

***Einhornia*, a new genus for electrids formerly classified as the *Electra crustulenta* species group (Bryozoa, Cheilostomata)**

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The new genus *Einhornia* is established for five species of cheilostome bryozoans formerly assigned to the genus *Electra*. The diagnostic features of the new genus are the presence of a single proximodorsal spine in zooids and a calcified operculum. These characters clearly distinguish *Einhornia* from the genus *Electra*, originally diagnosed by the presence of many spines in the zooids. The morphological findings are congruent with recently reported molecular genetic evidence for distinction of this genus.

Taxonomy, zoology, DNA barcoding, Electridae, taxonomic revision

INTRODUCTION

Of all electrids, the genus *Electra* Lamouroux, 1816 is the morphologically most heterogeneous. It consists of about 20 species, which are very different. A proximal median spine, the protrusion of gymnocyst, is the main common feature in zooids of *Electra*. However, it was unclear how phylogenetically informative is this main diagnostic character (Nikulina and Schäfer 2008). A molecular phylogenetic study based on mitochondrial and nuclear ribosomal genes could not support the monophyly in the genus *Electra* regardless of the data sets or phylogenetic methods. Moreover, topological tests strongly rejected the assumption of the monophyly (Nikulina and Schäfer 2008). Species characterized by a single spine and a calcified oper-

culum in the zooids, formed a separate highly supported monophyletic group. This group was paraphyletic to other species of *Electra* with zooids that possess many spines and an uncalcified operculum. Only the latter group of *Electra* species is morphologically consistent with the original description of the genus – with *Electra verticillata* (Ellis et Solander, 1786) as the type species – pointing to presence of many spines in zooids beside of the long proximodorsal spine (“Polypier remeux; cellules campanulées, ciliées en leur bords et verticillées”). Thus, both genetic and morphologic data suggest that the group with the single spine in the zooids should be excluded from the genus *Electra*.

These species, reclassified here as *Einhornia* gen. nov., were formerly considered as the single species *Electra crustulenta* (Pallas, 1766) and later as a group of several closely related species (Nikulina 2004, 2007, 2008) (Fig. 1). *E. crustulenta* is one of the oldest known North-Atlantic bryozoans (Baster 1762) described in the 18th century from the North Sea, Zeeland (Pallas 1766) (Fig. 1A-C). Because the type material is missing, the identity of the species was uncertain for a long time before it was clarified by Borg who analysed both the text and the study area of Pallas (Borg 1931). Unfortunately, Borg did not designate a neotype. Additionally, he delineated six varieties of *E. crustulenta* from which only *arctica* was later supported as a separate species *E. arctica* Borg, 1931 (Nikulina 2007, 2008).

The species *Electra arctica* Borg, 1931 (Fig. 1D, E) has long been treated as a variety of *E. crustulenta* (Kluge 1962; Powell and Crowell 1967; Denisenko 1983; Kuklinski 2002) or subspecies (Ryland 1969), although already Powell (1968b) elevated it to species rank. The species status of *E. arctica* was validated by a comparative study of nuclear and mitochondrial ribosomal genes that revealed deep divergence from and paraphyletic relation to *E. crustulenta* (Nikulina 2007). Because a holotype was not selected by Borg, the material from the type locality, Spitsbergen and the Russian Barents Shelf, was used to clarify the identity of *E. arctica* (Nikulina 2007).

Electra korobokkura Nikulina, 2006 and *Electra* sp. (a new species described in Nikulina 2008) were also discovered by the application of genetic methods (Nikulina 2004) (Fig. 1F-K). These two species were formerly classified as *E. crustulenta*

(Mawatari 1974; Kubanin 1976). *E. korobokkura* was described because of their clear size distinction from other species (Nikulina 2006). The formal taxonomic description of *Electra* sp. was possible after obtaining the evidence for their reproductive isolation from *E. arctica* – the closest relative and morphologically almost identical species (Nikulina 2008).

Electra venturaensis Banta et Crosby, 1994 was analogously to other species of these group treated as *E. crustulenta* (Soule et al. 1995) (Fig. 1L). The presence of special kenozooids allowed the recognition of the species as new (Banta and Crosby 1994).

Bryozoans of the genus *Einhornia* gen. nov. combine a conserved morphology with high intraspecific variability. The presence of geographic or ecological variation can additionally complicate their identification. Therefore these species are difficult to discriminate using morphological features alone and genetic identification can be necessary. The use of DNA sequences as a tool facilitating taxonomic identification of species played an increasingly important role over the past decade (Tautz et al. 2003; Blaxter 2003). Cryptic speciation, which has been found in most living taxa, is an additional argument for the necessity of complementing morphology with genetic data (Palumbi 1992; Knowlton 1993; Westheide and Schmidt 2002). The previous taxonomic assignments based on morphological, morphometric and genetic analyses of nuclear and mitochondrial rRNA gene sequences of type specimens of the *Einhornia* species provided an identification key using morphology and a list of reference sequences necessary for DNA-based identification (Nikulina 2004-2008).



Figure 1 Species of *Einhornia* gen. nov. A-C – *E. crustulenta* (A – neotype, SMF 1730; B – colony; C – bleached zooid). D, E – *E. arctica* (D – neotype, SMF1731; E – a colony, PIN RAN 146/3001). F, G – *E. korobokkura*, holotype, SMF 1724 (F – the colony; G – bleached zooids). H-J – *Einhornia* sp., (H – colonies, SMF 1725; I – fragment of a colony, SMF 1726; J – single zooid). L – *E. venturaensis*, SMF 1727. M-P – opercula (M – *E. crustulenta*; N – *E. korobokkura*; O – *Einhornia* sp.; P – *E. arctica*).

METHODS

Mitochondrial 16S rDNA sequences published in the European Molecular Biology Laboratory database (EMBL) as *Electra* were analysed (Suppl. 1). All sequences, including key sequences, were assembled and edited using SeqMan and EditSeq (DNASTAR Lasergene software). ClustalX (Thompson et al. 1997) with default settings was used for sequence alignment. Phylogenetic analyses were prepared with MEGA v3.0 software (Kumar et al. 2004). To compare likelihoods of different models of DNA substitution, implemented in MEGA, we used the program MODELTEST v3.6 (Posada and Grandal 1998), which utilizes also likelihood ratio tests. In accordance with result of this selection, genetic distances from pairwise com-

parisons among all sequences were estimated by the Tamura-Nei model (Tamura and Nei 1993) with gamma distribution shape parameter 0.2556. The resulting distance matrix was subjected to a neighbor-joining analysis implemented with MEGA. The reliability of the inferred tree was estimated by bootstrapping (Felsenstein 1985) with 2000 pseudoreplicates. Identification of each sample was based on inclusion in a well-supported (>80% bootstrap value) clade containing key reference sequences. These clades were treated as species corresponding to the reference sequences. In addition, the phylogenetic tree provided a graphic display of the patterns of divergence among the species.

RESULTS

In the 62 sequences analysed, 16 haplotypes were found. The NJ tree based on 401 bp (due to the short sequence of *E. venturaensis*) showed five clusters that included key references sequences of five species of *Einhornia* gen. nov. (Tab. 1, Fig. 2). All species were resolved as reciprocally monophyletic clades

with 96-100% bootstrap support. Mean interspecific and intraspecific distances were 17.1% (range 2.4-21.2%, standard error [SE]=0.12%) and 0.02% (range 0.0-0.08, SE=0.005), respectively (Tab. 1). Geographic origin of the sequences is summarised in Figure 3.

TAXONOMY

Einhornia gen. nov.

Diagnosis: Electrids with a single proximedial spine, which can be reduced in many zooids, and an operculum impregnated with calcium carbonate; gymnocyst developed or

reduced. Colonies encrusting, uni- to multiserial.

Type species: *Eschara crustulenta* Pallas, 1766: 39.

Etymology: From the German word for unicorn.

Table 1 Sequence data analysed. Species, number of sequences (S) and haplotypes (H), and sequence length (L) are shown. Distance matrix presents mean distances between (lower right) and within species (italics). Standard errors are reported in text.

| Species | S | H | L | Distance matrix (%) | | | | |
|------------------------|----|---|---------|---------------------|------------|------------|-------------|-----------|
| | | | | <i>Ec</i> | <i>Ea</i> | <i>Em</i> | <i>Ek</i> | <i>Ev</i> |
| <i>E. crustulenta</i> | 41 | 4 | 477 | <i>0.1</i> | | | | |
| <i>E. arctica</i> | 7 | 5 | 478 | 20.4 | <i>0.2</i> | | | |
| <i>Einhornia. sp.</i> | 11 | 5 | 476-477 | 20.3 | 2.7 | <i>0.3</i> | | |
| <i>E. korobokkura</i> | 2 | 1 | 485 | 12.0 | 19.6 | 20.5 | <i>0.00</i> | |
| <i>E. venturaensis</i> | 1 | 1 | 395 | 19.3 | 20.8 | 20.6 | 20.6 | - |

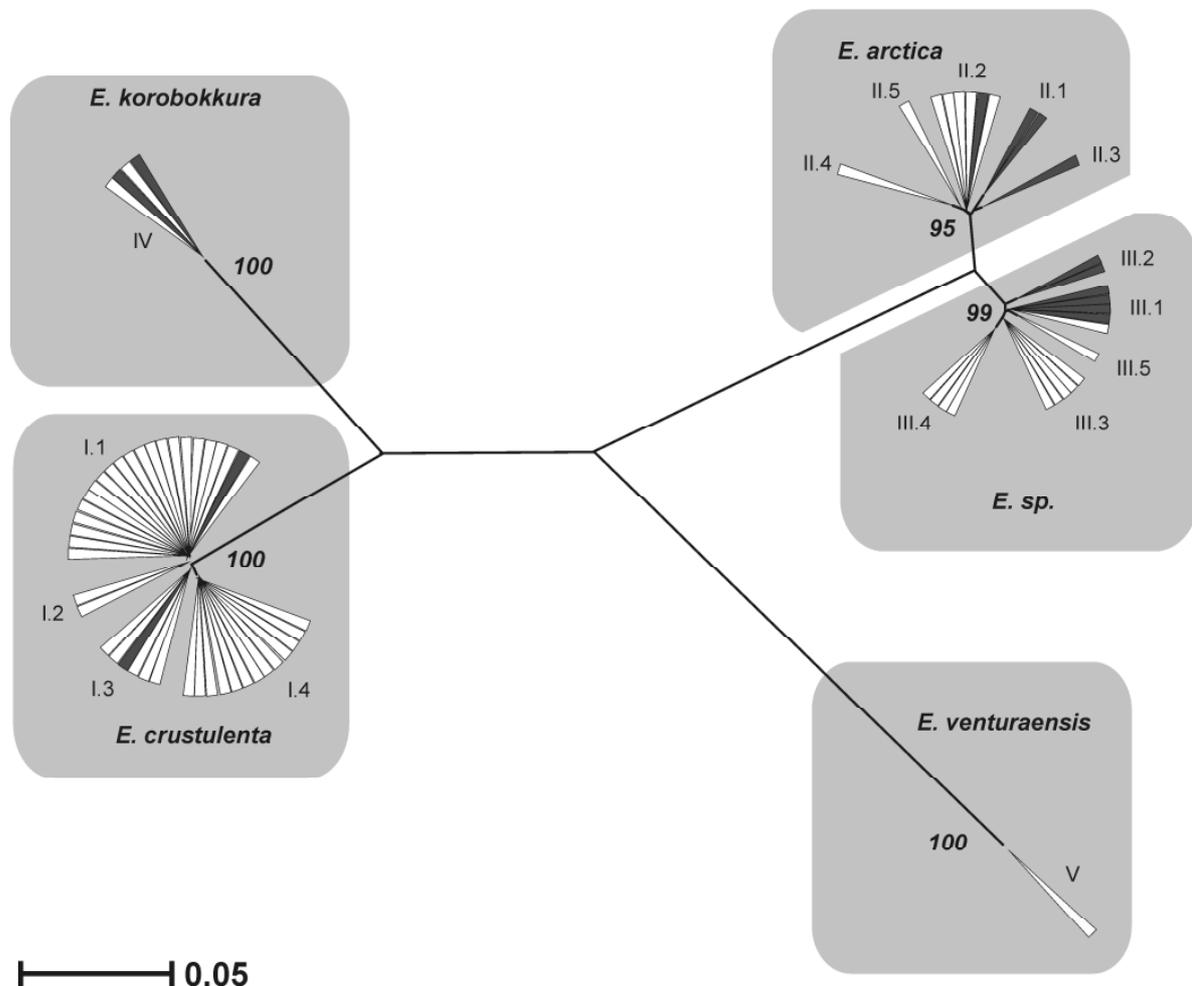


Figure 2 Unrooted neighbor-joining phylogram based on TN+G distances; key reference sequences are indicated in black. Designation of haplotypes corresponds to those shown in Supplement 1.

Species assignable

Einhornia crustulenta (Pallas, 1766), comb. nov. [*Eschara crustulenta* Pallas, 1766]; type not selected; neotype selected herein: Senckenberg Museum SMF 1730; type locality: western Baltic Sea, Kiel Bay, south-western coast of Lolland, 6 m; reference sequences in the EMBL: AM924134 (18S nDNA) and AJ853844 (16S mtDNA). (Fig. 1A-C, M)

Einhornia arctica (Borg, 1931), comb. nov. [*Electra crustulenta* var. *arctica* Borg, 1931]; type not selected; neotype selected herein: SMF 1731, type locality: White Sea, Kandalaksha Bay, Biological station of the Moscow State University, 10 m; reference DNA sequences in the EMBL: AM492519 (18S nDNA) and AM492511 (16S mtDNA). (Fig. 1D, E, P)

Einhornia korobokkura (Nikulina, 2006), comb. nov. [*Electra korobokkura* Nikulina, 2006]; holotype SMF 1723; reference DNA sequences in the EMBL: AM158086 (18S nDNA) and AJ853948 (16S mtDNA). (Fig. 1F, G, N)

Einhornia sp., reference DNA sequences in the EMBL: AM492506 and AM492514 (Nikulina 2008). (Fig. 1H-J, O)

Einhornia venturaensis (Banta et Crosby, 1994), comb. nov. [*Electra venturaensis* Banta et Crosby, 1987]; holotype: United States National Museum of Natural History USNM 477681; reference DNA sequences obtained from a topotype SMF 1727: AM903323 (16S mtDNA). (Fig. 1L)

Species identification key is presented in Supplement 2. Genetic data, necessary for DNA based identification of the species are summarised in Supplement 1.

Comparison with related genera

The new genus can be distinguished from all other electrids by its single proximodorsal spine - gymnocyst protrusion proximally from the operisium. Additionally, the operculum in *Einhornia* gen. nov. is more or less impregnated with calcium carbonate – a feature that is unique to this genus.

Notes on phylogenetic relationships within the genus

The obtained phylogenetic tree demonstrates close relationship between *E. crustulenta* and *E. korobokkura* as well as between *E. arctica* and *Einhornia* sp. (Fig. 2). The concordant result was obtained by analyses of nuclear 18S rDNA (Nikulina 2008).

E. arctica and *Einhornia* sp. are very young sister species, as follows from both genetic and morphological distances. This is a pair of a cold (*E. arctica*) and a temperate (*Einhornia* sp.), and their divergence may have been conditioned by climatic oscillations of the Quaternary period: Allopatric fragmentation of an ancestral population during a glacial period was followed by local adaptation to different ecoclimatic conditions (Nikulina 2008).

Notes on the geographic distribution of the genus

The complete geographic range of the species of *Einhornia* gen. nov. is unknown. The origin of DNA sequences demonstrates the distribution of *E. crustulenta* in the North and Baltic Seas (NE Atlantic), in the Barents and White Seas (Arctic), and on the Pacific coast of Oregon (NE Pacific); *E. arctica* in the Barents and White Sea, at Spitsbergen, and in the

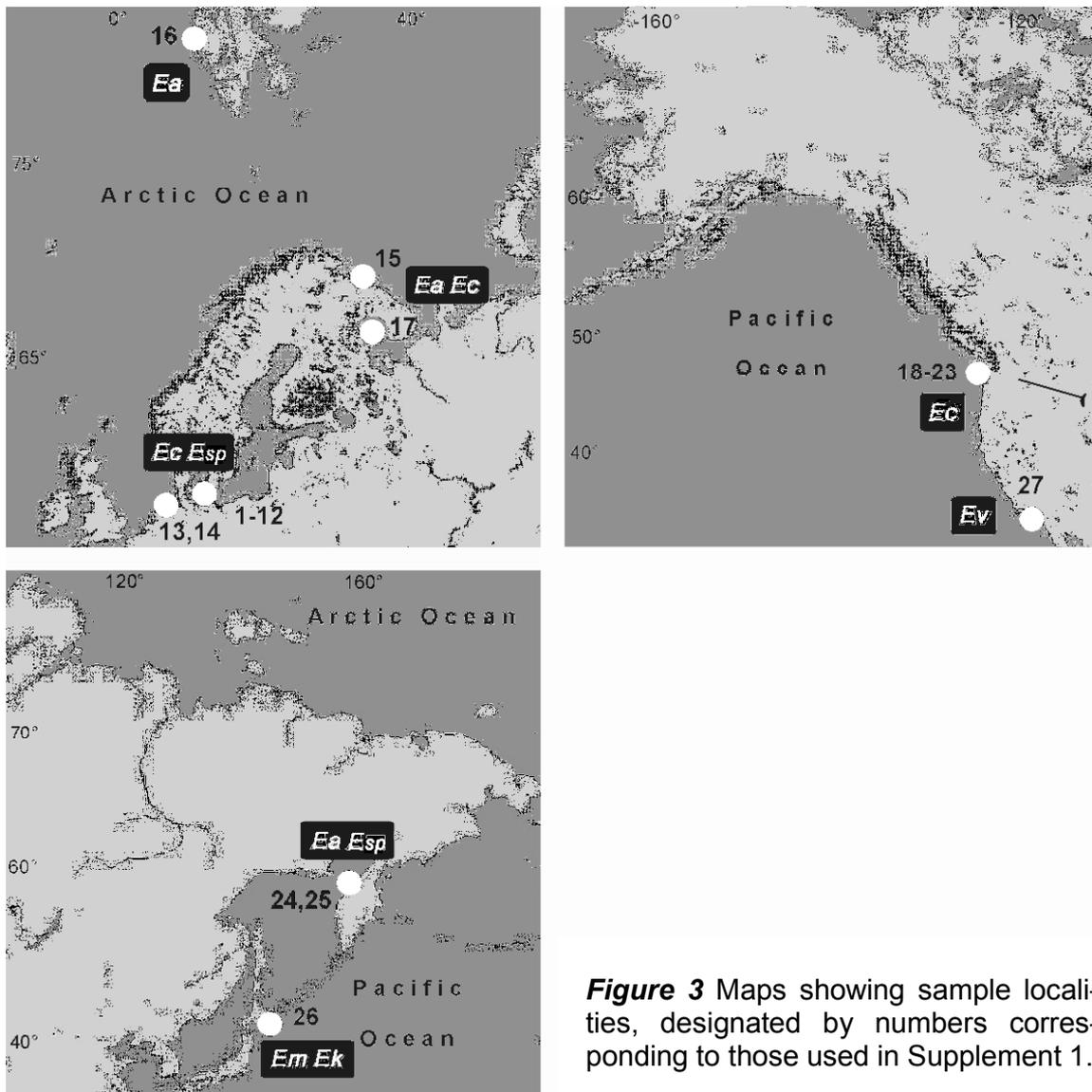


Figure 3 Maps showing sample localities, designated by numbers corresponding to those used in Supplement 1.

Sea of Okhotsk (NW Pacific); *Einhornia* sp. in the Western Baltic Sea and Sea of Okhotsk, and on the Pacific coast of Japan; *E. korobokkura* at Akkeshi Bay; and *E. venturaensis* on the coast of southern California (NE Pacific). Taking into account the trans-Arctic distribution of the *E. crustulenta* – *E. korobokkura* and *Einhornia* sp. – *E. arctica* pairs of closely related species, a dispersal event via the Bering Strait probably

took place at some time after the opening of this gateway in the Late Miocene or earliest Pliocene between 4.8 and 7.3-7.4 Ma (Marincovich and Gladenkov 1998).

Taking into account the morphological similarity of these species and their sympatry in most of the geographic regions studied, a DNA based study can be necessary for their reliable taxonomic identification.

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Supplement 1 Collection of data from the EMBL data base demonstrating the geographical distribution of the species of the genus *Einhornia*. Haplotype group designations (HT) correspond to those in Figure 2 and station numbers (StNo) – in Figure 3. The reference sequences are in bold.

| Accession No | Region | Locality | StNo | HT |
|------------------------------|-------------|--------------------------------------|------|-----|
| <i>E. crustulenta</i> | | | | |
| AJ853844 | NE Atlantic | Western Baltic, Lolland | 1 | I.1 |
| AJ853893 | | | 1 | I.1 |
| AJ853894 | | Western Baltic, Friedrichsort | 2 | I.1 |
| AJ853895 | | | 2 | I.1 |
| AJ853896 | | | 3 | I.1 |
| AJ853897 | | Western Baltic, Kattegat | 4 | I.1 |
| AJ853898 | | | 4 | I.1 |
| AJ853899 | | | 5 | I.1 |
| AJ853900 | | | 5 | I.1 |
| AJ853901 | | Western Baltic, Bülk | 6 | I.1 |
| AJ853904^{c)} | | | 6 | I.1 |
| AJ853902 | | | 6 | I.1 |
| AJ853903 | | | 7 | I.1 |
| AJ853906 | | | 8 | I.1 |
| AJ853965 | | | 9 | I.1 |
| AJ853907 | | Western Baltic, Poel | 10 | I.1 |
| AM263556 | | | 10 | I.2 |
| AJ853966 | | Western Baltic, Salzhaff | 11 | I.1 |
| AJ853967 | | | 11 | I.1 |
| AM263557 | | | 11 | I.1 |
| AJ853905 | | North Sea, Helgoland | 13 | I.1 |
| AM263558 | | | 13 | I.1 |
| AM412569^{e)} | | North Sea, Lauwersmeer | 14 | I.3 |
| AJ853964 | Arctic | Barents Sea, Mogilnoe Lake | 15 | I.1 |
| AJ867424 | NE Pacific | Oregon, Jarvis Range Markers | 18 | I.4 |
| AJ867421 | | | 18 | I.4 |
| AJ867422 | | | 18 | I.4 |
| AJ867425 | | | 18 | I.4 |
| AJ867426 | | | 18 | I.4 |
| AJ867427 | | | 18 | I.4 |
| AJ867435 | | | 19 | I.3 |
| AJ867428 | | Oregon, Coos Bay City Dock | 20 | I.4 |
| AJ867429 | | Oregon, Empire at Eureka | 21 | I.4 |
| AJ867430 | | | 21 | I.4 |
| AJ867431 | | | 21 | I.4 |
| AJ867432 | | | 21 | I.4 |
| AJ867433 | | | 21 | I.4 |
| AJ867423 | | | 21 | I.2 |
| AJ867434 | | | 21 | I.4 |
| AJ867436 | | Oregon, Citrus Army Corp and Central | 22 | I.3 |
| AJ867437 | | | 22 | I.3 |

| | | | BIOLOGY | |
|-------------------------------|-------------|--|----------------|-------|
| AJ867438 | | | 22 | I.3 |
| AJ867439 | | | 22 | I.3 |
| <hr/> | | | | |
| <i>E. arctica</i> | | | | |
| <hr/> | | | | |
| AM492512^{e)} | Arctic | Spitsbergen, Ny Alesund | 16 | II.1 |
| AM412568^{d)} | | | 16 | II.2 |
| AM492511^{e)} | | White Sea, Kandalaksha Bay | 17 | II.1 |
| AM412567^{d)} | | | 17 | II.3 |
| AJ853958 | NW Pacific | Sea of Okhotsk, W. Kamchatka, Rodino | 24 | II.2 |
| AJ853957 | | | 24 | II.4 |
| AJ853959 | | | 24 | II.2 |
| AJ853960 | | | 24 | II.2 |
| AJ853961 | | | 24 | II.5 |
| AJ853952 | | Sea of Okhotsk, W. Kamchatka, Ptichii Island | 25 | II.2 |
| AJ853953 | | | 25 | II.2 |
| <hr/> | | | | |
| <i>Eihornia</i> sp. | | | | |
| <hr/> | | | | |
| AM492506^{e)} | NE Atlantic | Baltic Sea, Kiel Bay | 12 | III.1 |
| AM408317^{a)} | | | 12 | III.2 |
| AM492507^{e)} | | | 12 | III.1 |
| AM492508^{e)} | | | 12 | III.1 |
| AM492509^{e)} | | | 12 | III.1 |
| AM492510^{e)} | | | 12 | III.2 |
| AJ853845 | NW Pacific | Pacific coast of Hokkaido, Akkeshi Bay | 26 | III.3 |
| AJ853941 | | | 26 | III.3 |
| AJ853942 | | | 26 | III.3 |
| AJ853943 | | | 26 | III.3 |
| AJ853846 | | | 26 | III.4 |
| AJ853944 | | | 26 | III.4 |
| AJ853945 | | | 26 | III.4 |
| AJ853954 | | Sea of Okhotsk, W. Kamchatka, Ptichii Island | 25 | III.4 |
| AJ853955 | | | 25 | III.5 |
| AJ853962 | | Sea of Okhotsk, W. Kamchatka, Rodino | 24 | III.1 |
| AJ853963 | | | 24 | III.3 |
| <hr/> | | | | |
| <i>E. korobokkura</i> | | | | |
| <hr/> | | | | |
| AJ853947^{b)} | NW Pacific | Hokkaido, Akkeshi Bay | 26 | IV |
| AJ853948^{b)} | | | 26 | IV |
| AJ853946 | | | 26 | IV |
| AJ853949 | | | 26 | IV |
| <hr/> | | | | |
| <i>E. venturaensis</i> | | | | |
| <hr/> | | | | |
| AM903323 | NE Pacific | Southern California | 23 | V |
| <hr/> | | | | |

Sources: a) – Nikulina and Schäfer (2006); b) – Nikulina (2006); c) – Nikulina et al. (2007); d) – Nikulina (2007); e) – Nikulina (2008). The remaining sequences are collected from the EMBL database (submitted by Nikulina 2004, unpublished).

Supplement 2 Key to species of *Einhornia* gen. nov.

| | | |
|---|---|-------------------------------|
| 1 | <i>Small pores or kenozooids present in distal-lateral parts of zooids</i> | <i>E. venturaensis</i> |
| | Zooids pyriform, gymnocyst are developed, operculum is slightly wider than high, proximal border is straight, a medial-proximal spine is well-developed. Colonies encrusting, oligo- or multiserial. Boreal species; NW Pacific. (Fig. 1L) | |
| - | <i>Pores or kenozooids absent</i> | 2 |
| 2 | <i>Gymnocyst is reduced, operculum with a slightly concave transverse proximal border; height of operculum is distinctly smaller than their width</i> | <i>E. crustulenta</i> |
| | Zooids oval, aperture and mural rim occupy full frontal surface, cryptocyst narrow, a medial-proximal spine is small and rare. Operculum with a slightly concave transverse proximal border; ratio of operculum height to width is 0.6. Colonies encrusting, multiserial. Boreal species; NE Atlantic, NE Pacific, European Arctic. (Fig. 1A-C, M) | |
| - | <i>Gymnocyst developed, operculum is slightly wider than high, proximal border is straight</i> | 3 |
| 3 | <i>Zooids small, about 500 µm in length</i> | <i>E. korobokkura</i> |
| | Zooids pyriform, small, ratio of opesium to gymnocyst length about 1:1, cryptocyst narrow, a tiny medial-proximal spine present in some or many zooids. Operculum with a straight transverse proximal border; ratio of operculum height to width is about 0.8. Colonies encrusting, uniserial to oligoserial. Boreal species; NW Pacific. (Fig. 1F, G, N) | |
| - | <i>Zooid length is more than 500 µm</i> | 4 |
| 4 | <i>Length of gymnocyst is about 1/2 of zooid length</i> | <i>E. arctica</i> |
| | Zooids pyriform, 1:1 ratio of opesium to gymnocyst length, cryptocyst narrow, a small medial-proximal spine present in many zooids. Operculum with a straight transverse proximal border; ratio of operculum height to width is 0.8. Colonies encrusting, usually oligoserial. High-arctic-high-boreal species: Arctic, NW Pacific. (Fig. 1D, E, P) | |
| - | <i>Length of gymnocyst is about 1/3 of zooid length</i> | <i>Einhornia</i> sp. |
| | Zooids pyriform to ovoid, ratio opesium to gymnocyst length is about 2:1, a conspicuous medial-proximal spine present in most zooids. Operculum with a straight transverse proximal border; ratio of operculum height to width is about 0.8. Colonies encrusting, oligo- to multiserial. Boreal species; NE Atlantic, NW Pacific. (Fig. 1H-K, O) | |