)	78922	299	AM183135	MG136789	nd
<i>Leuckartaria longicalcar</i>	48.54514, -123.01206	USA, San Juan Island	0.5	20.05.2011	m	this study	78922	869	MG136731	MG136783	nd
<i>Leuckartaria octona</i>	nd	Germany, North Sea	nd	nd	p	Schuchert (2007)	49100	487	AM411422	MG136777	nd
<i>Leuckartaria octona</i>	50.33385, -4.16725	England, Plymouth	0	28.06.2007	m	Schuchert (2007)	63261	564	MG136737	MG136790	nd
<i>Leuckartaria octona</i>	55.035, -1.4286	England, Cullercoats	nd	00.03.2008	p	10.5281/zenodo.896089		734	MG136738	MG136791	nd
<i>Leuckartaria octona</i>	55.035, -1.4286	England, Cullercoats	nd	26.03.2008	p	10.5281/zenodo.896111		721	MG136739	MG136792	nd
<i>Leuckartaria octona</i>	60.275, 5.200	Norway, Raunefjord	10	22.05.2012	m	10.5281/zenodo.896123		915	MG136743	MG136795	nd
<i>Leuckartaria octona</i>	58.25362, 11.39031	Sweden, Gullmarsfjorden	5-50	29.09.2014	p	this study	89890	1055	MG136755	MG136808	nd
<i>Leuckartaria octona</i>	58.24385, 11.43231	Sweden, Kristineberg	0	03.10.2014	m	10.5281/zenodo.896290		1061	MG136756	MG136809	nd
<i>Leuckartaria octona</i>	43.686, 7.317	France, Villefranche-sur-Mer	0-70	04.04.2005	m	10.5281/zenodo.896334		354	AM411421	MG136810	nd
<i>Leuckartaria octonema</i>	34.47806, 136.8675	Japan, Toba City	0	09.05.2014	m	this study	97018	1208	MG136758	MG136813	nd
<i>Leuckartaria spec.</i>	48.54514, -123.01206	USA, San Juan Island	0.5	22.05.2011	m	this study	78921	871	MG136736	MG136788	nd
<i>Neoturrus abyssus</i>	60.275, 5.200	Norway, Raunefjord	10	22.05.2012	m	this study	82129	953	MG136742	nd	MG136768
<i>Neoturrus abyssus</i>	60.20833, 5.20261	Norway, Korsfjord	0-20	23.05.2012	m	this study		916	MG136744	MG136796	MG136769
<i>Neoturrus abyssus</i>	60.20833, 5.20261	Norway, Korsfjord	0-20	23.05.2012	m	this study		917	MG136745	MG136797	MG136770
<i>Neoturrus abyssus</i>	60.20833, 5.20261	Norway, Korsfjord	0-20	23.05.2012	m	this study		918	MG136746	MG136798	MG136771
<i>Neoturrus abyssus</i>	60.20833, 5.20261	Norway, Korsfjord	0-20	23.05.2012	m	this study		919	MG136747	MG136799	nd
<i>Neoturrus abyssus</i>	60.20833, 5.20261	Norway, Korsfjord	0-20	23.05.2012	m	this study		954	MG136748	MG136800	nd
<i>Neoturrus abyssus</i>	60.24079, 5.22941	Norway, Fanaefjord	0-30	24.04.2015	m	this study		1119	MG136749	MG136801	MG136772
<i>Neoturrus abyssus</i>	60.33802, 5.18163	Norway, Raunefjord	32-42	16.09.2008	p	this study		935	MG136750	MG136802	MG136773
<i>Neoturrus abyssus</i>	60.33802, 5.18163	Norway, Raunefjord	32-42	16.09.2008	p	this study		936	MG136751	MG136803	nd
<i>Neoturrus abyssus</i>	60.30282, 5.2016	Norway, Raunefjord	45-100	19.09.2008	p	this study		694	MG136752	MG136804	MG136774
<i>Neoturrus abyssus</i>	60.435, 5.122	Norway, Hauglandosen	135-151	19.09.2008	p	this study	62572	695	MG136753	MG136805	MG136775
<i>Neoturrus abyssus</i>	60.435, 5.122	Norway, Hauglandosen	135-151	19.09.2008	p	this study	62572	704	MG136754	MG136806	nd
<i>Neoturrus brevicornis</i>	48.54514, -123.01206	USA, San Juan Island	0	16.05.2011	m	this study		949	MG136726	MG136778	MG136761
<i>Neoturrus brevicornis</i>	48.54514, -123.01206	USA, San Juan Island	0.5	19.05.2011	m	this study		882	MG136728	MG136780	MG136763

species	WGS84 coordinates	locality	depth [m]	date collected	polyp medusa	comments, reference or DOI of photos	MHNG-INVE- voucher	DNA isolate	16S	COI	ITS
<i>Neoturris pileata</i>	43.685, 7.31567	France, Villefranche-sur-Mer	0-30	11.04.2017	m	this study	97957	1280	MGI36759	MGI36814	MGI36776
<i>Oceania armata</i>	43.686, 7.317	France, Villefranche-sur-Mer	0	21.01.2014	m	Schuchert (2016)	87094	956	KP776815	KX096599	nd
<i>Pandea conica</i>	43.685, 7.31567	France, Villefranche-sur-Mer	0-30	14.04.2017	m	10.5281/zenodo.896414	97958	1281	MGI36760	MGI36815	nd
<i>Pandopsis ikarii</i>	34.47812, 136.867	Japan, Toba City	0	nd	m	this study	92017	1129	MGI36757	MGI36812	nd
<i>Stomotoca atra</i>	48.54514, -123.01206	USA, San Juan Island	0.5	20.05.2011	m	10.5281/zenodo.998730		884	MGI36732	MGI36784	nd
<i>Stomotoca atra</i>	48.54514, -123.01206	USA, San Juan Island	0.5	20.05.2011	m	this study		960	MGI36733	MGI36785	nd
<i>Stomotoca atra</i>	48.54514, -123.01206	USA, San Juan Island	0.5	20.05.2011	m	this study		961	MGI36734	MGI36786	nd

Table 2: Selected clade divergences of 16S, COI, and ITS sequences calculated as p-values in %

clades	Maximal intraclade p-values in % (= intraspecific or intrageneric divergences)	
	16S	ITS
<i>Neoturris pileata+abyssi</i>	0.50	1.50
<i>Neoturris abyssii</i> Norway population	0.50	1.51
<i>Neoturris brevicornis</i>	0.52	1.51
<i>Leuckartiara octona</i>	0.50	1.68
<i>Stomotoca atra</i>	0.33	0.90
genus <i>Catablema</i>	0.34	3.77

clades	Minimal p-values in % of selected clade pairs (= interspecific divergences, barcode gaps)	
	16S	ITS
<i>Neoturris abyssii</i> Norway vs. <i>N. pileata</i>	0.17	0.30
<i>N. pileata+abyssi</i> vs. <i>N. brevicornis</i>	2.01	8.89
<i>L. octona</i> vs. <i>L. cf. octonema</i>	2.01	8.56
<i>L. octona</i> vs. <i>L. longicalcar</i>	1.17	5.72
<i>Catablema multicirratum</i> vs. <i>C. vesicaria nodulosum</i>	0.17	2.41
<i>A. dinema</i> Atlantic vs. <i>A. dinema</i> Pacific	15.1	16.1

Most importantly, however, the sequences placed the Mediterranean sample of *N. pileata* clearly within this *L. abyssi* clade, this with very little sequence divergence. The sequence differences of the *N. pileata* sample to *N. abyssi* are smaller than the maximal intrapopulation differences of *Neoturris abyssi* (Figs 3-4, Table 2). This is thus good evidence that *L. abyssi* is conspecific with *N. pileata*. Therefore, despite the morphological differences (compare Figs 3-6; colour of manubrium, bell-size, and proportion of height to width), the genetic data confirm the suspicion of Edwards (1965) that both are conspecific. Edwards (1965) hesitated to synonymise the two names because also another *Neoturris* medusa, *N. breviconis* (Murbach & Shaerer, 1902), had been reported from the North Sea by Hartlaub (1914) and later by Kramp & Damas (1925; as *Leuckartiara breviconis*) for the Bergen region. *Neoturris breviconis* was originally described from the NE Pacific (Fig. 10) and until the re-description of Arai & Brinckmann-Voss (1980) it was not well known and misidentified by a number of authors. The Atlantic animals depicted by the influential Russell (1953) and Kramp (1959) were clearly not *N. breviconis*. Re-examination of part of their material showed it to belong to other *Leuckartiara*, *Neoturris*, and *Catablema* species (Schuchert, 2007, 2012). The *Neoturris abyssi* medusae of this study observed near Bergen mostly lacked a distinct apical process, which is often very variable in Pandeidae. The medusae observed by Kramp & Damas (1925) in the same region and identified as *Leuckartiara breviconis* were likely the same and the insignificant apical process may have prompted them to separate them from their *N. pileata* originating from Iceland and the North Sea. True *Neoturris breviconis* originating from the NE Pacific (Fig. 10) appear quite distinct from typical *N. pileata* (Figs 3-6), but the diagnostic differences are much more difficult to formulate, in particular also criteria that can be used for preserved material (see below in Taxonomy section). The molecular data, however, clearly separate the two species (Figs 8-9, Table 2).

The differences (colour, size) of the *Neoturris* medusae from Norway and the Mediterranean observed in the present study are thus likely primarily due to age differences for the size and form, and environmental conditions for the colour differences (primarily the consumed food). A *Neoturris* medusa from Sweden (Fig. 5) had a much darker manubrium, despite being not much larger than the Norwegian ones. The developmental stages documented by Edwards (1965) agree nicely with these observations and also Hartlaub (1914), Russell (1953), and Schuchert (2007) mentioned the high degree of variability of the medusa. Hartlaub (1914) found that the apical process can be well developed but also lacking, the proportion of the manubrium length to bell-height is also very variable, like the number of tentacles (see also Kramp, 1926).

The medusae observed in Norway (Figs 3-4) with sizes of 8-12 mm had not attained the adult size of typical

N. pileata (2-4 cm, Figs 5-6). Gametes (oogonia) could only be found in the folds of larger medusae (>10 mm, the eggs in mature Mediterranean specimens are also not so easy to observe, they are quite small with a diameter of about 60 µm). In the Mediterranean, juvenile medusa stages have rarely been documented, but those shown in Hartlaub (1914) correspond well to the ones from Norway shown here (Fig. 3). Mediterranean polyps of *N. pileata* are even more rare, having only been reported by Bavestrello (1985). Contrary to the Atlantic counterparts, they were not found on *Nucula* species or scaphopods, but on shells of the hermit crab *Paguristes oculatus* (Fabricius) [now accepted as *Paguristes eremita* (Linnaeus)].

Although the 16S and COI sequence data unambiguously link *L. abyssi* from Norway and *Neoturris pileata* from the Mediterranean, these results depend to some degree on the reliability of these markers to distinguish biological species. While so far they proved to be very reliable for other hydrozoan groups (see Schuchert, 2016; Schuchert *et al.*, 2017 and references therein), the 16S results obtained in this study for three nominal *Catablema* species showed very little divergences, in part less than the maximal intraspecific divergence of other Pandeidae (Table 2, Fig. 8). This apparently puts into question to some degree the general usefulness of 16S as barcoding marker. However, as explained in the next section, the problem is more likely founded in the problematic identifications of the *Catablema* species and to a lesser degree the 16S marker.

Catablema species

16S and COI Sequences of all three currently accepted *Catablema* species could be analysed. The identification details of the new material are given below in the Taxonomy section, but the general results and interpretations must also be discussed in the context of the *Neoturris* section above. In addition to the two forms sequenced for this study, a few sequences of *C. vesicarium* from GenBank were also available and which are derived from correctly identified material (see Taxonomy section).

The surprising result was that the 16S sequences of all three morphotypes had very little sequence variation (Fig. 8), being in the range of intraspecific divergences of other species of the family (Table 2). COI has about three times higher divergence values than 16S (Table 2) and for this marker more structure is seen in the tree (Fig. 9). The Pacific *Catablema multicirrata* as well as the Svalbard *C. multicirrata* diverge clearly from the rest of the sampled specimens, but nevertheless do not reach minimal interspecific values seen for other species pairs (Table 2). The ITS data were similar (not shown).

These results could be used as an argument that 16S is not a suitable DNA barcoding marker for the Pandeidae as some species might not be separated into distinct

clades. However, the problem here seems more likely due to the taxonomy of the *Catablema* species than the 16S marker. As argued in the Taxonomy section, *Catablema nodulosa* is likely only a form of *C. vesicarium* with fewer tentacles. It is well possible that also the medusa identified here as *C. multicirrata* is in fact only a large form of *C. vesicarium* with a high number of tentacles. To conclude, the low divergences of the *Catablema* samples cannot be a priori used to question the value of the molecular data and results linking and synonymising of *N. abyssi* and *N. pileata*.

TAXONOMY

Remarks: Some of the material listed in Table 1 and used for the phylogenetic trees in Figures 8-9 has already been described in Schuchert (2007). Only pandeid species for which new information or interpretations have become available are discussed here.

Genus *Neoturris* Hartlaub, 1914

Type species: *Medusa pileata* Forsskål, 1775 (Kramp, 1959).

Remarks: For the diagnosis see Schuchert (2007). The genus comprises the species *Neoturris pileata* (Forsskål, 1775); *N. brevicornis* (Murbach & Shaerer, 1902); *N. papua* (Lesson, 1843); *N. bigelowi* Kramp, 1959; *N. crockeri* Bigelow, 1940; *N. fontata* (Bigelow, 1909a); *N. pelagica* (Agassiz & Mayer, 1902). The first three are well described species, the others all need to be redescribed and some of them are rather doubtful.

Neoturris pileata (Forsskål, 1775)

Figs 1-7

Medusa pileata Forsskål, 1775: 110. – Forsskål, 1776: pl. 33, fig. D.

Oceania Lesueurii Péron & Lesueur, 1810: 345. – Goy, 1995: 244, plate.

Carybdea pisifera Oken, 1815: 125.

Oceania pileus de Blainville, 1830: 258.

Oceania ampullacea M. Sars, 1835: 22, pl. 4 fig. 8. – Haeckel, 1879: 58, synonym.

Tiaria papalis Lesson, 1843: 287. – Haeckel, 1879: 58, synonym.

Turris digitale Forbes, 1846: 286. – Hartlaub, 1914: 324, synonym.

Turris digitalis. – Forbes, 1848: 21, pl. 3 fig. 1. – Haeckel, 1879: 61, pl. 4 figs 2-3. – Kramp, 1955: 153, revision of Haeckel's material.

Oceania episcopalis Forbes, 1848: 27, pl. 2 fig. 1. – Haeckel, 1879: 58, synonym.

Oceania coccinea Leuckart, 1856: 20, pl. 2 fig. 3. – Haeckel, 1879: 58, synonym.

Oceania constricta Patterson, 1859: 279, figs.

Tiaria pileata. – Haeckel, 1879: 58, pl. 3 figs 6-8.

Turris coeca Hartlaub, 1892: 19, fig. 1. – Hartlaub, 1914: 329, synonym.

in part *Turris pileata*. – Mayer, 1910: 123, pl. 12 fig. 4, pl. 13 fig. 6.

Tiaria pileata. – Le Danois, 1914: 17, fig. 4.

Perigonimus abyssi G.O. Sars, 1874: 126, pl. 5 figs 27-30. **new synonym**

Neoturris pileata. – Hartlaub, 1914: 326, figs 270, 273, 274-281. – Kramp, 1926: 92, fig. 37, pl. 2 figs 13-14, chart XVIII. – Russell, 1953: 203, figs 104-106, pl. 12 fig. 1. – Edwards, 1965: 461, figs 1-4, life cycle. – Schuchert, 2007: 333, figs 59-60, review.

in part *Leuckartiara brevicornis*. – Hartlaub, 1914: 304, figs 254-256. [incorrect subsequent spelling]

in part *Leuckartiara brevicornis*. – Kramp, 1926: 80, pl. 2 fig. 8. – Russell, 1953: 198, pl. 12 fig. 2. – Kramp, 1959: 120, fig. 121. [not *Neoturris brevicornis* (Murbach & Shaerer, 1902)]

Leuckartiara brevicornis. – Kramp & Damas, 1925: 280 [not *Neoturris brevicornis* (Murbach & Shaerer, 1902)]

Leuckartiara abyssi. – Rees, 1938: 19, fig. 6a-d, part of life cycle. – Rees, 1956: 114, re-examination of type material, lectotype designation. – Schuchert, 2007: 330, fig. 57, redescription, status.

Type locality: Mediterranean

Material of *N. abyssi*: All specimens came from Bergen area in Norway. See also Table 1 for GenBank numbers. If no museum accession number is given, there is no material in a permanent collection.

Hydroid stage:

MHNG-INVE-54693; without gonophores on *Nucula* spec; Herdla fjord, 60.503° 5.2152°, 375-440 m depth; collection date 20.04.2007. – MHNG-INVE-54695;

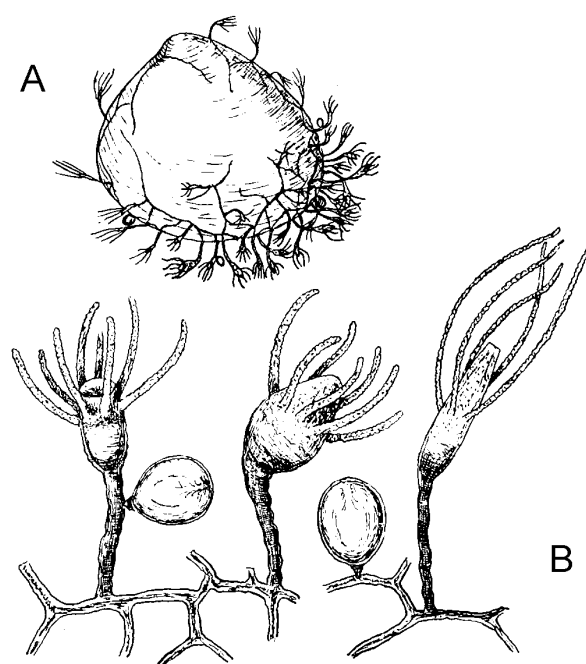


Fig. 1. *Neoturris abyssi*, original illustration of Sars (1874).

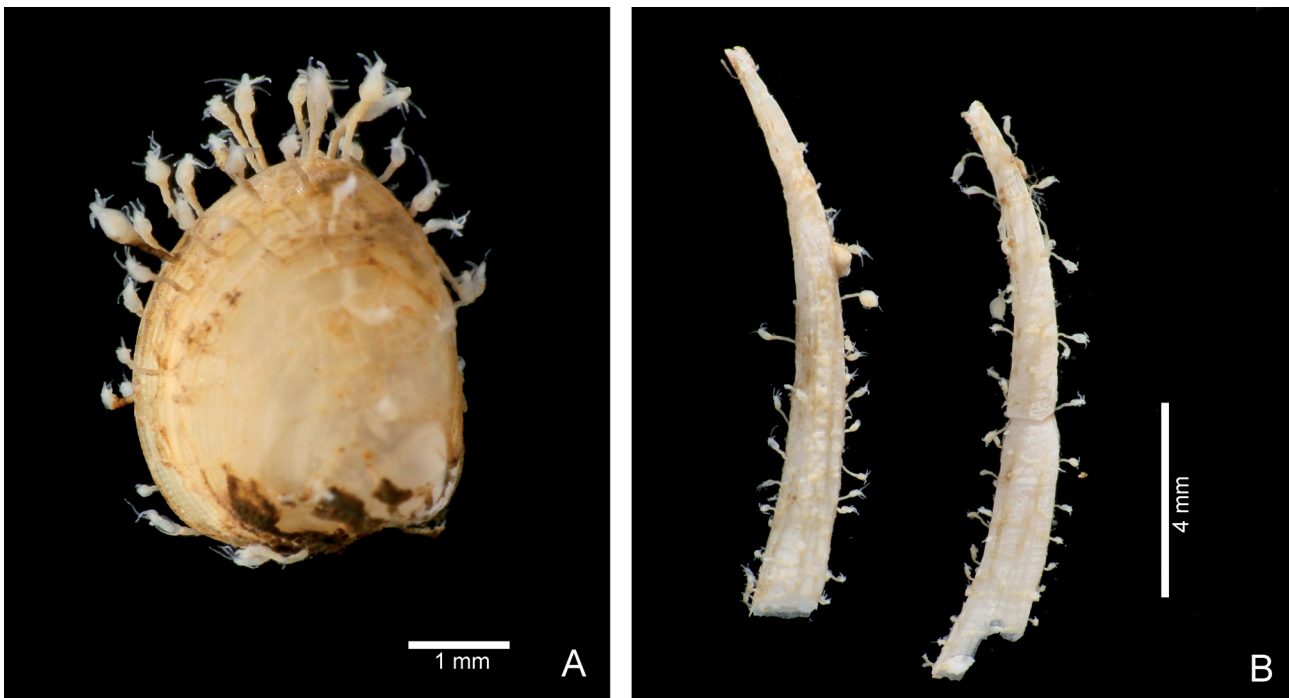


Fig. 2. *Neoturris abyssis* (= *Neoturris pileata*), preserved polyp specimens from Norway, Bergen area, Hauglandsosen. (A) MHNG-INVE-54695 on *Nucula* spec. (B) MHNG-INVE-62572, on a scaphopod, (DNA 695, see Table 1).

without gonophores on *Nucula* spec.; Hauglandsosen, 60.433° 5.1167°, 180 m depth; collection date 15.08.2007. – Hydroid without gonophores on scaphopod of about 5 mm size; Raunefjord, Vatløstraumen, 60.33802° 5.18163°, 32–42 m depth; collection date 16.09.2008; DNA isolate 935. – Hydroid without gonophores on *Nucula* spec.; Raunefjord, Vatløstraumen, 60.338017° 5.181633°, 32–42 m depth, temperature °C; collection date 16.09.2008; DNA isolate 936. – Hydroid without gonophores on sipuncule in *Antalis entalis*; Raunefjord, Flesland, 60.30282° 5.2016°, 45–100 m depth; collection date 19.09.2008; DNA isolate 694. – Hydroid without gonophores on *Nucula* spec; Hordaland, Hauglandsosen, 60.435° 5.122°, 135–151 m depth; collection date 19.09.2008; DNA isolate 695. – Hydroid without gonophores on *Nucula* spec; Hordaland, Hauglandsosen, 60.435° 5.122°, 135–151 m depth; collection date 19.09.2008; DNA isolate 704.

Medusa stage:

Raunefjord, 60.275° 5.200°, 10 m depth; collection date 22.05.2012; DNA isolate 953. – MHNG-INVE-82129; Korsfjord, 60.20833° 5.20261°, 0–20 m depth; collection date 23.05.2012; DNA isolate 916. – Korsfjord, 60.20833° 5.20261°, 0–20 m depth; collection date 23.05.2012; DNA isolate 917. – Korsfjord, 60.20833° 5.20261°, 0–20 m depth; collection date 23.05.2012; DNA isolate 918. – Korsfjord, 60.20833° 5.20261°, 0–20 m depth; collection date 23.05.2012; DNA isolate 919. – Korsfjord, 60.20833° 5.20261°, 0–20 m depth; collection date 23.05.2012; DNA isolate 954. –

Fanafjord, 60.24079° 5.22941°, 0–20 m depth; collection date 24.04.2015; DNA isolate 1119.

Material of *N. pileata*: MHNG-INVE-97957; France, Bay of Villefranche-sur-Mer, 43.685° 7.315667°, 0–30 m depth; collection date 11.04.2017; DNA isolate 1280. Additional examined material is given in Schuchert (2007).

Diagnosis: *Neoturris* medusa with bell that is usually higher than wide, height 2–4 cm, no exumbrellar nematocyst ridges, with or without apical projection, no apical canal, with up to 60–90 tentacles. Manubrium usually longer than half the subumbrella height, interradial gonad region large and without folds but with many gonadal pits (>20 per quadrant), eight adradial rows of horizontal gonads folds, folds appear directed towards interradial; no papillae on gonads, radial canals jagged, tentacle bases without abaxial spurs, no ocelli. Colours depending on age and environment, manubrium in younger ones yellow-orange, in fully grown medusae pink to ruby-red; tentacle-bases yellowish.

Hydroids usually on scaphopods and *Nucula* shells, colonial, arising from creeping stolons; hydrocauli covered by perisarc, not branched, monosiphonic. Perisarc extends onto hydranth body as a more or less gelatinous pseudohydrotheca which does not envelop the tentacles. Hydranths with conical hypostome, one whorl of filiform tentacles. Gonophores develop on cauli or stolons, enclosed in thin perisarc membrane. Gonophores liberated as free medusae with four tentacles.

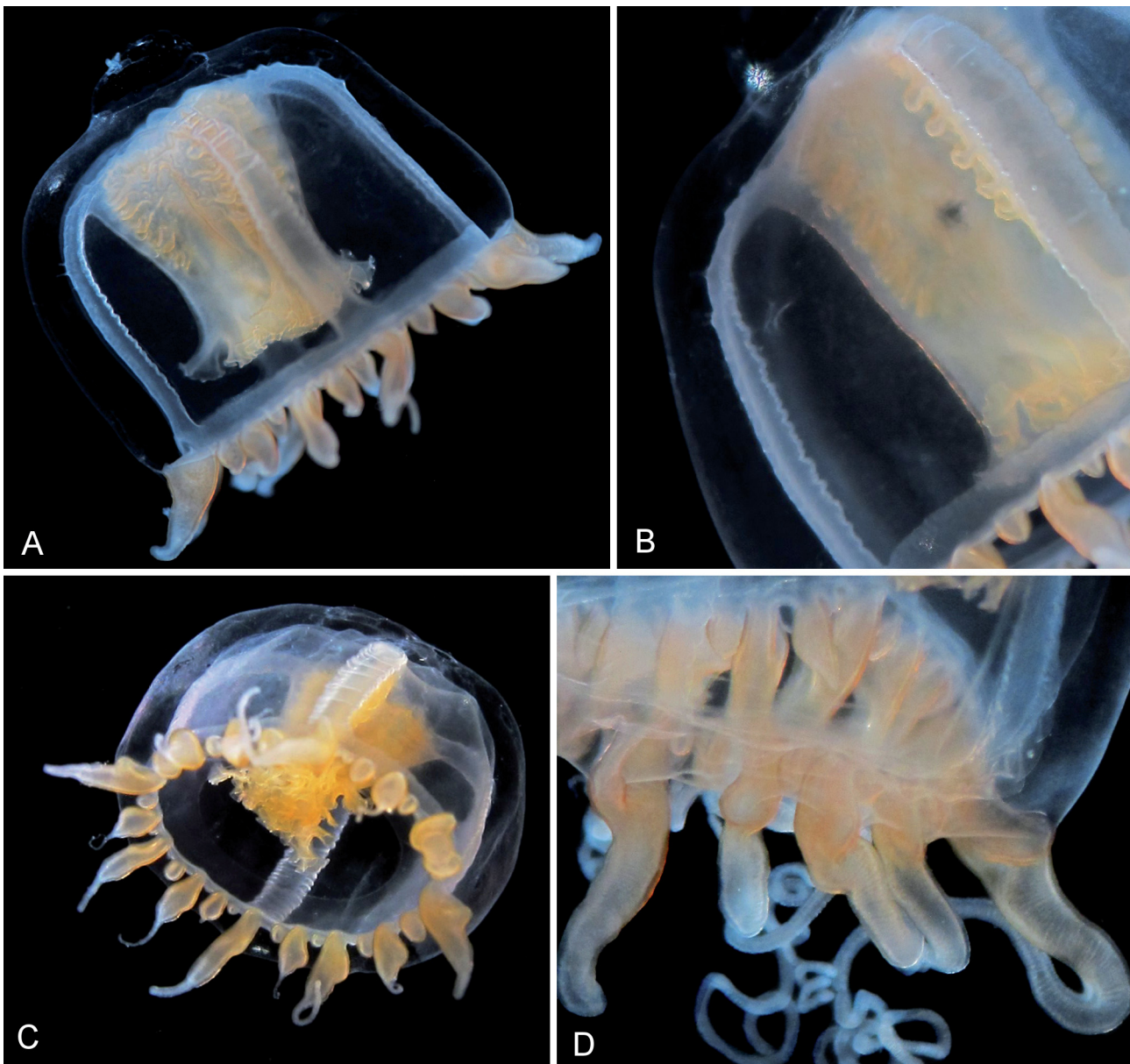


Fig. 3. *Neoturris abyssis* (= *Neoturris pileata*) medusae from Norway, photographs of living, relatively young stages. (A) Lateral view of medusa with bell size 7 mm (DNA 953, see Table 1). (B) Same as A, detail of radial canals and folds of stomach wall. (C) Same as A, oblique view from below. (D) Animal of bell size 9 mm, detail of tentacle bases, note the absence of ocelli.

Description: See Schuchert (2007).

Remarks: As already suspected by Edwards (1965), the 16S and COI sequence comparisons presented above are evidence that the hydroid *Leuckartiara abyssis* G.O. Sars, 1874 must belong to *Neoturris pileata* (Forsskål, 1775). The hydroid of *L. abyssis* from near the original collecting site of Sars belongs unambiguously to *Neoturris* medusae found at the same locality. These *Neoturris* medusae were smaller than those of adult Mediterranean ones (largest ones seen about 15 mm high), but the morphology of the manubrium with its numerous interradiial pits and the adradial folds

(Fig. 4B) comes close to the ones in more southern waters (comp. Figs 6-7). The colour of the manubrium was, however, never as red as found in medusae south of Norway to the Mediterranean. A *Neoturris* medusa from Sweden (Fig. 5) had a much darker manubrium, despite being not much larger than the Norwegian ones. The yellowish *Neoturris* medusae occur regularly in the Bergen region (see also Hosia & Båmstedt, 2007; as *N. pileata*) and must also have been seen by Kramp & Damas (1925) who attributed them to *N. brevicornis*. The sequence comparison made here (Figs 8-9), however, show that this cannot be the case as *N. brevicornis* is well separated from the *N. abyssis*+*N. pileata* clade.

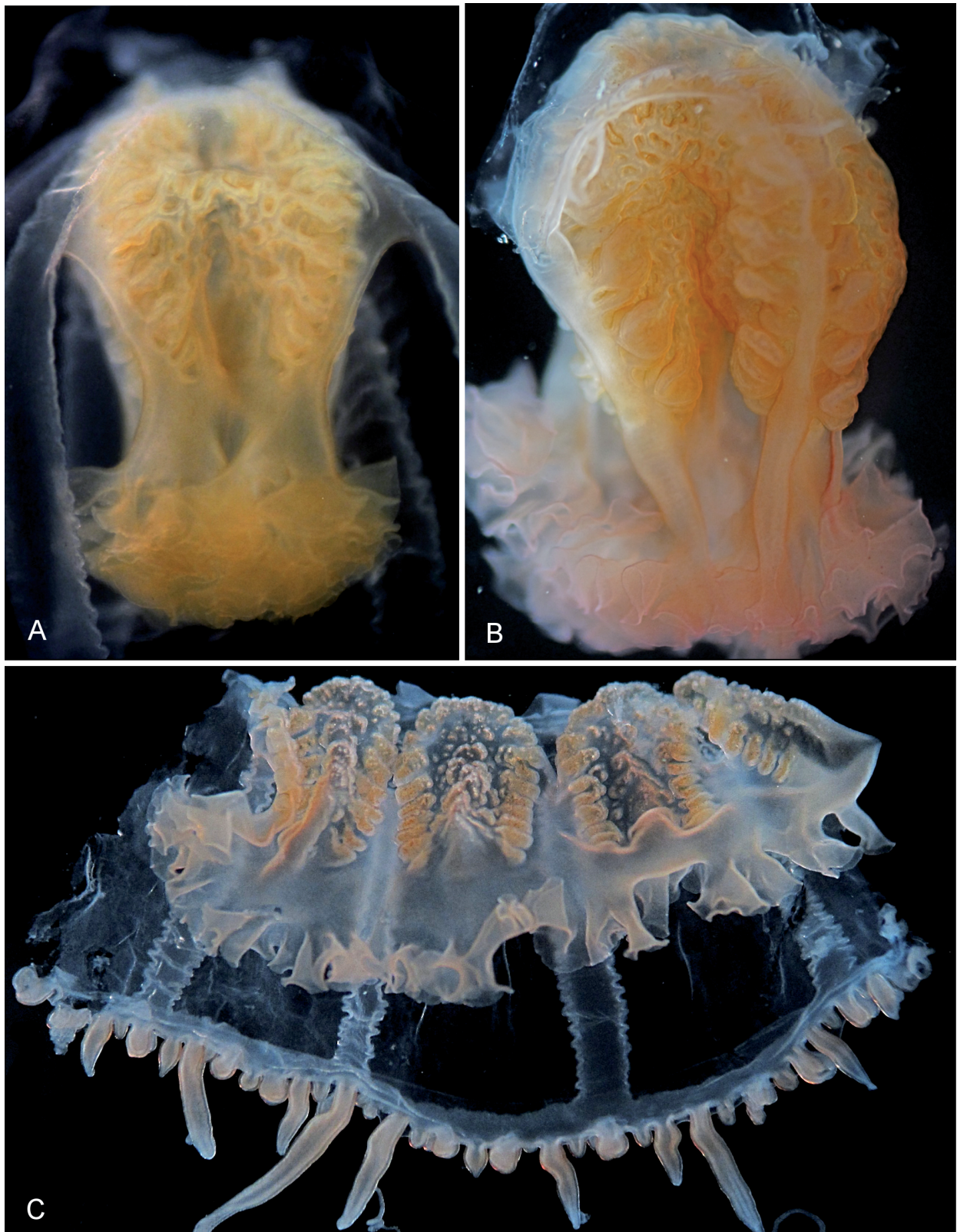


Fig. 4. *Neoturris abyssis* (= *Neoturris pileata*) medusae from Norway, photographs of the most advanced stages found. (A) Lateral view of manubrium of a medusa with bell size 9 mm (DNA 918, see Table 1). No gametes could be seen when examining the gonad fold under a compound microscope. (B) Manubrium of a medusa with bell size 12 mm (DNA 916, see Table 1); note the increased number of gonadal folds and pits. Small oocytes were present in the gonads folds. Except for the colour this animal closely resemble Mediterranean specimens (Figs 6-7). (C) Medusa of about 10 mm height (DNA 919, see Table 1) cut open and spread to visualise anatomical details (inner side of stomach facing observer).



Fig. 5. *Neoturris abyssis* (= *Neoturris pileata*), living medusa from the Swedish coast, photo taken by Fredrik Pleijel and reproduced with the permission of the author. The manubrium is contracted, feigning a horizontal gonadal fold on the manubrium resembling the permanent one seen in some *Leuckartiara* species. This photo is copyright protected and it must not be reproduced without the consent of the author.

The hydroid of *N. pileata* without medusa buds is not readily distinguishable from *Leuckartiara octona*, the only other pandeid known from the region (Hosia & Båmstedt, 2007). The only character to reliably distinguish the two is found in the newly released medusae, which have four tentacles instead of the two tentacles present in *L. octona*. A less reliable character is the absence of branching of the stems, which in fully grown colonies of *L. octona* are quite regularly branched once, but not so in *N. abyssis*. The Norwegian hydroids here assigned to *L. abyssis* lacked medusa buds, but were nevertheless assigned to *L. abyssis* because they came from close to the type locality, they grew on the typical substrate, the pedicels were never branched, and they occurred in relatively deep waters. Their sequences separated them immediately from *L. octona* medusae collected at the same locality (Figs 8-9). An infertile pandeid hydroid on a *Nucula* shell collected in 5-50 m depths along the Swedish coast (DNA 1055, Table 1) was initially also identified as *L. abyssis*, but the DNA data clearly identified it as *L. octona* and it was reclassified accordingly.



Fig. 6. *Neoturris pileata*, preserved specimen (MHNG-INVE-35522) from the Mediterranean, collected before 1895 and identified by C. Hartlaub. Note that the bell shape is not elongated as often seen in other illustrations (e.g. Fig. 7), but nevertheless lies within the range of variation for Mediterranean specimens. Moreover, the bell is somewhat flattened in this preserved sample.

Neoturris brevicornis (Murbach & Shaerer, 1902)

Fig. 10A-E

Turris brevicornis Murbach & Shearer, 1902: 73. – Murbach & Shearer, 1903: 170, pl. 18 figs 1-2. – Mayer, 1910: 127. in part *Leuckartiara brevicornis*. – Hartlaub, 1914: 304, figs 254-256. [subsequent incorrect spelling]
not *Leuckartiara brevicornis*. – Kramp & Damas, 1925: 280. [= *Neoturris pileata* (Forsskål, 1775)]
in part or not *Leuckartiara brevicornis*. – Kramp, 1926: 80, pl. 2 fig. 8. – Russell, 1953: 198, pl. 12 fig. 2. – Kramp, 1959: 120, fig. 121. – Kramp, 1961: 103. – Kramp, 1968: 4, fig. 124. – Russell, 1970: 246.
not *Perigonimus brevicornis*. – Naumov, 1969: 204, fig. 72. [= *Catablema multicirratum*]
Neoturris brevicornis. – Arai & Brinckmann-Voss, 1980: 57, figs 31-33, new combination.
in part *Neoturris brevicornis*. – Schuchert, 2007: 338, fig. 61A-B, not 61C-E.

Type locality: St. Paul Island, Pribilof Islands, Bering Sea.

Material examined: MHNG-INVE-82207, 1 mature specimen in ethanol; Canada, Vancouver Island, 49.0.467°-124.5018°, 0 m depth; collection date 21.05.2012; leg. M. Galbraith. – Several specimens,



© Nicholas Samaras

Fig. 7. *Neoturris pileata*, living medusa photographed by Nicholas Samaras, location: Mediterranean, Greece, Chalkidiki Peninsula, depth 3 m. The photo shows a perfect and typical *N. pileata*, note the intense red colour of the manubrium as well as the folds and pits on it. Note that this photo is copyright protected and the right to reproduce it here was acquired by paying a royalty fee to the copyright holder Nicholas Samaras (www.underwater-photography.gr).

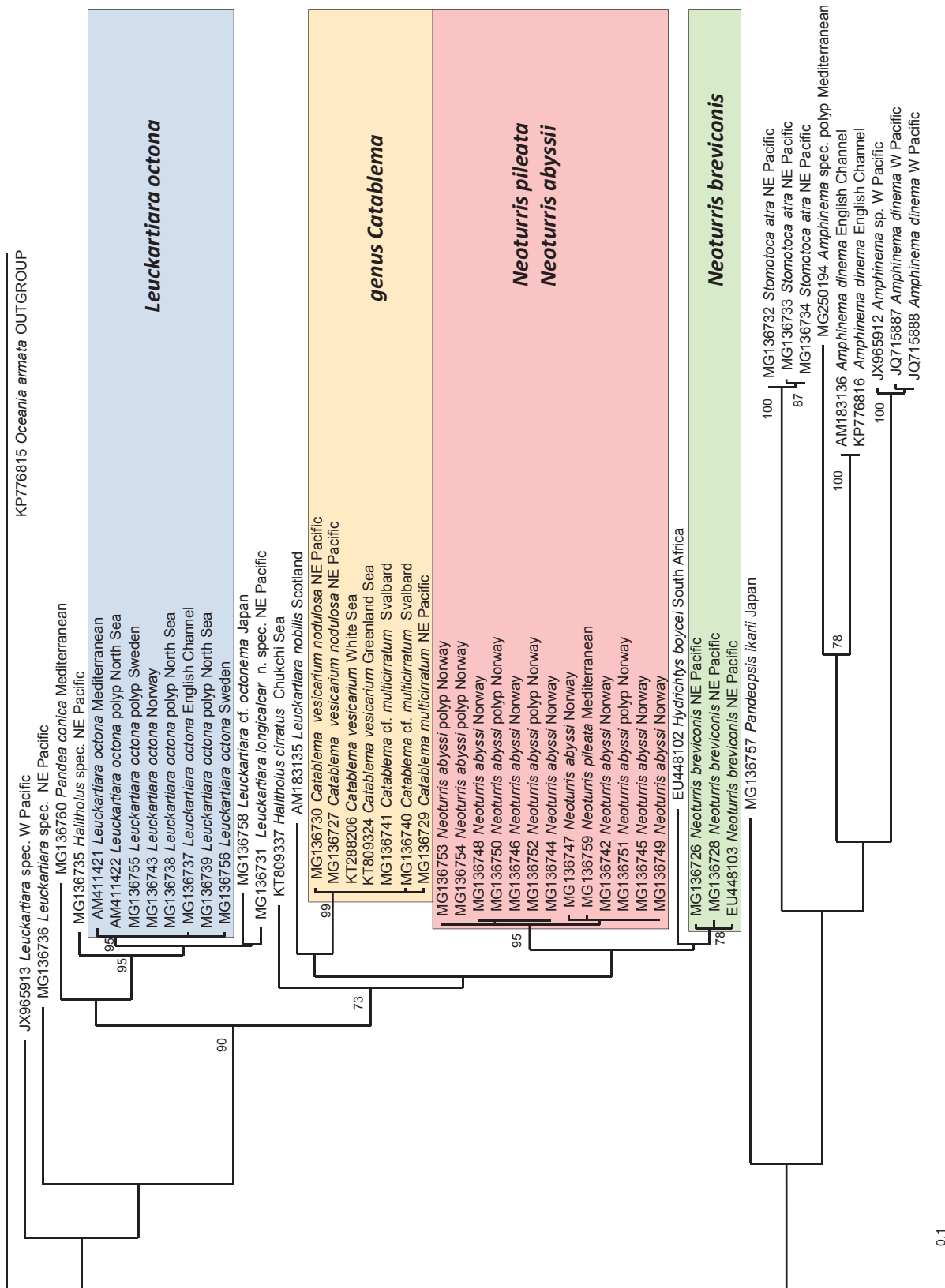


Fig. 8. 16S maximum likelihood phylogenetic tree of Pandeidae species obtained with PhyML (GTR+G+I model) and based on 595 bp positions of the mitochondrial 16S gene. Node-support values are bootstrap values of 100 pseudoreplicates (shown only if > 70%). For more details see text and Table 1.

Samples based on the polyp stage are indicated, all others are medusa samples.

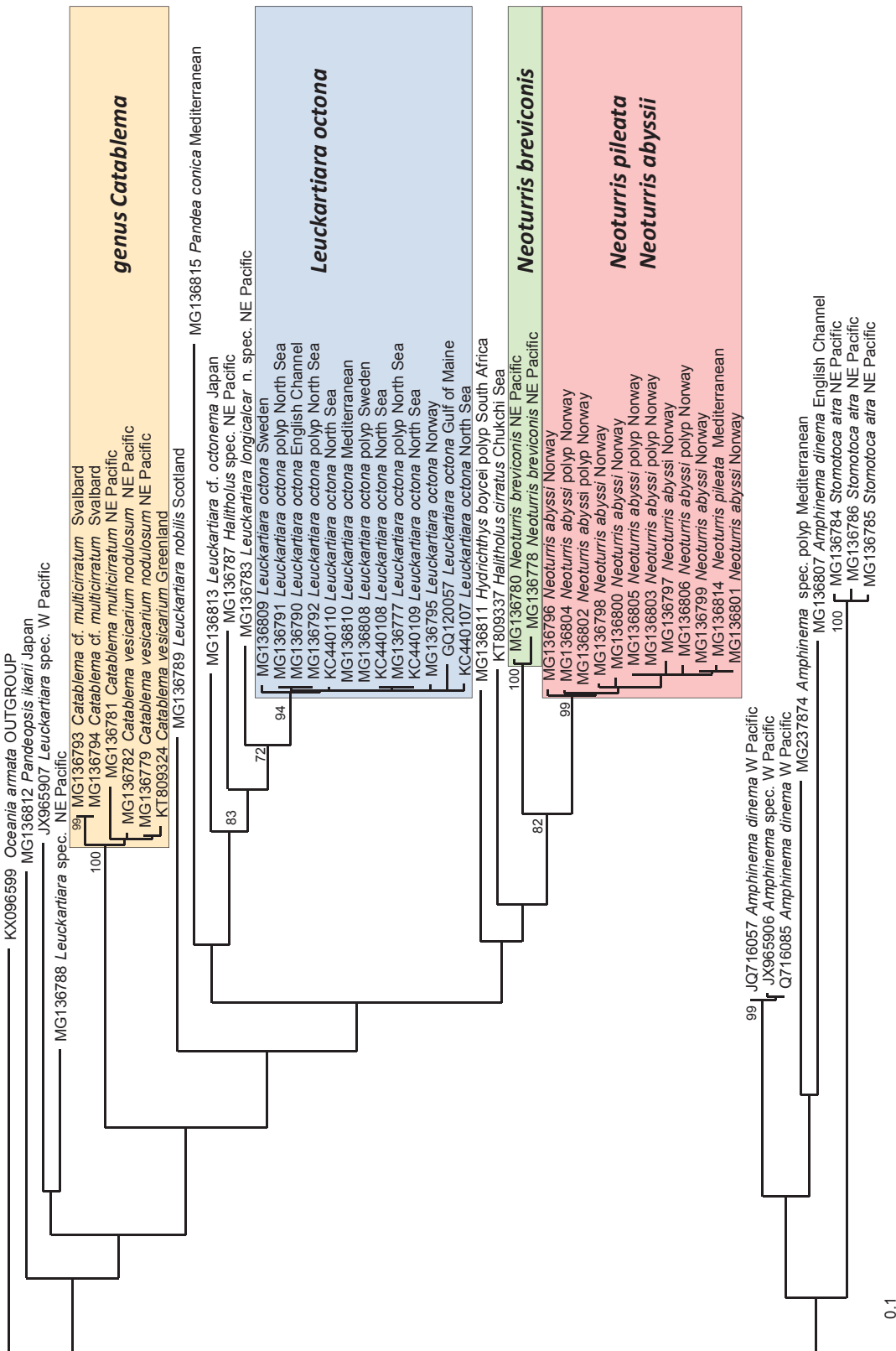


Fig. 9. COI maximum likelihood phylogenetic tree of Pandeidae species obtained with PhyML (GTR+G+I model) and based on 664 bp positions of the mitochondrial COI gene. Node-support values are bootstrap values of 100 pseudoreplicates (shown only if > 70%). For more details see text and Table 1.

Samples based on the polyp stage are indicated, all others are medusa samples.

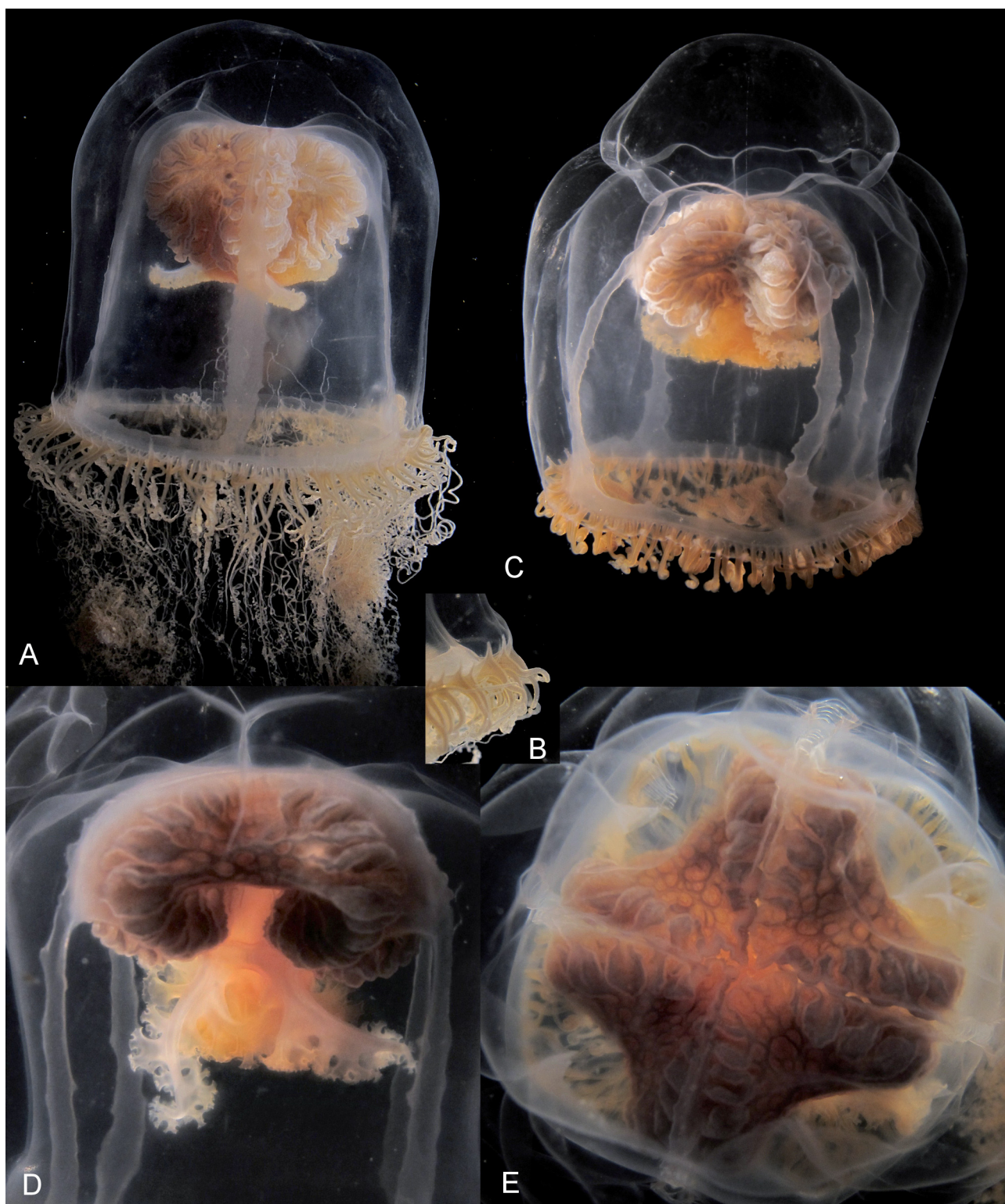


Fig. 10. *Neoturris breviconis*, living individuals from Friday Harbor, USA. (A) Fully grown animal without apical process, height approximately 3–4 cm. (B) Tentacle bases, note abaxial spurs and the absence of ocelli. (C) Smaller animal than shown in A (not to scale) with an apical process. (D) Medusa of with dark pigment in gonad region. The H-form of the gonad-bearing part of the manubrium is rather characteristic, but only temporary as due to contraction. This H-shape of the gonads is reminiscent of some *Leuckartiara* species. (E) Same specimen as in D seen from aboral side. Note that the gonadal pits are more numerous and much better seen in this view.

not in permanent collection; USA, San Juan Island, Friday Harbor, 48.54514°-123.01206°, 0-0.5 m depth, collection date 16.05.2011; DNA isolates 949 and 882, photos Fig. 10, see also Table 1.

Presumed Atlantic material was examined for the publication Schuchert (2007).

Diagnosis: *Neoturris* medusa up to 45 mm high, broad, cylindrical bell, without or with shallow apical process, no exumbrellar ridges with nematocysts, manubrium voluminous, about half or less the height of subumbrella, 90-140 tentacles of similar size, interradial gonad region with 5-20 pits per quadrant, no papillae on gonads, radial canals jagged. Manubrium orange-brown sometimes with dark pigment granules at surface of gonads.

Description: Medusa up to 45 mm high and 35 mm wide, bell often rather cylindrical, top evenly rounded or with a shallow apical projection. Without exumbrellar ridges with nematocysts. Apical canal above manubrium absent or very thin. Aboral subumbrella often with distinct interradial pockets.

Manubrium broad and voluminous, about half the height of subumbrella or less; mesenteries variable in length, usually 1/3 of manubrium height; mouth margin crenulated or finely folded, periradial corners of often drawn out into long processes (Fig. 10A, D). Gonad tissue in upper two thirds of manubrium wall, this region with rows of horizontal folds along the radial canals, about 20 such folds per row, folds thick, and somewhat irregular, some also branched, most folds do not appear directed towards interradial (only those close to top, Fig. 10E), interradial region of gonads rather narrow and depressed, with 5-20 pits per quadrant. If disturbed, the animal can contract the manubrium, resulting in a temporary horizontal fold that looks like a connection of the gonad-folds as seen in the genus *Leuckartiara* (Fig. 10D).

Radial canals jagged and very broad. Ring canal smooth, broad. Up to 140 tentacles, densely crowded, no rudimentary tentacles but some smaller tentacles in development. Marginal tentacle bulbs elongated, laterally compressed conical and tapering rapidly, base grasping margin with or without abaxial spur (Fig. 10B), no ocelli. Tentacles without permanent row of folds.

Color of living specimens, gonads and manubrium pale orange-brown, surface of gonads sometimes with dark red to purple pigment granules (Fig. 10D-E).

Younger animals with short gonad-zone, low number of shallow folds, few interradial pits (figures 31-32 in Arai & Brinckmann-Voss (1980)).

Hydroid not known.

Remarks: When describing *N. brevicornis*, Murbach & Shearer (1903) already noted the similarity of this species to *N. pileata*, but the illustration depicting the medusa seen from the side was somewhat inaccurate and they did not mention the interradial pits. In his revision of the Pandeidae, Hartlaub (1914) deplored

these inaccuracies, but hesitatingly also attributed some badly preserved medusae from the northern North Sea to this species. His specimens were smaller (23 mm in height) and the gonad folds resembled more the ones in the genus *Leuckartiara*. Therefore, he introduced the new combination *Leuckartiara brevicornis* (Murbach & Shaerer, 1902). There were no ocelli present, but his material had been preserved for a long time and the pigment of ocelli disappears after a few months in formalin. Later, also Kramp (1926, 1959) and Russell (1953) thought to have found Atlantic specimens of this species. Their illustrations, however, were not *N. brevicornis*. Schuchert (2007, 2012), after re-examination of some of Hartlaub's and Kramp's medusae, found that they are unlikely *N. brevicornis*, perhaps rather large *Leuckartiara nobilis*, other *Neoturris*, or *Catablema* species.

After examination of medusae from the NE Pacific, Arai & Brinckmann-Voss (1980) found that the species closely resembles *N. pileata* (gonad structure, absence of ocelli) and they transferred it from the genus *Leuckartiara* to the genus *Neoturris*.

Living *Neoturris brevicornis* originating from the NE Pacific (Fig. 10) look quite distinct from typical *N. pileata* (Figs 3-6), but the diagnostic differences are much more difficult to formulate, in particular also criteria that can be used for preserved material. *Neoturris brevicornis* can be distinguished from *N. pileata* by the broader shape of the exumbrella, the relatively short manubrium, the smaller number of interradial pits on the manubrium (5-20 versus > 20 per quadrant), and the higher number of tentacles (fully grown 90-140 tentacles versus 60-80). Additionally, the apical projection if present is smaller, the adradial gonadal folds not clearly directed towards interradia (except the most aboral ones), and the tentacles bases may have short abaxial spurs. The 16S and COI sequence data clearly separate *N. pileata* and *N. brevicornis* (Figs 8-9).

While it is well possible that *N. brevicornis* is also present in the Atlantic, currently available evidence is insufficient to establish its presence in the Atlantic. New, living samples must be examined and ideally also their 16S or COI sequences compared with the data presented here.

There exist a few other, little known Pacific *Neoturris* species which are best distinguished using Kramp (1968).

Genus *Catablema* Haeckel, 1879

Type species: *Turris vesicaria* A. Agassiz, 1862 (Kramp, 1959).

Remarks: For the diagnosis see Schuchert (2007). The genus currently comprises the species *Catablema vesicarium* (A. Agassiz, 1862), *C. multicirratum* Kishinouye, 1910, and *C. nodulosum* Bigelow, 1913. According to Hartlaub (1914), Kramp (1959, 1961, 1968), Arai & Brinckmann-Voss (1980), and Schuchert (2007), the three can be distinguished as follows,

characteristics that were also used to identify the present material:

C. vesicarium – up to 32 tentacles, rarely 48, bell size up to 3 cm, in North Atlantic and Arctic Sea

C. nodulosum – 8 to 16 tentacles, bell-size up to 2 cm, in North Pacific

C. multicirratum – 80 to 160 tentacles, bell size up to 6 cm; in North Pacific and Arctic Sea.

***Catablema vesicarium* (A. Agassiz, 1862)**

? *Medusa campanula* Fabricius, 1780: 366.

Catablema campanula. – Haeckel, 1879: 63, pl. 4 figs 4-5. – Kramp, 1955: 153, re-examined material of Haeckel.

Turris vesicaria A. Agassiz, 1862: 97. – Agassiz, 1865: 164, figs 261-268.

in part *Turris vesicaria*. – Mayer, 1910: 126, pl. 12 figs 2-3 (not pl. 13. fig. 7 = *L. octona*).

Catablema vesicarium. – Bigelow, 1909b: 304, pl. 30 figs 3-4, pl. 31 fig. 6. – in part Hartlaub, 1914: 315, figs 263-267. – Kramp, 1926: 87, pl. 2 figs 10-11. – Kramp 1959: 122, 208-212, fig. 125. – Kramp, 1961: 96. – Kramp, 1968: 50, fig. 132. – Schuchert, 2007: 345, fig. 64, redescription. – Prudkovsky & Neretina, 2016: 533, figs 1-8, life cycle.

Catablema eurystoma Haeckel, 1879: 64, pl. 4 figs 6-7. – Kramp, 1955: 153, synonym.

Tiara conifera Haeckel, 1879: 59. – Kramp, 1955: 152, re-examination of type specimen, synonymy.

in part *Perigonimus vesicarius*. – Naumov, 1969: 202, ? not fig. 69.

Type locality: Nahant, Massachusetts Bay, USA.

Material examined: See Schuchert (2007). The molecular comparisons of this study included also 16S sequences of the material described in Prudkovsky & Neretina (2016), as well as of a medusa from the Nuuk-Fjord in Greenland (GenBank KT809324) collected 22 June 2010 and identified by Russell Hopcroft. It had about 28-30 tentacles, about as many rudimentary bulbs, and a large apical projection (after data and photos kindly provided by R. Hopcroft).

Diagnosis: *Catablema* medusa with bell up to 25 mm wide and 30 mm high, including the large, globular apical projection; gonads in long, irregular folds, oblique in lateral parts, almost perpendicular in middle part of each quadrant, with or without pits on gonad folds; 24-32 tentacles, rarely up to 48, often with small, rudimentary bulbs between two tentacle pairs; usually with small abaxial ocelli on at least some tentacles or bulbs, sometimes missing; mesenteries short.

Hydroid arising from reticulate stolons on bivalves, hydranths stolonal or with very short pedicel only, base of hydranth surrounded by a membranous pseudohydrotheca; hydranth fusiform, up to 0.75 mm long, conical hypostome, 3-8 filiform amphicoronate tentacles in a single whorl. Medusa buds arise from stolons, diameter reaches sizes similar as hydranths, young medusa released with two opposite tentacles only.

Description and illustrations: See Schuchert (2007) and Prudkovsky & Neretina (2015).

Distribution: An Arctic species, rarely penetrating into boreal regions.

Remarks: The medusae identified by Prudkovsky & Neretina (2016) had up to 28 tentacles, matched thus exactly the concept of *C. vesicarium* given in Kramp (1959) and also in the original description of Agassiz (1862, 1865). Likewise, the 16S and COI sequences of the sample from Greenland (GenBank KT809324) are derived from a typical specimen and can also be used as a reliably identified reference specimen and sequence. Although *C. nodulosum* is likely conspecific with *C. vesicarium*, it is discussed separately below to allow a better separation and clearer presentation of this morphotype.

***Catablema vesicarium nodulosum* Bigelow, 1913**

Fig. 11

Catablema vesicarium var. *nodulosum* Bigelow, 1913: 17, pl. 1 figs 8-9.

Catablema nodulosa. – Arai & Brinckmann-Voss, 1980: 45, fig. 21.

Type locality: Dutch Harbour, Unalaska Island, USA.

Material examined: 2 specimens, not in permanent collection; USA, San Juan Islands, Friday Harbor, 48.5451° -123.01206°; collection date 16.05.2011 and 20.05.2011; collected at water surface with a dipping jar; DNA isolates 932 and 957; GenBank numbers see Table 1.

Diagnosis: North Pacific *Catablema* medusa, up to 25 mm in size, including the apical projection of variable size and shape; gonads in long, irregular folds, oblique in lateral parts, almost perpendicular in middle part of each quadrant, gonadal folds usually without pits, rarely a few present; With 8 to 16 tentacles, rarely up to 25, with 2-6 small, rudimentary bulbs between adjoining tentacles, usually with small, inconspicuous abaxial ocelli on the rudimentary bulbs, fully formed tentacles lack ocelli; mesenteries about 1/3 of manubrium height. Manubrium gold-brown or peach colour in living specimens.

Hydroid unknown.

Description: See Arai & Brinckmann-Voss (1980).

Remarks: In the examined material, only the smaller tentacles and the rudiments had small ocelli, the fully developed tentacles lacked them.

Bigelow (1913) found that some *Catablema* medusae from the North Pacific differed in tentacle numbers and gonad structure from *C. vesicarium* he had seen in the North Atlantic. Although he states that they were probably still within the extremes of the nominal species and no morphological discontinuity existed, he treated them as



Fig. 11. *Catablema vesicarium nodulosum*, living medusa from Friday Harbor, WA, USA, bell height about 2 cm, the individual was used to obtain one of the DNA sequences of this study.

a variant of *C. vesicularium* and named it *Catablema vesicarium* var. *nodulosum*. Bigelow observed tentacle numbers of 14-25 tentacles, but the numbers were often difficult to establish as there was a continuum of sizes from mere knobs to fully grown tentacles. Hartlaub (1914: 321), Foerster (1924), and Kramp (1926, 1968) regarded *Catablema vesicarium* var. *nodulosum* Bigelow, 1913 as a synonym of *C. vesicarium*.

Arai & Brinckmann-Voss (1980) did not agree and raised the variant to full species level. They distinguished *Catablema nodulosum* from *C. vesicarium* solely on account of the lower tentacle number, being only 8-16 instead of 32. The shape of the gonads as argued by Bigelow (1913) was deemed unsuitable to distinguish the two species and I concur. Arai & Brinckmann-Voss (1980) based their decision on medusae from the southern limit of this genus, thus perhaps with a suboptimal growth. This could perhaps also explain the lower tentacle number compared to *C. vesicarium*, which is an Arctic species. Bigelow (1913), who had medusae from cooler waters (Aleutian Islands), founded his variety on animals having up to 25 tentacles. It is therefore reasonable to follow Bigelow, Hartlaub, and Kramp and regard *C. nodulosum* only gradually different from *C. vesicarium*, representing a local variant only. Moreover, tentacle numbers in Pandeidae medusae vary considerably and are deemed mostly unsuitable to delimit

species. The COI sequence data did not show significant differences between the *nodulosum* form from the NE Pacific and typical *C. vesicarium* from the Greenland Sea (Fig. 9; the 16S data show very little divergences within this genus). *Catablema nodulosum* should therefore be regarded as conspecific with *C. vesicarium*, or at most be treated as a subspecies of the latter. According to the ICZN (§45.6.4), a name introduced as variety before 1961 gets the rank of subspecies.

Catablema multicirratum Kishinouye, 1910

Figs 12-13

Catablema multicirrata Kishinouye, 1910: 24.

Catablema multicirrata. – Bigelow, 1913: 19, pl. 1 figs 4-7. – Hartlaub, 1914: 321. – Kramp, 1926: 91, pl. 2.– Uchida, 1927: 213. – Uchida, 1933: 130 fig. 6. – Uchida, 1940: 286. – Uchida, 1969: 286. – Arai & Brinckmann-Voss, 1980: 44, fig. 20. – Wang *et al.*, 2014: 99, fig. 12.

Catablema multicirratum. – Kramp, 1961: 96. – Kramp, 1968: 50, fig. 133

Perigonimus multicirratum. – Naumov, 1969: 204, fig. 71.

Perigonimus brevicornis. – Naumov, 1969: 204, fig. 72. [not *Neoturris brevicornis* (Murbach & Shaerer, 1902)]

Type locality: Paramushir Island, Kuril Islands, Pacific Ocean.

Material examined: 1 specimen, not in permanent collection; USA, Friday Harbor Laboratories, floating docks, 48.54514° -123.01206°, 0.5 m depth; collection date 19.05.2011; depicted in Fig. 12; DNA isolate 868; GenBank numbers of sequences see Table 1. – Tissue samples and photos of two medusae here identified as *Catablema* cf. *multicirratum* from north of Svalbard obtained from Aino Hosia (University Museum of Bergen); the rest of the medusae in the collections of the Bergen Museum. The collection data are given in Table 1.

Diagnosis: *Catablema* medusa with umbrella height and diameter 30 to 65 mm including large dome-like apical projection corresponding to about half the total bell height. Manubrium with very broad, quadrangular base, long mesenteries, mouth margin variably folded, gonadal folds oblique to vertical, few or no pits. Mature animals with 80 to 160 tentacles, without or only few marginal bulbs between tentacles in adult specimens. Radial canals relatively short but very broad and with large, complex lateral outgrowths. No ocelli observed. Stomach and marginal bulbs light orange in living specimens.

Hydroid not known.

Remarks: *Catablema multicirratum* was somewhat inadequately described by Kishinouye (1910), with the sole diagnostic character distinguishing it from *C. vesicarium* being the tentacle number, given as “several hundreds.” This must certainly be erroneous.

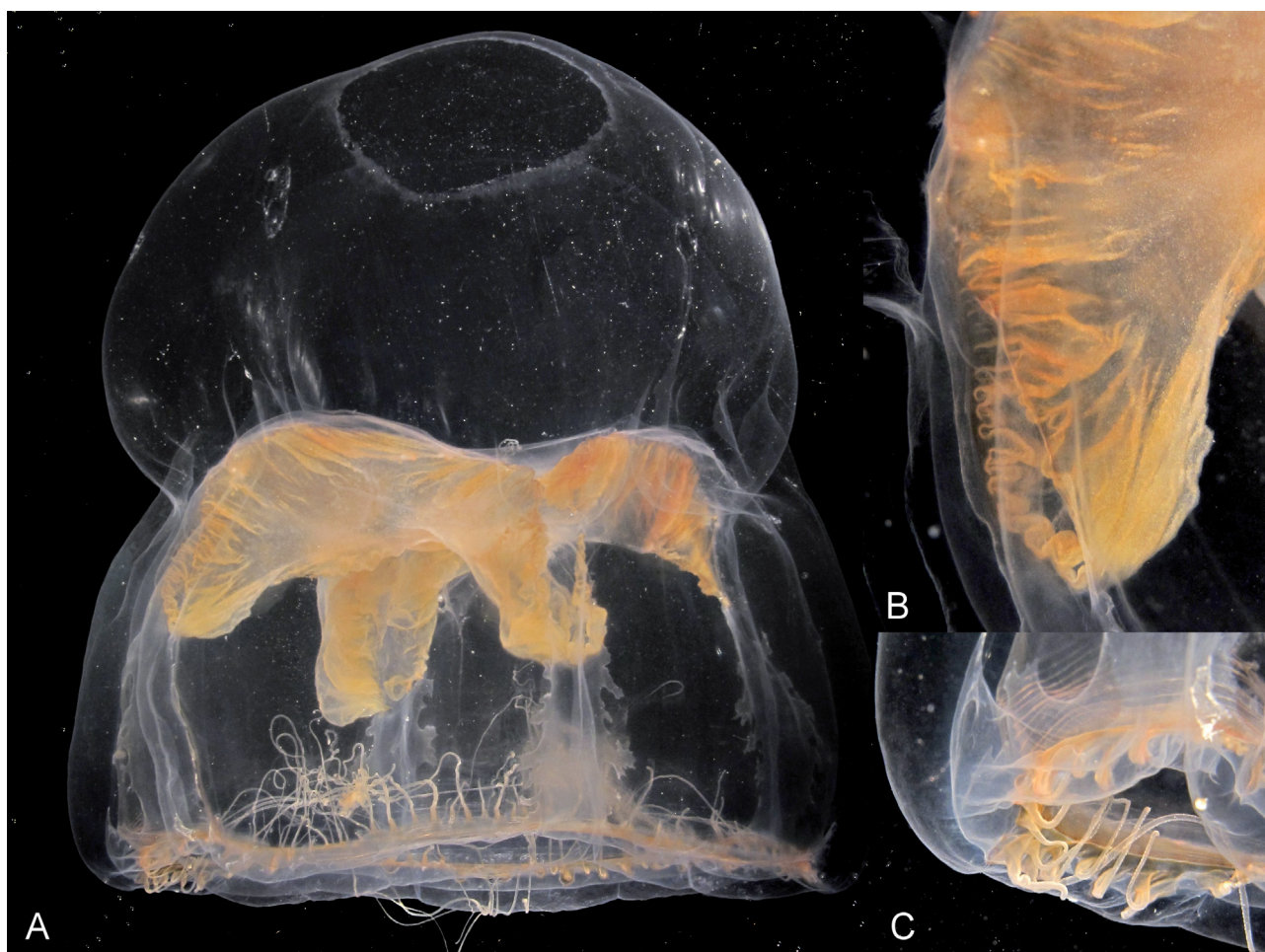


Fig. 12. *Catablema multicirratum*, living medusa from Friday Harbor, WA, USA, bell size about 6.5 cm, about 100 tentacles. The individual was used to obtain the DNA sequences of this study (DNA 868, see Table 1). (A) Whole medusa. The circular spot on the apical process is an area where the epidermis is lost due to contact with the water-air interface. (B) Detail of stomach with gonad folds. (C) Detail of umbrella margin with tentacles.

Bigelow (1913) then described and illustrated new material from the Bering Sea and the Gulf of Alaska. The species was subsequently also recorded from the west coast of Greenland by Kramp (1926). The Atlantic medusae were distinctly smaller, but had the same high number of tentacles. Although the species has been reported regularly (see Arai & Brinckmann-Voss, 1980; Wang *et al.*, 2014), only a few specimens have been documented. It seems that it has sometimes also been confused with *N. brevicornis* (e.g. Naumov, 1969). According to our current knowledge the tentacle number permits a reliable separation of *C. vesicarium* and *C. multicirratum*.

The Pacific specimen of *Catablema multicirratum* used for this study was identified based on Arai & Brinckmann-Voss (1980). The single animal was very large, reaching 6.5 cm in height (Fig. 12) and had approximately 100 tentacles. It was thus easily separable from the *Catablema vesicarium nodulosum* (Fig. 11) found at the same place. The two medusae from Svalbard (Fig. 13) were smaller

and had denser tissues with a darker orange colour than the Pacific specimen.

While morphologically separable, the status of the species remains somewhat problematic when using 16S, COI, and ITS sequence data. 16S and ITS sequences cannot be used to separate *C. multicirratum* from *C. vesicarium* (Fig. 8; Table 2). COI has about three times higher divergence values than 16S and permits to discern somewhat more structure in the *Catablema* clade (Fig. 9). The Pacific *Catablema multicirratum* separates from both, *C. vesicarium* and the Atlantic *C. multicirratum*. The Atlantic form is thus perhaps also an independent lineage and it was therefore named here *C. cf. multicirratum*.

The BOLD barcode database contains some additional COI sequences of *Catablema* samples, mostly identified as *C. vesicarium*. The origin of the material is from the Pacific and Atlantic coasts of Canada, but unfortunately the identifications are unreliable and the accompanying photos virtually useless. Due to the doubtful identities,



Fig. 13. *Catablema* cf. *multicirrata*, living subadult specimen from Svalbard (sample DNA 1139), photo courtesy of Aino Hosia. Note the high number of tentacles, estimated about 140 combined with the moderate size of the bell.

these sequences were therefore not included in the analyses of this study. Adding nevertheless these sequences to the ML-analysis (results not shown), the results remain similar to the one shown in Fig. 9. *Catablema* appears to be split into three clades with relatively low divergences: *C. vesicarium*, *C. multicirratum*, and *Catablema* from Svalbard.

However, it must be concluded that more *Catablema* samples with a thorough documentation and identification of the specimens are needed before any reliable conclusion is possible. Markers with more resolving power (e.g. microsatellites) might be necessary to settle the status of all nominal *Catablema* species. It is still possible that they all represent only different age groups and local variants.

Genus *Leuckartiara* Hartlaub, 1914

Type species: *Geryonia octona* Fleming, 1823 (Kramp, 1959).

Remarks: For the diagnosis see Bouillon *et al.* (2006) or Schuchert (2007). A key to all species is provided by Xu & Huang (2004), a comparative table of the species is also presented in Pagès *et al.* (1992). A list of all species, including also the ones described after 2004, is given in Schuchert (2017b).

Leuckartiara cf. *octonema* Xu, Huang & Guo, 2007

Leuckartiara octonema Xu, Huang & Guo, 2007: 70, fig. 5.

Type locality: Upwelling zone in the southern part of the Taiwan Strait (21°40'–23°51'N 116°47'–118°56'E).

Material examined: MHNG-INVE-97018; hydroid colony, young medusae, and medusae cultivated to maturity (31 days) by Takanori Suehiro; Japan, Mie, Honshu, Toba City, intertidal zone, 34.47806°N 136.8675°E; date collected 09.05.2014; DNA sample 1208; for GenBank number of sequences see Table 1.

Remarks: The material used to obtain the DNA sample and the details of the life cycle will be described by Suehiro & Kubota (2018).

The morphology of the adult medusa corresponds to *Leuckartiara octonema*, except for the presence of ocelli on the rudimentary bulbs. Therefore, the species was provisionally identified as *Leuckartiara* cf. *octonema* only, pending further sequence comparisons with specimens from near the type locality.

Leuckartiara longicalcar n. spec.

Figs 14, 15A–C, 16

in part *Leuckartiara octona*. – Mackie & Mackie, 1963: 68. [not *L. octona* (Fleming, 1823)]

Leuckartiara species. – Arai & Brinckmann-Voss, 1980: 56, fig. 30.

Holotype: MHNG-INVE-98638; female; USA, San Juan Island, Friday Harbor, 48.54514° -123.01206°,



Fig. 14. *Leuckartiara longicalcar*, n. spec., living animal (paratype), total height about 15 mm.

depth 0.5 m; collection date 20.05.2011; preserved in formalin, subsequently transferred to ethanol.

Paratypes: MHNG-INVE-78922, 9 specimens; USA, San Juan Island, Friday Harbor, 48.54514° -123.01206°, depth 0.5 m; collection date 20.05.2011; one specimen used to isolate DNA 869; for GenBank numbers of sequences see Table 1. – MHNG-INVE-82312, 2 specimens; Canada, British Columbia, Salish Sea, 49.2505° -123.74867°, depth 0-50 m; collected by Moria Galbraith; preserved in formalin, subsequently transferred to ethanol.

Additional data: Several photographs of living medusae taken by Kevin Lee off the coast of Palos

Verdes, California, USA, 33.8211° -118.4569°, one of the photos is reproduced here in Fig. 16.

Etymology: From the Latin *longus*, long, and *calcar*, spur, referring to the long abaxial spurs of the tentacle bulbs.

Type locality: USA, San Juan Island, Friday Harbor, 48.54514°N 123.01206°W.

Diagnosis: *Leuckartiara* medusa 15-20 mm total height, with large pointed apical process of about 1/3 to 2/5 of total bell height, umbrella higher than wide; up to 16-24 tentacles, between each tentacle pair 1-3 small, rudimentary bulbs, perradial and interradial tentacles with conspicuous, long, pointed abaxial spurs

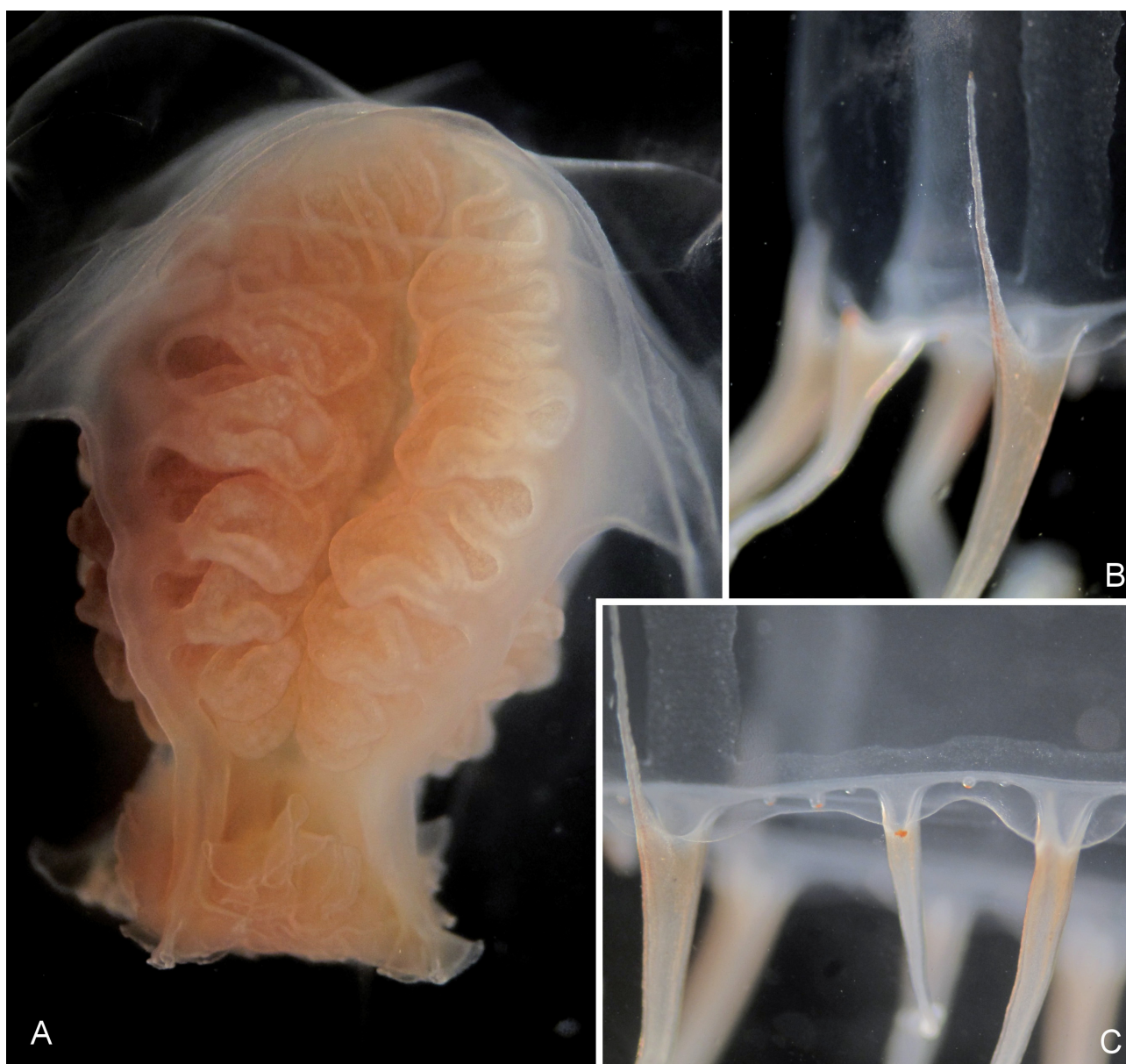


Fig. 15. *Leuckartiara longicalcar*, n. spec., living animal. (A) Details of manubrium with gonad folds. (B) Lateral view of a perradial tentacle with the characteristic, long, abaxial spur. (C) Adradial tentacles and rudimentary bulbs, note red ocelli.

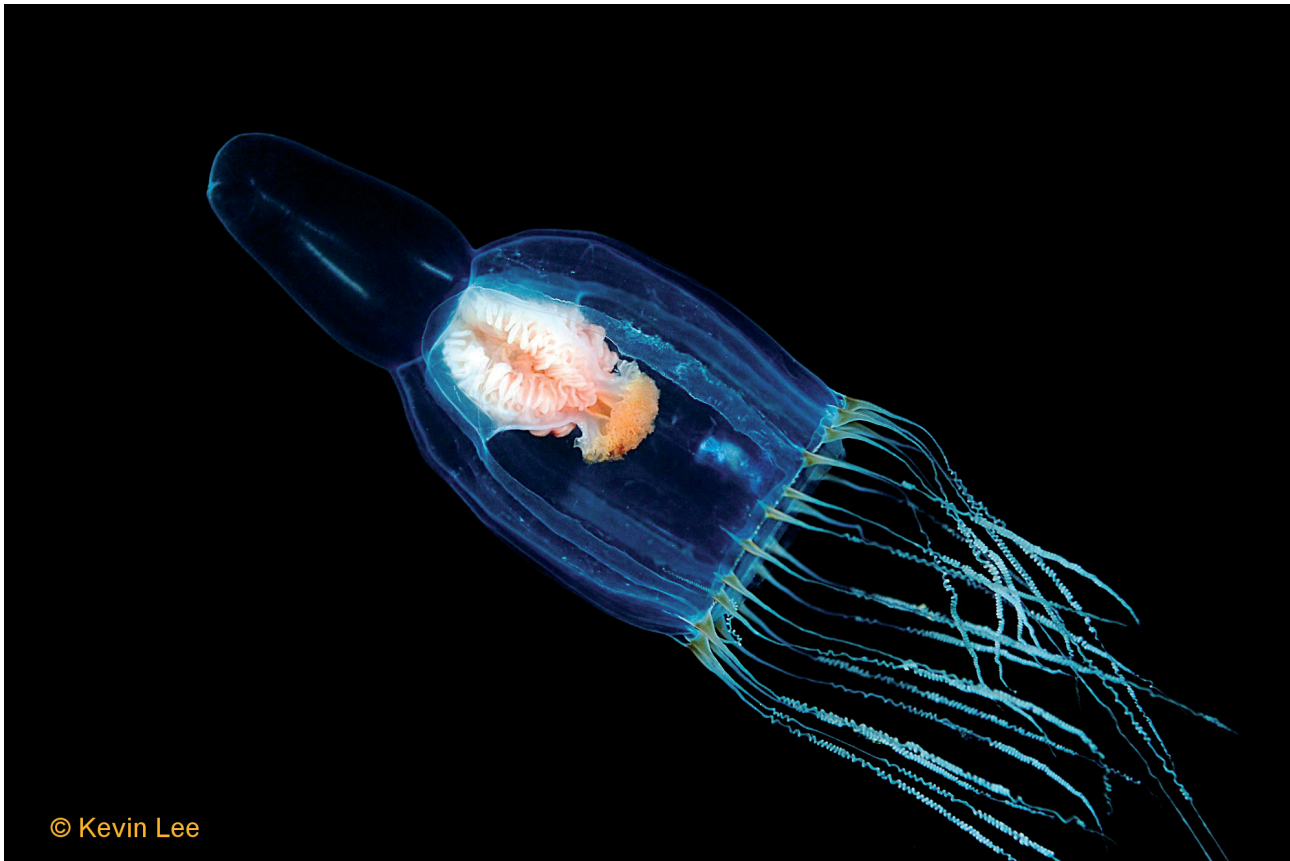


Fig. 16. *Leuckartiara longicalcar*, n. spec., living animal, height 13-18 mm, photographed by Kevin Lee off Los Angeles, California, USA. Note, this photograph is copyright protected and permission to use it here has been obtained by paying a royalty fee to the author and copyright holder Kevin Lee (www.diverkevin.com).

reaching up to 1/6 of the bell height; tentacles and bulbs lacking tentacles usually with small red abaxial ocelli. Manubrium about 1/2 of subumbrellar height, pale-orange, with long mesenteries, mouth cruciform, mouth margin moderately ruffled. Gonads in adradial series of horizontal folds, distinct interradianal connecting fold absent.

Description: *Leuckartiara* medusa up to 15-20 mm in height and about 10 mm in diameter when mature, with a large, pointed apical process of about 1/3 to 2/5 of total bell height, umbrella higher than wide. Interradianal, subumbrellar pockets of variable size present.

Manubrium about half the height of the subumbrellar height, shaped like inverted vase, connected to radial canals via long mesenteries (about 1/3 of manubrium height). Manubrium base and mouth opening cruciform, mouth rim moderately ruffled.

Gonad tissue in 8 series of broad, adradial, horizontal folds, 8-12 folds in an adradial series, many folds with a central depression and resembling a loop or simply bifurcated (Fig. 15A). The two series of gonad folds of one quadrant usually not connected by a fold across the interradianal region as in other congeners (thus without the H-form of the gonad folds, often described as “horse-

shoe shape” in older publications, comp. Fig. 17C). Sometimes an inconspicuous interradianal connection of the two rows of folds may be present at the aboral end of the manubrium. No gonadal pits. Egg size about 0.1 mm. Radial canals slightly jagged and broad. Ring canal smooth, broad.

Tentacles usually 16, sometimes up to 24, between each tentacle pair 1-3 small, rudimentary bulbs without tentacles. Bases of tentacles laterally compressed, clasping bell margin. Perradianal and interradianal tentacle bases (oldest tentacles) with long, pointed abaxial spurs, reaching up to 1/6 of the bell height (Fig. 15B), shorter (younger) tentacles with short spur or no spur. Spurs appear solid, without internal canal. A small red ocellus present on most tentacles and also rudimentary bulbs, situated on abaxial side at interface of tentacle to exumbrella, in tentacles with long abaxial spurs ocelli at end of spur.

Colour: Manubrium pale orange, proximal parts of tentacles pale orange to yellowish, ocelli orange-red.

Nematocysts of tentacles microbasal heteronemes, ca. 4 x 7 μ m.

Distribution: North-eastern Pacific, from Vancouver Island to Southern California.

Remarks: This species was described by Arai & Brinckmann-Voss (1980: 56) as *Leuckartiara* species distinct from *L. octona*. Dr Anita Brinckmann-Voss (pers. com., 2013) told me that she initially intended to name it in a subsequent publication, but was now unable to do it and encouraged me to do it myself.

Leuckartiara longicalcar does not match any of the known species (Kramp, 1968; Pages *et al.*, 1992; Xu & Huang, 2004; Schuchert, 2017). It has previously been misidentified as *L. octona* (Fleming, 1823) and been considered related to *L. zaca*e Bigelow, 1940 (see Arai & Brinckmann-Voss, 1980).

Leuckartiara octona is indeed similar in appearance, but lacks the long abaxial spurs and regularly has a fold across the interradial region connecting the adradial series of folds. The 16S and COI sequence data (Figs 8-9) clearly separated *L. longicalcar* from the Atlantic *L. octona*, although they are closely related.

*Leuckartiara zaca*e Bigelow, 1940 is a rare species first found in the Gulf of Panama. It is somewhat larger than *L. longicalcar* and has about the same number of tentacles. The most prominent difference is the length of the tentacle spurs: they are much longer and extend up to 2/3 of the bell height. Bigelow (1940) described them as exumbrellar ribs containing a thin gastrodermal canal. Additionally, *L. zaca*e has no apical process (but Kramp (1965) observed a small process in a juvenile specimen from Indonesia, the identity of this material is perhaps questionable), the umbrella without the process is larger

(21 versus 12 mm), the manubrium is more voluminous and has more gonadal folds. It is only known to occur in tropical seas (Kramp, 1965).

Other *Leuckartiara* species with tentacle spurs are *L. gardineri* Browne, 1916, *L. acuta* Brinckmann-Voss, Arai & Nagasawa, 2005, and *L. fujianensis* Huang, Xu, Lin & Qiu, 2008. All three have only four fully formed tentacles.

Kevin Lee (2017) published a series of magnificent photos of *Leuckartiara* medusae observed off Los Angeles, California. One of them is reproduced here (Fig. 17). These medusae must clearly be referred to *L. longicalcar* n. spec. Some of the individuals are almost identical to the ones shown here, while others (Fig. 17) appear somewhat larger, with up to 24 tentacles, and a more voluminous stomach. Some of the individuals have a more intense colour, appearing more reddish, and also the tentacle bases show some reddish pigments. The distribution of the species extends thus from Vancouver Island to Southern California.

***Leuckartiara* spec.**

Fig. 17A-C

Material examined: MHNG-INVE-78921, 1 of originally 2 specimens; USA, San Juan Island, Friday Harbor, 48.54514° -123.01206°, depth 0.5 m; collection date 22.05.2011; one medusa used to extract DNA,

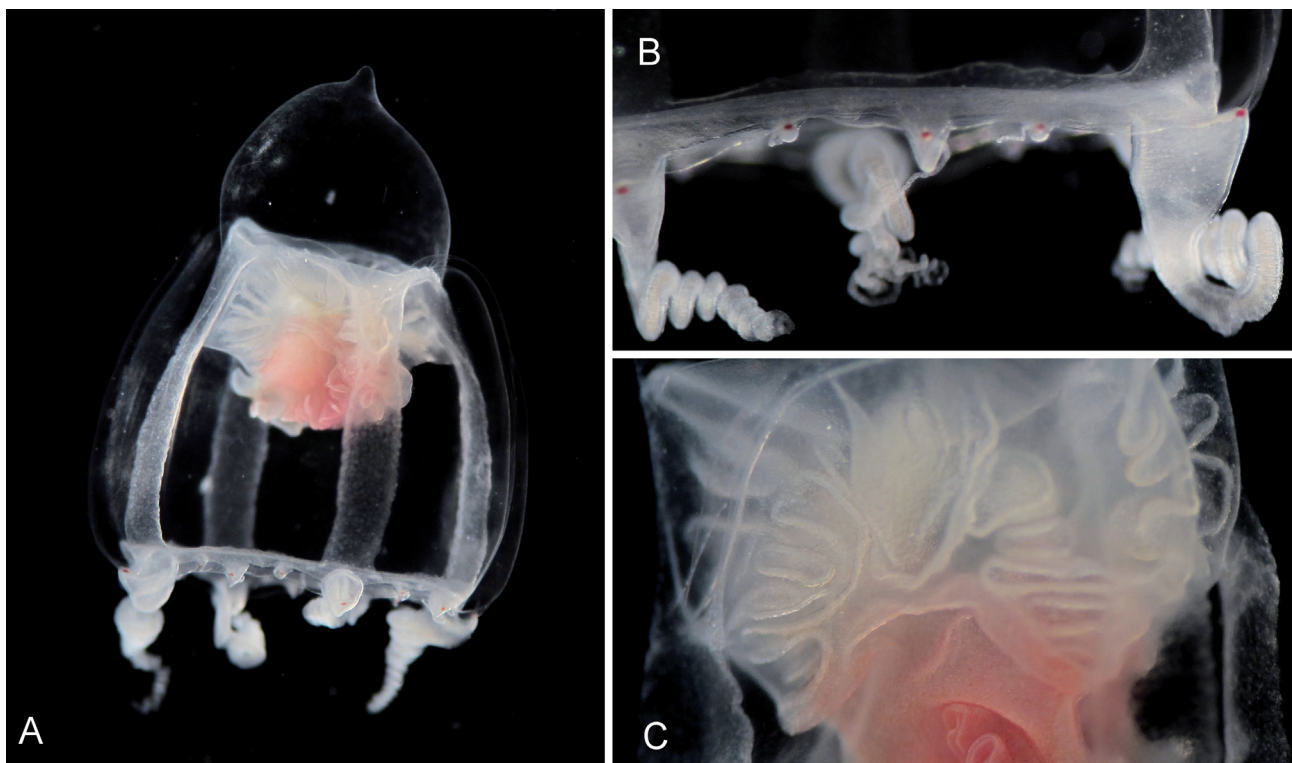


Fig. 17. *Leuckartiara* spec., living animal. (A) Whole medusa. (B) Bell margin with tentacle bases. (C) Details of manubrium with gonad folds (immature?).

isolate 871; for GenBank numbers of sequences see Table 1.

Diagnosis: Subadult *Leuckartiara* medusa up to 8 mm total height, with large pointed apical process of about 1/3 of total bell height, umbrella as wide as high. 4 perradial tentacles, 4 shorter interradial tentacles, 8 adradial small stumps or thin- and short tentacles. Tentacle bases not much laterally compressed, not clasping bell margin, without abaxial spurs, with red abaxial ocelli. Manubrium base and mouth cruciform, mouth margin with some folds. Gonad folds on manubrium in typical H-like arrangement, adradial folds directed perradially. Radial canals very broad, smooth, long mesenteries present (about 1/2 of manubrium height).

Remarks: These two medusae were obviously not fully developed and they could not positively be identified with any species described in Arai & Brinckmann-Voss (1980). Most probably it belongs to the species identified as *L. nobilis* Hartlaub, 1914 by Foerster (1924) and Arai & Brinckmann-Voss (1980). The latter authors report that their adults reached 22 mm in height. The younger specimens described by Foerster (1924) agreed with the current material. The 16S and

COI sequences, however, did not match the Atlantic specimen of *L. nobilis*. The latter was described in Schuchert (2007), but it was also not fully mature. More Atlantic and Pacific specimens fitting the description of this rare species must be examined in order to get a clearer picture of its identity.

Genus *Halitholus* Hartlaub, 1914

Type species: *Halitholus pauper* Hartlaub, 1914 (Kramp, 1959).

Remarks: For the diagnosis see Bouillon *et al.* (2006) or Schuchert (2007).

Halitholus spec.

Fig. 18A-C

in part *Leuckartiara octona*. – Mackie & Mackie, 1963: 68 [not *L. octona* (Fleming, 1823)]

Halitholus species I. – Arai & Brinckmann-Voss, 1980: 48, figs 23-24.

Material examined: 1 female specimens, not in permanent collection; USA, San Juan Island, Friday

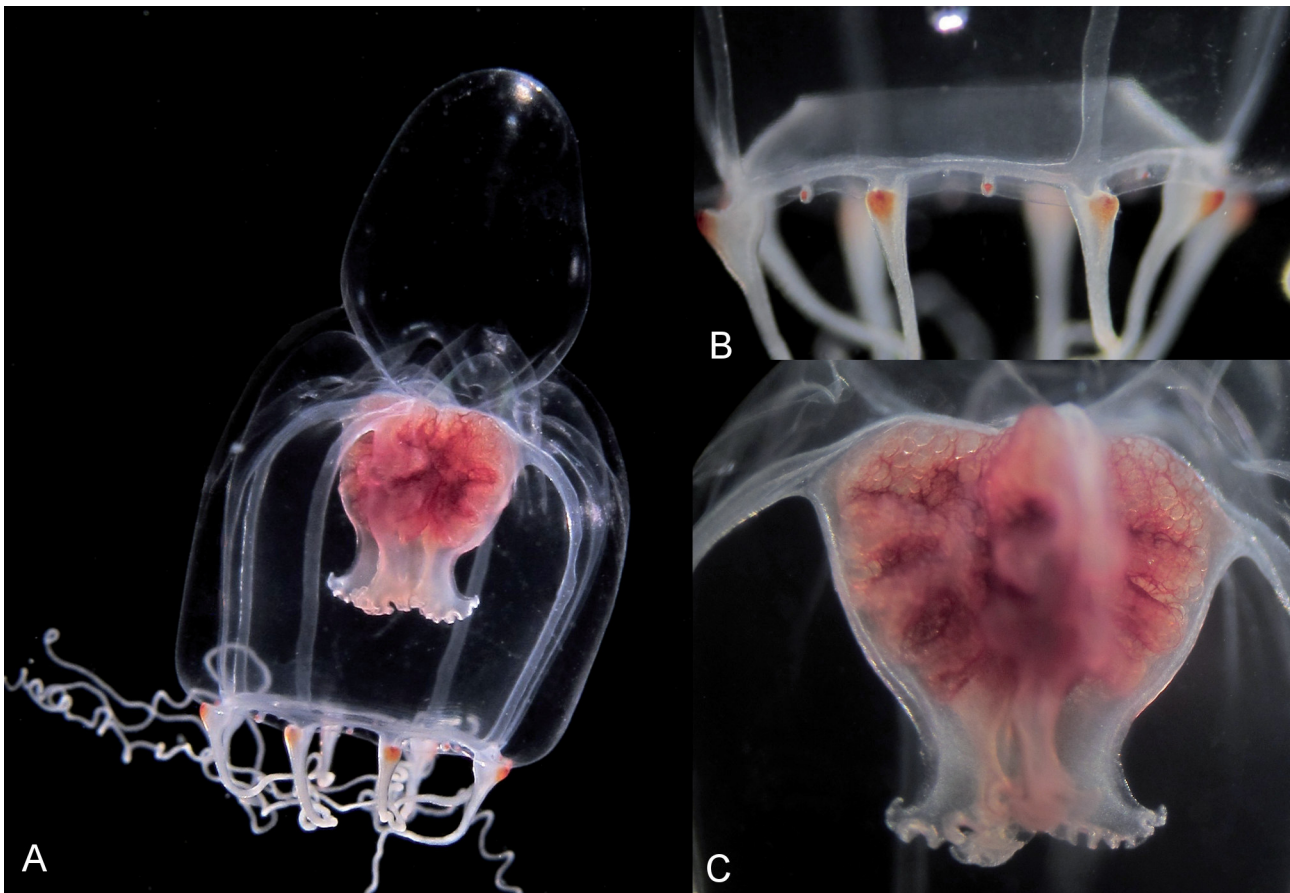


Fig. 18. *Halitholus* spec., living animal. (A) Whole medusa. (B) Bell margin with tentacle bases. (C) Details of manubrium with gonad folds. Note the clearly visible oocytes.

Harbor, 48.54514° -123.01206°, depth 0.5 m; collection date 20.05.2011; DNA isolate 870; for GenBank numbers of sequences see Table 1.

Remarks: This species was described by Arai & Brinckmann-Voss (1980) as *Halitholus* species I. They kept it distinct from *H. pauper* Hartlaub, 1914 on account of the lacking interradial gonad-fold connection and the smoother radial canals. However, the latter character appears not so convincing. As they also found a second similar morphotype (as *Halitholus* species II) they did not describe it as a new species.

I suspect that *Halitholus* species I and *Halitholus pauper* in Arai & Brinckmann-Voss (1980) are the same, but it is not clear if they are really conspecific with *H. pauper* from the Atlantic Ocean (see Schuchert, 2007 for description). In Atlantic *H. pauper*, the interradial tentacles are usually not fully developed and the deep interradial subumbrellar pockets as visible in Fig. 18A have not been reported. Good photographs and also DNA barcode sequences of the Atlantic form are needed to evaluate more precisely the specific identity of the present material.

Even though the present medusa had no distinct mesenteries (Fig. 18A), the only character that distinguishes the genus *Halitholus* from *Leuckartiara*, the 16S and COI sequences placed it close to *Leuckartiara octona*, type species of the genus (Figs 8-9). The other *Halitholus* species used in the analysis, *H. cirratus*, did not cluster with the *Halitholus* from Friday Harbor, which casts some doubts on the validity of the genus.

ACKNOWLEDGEMENTS

This study would not have been possible without the generous gifts of samples by my colleagues. I owe special thanks to Maciej Manko for collecting and giving me the *Neoturris* and *Pandea* samples from Villefranche-sur-Mer. Likewise, I thankfully acknowledge Christane Todt (Bergen University) for the epibenthic sampling of *Neoturris* polyps. I'm grateful to Takanori Suehiro for letting me have *Pandeopsis* and *Leuckartiara* medusae, and Aino Hosia for the gift of *Catablema* tissue samples. Paulyn Cartwright (Kansas University) covered the larger part of the costs of my stay at the Friday Harbor Laboratories and I wish to thank her for this generosity.

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