RESEARCH PAPER

PHYLOGENETIC PHYTOGEOGRAPHY OF SELECTED GROUPS OF SEAGRASSES (MONOCOTYLENDONEAE - ALISMATALES) BASED ON ANALYSING OF GENES 5.8S rRNA AND RUBISCO LARGE SUBUNIT.

Anton A. Iurmanov[1*]
[1]Main Botanical Garden named after N. V. Tsitsin RAS, st. Botanicheskaya, 4, Moscow, 127276, Russia
***Corresponding author:** yurmanov–anton.ya.ru@yandex.ru

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ABSTRACT. Seagrasses are representatives of the families Cymodoceaceae, Posidoniaceae, Zosteraceae, Hydrocharitaceae (Monocotylendoneae - Alismatales), adapted to growing in seawaters and all their important life circle events are taking place under the water including pollination and distribution of diasporas. Seagrasses are widespread in the littoral areas of the World Ocean, except for Antarctica, and play an important ecosystem role. Due to the insufficiently studied history of dispersal and formation of modern seagrasses habitats, we carried out a phylogenetic analysis of representatives of the families Cymodoceaceae (*Amphibolis, Halodule, Syringodium, Cymodocea,* and *Thalassodendron*), Posidoniaceae (*Posidonia*), Zosteraceae (*Zostera*, and *Phyllospadix*), and Hydrocharitaceae (*Enhalus, Halophila*, and *Thalassia*). The cladograms constructed based on molecular data analysis of the 5.8S ribosomal RNA and ribulose–1,5–bisphosphate carboxylase/oxygenase large subunit genes are used as the basis for reconstructing the history of dispersal of the studied taxa. It is found that the main stages of dispersal of selected groups of seagrasses took place in the Late Cretaceous period. The main track of historical distribution is largely predetermined by the modern ranges of almost all genera of seagrasses, stretches from the southwestern waters of eastern Gondwana to the northwestern waters of the Eurasian part of Laurasia. The main route of movement of diasporas and seagrasses populations was the Tethys water area, which was modified in the Late Mesozoic and early Cenozoic. It was revealed that the main method of dispersal of seagrasses was long-distance dispersal, which is confirmed by both molecular genetic data and very fast (on a geological time scale) processes of penetration into new water areas, and analysis of the features of dissemination of modern representatives. An alternative vicar scenario was proposed only for the reconstruction of the formation of the *Posidonia* range.

KEYWORDS: seagrasses, higher Alismatales, phylogenetic phytogeography, dispersal history, cladistic analysis, molecular data

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INTRODUCTION

Seagrasses are an ecological group of marine monocotyledonous angiosperms (Monocotylendoneae - Alismatales) adapted for growing in seawater. This ecological group includes representatives of the families Cymodoceaceae (*Amphibolis, Halodule, Syringodium, Cymodocea, Thalassodendron*), Posidoniaceae (*Posidonia*), Zosteraceae (*Zostera, Phyllospadix*), and Hydrocharitaceae *(Enhalus, Halophila, Thalassia*) and the plants grow, become pollinated, and produce fruits underwater (Hogarth 2015; Iurmanov et al. 2021). Seagrasses grow in estuaries, bays, lagoons, banks, and shoals in most parts of the Northern and Southern Hemispheres, except for the coastal areas of Antarctica (Hemminga and Duarte 2000). The distribution of seagrasses depends on the ability to spread and occupy new areas of the littoral and sublittoral zones. The highest diversity of seagrasses is observed in Southeast Asia and in different directions out of this area the number of species decreases along the trajectory of the main ocean currents (Green and Short 2003). Seagrasses populations are productive ecosystems that create three-dimensional shelter structures and generally form the habitat for dozens of companion species (Nagelkerken and van der Velde 2004), including breeding and growth sites, the final link in the food chain in such ecosystems often become marine mammals (Irlandi and Peterson 1991). They are important carbon sinks, accounting for about 10% of the total carbon sequestration in the atmosphere. Seagrasses communities sequester about 27.4 million tons of CO_2

annually (Macreadie et al. 2013). All this gives reason to consider them as habitat formers, similar in importance to the meadows of monocotyledonous plants on land (Coleman and Williamc 2002). Since a decrease in the cover of seagrasses in the sublittoral leads to a decrease in biodiversity and a change in the entire structure of coastal marine biocoenosis, they require attention not only as biological species but also as biotopes (Hemminga and Duarte 2000).

The available information on seagrasses fossils is not very extensive, but it is sufficient to establish in general terms the main stages of their evolution. Reliable fossils records are present for two extinct genera, *Thalassocharis* and *Archeozostera*, from the Upper Cretaceous of Western Europe (Voigt 1981) and Japan, respectively (Larkum and den Hartog 1989), and the modern genus *Posidonia* is represented by fossil species *Posidonia cretacea* Hosius von der Marck found in the sediments of the Upper Cretaceous, belonging to the Late Santonian - Campanian stages (84.9 - 70.6 Ma ago), in Ahrenfeld (Germany) (Hosius and Von der Marck 1880). Presumably, the first angiosperms returned to the marine environment during the Cretaceous, about 100 to 70 million years ago (Les et al. 1997). However, early Cretaceous seagrasses are almost unknown. The initial invasion of the ancestral sea by seagrasses occurred later, presumably in the Tertiary along the shores of the Tethys in parallel with the evolution of mangrove forests (Hogarth 2015). After that, the change in the areas of distribution of seagrasses was a consequence of tectonic movements, the emergence of barriers (landmasses), fragmenting the Tethys into partially isolated water areas. These barriers explain, for example, the phenomenon of habitation of morphologically closely related pairs of species from the genera *Thalassia, Halodule*, and *Syringodium* in the Pacific Ocean and the Caribbean, as well as *Zostera* and *Phyllospadix* on opposite coasts of the Pacific, separated, respectively, by the Isthmus of Panama and the vast water area of the central part of the Pacific. Throughout history, seagrasses have probably never been represented by a large number of species. Some of the fossil species are now extinct, but it is unlikely that the total number of species ever exceeded 50-60. Supposedly this can be explained by the reproductive limitations of life in the marine environment and the tendency for the spread of seagrasses by vegetative reproduction (the phenomenon of "vegetative mobility"), and not with the help of seed reproduction.

At the end of the Eocene, about 40 million years ago, most modern seagrasses genera evolved, including *Thalassia, Thalassodendron, Cymodocea*, and *Halodule*, while *Enhalus* and *Phyllospadix* appeared more recently (Larkum and den Hartog 1989). The origin of the seagrasses remains unclear as far as the fossil data are insufficient, and the modern molecular data support the concept that the life form of seagrasses arose during the evolution of monocots of the order Alismatales s. l. more than once and independently. The genera of Hydrocharitaceae (*Enhalus, Halophila*, and *Thalassia*) are most likely descended from freshwater ancestors that acquired salt tolerance and spread downstream into the sea; whereas the representatives of other families of seagrasses (Posidoniaceae, Cymodoceaceae, Zosteraceae) descended from freshwater or marine ancestors independently from each other and developed largely in parallel (Les et al. 1997). To model the history of dispersal of seagrasses the relationship between the genera of seagrasses was revealed based on molecular genetic data and taking into account paleobotanical data. The resolving of the evolutionary relationships of various species of key genera and families of seagrasses will make it possible to reconstruct the processes of their dispersal and to gain an idea of the hypothetical origin of modern marine biosystems.

MATERIALS AND METHODS

For the reconstruction of the dispersal history of the seagrasses, it was analyzed almost half (35 out of a total of 70 species) of the representatives, distributed in different parts of the range. As outgroup was selected *Hydrocharis morsus-ranae L.* (Hydrocharitaceae) and *Scheuchzeria palustris* F. Muell. (Scheuchzeriaceae) sister to seagrasses (APG IV 2016). Molecular genetic data was not enough to create a single cladogram of these species, so it was necessary to separate molecular cladograms for two groups of species. For molecular genetic analysis of genes was selected the data are shown in GenBank: 5.8S ribosomal RNA gene, and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) large subunit gene (Table 1.). Because of the formation of two groups of analyzed species, we created several phylogenetic scenarios (for greater representativeness of the results obtained) for most families. These two concepts of evolutionary relationships served as the basis for the reconstruction of a unified dispersal history model.

Table 1. Selected species for the study, sequenced for the 5.8S ribosomal RNA gene and ribulose–1,5–bisphosphate carboxylase/oxygenase large subunit gene

Gene	5.8S ribosomal RNA gene	ribulose–1,5–bisphosphate carboxylase/oxygenase large subunit gene
Species	*Hydrocharis morsus–ranae L., Scheuchzeria palustris F. Muell., Posidonia australis Hook. f., P. oceanica (L.) Delile, Zostera asiatica Miki, Z. capricorni Asch., Z. japonica Asch. & Graebn., Z. marina L., Z. mucronata Hartog, Z. noltii Hornem., Z. novazelandica Setch., Phyllospadix torreyi S. Watson, Cymodocea nodosa* (Ucria) *Asch., C. serrulata* (R. Br.) *Asch. & Magnus, Halodule pinifolia* (Miki) *Hartog, H. uninervis* (Forssk.) *Boiss., H. wrightii Asch., Thalassia hemprichii* (Ehrenb.) *Asch., Th. testudinum Banks ex K. D. König, Enhalus acoroides* (L. f.) *Royle, Halophila australis Doty & B. C. Stone, H. beccarii Asch., H. decipiens Ostenf., H. engelmannii Asch., H. hawaiiana Doty & B. C.Stone, H. major* (Zoll.) *Miq., H. ovalis* (R.Br.) *Hook. f., H. tricostata M. Greenway*	*Hydrocharis morsus–ranae L., Scheuchzeria palustris F. Muell., Posidonia australis Hook. f., P. oceanica (L.) Delile, Zostera angustifolia* (Hornem.) *Reichenb., Z. asiatica Miki, Z. capensis Setch., Z. capricorni Asch., Z. japonica Asch. & Graebn., Z. marina L., Z. mucronata Hartog, Z. noltii Hornem., Phyllospadix scouleri Hook., Ph. torreyi S. Watson, Ph. japonicus Makino, Amphibolis antarctica* (Labill.) *Asch., A. griffithii* (J. M. Black) *Hartog, Cymodocea serrulata* (R. Br.) *Asch. & Magnus, Halodule pinifolia* (Miki) *Hartog, H. uninervis* (Forssk.) *Boiss., H. wrightii Asch., Syringodium filiforme Kütz., S. isoetifolium* (Asch.) *Dandy, Thalassodendron pachyrhizum Hartog, Thalassia hemprichii* (Ehrenb.) *Asch., Th. testudinum Banks ex K. D. König , Enhalus acoroides* (L. f.) *Royle, Halophila decipiens Ostenf., H. engelmannii Asch., H. ovalis* (R.Br.) *Hook. f.*

Using DNA sequences data from GenBank, phylogenetic molecular genetic cladograms were constructed for genera of families of seagrasses and outer groups. After that, the encoded gene portions are copied into one file, which has loaded into the WinClada program to build a phylogenetic cladogram (Hall 2011). Selected portions of the DNA sequence are quite large, their length is in selected species from 477 to 670 (5.8S ribosomal RNA gene) and from 619 to 970 (RuBisCo large subunit) nucleotides.

The alignment for non-homologous portions was performed in Jalview Version: 2.9.0b2 (Waterhouse et al. 2009) using the Clustal W algorithm with standard parameters. The end portions that were not present in all sequences were excised. As a result, an alignment was obtained with a length of 670 (5.8S ribosomal RNA gene) and 970 (RuBisCo large subunit) nucleotides. The resulting alignment was also saved in FASTA format and processed in WinClada ver. 1.00.08 using the NONA ver. 2. Using bootstrap analysis, phylogenetic trees were constructed for 26 and 27 species seagrasses with outer groups.

RESULTS

As a result of our research, cladograms were obtained (Fig. 1, A, and B, respectively). The resulting cladograms are generally similar with high at most nodes bootstrap index, but there are differences in the topology of cladogram A, which reflects the relationship of 26 species of seagrasses (analysis of the RuBisCo large subunit gene) and cladogram B, reflecting the relationship of 27 species of seagrasses (analysis of the 5.8S ribosomal RNA gene).

Basal in both of these cladograms is the outgroup species - *Hydrocharis morus-ranae*. This is followed by a division into two large clades. The first consists of the seagrasses Hydrocharitaceae family species. The basal taxon in clades A and B is *Enhalus acoroides*. The sister species *Thalassia hemprichii* and *Thalassia testudinum* are closest to it. Also in this segment of the cladogram is the *Halophila* group of species. The second clade consists of the families Cymodoceaceae, Posidoniaceae, and Zosteraceae. A similar feature is the proximity of the Halodule and Posidonia clades. In the group of species of the Zosteraceae family, the clade begins with the species of the genus Phyllospadix, and the terminal taxa are the group of species of the genus Zostera.

Also, in the topology of cladograms, there are differences. The first distinguishing feature of the two phylogenetic trees is the topology of the genus *Halophila*. In contrast to cladogram A, in cladogram B, *Halophila decipiens* and H. *ovalis* have a sister, not a daughter position.

The position of one of the species of the outgroup, the *Sheuchzeria palustris* group, which in cladogram B goes immediately after the group of the genus *Halophila*, also differs. On cladogram A, before *Sheuchzeria palustris*, there are groups in sister relationships with a high bootstrap index (minimum 99); they form the species *Cymodocea nodosa* and C. *serrulata*, followed by closely related groups *Posidonia australis* and P. *oceanica*, as well as the clade of the genus *Halodule*, which includes all 3 studied species - H. *pinifolia*, H. *uninervis*, H. *wrightii*. While H. *pinifolia* and H. *wrightii* occupy a daughter position to H. *uninervis* in cladogram B.

In cladogram A, the representative of the outgroup, *Sheuchzeria palustris*, is followed by *Phyllospadix torreyi*. It, in turn, is followed by the terminal genus *Zostera* on the cladogram, in which the basal *Zostera marina* is sister to all other studied species. The next bifurcation is represented by sister Z. *noltii* and closely related species of the genus (Z. *asiatica* and Z. *japonica*), as well as three species – Z. *capricorni*, Z. *mucronata*, Z. *novazelandica*. In cladogram B, a similar clade begins with *Phyllospadix japonicus*, inhabiting the northwest of the Pacific followed by a sister group of species from *Ph. torreyi* and *Ph. scouleri*, distributed in the northeast of the Pacific. The terminal taxon in the cladogram, as in the previous case, is *Zostera*; however, the relationships between the species of the genus are markedly different. The *Zostera* species form two sister clades, the first of which includes Z. *asiatica* and the sister species Z. *angustifolia*

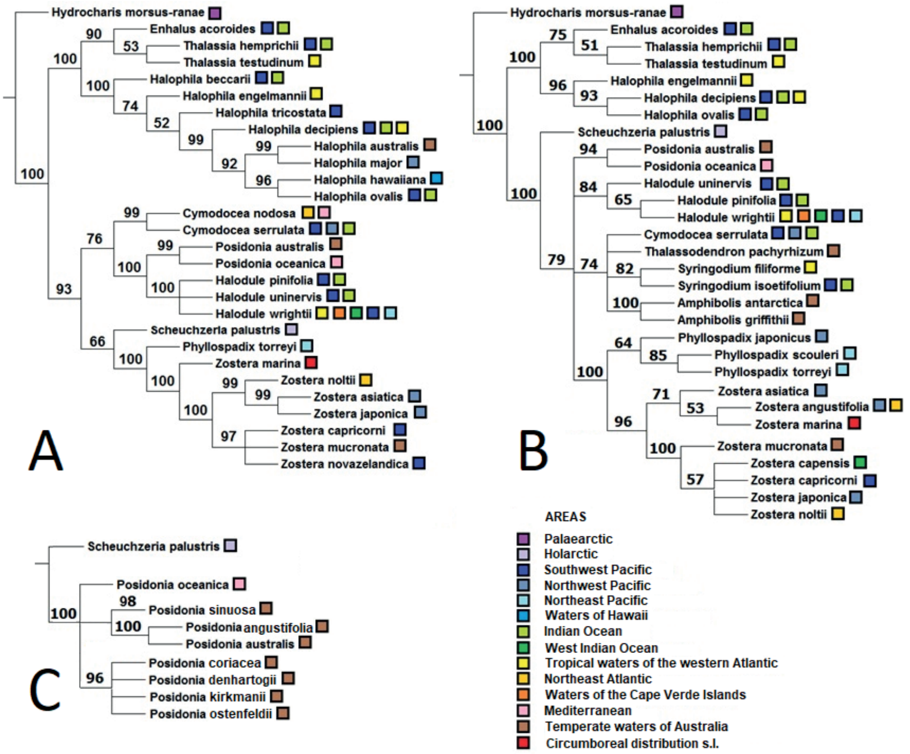

Fig. 1. Original phylogenetic trees of selected representatives of seagrasses, indicating their range, numbers - bootstrap index: A - 26 species (analysis of the RuBisCo large subunit gene), B - 27 species of sea grasses (analysis of the 5.8S ribosomal RNA gene), C - 8 species of the Posidoniaceae family (5.8S ribosomal RNA assay)

and Z. *marina*; in the second clade, the basal species is Z. *mucronata*, sister to which is a polytomy of four species: Z. *capensis*, Z. *capricorni*, Z. *japonica*, Z. *noltii*. This position of the freshwater representative of the outgroup *Sheuchzeria palustris* in both cladograms of the Zosteraceae family indicates the origin of the family independent of other seagrasses.

DISCUSSION

To begin with, it is necessary to turn to the individual stages of the geological history of the Earth and the change in the position of the continents during the collapse of Gondwana, that is, the opening of new water areas, littoral bridges for the settlement of seagrasses. The penetration of seagrasses through the littoral zones into new water areas is associated with the disintegration of Eastern Gondwana, which included Australia, Antarctica, Madagascar, and India, as well as the course of such geological processes as the end of the Messinian crisis (5 million years ago) and the separation of Japan from Eurasia (15 -20 million years ago) (Barnes 2003; Garcia-Castellanos et al. 2009).

The complete separation of the southern part of Gondwana took place 120-100 million years ago, leaving the Subantarctic Islands as the only connecting link between Antarctica and South America (Gabdullin 2005), the littoral of which could have become through the dispersal of seagrasses from the water area of Australia. Dissemination of seagrasses occurs both vegetatively and by seeds, but, unfortunately, has not yet been any studies that determine the time that the fruit can be held in the water and does not rot, as well as how long may be part of the plants by the roots and leaves is a substrate (that is, to vegetate in the water column, drawn by currents, until the moment when they can be fixed in the substrate). That is, the distance that seagrasses can travel in one "act of resettlement" is not known. In this regard, an interesting example is *Halophila hawaiiana*, an endemic to the Hawaiian Islands, which, according to our data, penetrated its present range from the southwestern Pacific. The flora of the Hawaiian Islands - both terrestrial and coastal - was formed due to the influx of new species and their further evolution in isolation for a long time (70 million years ago - the formation of the first islands, and the age of the oldest of the current surface, Midway - 28.3 million years ago (Clague & Dalrymple 1987). The remoteness and isolation of the Hawaiian Islands predetermined that the first marine monocots were able to make a long way to them without intermediate "stops". The proposed seagrasses dispersal model is based on phylogenetic trees (Fig. 1) and paleobotanical data (Fig. 2).

Seagrasses Hydrocharitaceae family

In addition to small families formed only by taxa of seagrasses, there is the freshwater family Hydrocharitaceae, in which there are three genera of marine (Cook 1998). The genus *Enhalus* (family *Hydrocharitaceae*) includes a single species, *Enhalus acoroides* (L. f.) Royle is a perennial herb (Cook 1998). *Enhalus* lives in the tropical coastal waters of the Indian and western Pacific oceans. The genus *Halophila* (family *Hydrocharitaceae*) includes 19 species of perennial or annual rhizome herbaceous plants (Cook 1998). Dissemination of seeds is hydrochory (pericarp rots underwater) or endozoochory (juicy fruits are eaten by aquatic animals). The genus *Halophila* is widespread in tropical waters, but its range also covers subtropical and temperate waters, primarily the Indian and Pacific Oceans. Also, representatives of the genus grow on the coasts of the Mediterranean and Caribbean Seas, the Gulf of Mexico. The genus *Thalassia* (family *Hydrocharitaceae*) includes 2 species: *Thalassia hemprichii* (Ehrenb.) Asch. and *Thalassia testudinum* Banks ex K. D. König is a perennial rhizome herbaceous plant (Cook 1998). Dissemination is hydrochloric (conical seeds with a massive basal part have a displaced center of gravity). Two species belonging to the genus Thalassia have a non-overlapping range. *Thalassia hemprichii* (Ehrenb.ex Solms) Asch. grows in the littorals of the tropical zone of the Indian Ocean and the western part of the Pacific, and *Thalassia testudinum* Banks & Sol. ex K. D. Koenig lives in the littoral areas of the Caribbean Sea.

Unfortunately, now, fossil marine representatives of this family, represented mainly by freshwater forms, have not been found. Our data suggest that the family settled in the Southwest Pacific/Indian Ocean, where most species of the genus still live (Fig. 3). In particular, the basal Enhalus acoroides and the closely related genus *Thalassia*, possibly widespread earlier. Later, the formation of land barriers divided the common range into two

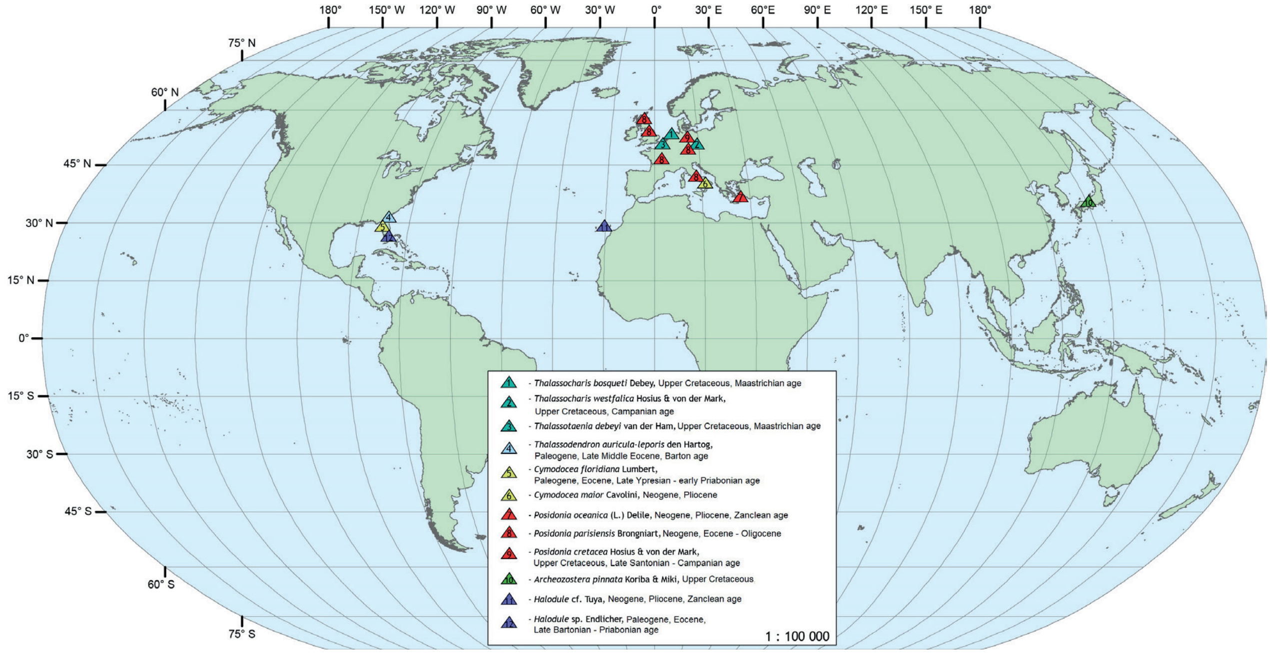

Fig. 2. Localities of discovery of fossils seagrasses

species growing in the warm waters of the Southwest Pacific, Indian Oceans (*Thalassia hemprichii*), and in the Caribbean Sea with adjacent waters (*Thalassia testudinum*). As for the numerous and widespread genus *Halophila*, representatives of the genus inhabited the tropical waters of the Atlantic twice independently (*Halophila engelmannii* and *Halophila decipiens*), two other tracks of the *Halophila* dispersal reached the center/northwest of the Pacific Ocean (*Halophila* major) and temperate waters of Australia (*Halophila australis*). The sister position of *Halophila hawaiiana*, which reached the water area of the Hawaiian Islands, in relation to *Halophila ovalis*, testifies to the fact that this species, whose range is noticeably isolated from other taxa of the genus, settled from the southwestern Pacific (Iurmanov 2018).

Family Cymodoceaceae

The Cymodoceaceae family includes 5 genera (17 species): *Amphibolis* (2 species), *Cymodocea* (4 species), *Halodule* (6 species), *Syringodium* (2 species), *Thalassodendron* (3 species) (APG IV) of perennial herbaceous plants (Kuo and McComb 1998a). Hydrochory dissemination (*Amphibolis* fruits with 4 hard blades covered with thin thorns can spread epizoochory, juicy bracteole of *Thalassodendron* fruits can be eaten by fish), for *Cymodocea nodosa* (Ucria) Asch. autobasichoric is characteristic.

The genus *Amphibolis* is distributed along the western and southern coasts of Australia and Tasmania. The genus *Cymodocea* is widespread in the littorals of the tropical and subtropical seas of the Old World. The genus *Halodule* is widespread on tropical and subtropical coasts. It is found, in fact, on most continents, except for Europe and Antarctica. Representatives of the genus Syringodium are found on the coasts of the Indian and Pacific Oceans, the Caribbean, and the Gulf of Mexico. The genus *Thalassodendron* grows in the western part of the Indian Ocean and the western part of the Pacific Ocean - *Thalassodendron ciliatum* (Forssk.) Hartog littoral of the tropical seas of the Indian and western Pacific oceans, *Thalassodendron pachyrhizum* Hartog littoral of the temperate coast of Western Australia, Thalassodendron leptoarteaule Mariade , sp. nov. littoral of the coast of Mozambique and KwaZulu-Natal.

The evolution of this family is relatively well documented by fossil remains (Fig. 2). For example, there are reliably dated and well-preserved fossils of the genus *Halodule* on the western and eastern sides of the Atlantic. Fossils of the genus *Halodule* have long been represented only by finds of *Halodule sp*. from the Paleogene, Eocene, Late Bartonian - Priabonian stages (40.4 - 37.2 Ma ago) in Florida (USA) (Taylor 2008). However, in 2017, fossils of this genus were discovered in the Canary Islands. The presence of fossil remains of *Halodule sp*., on the island of Gran Canaria (Canary Islands), was explained by warm currents during the Neogene, Pliocene, Zanklian stage (5.332 - 3.6 million years ago). The identification was confirmed by the presence of fossilized plagiotrophic rhizomes and fruits in the sedimentary deposits of the Early Pliocene. This fossil material from seagrasses is the first on the West African coast (Tuya et al. 2017). *Thalassocharis bosqueti* Debey was discovered in Limburg (Netherlands) and dates from the Upper Cretaceous, more precisely, its Maastrichtian stage (70.6 - 66.043 million years ago) (Voigt 1981). Also *Thalassocharis westfalica* Hosius von der Marck, dated to the Campanian Stage (83.6 - 72.1 Ma ago), was discovered in Westphalia (Germany) (Hosius and Von der Marck 1880). *Thalassodendron auriculaleporis* den Hartog occurs in sediments of the Paleogene, Late Middle Eocene, Bartonian Stage (40.4 - 37.2 Ma ago) in Florida (USA) (Lumbert et al. 1984). The appearance of the first reliable fossil representatives of this genus in America, where it is now widespread, corresponds to the concept of den Hartog (1970), according to which the range of this genus is relict. It is also worth noting *Thalassotaenia debeyi* van der Ham from the Maastrichtian Stage, the Upper Cretaceous System (70.6 - 66.043 Ma ago), found in Belgium (van der Ham et al. 2007). Various fossils and possibly existing species represent the genus *Cymodocea*. These are, for example, *Cymodocea floridana Lumbert*, which occurs in sediments of the Paleogene, Late Middle Eocene, Bartonian Stage (48.6 - 37.2 Ma ago) in Florida (USA) (Lumbert et al. 1984), and *Cymodocea maior* Cavolini, belonging to the Neogene, Pliocene (5.332 - 2.558 Ma ago), discovered in Copanello (Italy) (Ruggieri 1952).

The dominant in the processes of dispersal of the genera Cymodoceaceae was probably the same track as

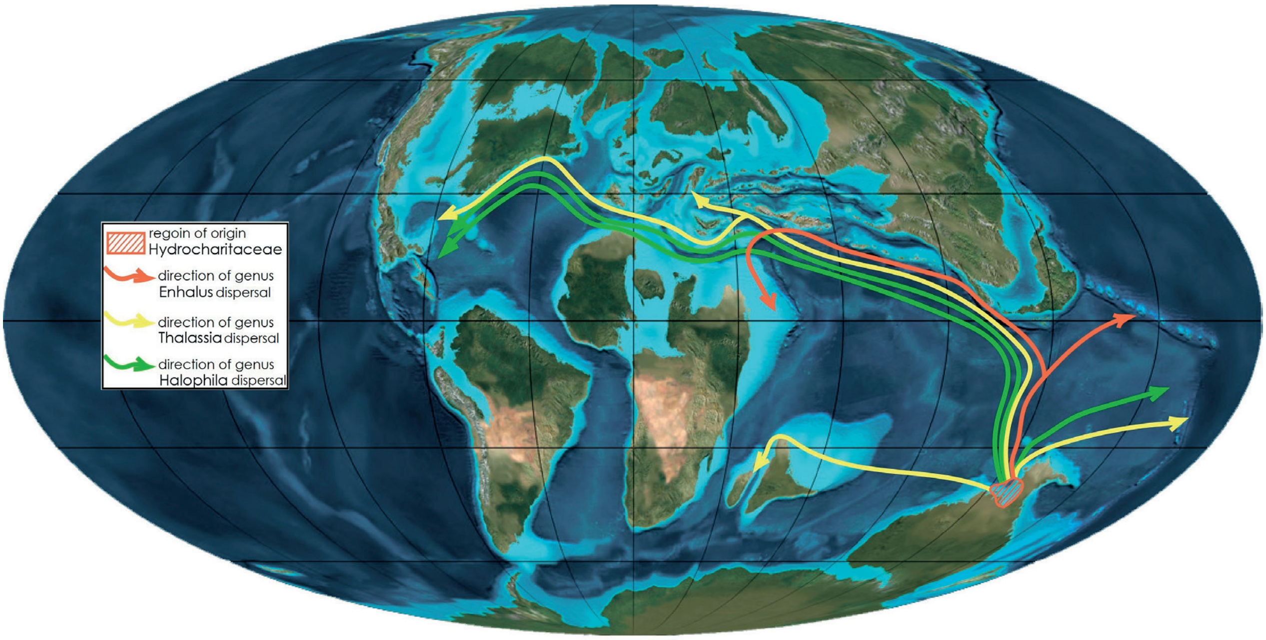

Fig. 3. Formation of the range of marine representatives of the Hydrocharitaceae family. Hereinafter: The position of the land corresponds to the Upper Cretaceous period (90 million years ago). Basemap - Paleogeographic map by Ron Blakey, Colorado Plateau Geosystems (2011)

in the marine taxa Hydrocharitaceae (Fig. 4). Halodule from the waters of the southwestern Pacific, where the genus probably arose (which is confirmed by the distribution of the basal species here - *Halodule uninervis*), spread to the Indian and South Pacific Ocean (*Halodule uninervis* and *Halodule pinifolia*), reaching even the western coast of Central America (*Halodule wrightii*). Another track was the settlement of *Halodule* in the tropical waters of the Atlantic across the waters of western Africa, where the *Halodule wrightii* range now remains. The dispersal of other Cymodoceaceae taxa proceeded according to a different scenario. The genus *Syringodium (Syringodium filiforme)*, which appeared in this water area, settled from the tropical waters of the western Atlantic, from which, upon reaching the western coast of Africa (*Syringodium isoetifolium*). The genus *Cymodocea (Cymodocea serrulata)* probably originated, and then - and the genera *Thalassodendron (Thalassodendron pachyrhizum)* and *Amphiobolis (Amphiobolis antarctica* and *Amphiobolis griffithii*), which reached the southern coast of Australia during further dispersal (Iurmanov et al. 2020a).

Family Posidoniaceae

The Posidoniaceae family includes 1 genus (9 species) - *Posidonia* (APG IV) of perennial herbaceous rhizome plants (Kuo and McComb 1998b). Dissemination is hydrochory, fish and seabirds eat juicy fruits. The genus Posidonia has a disjunctive range - the Mediterranean Sea and the Atlantic coast of the Iberian Peninsula (*Posidonia oceanica* (L.) Delile), as well as the southern coast of Australia and Tasmania.

According to den Hartog (1970), two *Posidonia* fossils are known (Fig. 2): *Posidonia cretacea* Hosius and Von der Marck (Hosius and Von der Marck 1880), and *Posidonia parisiensis* (Brongt.) Freitel (= *Caulinites parisiensis Brongt. Posidonia perforata* Saporta & Marion) from the Eocene of the Paris Basin, Germany, Italy, Great Britain, also found in the Oligocene deposits of Great Britain (48.6 - 23.03 million years ago) (Stockmans 1932). These species have thick rhizomes with short internodes and flattened pointed leaves. Among other things, in the sediments of the Neogene, Pliocene, Zanklian stage (5.332 - 3.6 million years ago), on the territory of the island of Rhodes (Greece), a recent species, *Posidonia oceanica*, was found (Moissette et al. 2007).

To analyze the hypothetical history of the settlement of the Posidoniaceae family and to better understand the relationships within the family, it is necessary to refer to a separately constructed cladogram (Iurmanov, 2017) (Fig. 1C). The basal species in this cladogram is *Posidonia oceanica*, probably the oldest taxon of the genus (and family). Fossils of *Posidonia* are found only in Europe. The described cladogram considers two equally probable scenarios: the first, within the framework of the long-distance dispersal concept, and the second, based on the vicar paradigm. (Fig. 5). The first scenario assumes that the genus *Posidonia* originated in the water area that corresponds to the modern Mediterranean Sea, no later than the Cretaceous period. Probably, at the end of the Cretaceous through the water area of the increasing Tethys Ocean, the species of the genus migrated to the Southern Ocean, where they settled in the water areas adjacent to Australia. The content of the second scenario is that at the end of the Cretaceous period, the hypothetical "progenitor species" *Posidonia* inhabited almost the entire Tethys basin. *P. oceanica* is probably closest to this basal species since the relatively stable conditions of the Mediterranean basin contributed to the conservation of the traits of the "progenitor species," whose descendants are so widely represented as fossil remains in Europe. Taxa of the genus, distributed in the southeastern regions of Tethys (den Hartog 1970), evolved at a faster rate, transforming during the Tertiary period into riverine species of temperate waters in Australia and Tasmania (Iurmanov, 2017).

Family Zosteraceae

The Zosteraceae family includes 2 genera (22 species) of perennial herbaceous plants (Kuo and McComb 1998c): *Phyllospadix* (6 species) and *Zostera* (16 species) (APG IV). Dissemination is hydrochloric. The genus *Zostera* is generally widespread along the coast of most of the Northern Hemisphere, as well as Australia, New Zealand, Southeast Asia, and southern Africa. One species of the genus is *Zostera noltii* Hornem. – also grows in the waters of the enclosed Caspian Sea. *Phyllospadix* is native to the temperate North Pacific.

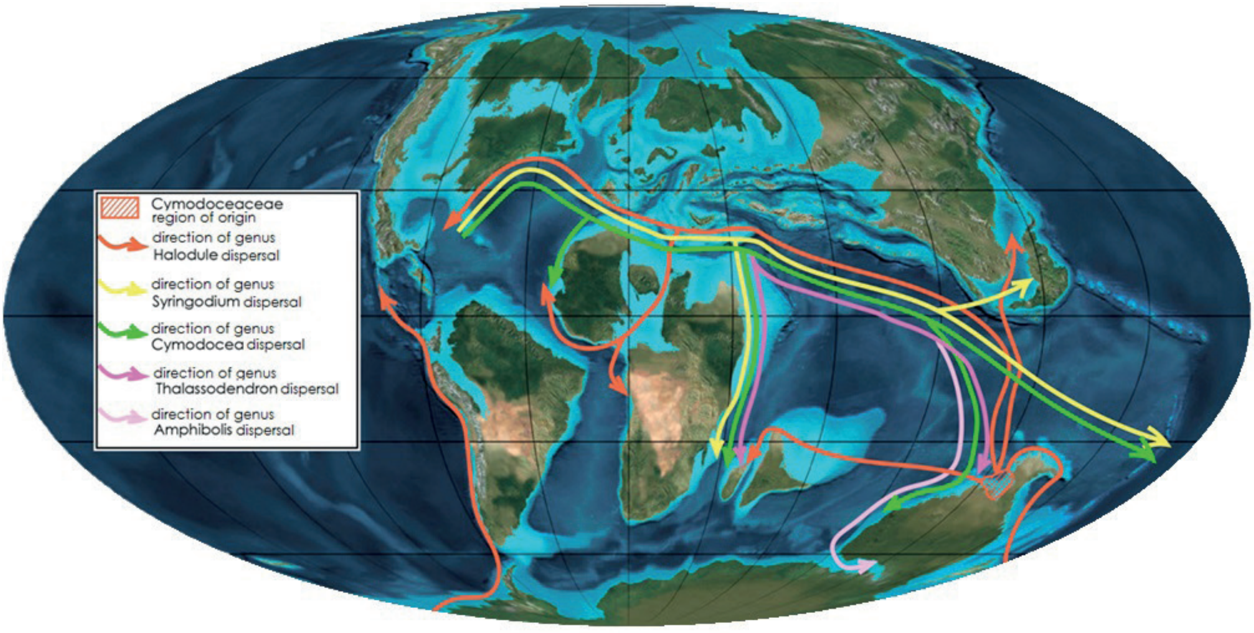

Fig. 4. Formation of the range of the Cymodoceaceae family

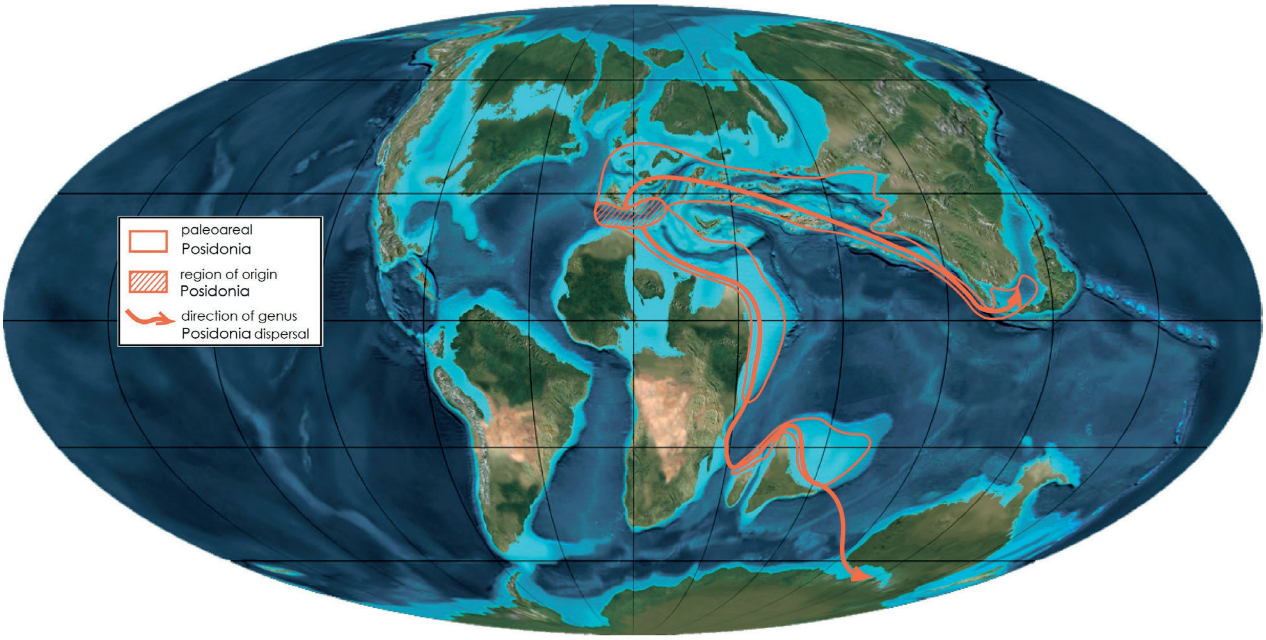

Fig. 5. Formation of the range of the Posidoniaceae family

The reconstruction of the history of distribution and settlement of this family is based on the results of original phylogenetic research and paleobotanical data (Fig. 6). Well-preserved fossils of Archeozostera Koriba & Miki (= *Archaeozostera*) (1931, 1958), have been described from the Upper Cretaceous (99.7 - 66.043 Ma ago) from several ranges of Japan, in particular Izumi, and were considered as protozosteroid - ancestral forms of modern Zosteraceae (den Hartog 1970; Larkum and den Hartog 1989). However, a re-examination of these fossils led to the conclusion that *Archeozostera* was not a marine monocot (Kuo and McComb 1989). They were inland freshwater so, we assume, that *Archeozostera pinnata* was a freshwater, this ancestor of modern Zosteraceae, which are quite resistant to desalination. Therefore, we can assume that the dispersal of representatives of this family began precisely from this range (Fig. 3).

The basal species in the cladogram (Fig. 1 B) is the species *Phyllospadix japonicus* inhabiting the waters of China, Korea, and Japan, while the *Phyllospadix* species from the west coast of North America are sister species to the first taxon. Presumably, the genus *Phyllospadix* settled on the North American coast of the North Pacific - from west to east; some species of the genus *Zostera* likely followed the same path, which also appeared, most likely, in the water area corresponding to the present Far Eastern seas. The genus *Zostera* could have two, not mutually exclusive, tracks in the future dispersal. The first is along the northern coasts of Eurasia. This fact is confirmed by the fact that the modern *Zostera* marina in this region overwinters under ice and endures desalination (water area of the White Sea), that is, *Zostera* may have been resettled along the "northern route" along the confluence of large rivers and further drift of diasporas to the west, which allowed representatives sort to reach the northeastern Atlantic and further - the northwestern part of its water area. At the same time, the sister species of *Zostera marina, Zostera angustiflora*, and *Zostera asiatica*, spread from west to east along the littorals of Beringia. Another dispersal track for Zostera passed through the southwest Pacific and the Indian Ocean, reaching Australia (*Zostera mucronata*), New Zealand and New Guinea (*Zostera capricorni*), and the east coast of Africa (*Zostera capensis*). Possible competition with other species of primordially tropical seagrasses (and, hypothetically, with abundant macrophyte algae in temperate waters of the Southern Hemisphere) could significantly complicate the dispersal of the genus *Zostera* in the waters of the southern Indian and Pacific oceans (Iurmanov et al. 2020b).

Phylogenetic phytogeography of selected groups of seagrasses

Thus, despite the presumably different regions of origin of the selected families of seagrasses, we can distinguish common tracks of their dispersal. The most important of them originate in the subtropical waters of Australia, follow through southern Asia to the east coast of Africa and the waters of Madagascar. The seagrasses colonization of the eastern coast of North America proceeded from the waters of eastern Asia through the waters of northern Europe, and from the basin, widely understood as Tethys, through the western coasts of Africa. But the settlement of the western coast of the Americas, most likely, was carried out from the waters of Eastern Eurasia through the waters of Beringia, as well as from the waters of eastern Australia along with the littoral islands of the Antarctic - up to the western coast of South America. Representatives of the genus Zostera performed the last track (Iurmanov and Romanov 2020).

CONCLUSIONS

1. The analysis of fossil remains of seagrasses concludes that the main families of this ecological group arose at the turn of the Early/Late Cretaceous and rather quickly settled in the World oceans, by the end of the Mesozoic having settled in tropical and subtropical littorals, as well as penetrated temperate waters in certain water areas.

2. The family Zosteraceae probably arose in the waters corresponding to the present northern part of the Pacific, and settled from there along the northern and southern coasts of the Eurasian landmasses, penetrating the waters of the Southern Hemisphere at least twice.

3. The region of origin of the only genus of the family Posidoniaceae was the water area of the Tethys, or only its extreme western part (corresponding to the modern Mediterranean basin). According to the most probable dispersal scenario, the ancient Posidonia species settled in the Southern Hemisphere, "moving" along the littoral zones of the western part of the Indian Ocean.

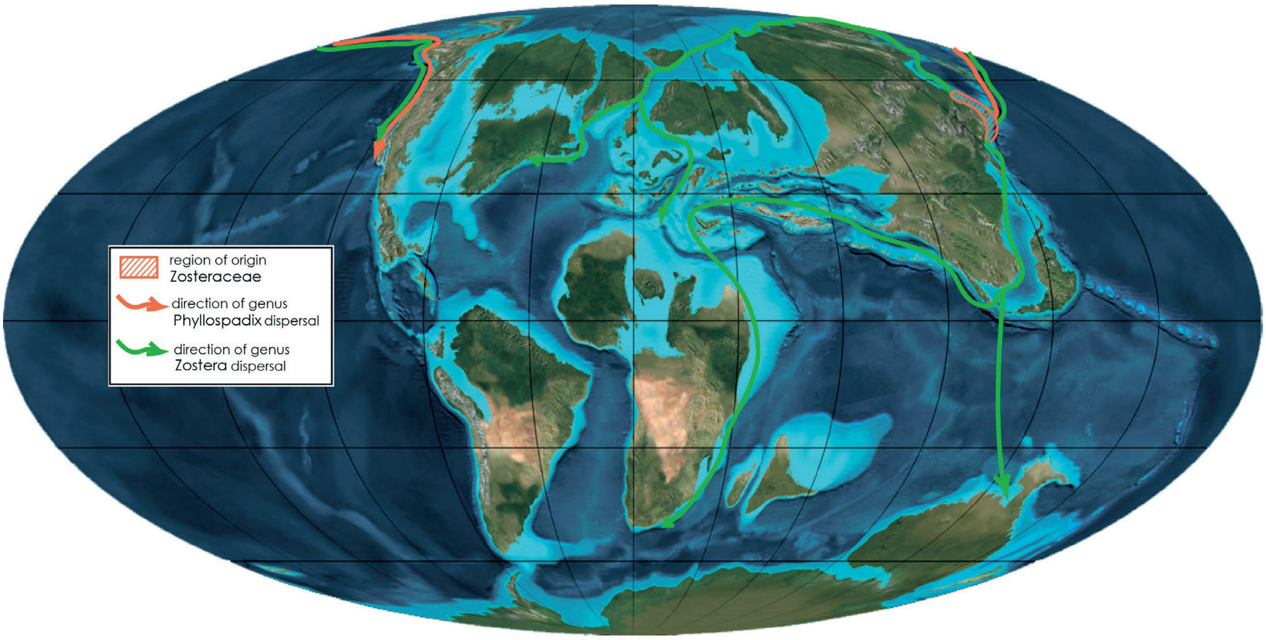

Fig. 6. Formation of the range of the Zosteraceae family

4. In the waters of the tropical part of East Gondwana (Australia + Antarctica), the Cymodoceaceae family arose. This most polymorphic (5 genera) group of seagrasses spread across the World Ocean through various tracks, the most important of which passed along the southern coast of the Eurasian continental masses (through the Tethys' residual basin) and then reached the water area of the modern Atlantic. The genera Cymodocea and Syringodium formed in these waters used the same track to populate the waters of the Indian Ocean and the southwestern Pacific, but "moving" along it, however, in the opposite direction.

5. Analogously, marine representatives of the Hydrocharitaceae family - the genera *Enhalus, Halophila*, and *Thalassia* - settled into the Atlantic from the waters of the tropical part of Eastern Gondwana. Just like in the representatives of Cymodoceaceae, seagrasses from the number of Hydrocharitaceae also spread along other, secondary tracks, penetrating the waters of the Pacific and the southwestern Indian Ocean.

6. The main method of dispersal of the studied groups of seagrasses was long-distance dispersal, which is confirmed not only by molecular genetic data and very fast (on the scale of geological time) penetration into new water areas but also by the analysis of the features of dissemination of modern representatives. Only for the reconstruction of the formation of the range of the genus *Posidonia* was an alternative vicar scenario proposed. ■

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