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A new species of *Cyanea* jellyfish sympatric to *C. capillata* in the White Sea

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Abstract *Cyanea* is a genus of large bloom-forming scyphozoans, including some of the most conspicuous representatives of megaplankton. Its taxonomy has been revised repeatedly throughout the last century due to the fact that most of the morphological characteristics of *Cyanea* species, such as color, structure of gastrovascular system and number of tentacles, may overlap greatly in different populations. Here, we report a new species of *Cyanea*, *Cyanea tzetlinii* sp. nov., from the White Sea, which is distinguishable from all previously described *Cyanea* species by an eye-spot-bearing bulb formed at the base of each rhopalium. This well-recognizable morphological characteristic is supported at the molecular level by a substantial genetic distance in mitochondrial (CO1: 9.6–10.6 %, 16S RNA: 3.1–3.5 %) as well as nuclear (ITS: 5.0 %, 18S RNA: 0.1 %) loci, making it the sister species to *Cyanea capillata*. Taking into account the young geological age of the White Sea and a substantial genetic divergence between *C. tzetlinii* sp. nov. and the nearest sister species, we suppose that *C. tzetlinii* sp. nov. has been advected to the White Sea from elsewhere and may also inhabit other Arctic seas. Past ecological studies in the White Sea and possibly in other Arctic Seas could have conflated *C. tzetlinii* sp. nov. with other species, which likely affected the analyses.

Keywords Scyphozoa · *Cyanea* · Medusae · Taxonomy · Rhopalium · Molecular genetics · Biodiversity

Introduction

Populations of pelagic animals are often depleted in barriers to reproduction and dispersal and therefore have a high proportion of widespread or cosmopolitan species, in particular those of scyphozoans (Mayer 1910; Kramp 1961; Brewer 1991; Palumbi 1992; Dawson and Jacobs 2001). Vast ranges, combined with often huge population sizes, contribute to a high level of within-species variability in morphological traits, sometimes complicating taxonomy (Sparmann 2012). One remarkable example is *Cyanea*, a genus of majestic bloom-forming scyphozoans which is widely distributed around the world, from the temperate to boreal and polar waters (Lamarck 1837, 1840; Agassiz 1862; Vanhöffen 1888; Mayer 1910; Naumov 1961; Dawson 2005), and includes the notorious lion's mane *Cyanea capillata* (von Linnaeus 1758). Over the last two centuries, the species-level taxonomy of genus *Cyanea* has been repeatedly and extensively revised (von Linnaeus 1758; Lamarck 1837, 1840; Agassiz 1862; Haeckel 1879; Mayer 1910; Bigelow 1926; Kramp 1961; Dawson 2005; Sparmann 2012). This has two main explanations. Firstly, the fragility of large-bodied scyphozoans leads to their frequent destruction during collection with nets and poor preservation of

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museum material (Hay et al. 1990; Raskoff et al. 2003, 2010). Scyphozoans (or fragments of their bodies) are often ignored or misidentified in ordinary zooplankton samplings with conventional plankton nets. Therefore, the information about their distribution, abundance and diversity is lacking (Naumov 1961; Ospovat 1985; Stepanjants 1989; Raskoff et al. 2003; 2010; Lilley et al. 2011).

The second explanation is a considerable morphological variability of *Cyanea* species (Mayer 1910; Bigelow 1926; Dawson 2005; Sparmann 2012). Several *Cyanea* species described in the 1700–1900s have been synonymized in the 1910–1960s, because in many of the morphological traits such as color, size, and organization of muscular and gastrovascular systems, the interspecies variation was found to be comparable or exceed the intraspecies variation (von Linnaeus 1758; de Lamarck 1840; Agassiz 1862; Mayer 1910; Bigelow 1926; Stiasny and van der Maaden 1943; Kramp 1961; Russell 1970; Dawson 2005). Reorganization of the genus *Cyanea* over the years has been reviewed in detail by Dawson (2005) and Sparmann (2012).

The advent of molecular methods has led to a revision of many of the previously distinguished taxa and, often, to a radical increase in the taxonomic diversity due to discovery of a large number of new species (Knowlton 1993, 2000; Hillis and Wiens 2000; Féral 2002; Graham and Bayha 2007). Molecular methods applied to jellyfish allow distinguishing species relatively easily, because the difference between the within- and the between-species genetic distances tends to be large. In the scyphozoans of the North Sea, the range of the intraspecies differences in the CO1 marker is 0–2.1 %, while the range of the differences between species in a genus is 7.5–20.7 % (Dawson and Jacobs 2001; Dawson 2004; Holst and Laakmann 2013).

Here, we describe a new *Cyanea* species found in the White Sea, where only *C. capillata* has been reported previously. Morphologically, *Cyanea* sp. nov. is easily distinguishable from all described *Cyanea* species by an eye-spot-bearing bulb in the basal part of each of the eight rhopalia upon the subumbrellar side of the bell, as well as by a unique combination of other morphological traits (the shape of bell margin, gastrovascular system, color and nematocyst clusters on the exumbrellar surface). At the molecular level, *Cyanea* sp. nov. is characterized by substantial differences from the sister species *C. capillata* both in nuclear and mitochondrial loci, which warrants distinguishing a new *Cyanea* species.

Materials and methods

Sampling location and processing

Specimens of *Cyanea* sp. nov. were collected in the Kandalaksha Bay of the White Sea (Fig. 1); five specimens were collected in July 2010 and 69 ones in July–August 2014. Medusae were collected by hand from the pier and from boat near the pier of the N.A. Pertsov White Sea Biological Station of the Moscow State University (66.55379N, 33.10473E), or by SCUBA diving approximately 6 km east of the Biological Station (66.54373N, 33.18399E), and transported to the laboratory in buckets with seawater. Both morphological and molecular investigations were conducted in laboratories of the White Sea Biological Station of the Moscow State University.

Morphological analysis

Live medusae were examined with the Leica M 165 C stereomicroscope. Digital images were captured by Leica DFC 290 camera with Leica Application Suite software. Particular attention was given to traits commonly used for *Cyanea* classification such as the color, shape of the bell marginal lobes, presence or absence of intrusions in circular musculature, anastomoses in the gastrovascular system, eye-spot-bearing bulbs, smoothness of bell surface and number of tentacles. For the molecular investigations, each animal was biopsied for a tissue sample of coronal musculature. These samples had been preserved in 96 % ethanol before medusae were fixed in 10 % formaldehyde buffered in seawater.

DNA amplification and sequencing

Genomic DNA was extracted from 49 jellyfishes. Diatom DNA Kit (IsoGene, Moscow, Russia) was used for tissue lysis and DNA purification following the manufacturer's protocol. Polymerase chain reaction (PCR) amplification of nuclear ITS1-5.8-ITS2 S rDNA and 18S regions and mitochondrial 16S and CO1 fragments was accomplished with the primers presented in Table 1. All primers were designed with M13 tails. Loci were amplified using Encyclo PCR kit (Evrogen Joint Stock Company, Russia, Moscow). Amplification was done in a total volume of 20 μL reaction mix containing 5× PCR buffer ScreenMix, 0.4 μL of 10 μM of primer pair mix, and 1 μL of template. Reaction mixtures were heated on Veriti® Thermal Cycler to 95 °C for 300 s, followed by 40 cycles of 15 s at 95 °C, 15 s at corresponding annealing temperature given in Table 1 and 60 s at 72 °C and then a final extension of 2 min at 72 °C. The Promega PCR Purification Kit protocol (Promega) was employed to purify the amplification products. Amplification products were sequenced in both directions using M13F and M13R primers. Each sequencing reaction mixture in a total volume of 10 μL, including 1 μL of BigDye (Applied Biosystems, Perkin-Elmer Corporation, Foster City, CA), 1 μL of 1 μM primer and 0.5 μL of DNA template, ran for 25 cycles of 96 °C (10 s),

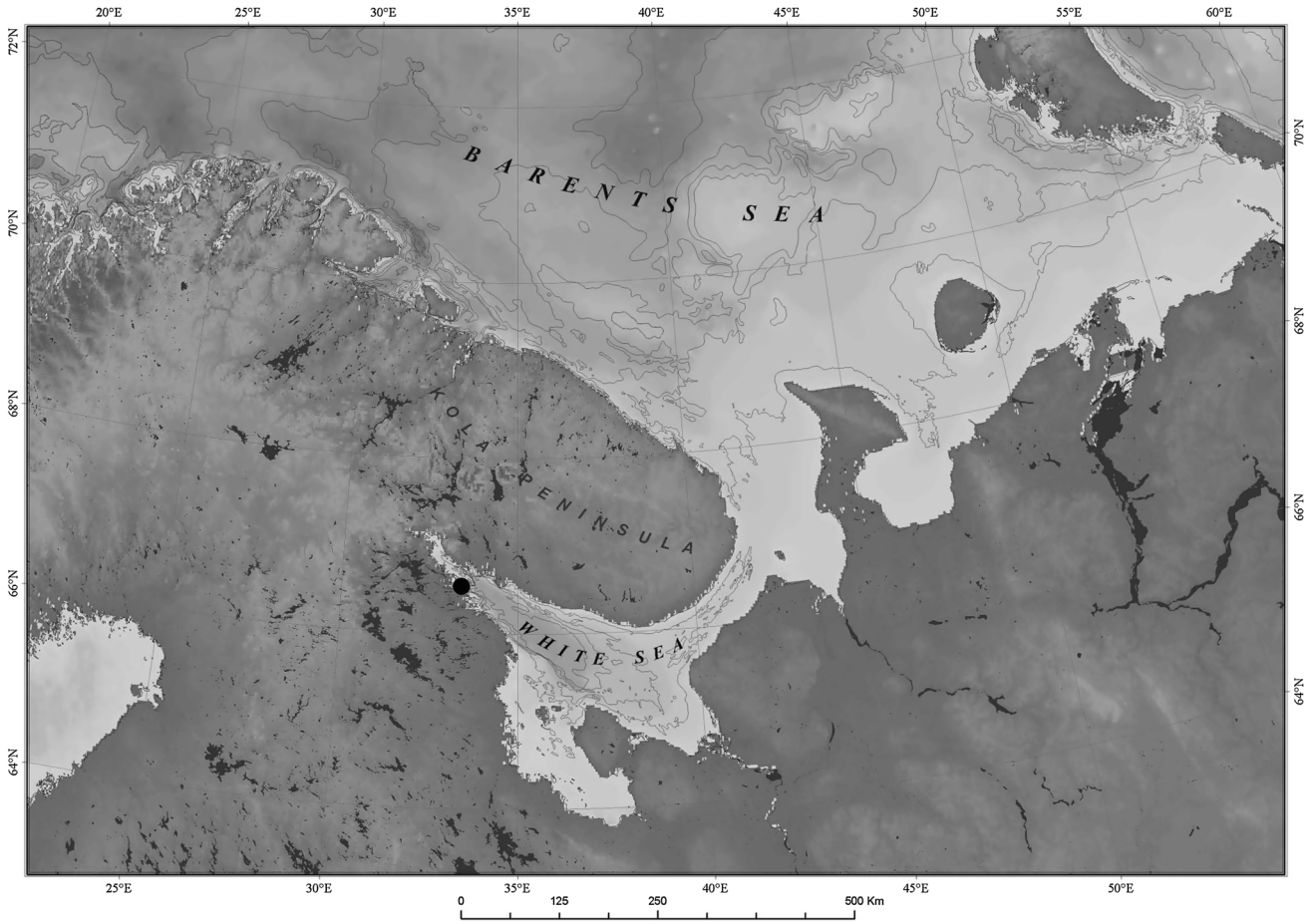

Fig. 1 Location of the study site

50 °C (5 s) and 60 °C (4 min). Sequences were purified by ethanol precipitation to remove unincorporated primers and dyes. Products were re-suspended in 12 μL formamide and electrophoresed in an ABI Prism 3500 sequencer (Applied Biosystems). GenBank accession numbers of sequences obtained in the present study are given in Table 3. To get 18S rDNA gene sequence, three overlapping fragments were sequenced and aligned.

Phylogenetic analysis

Nucleotide sequences were edited using the software CodonCode Aligner v. 5.0.2 (CodonCode Corporation, Dedham, MA, 2014) and checked for identity to nr database of GenBank by BLASTn (Altschul et al. 1990). Multiple nucleotide alignments were made with MUSCLE algorithm (Edgar 2004). Tree constructions and the calculation of pairwise genetic distances were performed using MEGA software package v. 6.06 (Tamura et al. 2013). CO1 phylogenetic tree was reconstructed with neighbor-joining algorithm (Saitou and Nei 1987) with distance matrix calculated basing on K80 model (Kimura 1980), while 16S, 18S and ITS trees were reconstructed with maximum likelihood algorithm basing on K80 model. All trees were bootstrapped 1000 times.

Results

Cyanea tzetlinii Kolbasova and Neretina sp. nov

Description

Bell of *C. tzetlinii* sp. nov. purple-red, rather flat, saucer-shaped, uniformly thickened at the central part and thin at the periphery. Bell margin divided by eight adradial clefts into eight bifurcating marginal lobes. Marginal lobes are rounded, with shallow tertiary cleft (Fig. 1a, b). The center of exumbrella smooth, without papillae, the periphery of exumbrella with small nematocyst clusters. Eight rhopalia located in deep club-shaped rhopalial pits on eight marginal lobes; each rhopalial stalk bears proximally an ectodermal bulb ~1/3 of the length of the rhopalium (Figs. 2a, b, 3a, b). Rhopalial bulb contains pit of the

Table 1 Primers used for PCR

Name	Sequence	Molecular marker	Length of PCR product (bp)	T_m (°C)	Source
18S1F	TGTAAAACGACGGCCAGTTACCTGGTTGATCCTGCCAGTAG	18S	966	49	Giribet et al. (1996)
18S5R	CAGGAAACAGCTATGACCTTGGCAAATGCTTTCGC	18S			
18S3F	TGTAAAACGACGGCCAGTGTTCGATTCCGGAGAGGGA	18S	963	49	Giribet et al. (1996)
18Sbi	CAGGAAACAGCTATGACGAGTCTCGTTCGTTATCGGA	18S			
18Sa2.0	TGTAAAACGACGGCCAGTATGGTTGCAAAGCTGAAAC	18S	683	52	Giribet et al. (1999)
18S9R	CAGGAAACAGCTATGACGATCCTTCCGCAGGTTCACCTAC	18S			
16SF (Cnidaria)	TGTAAAACGACGGCCAGTTCGACTGTTTACCAAAAACATAGC	16S	658	52	Bridge et al. (1992)
16SR (Cnidaria)	CAGGAAACAGCTATGACACGGAATGAACTCAAATCATGTAAG	16S			
dgLCO-1490-tailed	TGTAAAACGACGGCCAGTGGTCAACAAATCATAAAGAYATYGG	CO1	658	45	Meyer et al. (2005)
dgHCO-2198	CAGGAAACAGCTATGACTAAACTTCAGGGTGACCAAARAAYCA	CO1			
LR1	TGTAAAACGACGGCCAGTGGTTGGTTTCTTTTCCT	ITS	487	52	Gardes and Bruns (1993)
SR6R	CAGGAAACAGCTATGACAAGWAAAAGTCGTAACAAGG	ITS			

rhopalial canal and an accretion of pigmented cells (eye spot). Tentacles long, arranged in eight adradial groups, with about 20–23 tentacles in each group in small individuals [~3 cm bell diameter (bd)] and 61–65 in larger ones (12–17 cm bd). Tentacles long, located at subumbrellar surface, with length exceeding the bell diameter by several fold. Interradial gastric septa in the stomach absent; numerous gastric filaments arranged in four interradial groups. Gastrovascular sinus subdivided into 16 branched pouches; marginal canals of these pouches are most dendritic, without anastomoses (Fig. 2a, b). In large individuals (12–17 cm bd), pit-like intrusions of the gastrovascular sinus are present in interradial as well as in perradial and adradial muscular fields (Fig. 2c, d). In smaller specimens (3–7 cm bd), such intrusions found only in interradial and perradial muscular fields. Mouth central, four-cornered, surrounded by four brown-red oral arms. Length of each arm equal to the radius of the umbrella or slightly exceeds it. Four large interradial gonads located in folded pouches of the stomach and hang down from the subumbrellar surface.

Diagnosis

The bell red-purple, red or pale brown, 3.2–17.0 cm in diameter. Bell margin formed by eight bifurcating marginal lobes, with width twice as large as length. The center of exumbrella smooth, the periphery of exumbrella with nematocyst clusters. Eight rhopalia located in deep club-shaped rhopalial pits at the terminal part of the marginal lobes. Each rhopalial stalk bears the single eye-spot-bearing bulb proximally. Rhopalium terminates in knob-like part containing crystalline accretion. Pigmented cells forming the ocellar spots both in the rhopalial bulb and in the terminal part of rhopalium are yellow or dark brown. Mouth large, central, four-cornered. Gastrovascular system forms eight ocular and eight tentacular gastric pouches; terminal canals of these pouches dendritic, without anastomoses. Intrusions of the gastrovascular sinus penetrate the circular musculature of the bell. Oral arms are red-violet, and slightly exceed in length the bell radius. Tentacles organized into eight horseshoe-shaped groups. Gonadal sacs large, folded sack-shaped. Mature eggs deep purple; planula pink.

Differences from other northern Cyanea species

Cyanea tzetlinii sp. nov. is distinguishable from *C. ferruginea* Eschscholtz 1929 by the presence of exsumbrellar nematocyst clusters on the periphery of the bell, rounded marginal lobes with small (shallow) tertiary clefts, and the presence of rhopalial bulbs. It is distinguishable from *C. citrea* Kishinouye 1910 by purple-red color, presence of exsumbrellar nematocyst clusters on the periphery of the

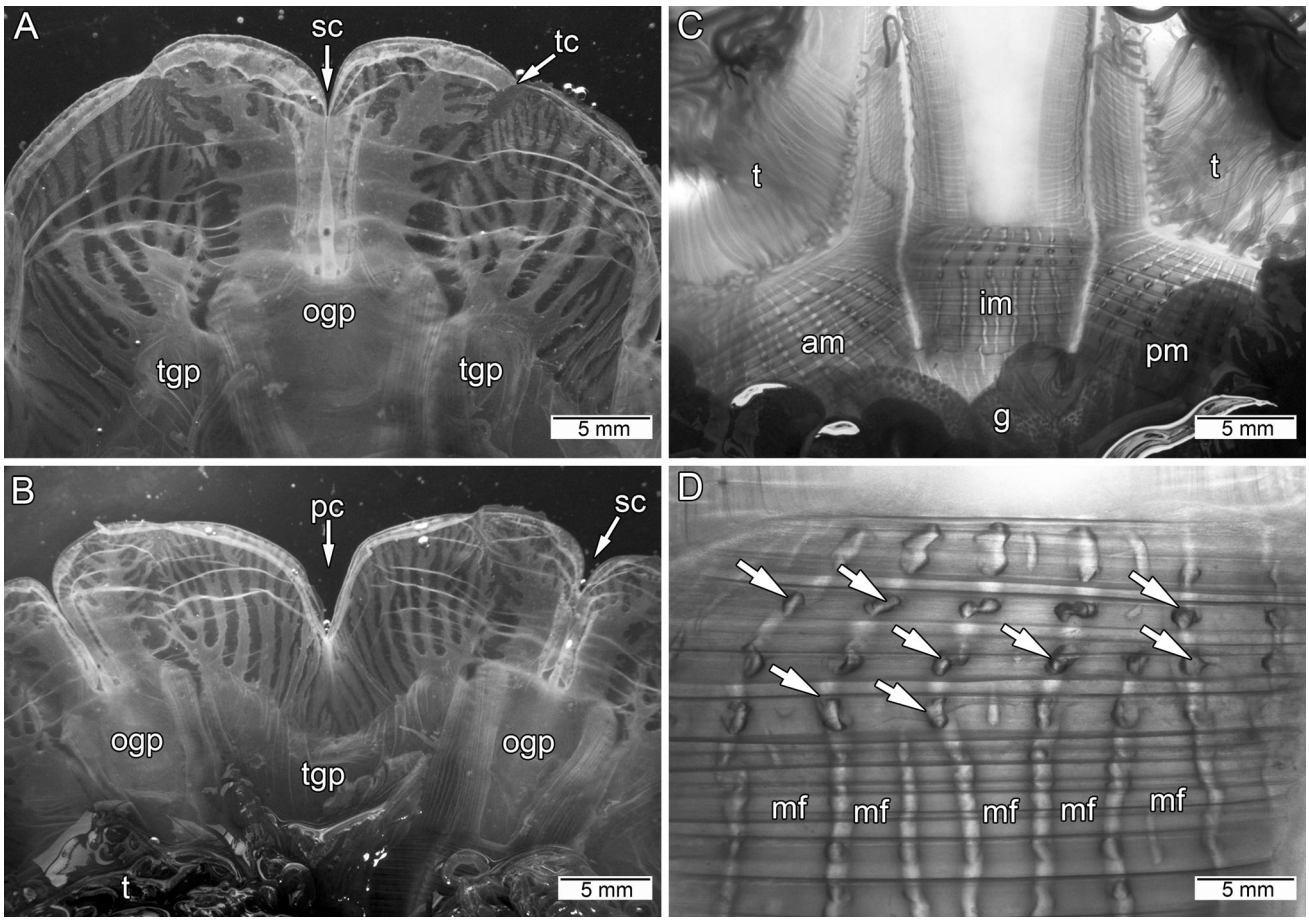

Fig. 2 Morphological characteristics of *Cyanea tzetlinii* sp. nov., subumbrellar side: **a**, **b** general view of marginal lobes of the bell, marginal canals of the gastrovascular system are dendritic, anastomoses are absent; **c**, **d** muscle folds with gastrovascular intrusions. *Abbreviations* am, adradial subumbrellar muscle field; g, gonads; im, irradial muscle field; mf, muscular fibers; ogp, ocular gastric pouch; pc, primary cleft of the marginal lobe; pm, perradial muscle field; sc, secondary cleft of the marginal lobe; t, tentacles; tc, tertiary cleft of the marginal lobe; tgp, tentacular gastric pouch; *white arrows* show intrusions of the gastrovascular system into the muscle folds

bell, presence of rhopalial bulbs, and absence of anastomoses between gastrovascular canals. It is distinguishable from *C. postelsi* Brandt 1838 by purple-red color, presence of exsumbrellar nematocyst clusters on the periphery of the bell, presence of rhopalial bulbs, and the shape of marginal lobes and gastrovascular canals. It is distinguishable from *C. lamarckii* Péron and Lesueur 1810 by purple-red color, absence of exsumbrellar papillae, presence of rhopalial bulbs and gastrovascular intrusions in muscle folds. Finally, from *C. capillata*, it is distinguishable by presence of rhopalial bulbs, by the shape of the rhopalial pits, which are club-shaped, compared to a cylindrical shape in *C. capillata* and by gastrovascular cannels without anastomoses (Figs. 3, 4).

Material examined

Holotype—WS3092, mature female 7 cm bd, collected at Kandalaksha Bay of the White Sea, Russia, in August 2014. Paratypes—WS85, WS87, WS3036, WS3076, WS3077, WS3080, WS3082, WS3086, WS3092, WS3097, WS3099, WS3100, WS3101, medusae 3.2–17.0 cm bd, Kandalaksha Bay of the White Sea, collected in June 2010 and August 2014.

Description of holotype specimen

Bell flat, circular, 7 cm bd, 15 mm thick at center, 10 mm at 1/3 bell radius from center, and 7 mm at 2/3 bell radius from center. Center of exumbrella transparent, smooth, periphery with small nematocyst clusters. Bell margin forms eight marginal lobes, and each lobe is 23 mm wide and 12 mm long. Eight rhopalia located at the bottom of deep pits, surrounded by two lips in subumbrella. Rhopalial pits club-shaped, 4.14 mm deep and 0.9 mm wide. Each rhopalium terminally bears yellow-colored pigmented cells and crystalline accretion. Rhopalial bulb located at the base of the rhopalial stalk. Each bulb is penetrate by the pit of rhopalial

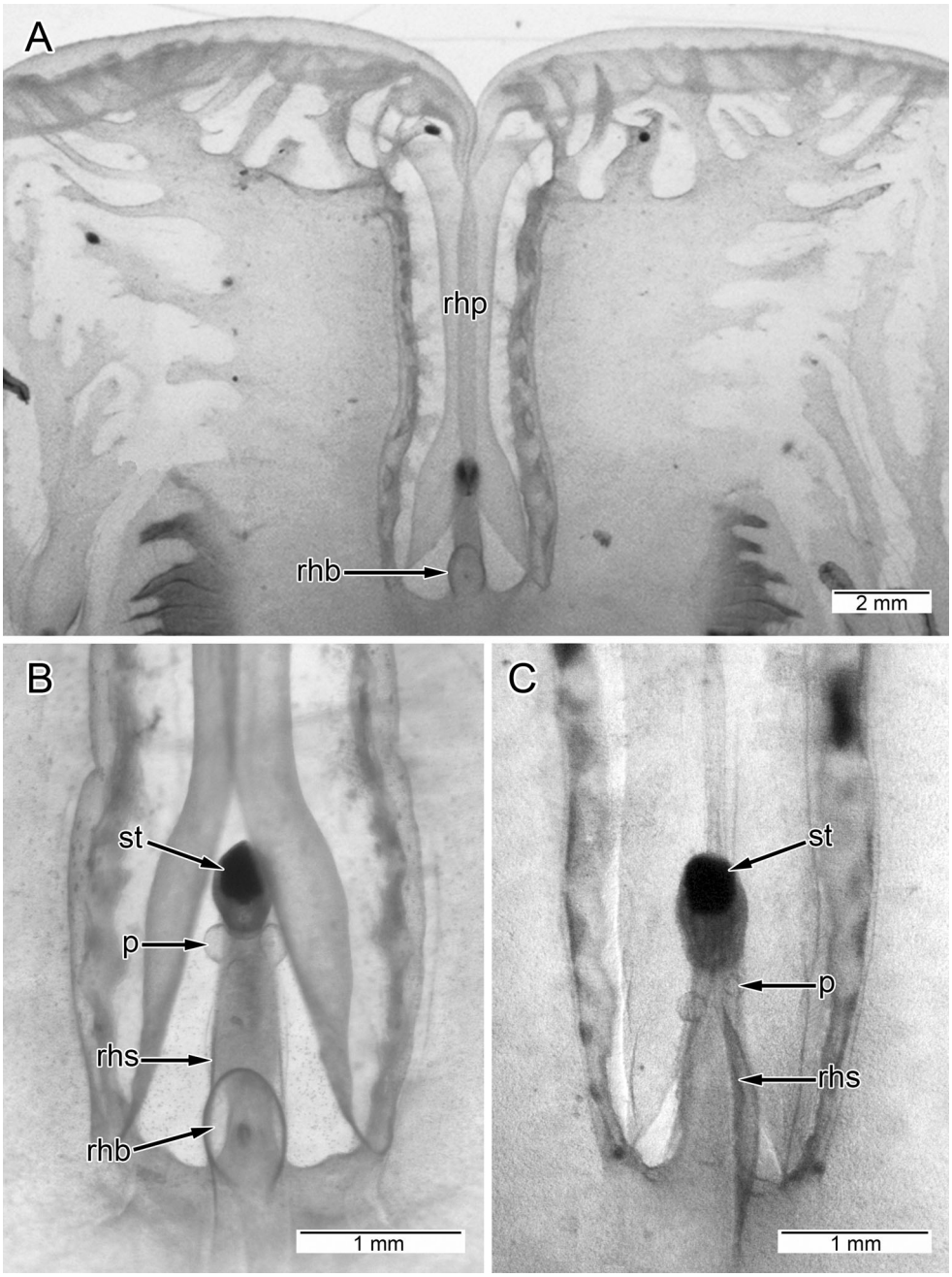

Fig. 3 Rhopalia of *Cyanea tzetlinii* sp. nov. and *Cyanea capillata*, subumbrellar side: **a**, **b** *C. tzetlinii* sp. nov., **c** *C. capillata*. *Abbreviations* p, papillae; st, statocysts; rhb, rhopalial bulb; rhp, rhopalial pit; rhs, rhopalial stalk

canal and contains an aggregation of yellow-colored pigmented cells. Tentacles assembled in eight horseshoe-shaped adradial groups with 66, 65, 36 (damaged), 60, 21 (damaged), 52, 64 and 65 tentacles. Tentacles at the proximal region of group are the largest and gradually decrease in size distally, so that tentacles closest to the bell margin are short and thin. Large four-cornered central mouth surrounded by four oral arms 27 mm long. Stomach contains numerous gastric filaments arranged in four interradial groups. Gastrovascular system bifurcates into eight ocular and eight tentacular gastric branched pouches; terminal canals of these pouches dendritic, without anastomoses. Gonadal sacs filled with mature dark-purple eggs.

Variation from type specimen

Medusae varied in size from 3.2 cm to 17 cm bd. The number of tentacles increased with the bell diameter, varying between 20 and 65 tentacles per group. Bell color varied from purple (21 samples) to pale brown (2 samples). The developmental stage of gonads varied from immature eggs to fully developed eggs or life planulae.

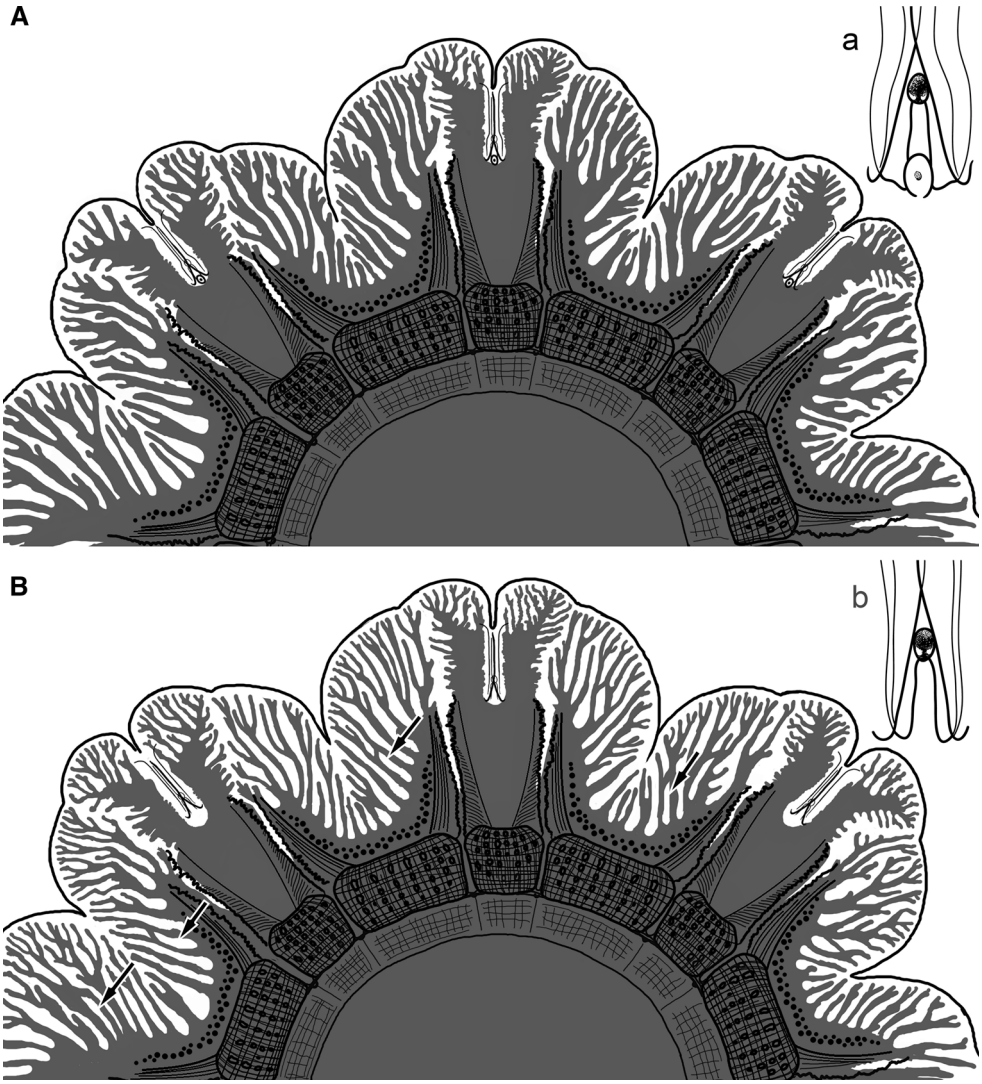

Fig. 4 General scheme of the similarities and main differences between *Cyanea tzetlinii* sp. nov. and *Cyanea capillata* from the White Sea, subumbrellar side. **A** *C. tzetlinii* sp. nov., marginal gastrovascular cannels without anastomoses, *a* rhopalium with eye-spot-bearing bulb, **B** *C. capillata* marginal gastrovascular cannels with rare anastomoses (*black arrows*), *b* rhopalium without bulb

Type locality

Velikaya Salma of the Kandalaksha Bay, White Sea, Arctic Ocean.

Habitat

Medusae are found at or near the surface and at the depth of 18 m in coastal waters.

Distribution

White Sea.

Etymology

Named after Professor Alexander Tzetlin.

DNA sequence

Nuclear 18S and 18S-ITS1-5.8S, and mitochondrial CO1 and 16S sequence data are available in GenBank under accession numbers [id to be obtained].

Phylogeny

In all four studied markers, two mitochondrial (CO1 and 16S) (Figs. 5, 6a) and two nuclear (18S and ITS1) (Fig. 6b, c), *C. tzetlinii* sp. nov. forms a distinct clade separate from all other species of *Cyanea* (Figs. 5, 6). In three of these markers, the bootstrap support for the clade is 100 % (Figs. 5, 6a, c). In the remaining highly conservative 18S marker, a distinct *C. tzetlinii* sp. nov. clade is also observed, although the genetic distance between *C. tzetlinii* sp. nov. and the nearest outgroup samples is very low (arising from just a single differing nucleotide), leading to low bootstrap support (Fig. 6b).

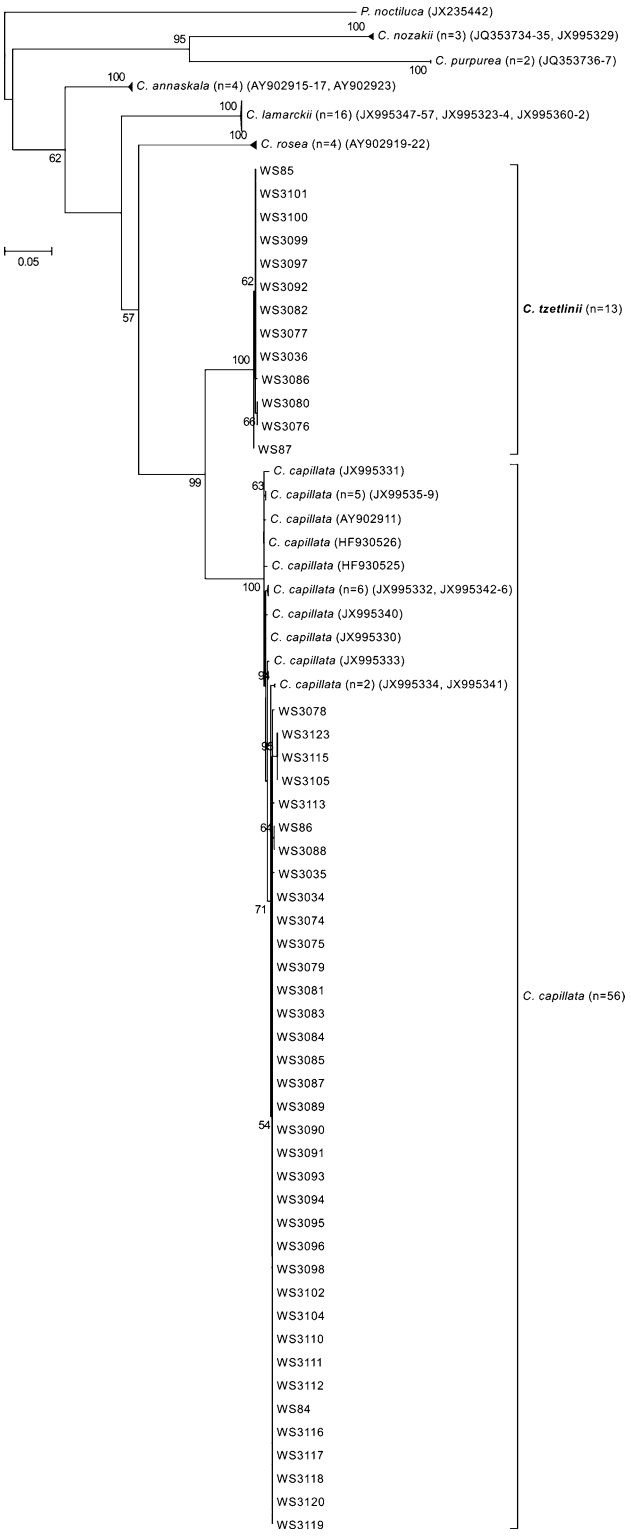

Fig. 5 Molecular genetic analysis of new sequences. neighbor-joining analysis of cytochrome oxidase subunit 1. Analysis was performed using model HKY+I+GK80 with 1000 bootstrap replication trials. Where available, GenBank sequences of *Cyanea* genus (*C. annaskala*, *C. capillata*, *C. nozakii*, *C. lamarckii*, *C. purpurea*, *C. rosea*) were included in the analysis. *P. noctiluca* was included as outgroup species

In the three robust phylogenies (CO1, 18S and ITS1), *C. tzetlinii* sp. nov. forms a sister clade to *C. capillata*, which includes the newly obtained *C. capillata* samples from the same location at the White Sea. The phylogeny of the remaining *Cyanea* species obtained from GenBank is consistent with the well-accepted topology (Holst and Laakmann 2013).

The genetic distances between *C. tzetlinii* sp. nov. and *C. capillata* are large (9.6–10.6 %, mean 10.1 %), compared to within-species distances. Specifically, for CO1, the ratio of this interspecies distance to the within-species genetic distances (barcoding gap) is 15.6 and 35.3 for within-species polymorphism of *C. tzetlinii* sp. nov. and *C. capillata*, respectively (Table 2).

Remarks

Color description of *C. tzetlinii* sp. nov. covers the same for *C. capillata* and *C. ferruginea* (purple-red, red or brown); *C. lamarckii* is blue, *C. postelsi* and *C. citrea* are colored mainly yellow. The center of exsumbrella of *C. tzetlinii* sp. nov. is smooth, periphery with nematocyst clusters, as in *C. capillata*. *C. lamarckii* has papillosed exumbrella, *C. ferruginea*, *C. postelsi* and *C. citrea* have smooth exsumbrella. The shape of gastrovascular canals of *C. tzetlinii* sp. nov. shows some morphological plasticity; in most investigated *C. tzetlinii* sp. nov. canals are dendritic as in *C. capillata* or *C. citrea* but lacking anastomoses, but several specimens demonstrate more or less straight canals, looks like those of *C. ferruginea*. Gastrovascular intrusions in muscle folds are present as in *C. capillata*, *C. ferruginea*, *C. postelsi* and *C. citrea* but not in *C. lamarckii*. Marginal lobes of *C. tzetlinii* sp. nov. are rounded as them of *C. capillata* and *C. citrea*, with small (shallow) tertiary cleft. *C. ferruginea* has square-shaped marginal lobes with a deep tertiary cleft. *C. postelsi* have extremely specific shapes of gastrovascular canals and marginal lobes, easily distinguishable from all this four species. The collected specimen of *C. tzetlinii* sp. nov. were somewhat smaller than *C. capillata* from the White Sea; none of the 74 *C. tzetlinii* sp. nov. specimens were exceeded 17 cm (bd), whereas *C. capillata* 27–30 cm (bd), collected in the same time in the same place, were present (Fig. 7).

In the ecological aspect, we can note that *C. tzetlinii* sp. nov. in summer 2014 appeared a bit later, than *C. capillata*. In July, we found *C. capillata* predominantly (8 *C. capillata* to 2 *C. tzetlinii* sp. nov.). Since the second half of August, the number of *C. capillata* gradually reduces (2 *C. capillata* to 8 *C. tzetlinii* sp. nov.) and in the period from 25 August to 10 September only *C. tzetlinii* sp. nov. was detected. Mature *C. capillata* form accumulations approximately from 25 July to 10 August. Mature *C. tzetlinii* sp. nov. form accumulations since from 10 to 25 August.

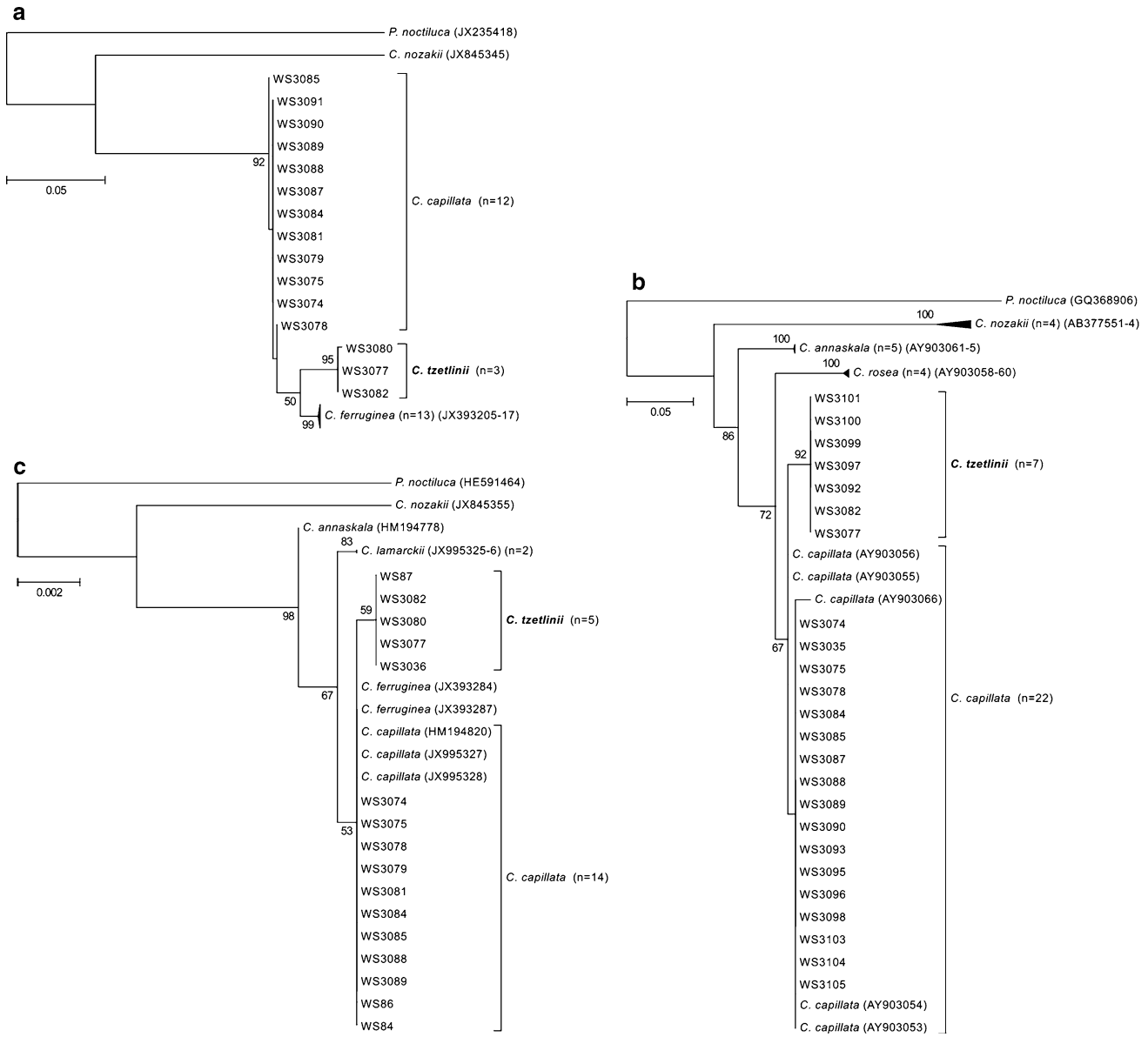

Fig. 6 Molecular genetic analysis of new sequences: **a** maximum likelihood analysis of internal transcribed spacer (ITS), **b** 18S rDNA, **c** 16S rDNA. Analyses were performed using for 16S model HKY+G, for ITS1 model K80+I and for 18S model TrN+I with 1000 bootstrap replication trials. Where available, GenBank sequences of *Cyanea* genus (*C. annaskala*, *C. capillata*, *C. ferruginea*, *C. nozakii*, *C. lamarckii*, *C. purpurea*, *C. rosea*) were included in the analysis. *P. noctiluca* was included as outgroup species

Discussion

The type species of the genus *Cyanea*, *C. capillata* (von Linnaeus 1758), was described from the North Sea (von Linnaeus 1758), and it has a North Atlantic, North Pacific and Circum-Arctic distribution (Mayer 1910; Naumov 1961; Dawson 2005; Sparmann 2012; Holst and Laakmann 2013). In addition to *C. capillata*, three *Cyanea* species were described from the North Atlantic and North Pacific, *C. postelsii*, *C. citrea* and *C. ferruginea* (Murdoch 1885; Mayer 1910; Sparmann 2012).

Traditionally, only *C. capillata* is detected in the White Sea (Yashnov 1948; Naumov 1961; Loginova and Perzova 1967; Hansson 1997; Saranchova and Flyachinskaya 2001; Malyutin 2010) or *Cyanea arctica* Peron and Leuseur (1809) (Vanhöffen 1888). *C. arctica* was described originally by Peron and Leuseur (1809) and then redescribed by Mayer (1910) as a synonym of *C. capillata*. According to Mayer (1910), *C. capillata* var. *arctica* distinguishes from *C. capillata* by the shape of rhopalial lappets, which are "…not project as far beyong the general contour of the bell margin as in *C. capillata*" and by larger size.

Table 2 Minimum and maximum pairwise genetic distances (%) for investigated specimens of *C. capillata* and *C. tzetlinii* based on (a) mitochondrial CO1 (alignment length 594 nucleotides), (b) mitochondrial 16S rDNA, (c) nuclear 18S rDNA and (d) nuclear ITS

	C. capillata	*C. tzetlinii*
a (*CO1*)		
C. capillata	0–1.85	
C. tzetlinii	8.42–9.76	0–0.34
P. noctiluca	22.56–23.74	24.41–24.75
b (*16S*)		
C. capillata	0–0.37	
C. tzetlinii	2.61–3.17	0–0.19
P. noctiluca	19.78–20.15	19.96–20.15
c (*18S*)		
C. capillata	0	
C. tzetlinii	0.06	0
P. noctiluca	2.01	1.95
d (*ITS1*)		
C. capillata	0–0.71	
C. tzetlinii	0.71–0.94	0
P. noctiluca	10.59–10.82	10.82

Aurelia aurita is the outgroup. Alignment lengths are (a) 594, (b) 536, (c) 1613, and (d) 715 nucleotides

From the literature about *Cyanea,* we have not find any mentions about bulbs on rhopalia (von Linnaeus 1758; Peron and Leuseur 1809; de Lamarck 1840; Agassiz 1862; Eimer 1878; Haeckel 1879; Vanhöffen 1888; Mayer 1910; Bigelow 1926; Stiasny and van der Maaden 1943; Yashnov 1948; Kramp 1961; Naumov 1961; Russell 1970; Malyutin 2010; Sparmann 2012). Only *C. capillata* var. *arctica* has some epithelial structures in the basal part of rhopalia: "…proximal half of club quite thick, with a well-developed swelling upon its lower (subumbrella) side; this swelling is cowered with wart-like elevations and provided with one or two papillae" (Mayer 1910). Sometimes, papillae and elevations are present in rhopalia of *Cyanea* (Fig. 2b, c). This elevations and papillae were also marked in Plate IV in Agassiz (1857), but in our opinion, neither Mayer's description nor Agassiz's drawing describes rhopalial bulbs (Figs. 2, 3). At the same time, rhopalial bulbs of *C. tzetlinii* sp. nov. are greatly observable and it is impossible that such a characteristic was overlooked by Eimer (1878), Agassiz (1857), Mayer (1910) and following researchers. Also, the original description of *C. arctica* by Peron and Lesueur did not mention any elevations, papillae or bulbs in the basal part of rhopalial stalks. Due to this and to the smaller bell size of *C. tzetlinii* sp. nov., we suppose that it is not *C. capillata* var. *arctica* of A.G. Mayor.

It seems that the differences between *C. tzetlinii* sp. nov. and *C. capillata* are not limited only by morphological characteristics. According to our observations in summer 2014, *C. tzetlinii* sp. nov. occurred in the plankton later than *C. capillata*. In July, we found *C. capillata* predominantly, since the second half of August *C. capillata* was gradually replaced by *C. tzetlinii* sp. nov. and from the end of August to September only *C. tzetlinii* sp. nov. was detected.

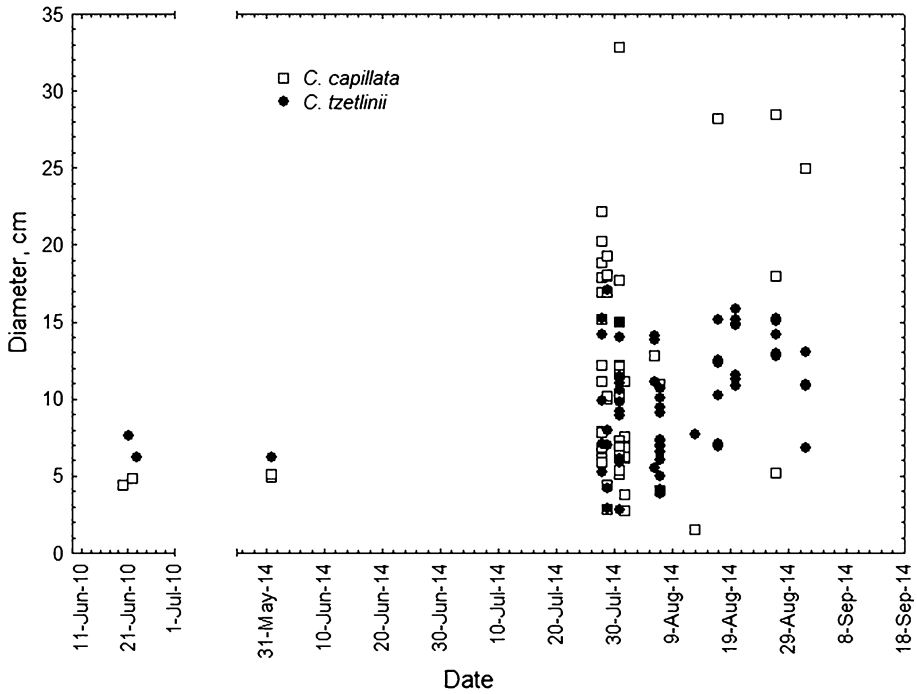

Fig. 7 The size of bell diameter of *Cyanea tzetlinii* sp. nov. and *Cyanea capillata* from the White Sea

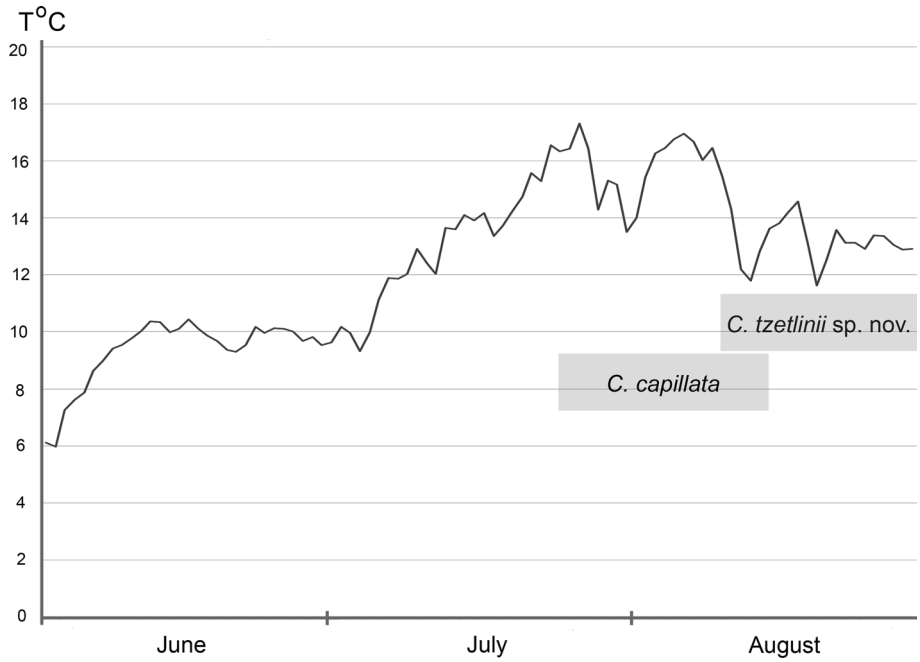

Fig. 8 The latest occurrence of mature *Cyanea tzetlinii* sp. nov. and the earliest occurrence of mature *Cyanea capillata* in summer 2014 (Kandalaksha Bay of the White Sea)

Mature *C. capillata* formed accumulations approximately from 25 July to 10 August, in period from 1 to 15 August, and it releases plaunlae. Mature *C. tzetlinii* sp. nov. form accumulations since from 10 to 25 August. Thus, the maturation of *C. capillata* took place ahead of *C. tzetlinii* sp. nov. (Fig. 8).

Molecular genetics

At the molecular level, the range and consistency of the observed genetic distances between *C. tzetlinii* sp. nov. and the nearest sister species *C. capillata* warrant species designation for the former. The molecular analyses consistently place *C. tzetlinii* sp. nov.as a monophyletic group, with low intergroup genetic distances, and high distance from *C. capillata.* While our sample of *C. capillata* clusters with other *C. capillata* samples from GenBank, *C. tzetlinii* sp. nov. forms a distinct clade. The distances are fully consistent between the mitochondrial (CO1 and 16S RNA) and nuclear (ITS and 18S RNA) loci and the morphological traits, with each studied individual falling unambiguously either into the *C. capillata* or the *C. tzetlinii* sp. nov. clade according to all five characteristics. Indeed, the magnitude of the differences in CO1 (~10 %) far exceeds the range of within-species differences observed in a wide range of scyphoid jellyfish (0.0–2.5 %) and falls within the range of differences between species within a genus (7.5–20.7 %) (Holst and Laakmann 2013).

The White Sea is characterized by young age since the end of the latest glaciation (~10,000 years; Naumov and Fedyakov 1993; Lambeck 1996) yearly ice cycles, and high degree of isolation, with dispersal into the Barents Sea limited by the narrowness of the Gorlo strait. All these characteristics contribute to the comparatively low level of species diversity as well as within-species genetic diversity within its boundaries.

The young age of the White Sea implies that sympatric divergence within its boundaries is improbable. Indeed, there are few species endemic to the White Sea. It is unlikely that the high genetic distance that separates *C. capillata* from *C. tzetlinii* sp. nov. was obtained in the course of only ~10,000 years since the origin of the White Sea. Therefore, like *C. capillata*, *C. tzetlinii* sp. nov. has probably originated elsewhere and may still be present outside the White Sea.

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