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Molecular Genetic Markers of Intra- and Interspecific Divergence within Starfish and Sea Urchins (Echinodermata)

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Abstract—A fragment of the mitochondrial *COI* gene from isolates of several echinoderm species was sequenced. The isolates were from three species of starfish from the Asteriidae family (*Asterias amurensis* and *Aphelasterias japonica* collected in the Sea of Japan and *Asterias rubens* collected in the White Sea) and from the sea urchin *Echinocardium cordatum* (family Loveniidae) collected in the Sea of Japan. Additionally, regions including internal transcribed spacers and 5.8S rRNA (*ITS1* – 5.8S rDNA – *ITS2*) were sequenced for the three studied starfish species. Phylogenetic analysis of the obtained *COI* sequences together with earlier determined homologous *COI* sequences from *Ast. forbesii*, *Ast. rubens*, and *Echinocardium laevigaster* from the North Atlantic and *E. cordatum* from the Yellow and North Seas (GenBank) placed them into strictly conspecific clusters with high bootstrap support (99% in all cases). Only two exceptions – *Ast. rubens* DQ077915 sequence placed with the *Ast. forbesii* cluster and *Aph. japonica* DQ992560 sequence placed with the *Ast. amurensis* cluster – were likely results of species misidentification. The intraspecific polymorphism for the *COI* gene within the Asteriidae family varied within a range of 0.2-0.9% as estimated from the genetic distances. The corresponding intrageneric and intergeneric values were 10.4-12.1 and 21.8-29.8%, respectively. The interspecific divergence for the *COI* gene in the sea urchin of *Echinocardium* genus (family Loveniidae) was significantly higher (17.1-17.7%) than in the starfish, while intergeneric divergence (14.6-25.7%) was similar to that in asteroids. The interspecific genetic distances for the nuclear transcribed sequences (*ITS1* – 5.8S rDNA – *ITS2*) within the Asteriidae family were lower (3.1-4.5%), and the intergeneric distances were significantly higher (32.8-35.0%), compared to the corresponding distances for the *COI* gene. These results suggest that the investigated molecular-genetic markers could be used for segregation and identification of echinoderm species.

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Key words: molecular evolution, population, speciation, Echinodermata, cytochrome oxidase subunit I gene, internal transcribed spacers

The main goal of molecular genetic studies of microevolution is investigation of biological diversity. For this purpose, new approaches for species identification are required that differ from traditional morphology-based methods. It is known that different parts of genomes evolve at different rates. The initial steps in studying microevolution require identification and structural characterization of genome regions undergoing rapid evolutionary changes and therefore exhibiting high levels of inter- and intraspecific polymorphism. These studies help understand the gene flow between geographically close or distant populations and evolutionary changes in geographically isolated populations. The results of such studies are now widely used for evaluation of biological diversity by the DNA barcoding methods [1]. The molecular genetic approaches developed could be used for identification of species and populations of various organisms, especially those used in industry or serving as indicators.

Studying biological diversity and mechanisms of speciation requires the use of various nuclear genome regions that would allow comparison of populations and species with different degrees of genetic isolation. Such regions might be noncoding repeats, in particular satellite DNAs of heterochromatin [2]. Other genetic markers widely used for identification and segregation of species are

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internal transcribed spacers of nuclear rRNA genes (*ITS1* and *ITS2*) and a 5′-fragment of the mitochondrial cytochrome *c* oxidase subunit I gene (~650 bp). The latter has become a standard gene for DNA barcoding of various animal species [1], including echinoderms [3-5] and mollusks [6-10]. The applicability of *ITS1* and *ITS2* for species identification has yet to be estimated; however, nuclear *ITS* sequences in combination with two plastid DNA regions (*rpL32-trnL* and *trnL-trnF*) have been successfully used for revision of the widely distributed group of *Allium saxatile* onions [11].

The aim of this work was to estimate the levels of inter- and intraspecific polymorphism of echinoderm species from the White Sea and the Sea of Japan by comparing rapidly evolving fragments of nuclear and mitochondrial genomes. Total DNA was isolated from 96% ethanol-fixed tissues from various echinoderm species, and fragments of the mitochondrial *COI* gene and the nuclear rRNA *ITS* region were amplified and sequenced. The resulting sequences were clustered by building phylogenetic trees, and the polymorphism within and between clusters was analyzed.

MATERIALS AND METHODS

Isolates of the sea urchin *Echinocardium cordatum* and starfish species *Asterias amurensis* and *Aphelasterias japonica* were collected in regions of Peter the Great Bay (Sea of Japan). Isolates of the starfish *Asterias rubens* were collected in Kandalaksha Bay (White Sea) in the vicinities of the White Sea Biological Station of Moscow State University. Total DNA was isolated from the ambulacral feet or, in some cases, gonads fixed in 96% ethanol using the NucleoSpin® Tissue DNA isolation kit (Macherey-Nagel, Germany) as recommended by the manufacturer.

A fragment of the mitochondrial *COI* gene was amplified using either a set of primers designed for sea urchins and starfish based on a set of primers universal for all multicellular animals [12] or a set of echinoderm-specific primers [13] developed based on new data on echinoderm sequences. To amplify the *ITS1*, *ITS2*, and 5.8S rDNA nuclear sequences, we designed and tested a set of primers specific for the genes coding for 18S, 5.8S, and 28S rRNA. The location of the primers and the amplification strategy for *ITS1*, *ITS2*, and 5.8S rDNA are shown in Fig. 1. The sequences of all designed and used primers are shown in Table 1.

Fragments of the mitochondrial *COI* gene were amplified by PCR using an Encyclo Plus PCR kit (Evrogen, Russia) as recommended by the manufacturer. The amplification reaction included initial denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 30 s, primer annealing at 45°C for 30 s, elongation at 72°C for 1 min, and final elongation at 72°C for 10 min. The conditions for the ITS amplification were the same except that primer annealing temperature was raised to 55°C (30 s).

PCR products were separated by electrophoresis in agarose gel. DNA bands of interest were cut out from the gel, purified using a DNA gel extraction kit (Cytokine, Russia), and sequenced (both chains) with an automated sequencer (Genom, Institute of Molecular Biology, Russian Academy of Sciences). The resulting sequences were deposited in GenBank under accession numbers KX592544-KX592561 (*COI*) and KX592562-KX592568 (*ITS1*, *ITS2*, and 5.8S rDNA).

Phylogenetic analysis, including building phylogenetic trees, clustering of sequences, and estimation of the inter- and intragroup levels of polymorphism, was performed using the MEGA6 suite of molecular genetic programs [14]. The evolutionary model for the analyzed sets of sequences was selected with the corresponding program from the MEGA6 suite; phylogenetic trees were constructed by the method of maximum likelihood based on the general model of reversible evolution [15]. The tree for the heuristic search was constructed using the NJ and BioNJ methods and the distance matrix calculated by the MCL method [15] with subsequent selection of the best topology. The inter- and intragroup distances were calculated using the Kimura two-parameter model [16].

RESULTS

Analysis of clustering of the *COI* and *ITS* genes. The obtained *COI* gene sequences were aligned with homologous *COI* sequences from other species of Atlantic and Pacific starfish (GenBank) and analyzed phylogenetically. *COI* sequences from the following species were used in analysis: *Aphelasterias japonica* (five sequences) and three

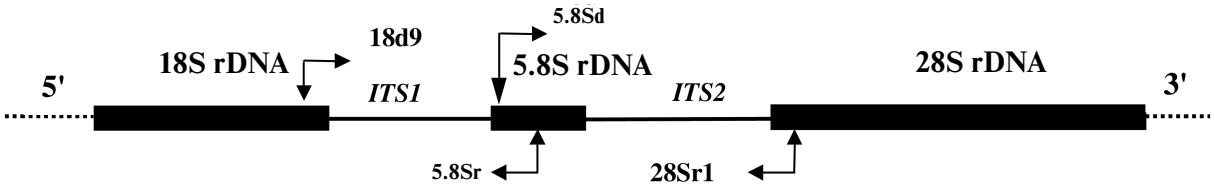

Fig 1. Scheme for amplification of internal transcribed spacers and a gene for 5.8S rRNA (*ITS1* – 5.8S rDNA – *ITS2*). Sites of primer annealing and direction of amplification are shown with arrows.

Table 1. Primers for amplification of the *COI* gene and internal transcribed spacer region (*ITS1* – 5.8S rDNA – *ITS2*)

Primer	Sequence
	Primers for *COI* gene from sea urchins
COIEcF1	5'-TTTCTACTAACCACAAGGACATCGG-3'
COIEcR1	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'
	Universal primers for echinoderm *COI* gene
COIurF1	5'-ACTGCCCACGCCCTAGTAATGATATTTTTTATGGTRATGCC-3'
COIurR2	5'-TCGTGTGTCTACGTCCATTCCTACTGTRAACATRTG-3'
	Primers for *COI* gene from starfish species of the Asteriidae family
AsCOIF1	5'-TTTCTACTAAACATAAGGACATTGG-3'
AsCOIR1	5'-CTTCAGGGTGTCCAAAAAATCA-3'
AsCOIR2	5'-ATAATCATAGTAGCGGCAGTAAAG-3'
	Primers for 18S, 5.8S, and 28S rRNA
18d9	5'-GTCGTAACAAGGTTTCCGTAGGTGAAC-3'
5.8Sd	5'-TCGATGAAGAACGCTGCCAGC-3'
5.8Sr	5'-GCCAAGAGCGTTCGAAATGTCGA-3'
28Sr1	5'-ATATGCTTAAATTCAGCGGGT-3'

of the most widely distributed species of the genus *Asterias* (family Asteriidae) – *Ast. forbesii* and *Ast. rubens* from the East and West Atlantic populations, and *Ast. amurensis* from several Pacific populations. *Distolasterias nipon* and *Forcipulatida* sp. (one sequence for each species) were used as an external group.

To identify monophyletic groups of sequences and to elucidate evolutionary relations between species of the genus *Asterias*, we constructed a phylogenetic tree for the *COI* sequences (Fig. 2). All three species formed a monophyletic group with a high bootstrap support (99%). *Asterias rubens* and *Ast. amurensis* clusters form a group with 76% bootstrap support; the *Ast. forbesii* cluster appeared to be equidistant from the two other clusters. In general, the grouping of sequences from all three *Asterias* species had 98% bootstrap support. Clusters of *Ast. rubens* and *Ast. forbesii* sequences were more homogenous than the *Ast. amurensis* cluster, in which two groups could be distinguished with bootstrap support of 90 and 86%, respectively Note that in this phylogenetic tree, the Pacific species *Ast. amurensis* and the North Atlantic species *Ast. rubens* were closer to each other than the two North Atlantic species, *Ast. rubens* and *Ast. forbesii*. The constructed phylogenetic tree had seven monophyletic groups. *COI* sequences from *Ast. rubens* specimens collected in the White Sea clustered within the homogenous group of the North Atlantic populations of this species.

The constructed tree had two artefacts. One of *Aph. japonica* sequences (DQ992560) was assigned to the group formed by sequences from *Ast. amurensis*. GenBank sequence DQ077915 that had been deposited as a sequence from *Ast. rubens* was found in a cluster formed by sequences from *Ast. forbesii* (see "Discussion" for a possible explanation).

A set of sea urchin-specific primers was used for amplification of *COI* genes from isolates of the heart-shaped sea urchin *Echinocardium cordatum* collected in various regions of the Sea of Japan (Vostok Bay, Ussuri Bay, Patroklus Bay, and Troitsa Bay). The phylogenetic tree constructed using amplified *COI* sequences together with *E. cordatum COI* sequences deposited in GenBank and *COI* sequences from other sea urchins of the Loveniidae family is shown in Fig. 3. All *COI* sequences from *E. cordatum* isolates from the Sea of Japan and from *E. cordatum* specimens collected at the Korean coast of the Yellow Sea (SY121347) formed a single cluster (Gp6) with high bootstrap support. The *COI* sequence of *E. cordatum* from the North Sea (FN562581) was located at a considerable distance from this cluster (Gp5). Another species of the same genius, *E. laevigaster* (AJ639913) from the Atlantic coast of England, was even more distant from the first two groups (Gp4) and formed with them a group with low bootstrap support. Groups Gp3 and Gp2 were formed by sequences from two other species of the

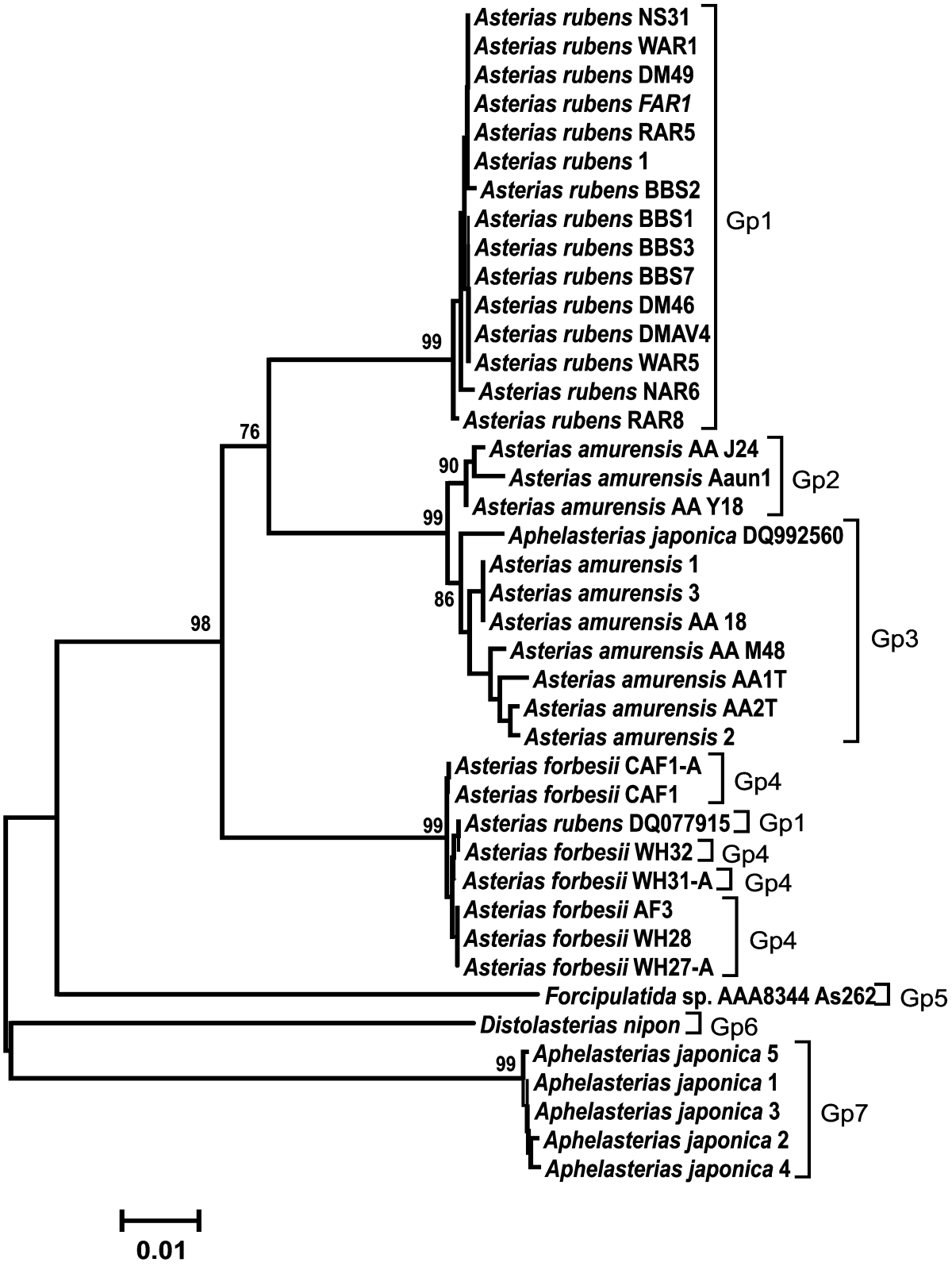

Fig. 2. Phylogenetic tree for the 5′-region of the mitochondrial *COI* gene from starfish of the Asteriidae family. The tree with the maximum logarithm of likelihood (−2417.442) is shown. The bootstrap values are shown on the branches leading to the corresponding clusters. The lengths of branches are proportional to the number of substitutions. Forty-one sequences (length, 614 positions) were analyzed considering all three codon positions.

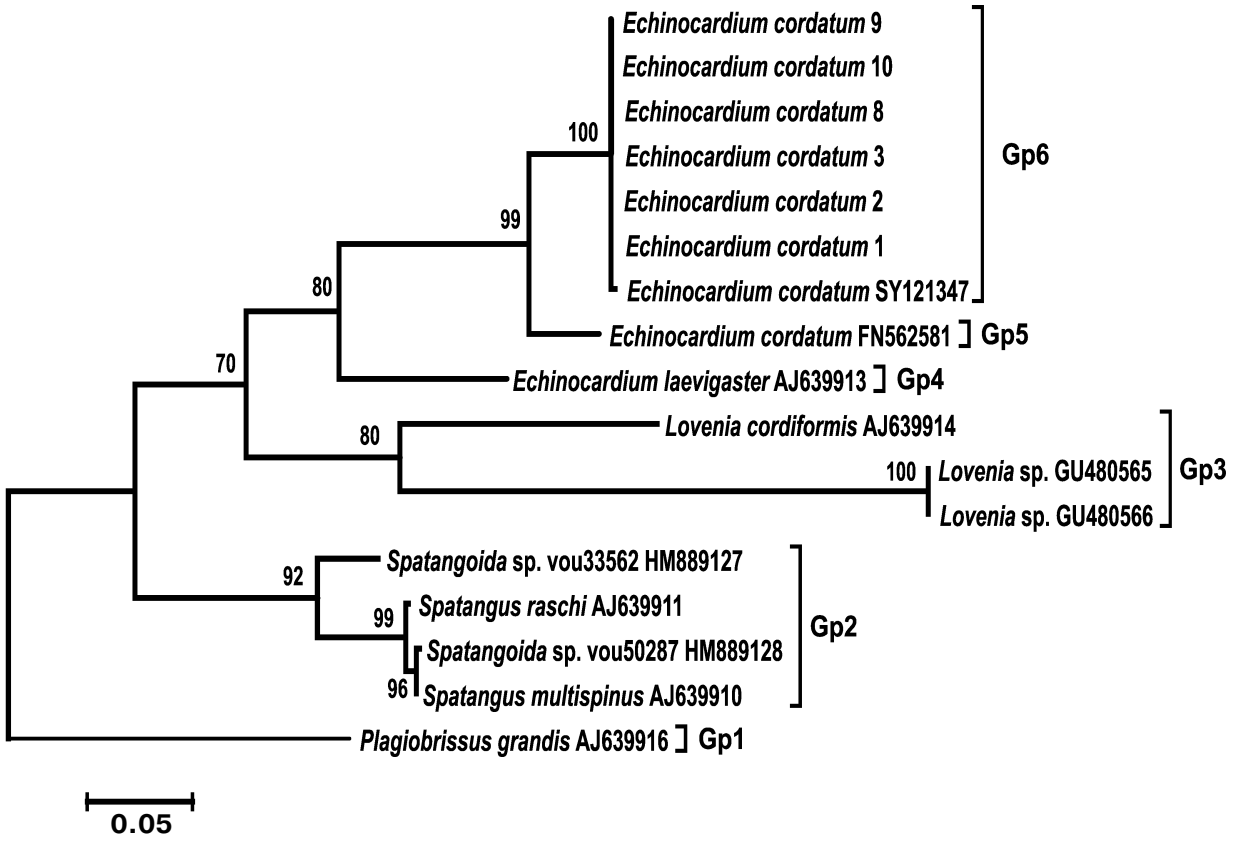

Fig. 3. Phylogenetic tree for the 5′-region of the mitochondrial *COI* gene from sea urchins of the Loveniidae family. The tree with the maximum logarithm of likelihood (−2555.0476) is shown. The bootstrap values are shown on the branches leading to the corresponding clusters. The lengths of branches are proportional to the number of substitutions. Seventeen sequences (length, 656 positions) were analyzed considering all three codon positions.

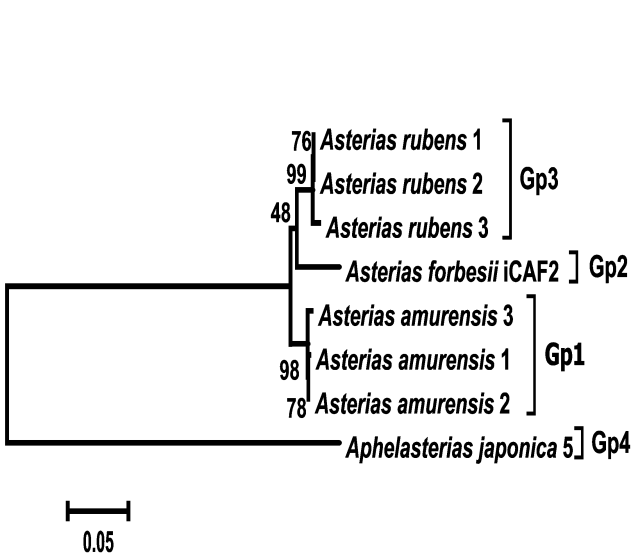

Fig. 4. Phylogenetic tree of the *ITS1*, *ITS2*, and 5.8S rDNA sequences from starfish of the Asteriidae family. The tree with the maximum logarithm of likelihood (−4008.5599) is shown. The bootstrap values are shown on the branches leading to the corresponding clusters. The lengths of branches are proportional to the number of substitutions. Eight sequences (length, 1600 positions) were analyzed.

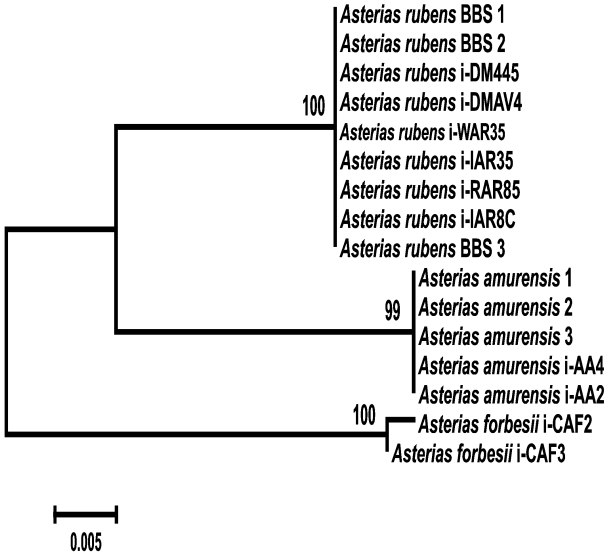

Fig. 5. Phylogenetic tree of the *ITS1* sequences from starfish of the Asteriidae family. The tree with the maximum logarithm of likelihood (−862.2724) is shown. The bootstrap values are shown on the branches leading to the corresponding clusters. The lengths of branches are proportional to the number of substitutions. Sixteen sequences (length, 488 positions) were analyzed.

Table 2. Intergroup divergence matrix for the mitochondrial *COI* genes from starfish of the Asteriidae family

Group	Gp1	Gp2	Gp3	Gp4	Gp5	Gp6	Gp7
Gp1	0	[0.015]	[0.016]	[0.018]	[0.028]	[0.029]	[0.032]
Gp2	0.104	0	[0.005]	[0.018]	[0.028]	[0.033]	[0.030]
Gp3	0.110	0.022	0	[0.019]	[0.027]	[0.033]	[0.031]
Gp4	0.121	0.114	0.121	0	[0.026]	[0.033]	[0.031]
Gp5	0.238	0.238	0.235	0.218	0	[0.031]	[0.036]
Gp6	0.228	0.259	0.258	0.262	0.255	0	[0.032]
Gp7	0.265	0.251	0.267	0.256	0.298	0.259	0

Notes: Distribution between groups was based on sequence clustering in the phylogenetic tree (Fig. 2): Gp1, *Ast. rubens*, 53 isolates; Gp2, *Ast. amurensis*, 6 isolates; Gp3, *Ast. amurensis*, 12 isolates; Gp4, *Ast. forbesii*, 20 isolates; Gp5, *Forcipulatida* sp., 1 isolate; Gp6, *Aph. japonica*, 5 isolates; Gp7, *Distolasterias nipon*, 1 isolate. Below the diagonal, intergroup divergence mean values expressed in number of nucleotide substitutions per site (after accounting for intragroup divergence); above diagonal (in brackets), standard errors. A total of 98 sequences were analyzed considering all three codon positions. Genetic distances were calculated using the two-parameter model of nucleotide substitutions [16].

same genus with bootstrap support of 80 and 92%, respectively. The *COI* sequence from another family of spatangoid sea urchins was at the base of the phylogenetic tree. These results demonstrate that nucleotide sequences of the mitochondrial *COI* gene form specific or, as in the case of *Ast. amurensis*, population clusters.

DNA sequences for the *ITS1* and *ITS2* nuclear spacers and 5.8S rDNA (total length, 1365-1466 nucleotides) were amplified and sequenced for three isolates of *Ast. amurensis* (1, 2, and 3), three isolates of *Ast. rubens* (BBS1, BBS2, and BBS3), and one isolate of *Aph. japonica*. These sequences were aligned together with a homologous sequence from the *Ast. forbesii* CAF2 isolate and analyzed phylogenetically. Similarly to *COI* genes, *ITS1*, *ITS2*, and 5.8S rDNA sequences from each of the species (*Ast. rubens* and *Ast. amurensis*) formed monophyletic groups with high bootstrap support (99 and 98%, respectively). The *Ast. forbesii* sequence was assigned to the *Ast. rubens* cluster with low bootstrap support (48%) (Fig. 4).

Analysis of 16 *ITS1* sequences (Fig. 5) showed with 100% bootstrap support that sequences from *Ast. rubens* isolates from the White Sea (BBS1, BBS2, and BBS3) belonged to a homogenous group of the North Atlantic population of this species. *Asterias amurensis ITS1* sequences also formed a cluster with high bootstrap support (99%) that was a sister cluster to *Ast. rubens*. The *ITS1* sequences of two *Ast. forbesii* isolates form a group (100% bootstrap) at the base of the tree.

Analysis of intra- and intergroup polymorphism. To determine the levels of intra- and intergroup polymorphism of the studied species, their sequences were clustered into groups based on the results of phylogenetic reconstruction, and the inter- and intragroup genetic distances were determined. We found that the levels of intergroup polymorphism for the *COI* gene in starfish species of the Asteriidae family varied from 0.003 to 0.009 (as estimated from genetic distances within the corresponding groups), while the levels of intergroup polymorphism varied from 0.104 to 0.298 (as estimated from genetic distances between specific and generic clusters) (Table 2).

The genetic distances between species of the genus *Asterias* were within the range 0.104-0.121; the corresponding values of the intergeneric divergence varied from 0.218 to 0.298 (Table 2). Therefore, the values for the intraspecific (0.003-0.009) and interspecific (0.104-0.121) genetic distances for the genus *Asterias* did not overlap.

Evaluation of the intragroup genetic distances for sea urchins of the Loveniidae family (Table 3) showed a considerably higher level of interspecific divergence of the *COI* gene in the genus *Echinocardium* (0.171-0.177) compared to starfish. Besides, the genetic distance (0.068) between the *COI* sequences from an *E. cordatum* specimen from the North Sea (Gp5) and specimens from the Sea of Japan and the Yellow Sea (Gp6) considerably exceeded the intraspecific genetic distances in starfish. The levels of the intrageneric divergence (0.146-0.257) were similar to those in starfish.

Estimation of genetic distances for the sequences of transcribed nuclear markers (*ITS1* – 5.8S rDNA – *ITS2*) in starfish of the Asteriidae family showed significantly lower level of intraspecific divergence (0.031-0.045) and higher level of intrageneric divergence (0.328-0.350) compared to the values obtained using the mitochondrial *COI* gene (Table 4).

DISCUSSION

Genetic analysis showed (Fig. 2) that *COI* gene sequences from different populations of the same starfish species form monophyletic clusters. Thus, sequences of

Table 3. Intergroup divergence matrix for the mitochondrial *COI* genes from sea urchins of the Loveniidae family (order Spatangoida)

Group	Gp1	Gp2	Gp3	Gp4	Gp5	Gp6
Gp1	0	[0.026]	[0.026]	[0.029]	[0.030]	[0.031]
Gp2	0.215	0	[0.020]	[0.022]	[0.022]	[0.022]
Gp3	0.190	0.149	0	[0.019]	[0.026]	[0.023]
Gp4	0.233	0.163	0.146	0	[0.024]	[0.023]
Gp5	0.251	0.193	0.195	0.177	0	[0.010]
Gp6	0.257	0.194	0.174	0.171	0.068	0

Notes: Distribution between groups was based on sequence clustering in the phylogenetic tree (Fig. 3): Gp1, *Plagiobrissus grandis* AJ639916, 1 isolate; Gp2, genus *Spatangus* and *Spatangoida* sp., 2 isolates each; Gp3, genus *Lovenia*, 3 isolates; Gp4, *Echinocardium laevigaster*, 1 isolate; Gp5, *E. cordatum*, 1 isolate; Gp6, *E. cordatum*, 6 isolates. Below diagonal, intergroup divergence mean values expressed in number of nucleotide substitutions per site (after accounting for intragroup divergence); above diagonal (in brackets), standard errors. Seventeen sequences were analyzed considering all three codon positions. Genetic distances were calculated using the two-parameter model of nucleotide substitutions [16].

Table 4. Intergroup divergence matrix for sequences of nuclear spacers *ITS1*, *ITS2*, and 5.8S rDNA from starfish from the Asteriidae family

Group	Gp1	Gp2	Gp3	Gp4
Gp1	0	[0.006]	[0.004]	[0.019]
Gp2	0.049	0	[0.005]	[0.020]
Gp3	0.031	0.045	0	[0.018]
Gp4	0.350	0.355	0.328	0

Notes: Distribution between groups was based on sequence clustering in the phylogenetic tree (Fig. 4): Gp1, *Ast. amurensis*, 3 isolates; Gp2, *Ast. forbesii*, 1 isolate; Gp3, *Ast. rubens*, 3 isolates; Gp4, *Aph. japonica*, 1 isolate. Below diagonal, intergroup divergence mean values expressed in number of nucleotide substitutions per site (after accounting for intragroup divergence); above diagonal (in brackets), standard errors. Eight sequences were analyzed; total number of positions (including gaps) was 1601. Genetic distances were calculated using the two-parameter model of nucleotide substitutions [16].

three studied species of the genus *Asteria*s form monophyletic groups with high bootstrap values (99%). Note that geographically distant North Atlantic (American), East Atlantic (European), and White Sea populations of *Ast. rubens* form a homogenous cluster in which no subgroups could be distinguished. The observed two artifacts (assignment of *Aph. japonica* DQ592560 sequence to the *Ast. amurensis* cluster and assignment of *Ast. rubens* DQ077915 sequence to the *Ast. forbesii* cluster) were most probably due to species misidentification. It is evident that the *COI* sequence from *Aph. japonica* DQ592560 belongs to the *Ast. amurensis* cluster, since sequences of all five other *Aph. japonica* specimens formed a single cluster positioned at the base of the phylogenetic tree and distant from the *Ast. amurensis* cluster. The sequence *Ast. rubens* DQ077915 most probably belongs to *Ast. forbesii*. Since species of the genus *Asterias* are difficult to be distinguished based on morphological features only, they could be easily misidentified, especially when their areas overlap. Indeed, two *Asterias* species were found in the North Atlantics. *Asterias forbesii* inhabits the shelf zone of the North American coast from Cape Hatteras to Cape Cod. The area of *Ast. rubens* lies north of the area of *Ast. forbesii*. The European population of *Ast. rubens* inhabits the Atlantic coast shelf from Iceland to West France. The areas of North American populations of *Ast. forbesii* and *Ast. rubens* overlap in a large shelf region around Cape Cod [17]. Therefore, the data (including the artefacts) show that the fragment of the *COI* gene could be used for species identification of starfish.

Analysis of relations at a higher taxonomic level showed that *Ast. amurensis* (North Pacific) and *Ast. rubens* (North Atlantic) are the closest species within the *Asterias* genus despite significant geographical remoteness of their areas. The proximity of these two species is emphasized by the number of common specific features: out of 36 phylogenetically informative sites, 13 supported clustering (vs. 7 sites for clustering of *Ast. rubens* and *Ast. forbesii*). At the same time, the North Atlantic species *Ast. forbesii* is equidistant from the other two species and localizes to the base of a group combining the three species of this genus. Positioning of sequences from

species of two other genera from the Sea of Japan (*Distolasterias* and *Aphelasterias*) at the base of the tree indicates considerable distance between these genera and *Asterias*. It should be noted that reliability of the estimation of relations between taxa higher than species based on the *COI* gene sequence might be questionable because of gene saturation with mutations due to the old evolutionary age of the taxa.

Identification of animal species and populations is based on the levels and character of the intra- and interspecific divergence of marker sequences. By now, extensive, but not sufficient, information has been accumulated on the divergence of the mitochondrial *COI* gene. In most cases, the intraspecific divergence of this gene stays below 1% and rarely exceeds 2% (such increased levels are mostly found in animals of the same species but from geographically distant regions or can be explained by the presence of cryptic species) [18]. The level of divergence can differ in different groups, although similar groups usually display similar values. Thus, for mollusks from the Vesicomyidae family, the levels of the intraspecific and interspecific divergence of the *COI* gene were estimated as 0.2-0.8 and 3.9-10.3%, respectively [6-8]. Similar values were found for mollusks of the Veneridae family [9]. In echinoderms, the levels of the *COI* gene intraspecific divergence vary within the 0.0-3.0% range (average, 0.62%); the levels of interspecific divergence vary within the 0.0-27.06% range (average, 15.33%) [3-5]. Analysis of the *COI* gene divergence for 22,266 populations and species of animals from various groups revealed the following *p*-distance values for taxa of different rank: 0.89 ± 0.16 – population; 3.78 ± 1.18 – for subspecies, semispecies, and sibling species; 11.06 ± 0.53 – morphologically distinct species; 16.60 ± 0.69 – species from different genera within a family; 20.57 ± 0.40 – families within the same order [19].

The values for the *COI* gene divergence in starfish of the Asteriidae family and sea urchins of the Loveniidae family determined in this study lie within the above-mentioned ranges. Moreover, the genetic distance between *COI* sequences of *E. cordatum* specimens from the Northern Sea and the Sea of Japan (6.8%) raises some doubt if these animals belong to the same species, although we cannot neglect the fact that their populations are very geographically distant [18].

We failed to confirm the applicability of the rRNA nuclear spacers for identification of animal species and populations. Apparently, the usefulness of genetic distances for this purpose is questionable because of the low levels of interspecific divergence (3.1-4.9%). It has been shown that the presence of species-specific compensatory replacements in the *ITS2* sequence that could be identified by analysis of the secondary structure might serve as a more reliable criterion in this case [20].

In conclusion, our results show that sequences of the transcribed nuclear spacers (*ITS*) and mitochondrial *COI* gene can be used as reliable markers for identification of echinoderm species from the Sea of Japan and the White Sea.

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