The first record of *Bougainvillia principis* (Steenstrup, 1850) (Hydrozoa: Anthoathecata) from the White Sea

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ABSTRACT: Hydroids are common components of fouling communities in the sea, but they are often inconspicuous and easily overlooked. In such cases, the appearance of their medusae in plankton is an obvious indicator of the species' presence in a locality. In this study, we present the first record of medusae *Bougainvillia principis* from the White Sea. We hypothesize that hydroids of the species *B. principis* inhabit the White Sea, as well, but they do not usually produce medusae and consequently the species does not exhibit sexual reproduction in the White Sea.
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KEY WORDS: *Bougainvillia principis*, medusa, first report, White Sea.

Первая находка медузы *Bougainvillia principis* (Steenstrup, 1850) (Hydrozoa: Anthoathecata) в Белом море

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РЕЗЮМЕ: Гидроидные — обычные обитатели морских сообществ обрастания. Однако многие виды сложно учитывать в естественных местообитаниях при мониторинговых исследованиях по причине их небольших размеров, а также сложности идентификации. В таких случаях появление медуз в планктоне может свидетельствовать о скрытом присутствии вида в исследуемой акватории. В данной работе мы представляем первую находку медуз *Bougainvillia principis* в Белом море. Мы предполагаем, что гидроидные полипы *B. principis* обитают в Белом море, но обычно не продуцируют медуз и не размножаются половым путём.
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КЛЮЧЕВЫЕ СЛОВА: *Bougainvillia principis*, медуза, первое обнаружение, Белое море.

Introduction

The fauna of the White Sea includes 19 species of hydromedusae (Naumov, 1960; Pertsova, 1979). *Bougainvillia superciliaris* (L. Agassiz, 1849) medusae were the only bougainvilliid medusae previously registered in the White Sea (Prudkovsky, 2012, 2013; Antsulevich, 2015). Medusae of another bougainvilliid species, *Bougainvillia principis* (Steenstrup, 1850), were recorded in the south-west part of the Barents Sea, in the near-shore branch of the North Cape Current (70°N, 35°E) (Zelickman, 1972). It is a rare species in the Barents Sea, and seems to be an immigrant from the west during periods of increase of the Gulf Stream.

In our paper, we present the first confirmed record of a *B. principis* medusa from the White Sea.

Material and methods

A tiny bougainvilliid medusa was found on 11 June 2014 near the Pertsov Biological Station of Lomonosov Moscow State University (Kandalaksha Bay, White Sea: 66°34' N, 33°08' E) (Fig. 1). It was collected by a plankton net from the surface of the sea. The surface water temperature was of about 10 °C at that time. The medusa was placed in a small dish and maintained at 4–6 °C until 24 August 2014. The water in the dish was changed every other day and the medusa was fed using concentrated zooplankton from the sea. The maintained medusa was photographed alive several times during the summer to follow its growth and development. Photographs were taken with a Canon camera equipped with macro lenses (100 mm and MP-E). When the medusa became an adult, it was preserved in 96% EtOH to allow molecular analysis. The preserved specimen was studied under a Lomo Biolam P1Y42 microscope to localize the position and form of its eye spots for accurate identification.

DNA amplification and sequencing were carried out according to Prudkovsky & Neretina (2016). All new sequences are available in GenBank (MK139153–MK 139156). The sequences were assembled and checked with the CodonCode Aligner software (www.codoncode. com/aligner). DNA sequences were then aligned using the ClustalW method implemented in the Molecular Evolutionary Genetic Analysis software, version 5.1 (MEGA 5.1) (Tamura *et al*., 2011). The evolutionary history was inferred using the neighbour-joining method (Saitou, Nei, 1987). The optimal tree with the sum of branch length = 1.11435471 is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004) and are in the units of the number of base substitutions per site. The analysis involved 19 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 505 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al*., 2016).

Results and Discussion

Bougainvilliid medusa was collected 11 June 2014. It was very young and flexible, with four interradial longitudinal furrows in the umbrella (Fig. 1A). There were reddish particles inside the gastral canals. The specimen was recognized to be distinct from the common species *B. superciliaris* because of the observed yellowish coloration of the tentacle bulbs and the oblong bell. A barnacle nauplius was identified inside the stomach of the collected medusa. During the next two and a half months the height of the medusa increased from 2.4 to 6.8 mm (Fig. 1A–C, Table 1). The number of marginal tentacles and dichotomous ramifications of oral tentacles also increased over the period (Table 1).

The peduncle of the manubrium was clearly seen in the young medusa but disappeared when the medusa became an adult (Fig. 1A–D). The oocytes became discernible within gonads after

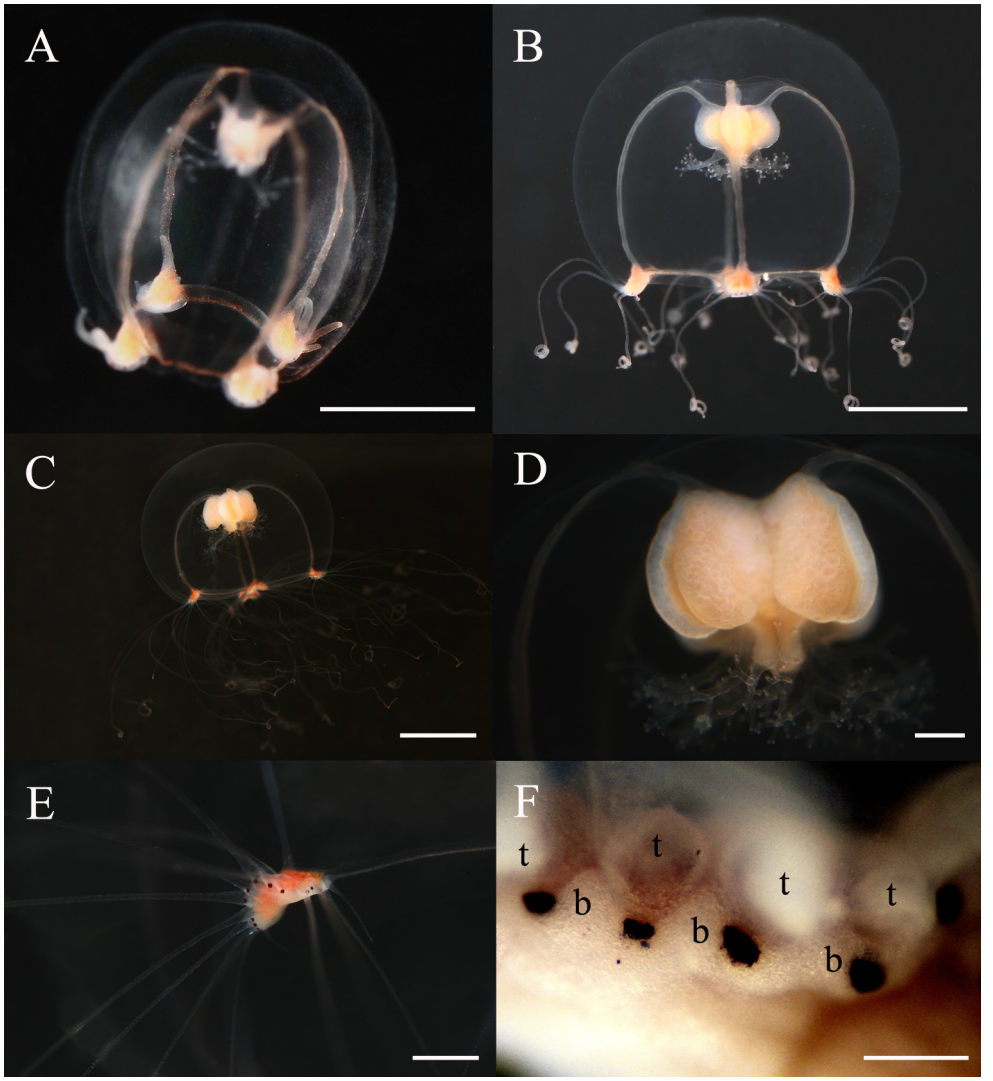

Fig. 1. The medusa *Bougainvillia principis* collected in the White Sea and maintained in the laboratory. A — the young medusa on 11 June 2014; B — the medusa on 7 July 2014; C — the female medusa with eggs over the manubrium on 24 August 2014; D — manubrium with eggs on 10 August 2014; E — marginal bulb with tentacles on 10 August 2014; F — ocelli at junctions of the tentacles with the marginal bulb on 24 August 2014.

Abbreviations: t — tentacle; b — tentacle base. Scale bar: A — 1 mm; B — 2 mm; C — 3 mm; D, E — 500 μm; F — 100 μm.

Рис. 1. Медуза *Bougainvillia principis*, пойманная в Белом море и культивируемая в лаборатории. A — молодая медуза 11 июня 2014 г.; B — медуза 7 июля 2014 г.; C — медуза с яйцами в гонадах на манубриуме 24 августа 2014 г.; D — манубриум медузы с яйцами 10 августа 2014 г.; E — щупальцевая бульба со щупальцами 10 августа 2014 г.; F — глазки на щупальцевой бульбе у оснований щупалец 24 августа 2014 г.

Обозначения: t — щупальце; b — основание щупальца. Масштаб: A — 1 мм; B — 2 мм; C — 3 мм; D, E — 500 мкм; F — 100 мкм.

Table 1. Morphology characteristics of maintained medusa *Bougainvillia principis*.
Таблица 1. Изменение морфологических признаков в процессе роста и развития культивируемой медузы *Bougainvillia principis*.

Date	Height of the bell	Width of the bell	Number of marginal tentacles per bulb	Number of branching points of oral tentacles
11 June	2.4	2.2	4	2–3
7 July	4.9	5.9	7	4–5
29 July	5.9	8.4	13–14	5–6
10 August	6.4	8.4	13–14	5–7
24 August	6.8	8.8	13–14	5–7

one and a half months of development (Fig. 1D). In August the medusa's stomach was orange-yellow and its marginal bulbs had a reddish colour with yellow borders and reddish ramifications in the direction of the tentacles. There were 13–14 tentacles at each marginal bulb of the grown medusa (Fig. 1E, Table 1). Round or oviform ocelli were situated at the junctions of the tentacles with the marginal bulb (Fig. 1E) and appeared on the bulbs in contracted specimens (Fig. 1F).

The 16S sequence analysis (Fig. 2) indicated that the medusa belonged to the clade including *B. principis* (AM183128) and *Bougainvillia multitentaculata* Foerster, 1923 (KP776799), and sequence of our medusa (WS2643, MK 139154) had 100% homology with two sequences of *B. principis* available in Genbank (AM183128, KP776760). There were no 16S fragment sequences of *B. superciliaris* available in GenBank. We consequently obtained our own sequences (WS20, MK 139153). The genetic distance between *B. principis* and *B. superciliaris* was more than 10% for the 16S sequences (MK 139153 and MK 139154) and approximately 5% for the 18S-ITS1-5.8S-ITS2-28S rDNA fragment sequences (MK 139155 and MK 139156).

The genus *Bougainvillia* includes about three dozen species (Kramp, 1959; Naumov, 1960; Kramp, 1961; Vanucci, Rees, 1961; Kramp, 1968; Millard, 1975; Schuchert, 1996; Bouillon, Boero, 2000; Schuchert, 2007; Mendoza-Becerril, Marques, 2013). Only a small number of species can be collected in the cold waters of the Northern Hemisphere, including *Bougainvillia britannica* (Forbes, 1841) (boreal), *B. superciliaris* (arctic), *B. principis* (northern boreal) and *B. multitentaculata* (boreal Pacific) (Vannucci, Rees, 1961; Schuchert, 2007). *Bougainvillia principis* medusae were recorded in the North Pacific and Bering Sea as well as in the North Atlantic and adjacent seas (Haeckel, 1879; Mayer, 1910; Hartlaub, 1911; Russell, 1938; Naumov, 1960; Vannucci, Rees, 1961; Edwards, 1966; Ballard, Myers, 1996, 2000; Sugisaki *et al*., 1998; Schuchert, 2007). The southernmost finding of medusae *Bougainvillia principis* in the Atlantic Ocean is from the Gulf of Maine (Pages *et al*., 2006). The most northern location is the Barents Sea (Zelickman, 1972; Dvoretsky, Dvoretsky, 2010). This is a rare species in the Barents Sea, although it has been observed in the western, south western and southern parts of the sea. It was not observed in the waters adjacent to the entrance to the White Sea or within the White Sea.

Previous findings of *B. principis* hydroids in the White Sea were not supported by any morphological or molecular evidence. It was accounted that hydroids *B. principis* occur everywhere, colonizing perisarc tubes of larger species such as *Tubularia indivisa* Linnaeus, 1758, *Eudendrium rameum* (Pallas, 1766), and sertulariids (Orlov, 1997). This erroneous point of view is the result of misidentification of the common species *B. superciliaris* (Prudkovsky, 2012). The hydroids of *B. principis* and *B. superciliaris* have similar morphology and can be easily misidentified (Schuchert, 2007). They can be accurately identified only after examination of the shape of the medusae buds. *B. super-*

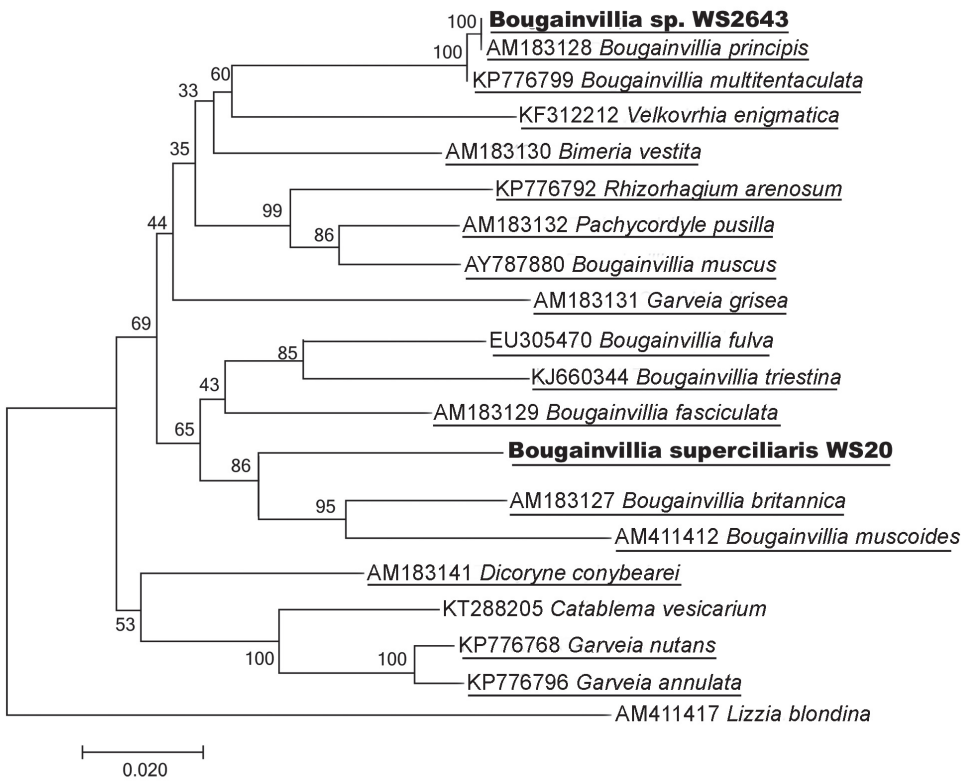

Fig. 2. Maximum likelihood phylogenetic tree of the bougainvilliid hydrozoans based on mitochondrial 16S rDNA data. Members of family Bougainvilliidae are underlined, bougainvilliids from the White sea are shown in bold. Sequences from the *Catablema vesicarium* (Pandeidae) and *Lizzia blondina* (Rathkeidae) were chosen as outgroups (Prudkovsky *et al*., 2017). Numbers above branches indicate bootstrap values (1000 replicates) (Felsenstein, 1985).

Рис. 2. Филогенетическое дерево гидроидных сем. Bougainvilliidae, построенное на основании анализа последовательностей 16S митохондриальной рДНК методом максимального правдоподобия. Виды относящиеся к Bougainvilliidae подчёркнуты, беломорские виды показаны жирным шрифтом. Последовательности 16S митохондриальной рДНК *Catablema vesicarium* (Pandeidae) и *Lizzia blondina* (Rathkeidae) использованы в качестве внешних групп (Prudkovsky *et al*., 2017). Над ветвями указаны индексы бутстрепа (1000 псевдореплик) (Felsenstein, 1985).

ciliaris medusae are abundant in the White Sea, but medusae buds on bougainvillia-like colonies were found only a few times. The colonies with medusae buds were collected in March and April (Margulis, Karlsen, 1985; Prudkovsky, 2012), and during the winter months (Prudkovsky, unpublished data). *Bougainvillia principis* hydroids with medusae buds or medusae had not yet been found in the White Sea previously. *Bougainvillia superciliaris* medusae are common in plankton during the period from April till June but were never observed in late summer. In our case the medusa *B. principis* was growing till the end of August in the laboratory. In the waters around the British Isles, newborn *B. principis* medusae have the size 1.26 x 1.08 mm (Edwards, 1966). These young medusae have four short oral tentacles, each dichotomously divided once or twice. Each marginal bulb of the youngest medusae bears three tentacles and three round black eyes. The medusa we collected was only slightly bigger. We hypoth-

esize that it was born not far from the sampling locality, because the only current that is obvious in the studied waters is tidal. The medusa was not delivered via ballast water because of low density of ship traffic in the region.

Therefore we conclude that the hydroids *B. principis* inhabit the White Sea but do not usually produce medusae. Irregularity of medusae appearance at the edge of the distribution range may be related to climatic variability. We assume that *B. principis* does not exhibit regular sexual reproduction in the White Sea, as is the case with the hydrozoan *Obelia geniculata* (Linnaeus, 1758) (Slobodov, Marfenin, 2005). Another case of abnormal reproduction in the White sea was demonstrated in the sea anemone *Aulactinia stella* (Verrill, 1864) which reproduced by ameiotic parthenogenesis (Bocharova, 2015). Lack of normal sexual reproduction should result in low genetic variation in such populations from the White Sea and thus they are worth further studying.

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