

Evolutionary Trends in *Hydrocharitaceae* Seagrasses

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Authors' contributions

This work was carried out in collaboration between both authors. Authors AB and SBH designed the study, wrote the protocol and interpreted the data. Author AB anchored the field study, gathered the initial data and performed preliminary data analysis. Authors AB and SBH managed the literature searches and produced the initial draft. Both authors read and approved the final manuscript.

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ABSTRACT

This paper provides evidences of the evolutionary pathway followed by one of the main groups of marine angiosperms, the *Hydrocharitaceae*. Current molecular data has confirmed the aquatic origin of these plants. The *Hydrocharitaceae* group has a cosmopolitan distribution and is well represented in the fossil record in Europe and North America. Morphological and phylogenetic data has shown dramatic differences between the *Hydrocharitaceae* and the other marine angiosperms. Furthermore, it supports the hypothesis that aquatic monocot ancestors were able to adapt to a continuously changing environment caused by widespread continental flooding in the Cretaceous Period when seagrasses first occur, to a gradual regression of inland seas during the Eocene leading to subsequent adaptation to a completely submerged marine environment within the subfamily *Hydriloideae*.

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1. INTRODUCTION

Marine angiosperms (seagrasses) are taxonomically confined to 60 species in 13 genera, assigned to five different monocot families within the single order *Alismatales*, aquatic subclass *Alismatidae* (alimatids). Seagrasses are known to inhabit the sea worldwide with the exception of Antarctica, representing the utmost adaptive radiation of freshwater plants on Earth [1]. Physical drivers, such as climate change, ocean currents and tectonic events, have been influential in their distribution. To date there are no studies examining patterns of biodiversity change of seagrasses over global or regional scales. According to Barret et al. [2] and Les [3] aquatic vascular plants in general have been reported as having a conservative macro-evolutionary pattern due to their low genetic variability and population differentiation below the species level.

Aquatic monocots have been indubitably present since the early Cretaceous as confirmed by the oldest fossils assigned to this clade, 110-120 million years old [4,5]. Monocot fossil records also confirm the early divergence of seagrasses during this time [5]. Using molecular clocks, the monocots have been dated between 124--141 MYA [6-11]. The origin and evolution of monocots and of those especially known to inhabit the seas is an intriguing subject that has evoked several hypotheses; Arber [12], Cronquist [13], Den Hartog [14], Les et al. [15], Wissler et al. [16] and Chen et al. [17]. The evolutionary pathway of seagrasses is still conjectural.

Arber [12] outlined very specialized features that were necessary for plants to have in order to exist and reproduce in marine environments. Her ideas were established from plant characteristics at the species level. She also suggested that marine angiosperms were indirectly derived from land plants that became long adapted to aquatic conditions and subsequently adapted further into salt tolerant or even brackish species, leading to completely submerged marine species. Cronquist [13] believed that the origin of monocots was aquatic and that terrestrial monocots are derived from aquatic pre-monocots. He also suggested that terrestrial monocots then conversely, gave rise, repeatedly, to aquatic groups. Among the new aquatic groups, some progressively adapted to a marine habitat. Den Hartog [14] on the other hand, by

examining monocot characteristics at the generic level came to a conclusion quite opposite that of Arber [12] and Cronquist [13]. Den Hartog [14] concluded that land plants, such as those that are found in mangrove communities, became salt tolerant in the first place and then established themselves in the marine environment. Furthermore, he speculated that the marine plants then evolved into brackish ones, and finally, into freshwater aquatics.

Using genetic analysis Les et al. [15], Wissler et al. [16], Chen et al. [17] and Ross et al. [18] concluded that seagrasses adaptation to complete submersion into the sea could have followed three separate adaptation lineages. This paper will concentrate on adaptations followed by the *Hydrocharitaceae* representatives.

2. METHODOLOGY

This study is largely based on a review of the available literature on the aquatic monocotyledons with emphasis on the *Hydrocharitaceae* Family including the four seagrasses: *Enhalus*, *Thalassia*, *Halophila* and the fossil *Thalassites* and three freshwater submerged genera: *Najas*, *Nechamandra* and *Vallisneria*; and observations made on our own (in part unpublished) material and results. The hydrocharitacean Floridian fossil seagrass *Thalassites parkavonensis* was examined for clues that would lead to the evolutionary trends in the *Hydrocharitaceae* seagrasses following the report of Benzecry [19] which compared the presence of paracytic stomata in members of the *Thalassia testudinum* (*Hydrocharitaceae* seagrass) and *Vallisneria* sp., a close freshwater submerged relative.

References will be made to representatives of the other seagrass families, Potamogetonaceae/ Zosteraceae, Cymodoceaceae, Posidoniaceae and Rupiaceae in order to better understand the evolutionary trends of the order *Alismatales*.

2.1 Molecular Phylogeny

Marine angiosperms polyphyletic origins were confirmed by molecular phylogenetic analyses at the family level [20,21]. Genetic studies of the *rbcl* gene [1,15,17,22,23] and complete plastid genomes [18] have determined that marine angiosperms evolved in three monophyletic clades: The *Hydrocharitaceae*, the Potamogetonaceae/ Zosteraceae, and the

Cymodoceaceae complex (Cymodoceaceae, Posidoniaceae and Ruppiaceae). Based on these results, Les et al. [15], Chen et al. [17] and Ross et al. [18] proposed that marine angiosperm ancestors were either freshwater plants or perhaps a mixture of freshwater and salt-tolerant species.

Comparisons of orthologous gene sequences of two seagrasses (*Posidonia oceanica* (L.) Delile and *Zostera marina* L.) within the order *Alismatales* and eight terrestrial angiosperms species by Wissler et al. [16] revealed that seagrass genes have diverged from their terrestrial counterparts via an initial aquatic stage characteristic of the order *Alismatales* and to the derived fully-marine stage characteristic of seagrasses. Sequence analyses of DNA by Les et al. [15], Chen et al. [17] and Ross et al. [18] have shown strong evidence of a monophyletic freshwater origin within the *Hydrocharitaceae*. Based on different phylogenetic analyses incorporating single and multiple gene sequences of cpDNA, mtDNA, nrDNA, rbcL and ndh, and morphological characteristics; Les & Tippery [1] and Ross et al. [18] confirmed their aquatic origin as well as the inclusion of the three marine genera (*Thalassia*, *Halophila* and *Enhalus*) into a single clade [1,15,18,24]. Contrary to other seagrasses in the Core Alismatids, pseudogenization of ndh genes between the seagrasses (*Thalassia*, *Halophila* and *Enhalus*) and their close freshwater relative *Vallisneria* was confirmed with 100% bootstrap support [18].

The *Hydrocharitaceae* group has a cosmopolitan distribution and is well represented in the fossil record in Europe and North America. The divergence time of *Hydrocharitaceae* is still a subject of debate and two competing ages (one much more recent than the other) have been proposed. Kato et al. [25] dated the seagrasses within *Hydrocharitaceae* at 119±11 MYA by analyses using the substitution rates of rbcL and matK. However, this time overlaps with the generally accepted age of the order *Alismatales* thus putting the validity of the results of that study into doubt [26]. Janssen & Bremer [10] placed the crown node age of this family in the Late Cretaceous (75 MYA) by analyses using rbcL and fossil calibrations also confirmed by Chen et al. [17].

2.2 Morphological Characters

The marine *Hydrocharitaceae* differ dramatically from other marine angiosperm lineages in

relation to the presence of stomata, their pollination mechanisms and pollen morphology as described by Chen, et al. [17], Waycott, et al. [27], and Tanaka et al. [28]. *Hydrocharitaceae* is a fully aquatic monocot family; which migrated from fresh water to the sea. It consists of 17 genera with approximately 127 species including freshwater, brackish water and marine representatives with paracytic stomata, a character considered to be of great importance for its identification of its freshwater representatives [13,29,30]. It is worthwhile to note that even though the other families containing marine representatives, Posidoniaceae, Cymodoceaceae, Ruppiaceae and Zosteraceae also contain freshwater and brackish water representatives; they are usually devoid of stomata [29,30]; indicating a different evolutionary adaptation trend.

Records indicate that stomatal apparatus development occurred at least four hundred million years ago (400 MYA) during the early evolution of plants [31]. At that time epidermal cells became interrupted by minute openings delimited by two specialized cells, the guard cells. Stomata are common to living plants in both land and fresh water environments, but previously unknown in seagrasses [14,29,30]. In terrestrial plants, it is believed that these specialized structures are involved in the exchange of gases between the plant and its environment due to the difference in osmotic pressure between the leaves and the roots. In submerged aquatic plants, these same specialized structures allow the plants to extrude water in a liquid form. However, only recently have we begun to understand and identify some of the environmental factors that control stomatal development [32-35].

The presence of more advanced stomatal types such as the paracytic stomatal complex (in which subsidiary cells that flank the stoma are parallel with the long axis of the guard cells) and the tetracytic stomatal complex (where guard cells are surrounded by four subsidiary cells) are common among freshwater monocots [36] but believed to be absent among marine species seagrasses [14,29,30]. A recent report [19] on the presence of paracytic stomata in *Thalassia testudinum* Banks ex König marine plants growing in the proximities of coastal freshwater intrusions raises the question of the possible evolutionary pathway of the *Hydrocharitaceae* seagrasses.

Further examination of the Floridian fossil seagrass *Thalassites parkavonensis* Benzecry & Brack-Hanes (*Hydrocharitaceae*) [37] which is also believed to have occurred in areas of “leaky coastal margins” [38,39], have shown to contain paracytic stomata (Fig. 1) similar to those of other *Hydrocharitaceae* species as described by [13,19,36,40-42] and others.

These discoveries as well as the current environmental conditions where seagrasses are found may indicate that phenotypic plasticity is operating in seagrasses just as it has in many aquatic and terrestrial plants [33,43-46]. To accurately determine patterns of plasticity and to investigate their ecological and evolutionary implications, we need to better understand the environmental context in which phenotypes are expressed.

2.2.1 Sample examined

Fossil, *Thalassites parkavonensis* Benzecry & Brack-Hanes [37], Fairleigh Dickinson University Paleobotanical Collection, specimen Holotype - AP Series # 267Ac and 267Bc (part and counterpart), Paratypes – AP Series # 268Ab, 268Ca, 268Cb, 268Cc, 268Cd, 268Ce, 275C, 276A. Samples collected at the dolomite/limestone quarry, approximately 1.5 miles south of Gulf Hammock (sec. 28, T14s, R16s), Levy County, Florida. Stratigraphy: Avon Park Formation, Claiborne Stage, Late Middle Eocene (38 MYA) [47].

2.3 Ecological and Environmental Conditions

The Atlantic Coastal Plain of Florida is underlain by a blanket of Miocene and post-Miocene siliciclastic deposits that overlie a thick sequence of Tertiary carbonates composed of Eocene to Miocene limestone and dolostone. Florida's limestone bedrock is continuously dissolved by moving water on the surface and underground, thus forming its karst topography. Submarine groundwater discharges (SGD) and other karst features present today along the Gulf of Mexico coasts and extending below sea level, occurred during a low stand of the Pleistocene sea when the top of the saturated zone stood lower than the bottom of the deepest natural wells [48-50]. Carruthers et al. [51] reported that outflow from submarine springs present both in Mexico and Florida may be influencing nutrient processes within *Thalassia testudinum* meadows. It is plausible, therefore, that an influx of freshwater in

a marine environment can cause a change in the marine plant's hydrostatic pressure, thereby inducing the phenotypic expression of stomata.

Several environmental factors such as light, temperature, dissolved oxygen and ionic concentration can, indeed, influence the expression of anatomical characters in plants. Allsopp [52] and Sculthorpe [53] reported that environmental factors such as water stress or a change in light conditions have induced a heterophyllous switch in freshwater aquatic plants and that those changes are reversible. Experiments by Ueno et al. [54], Bowes & Salvucci [55], Reiskind et al. [56] and Sultan [57], demonstrated that a change from C3 to C4 metabolism can occur within a plant when carbon dioxide is limited in the water. This biochemical change also causes structural changes such as the induction of Kranz anatomy in submerged amphibious plants [58].

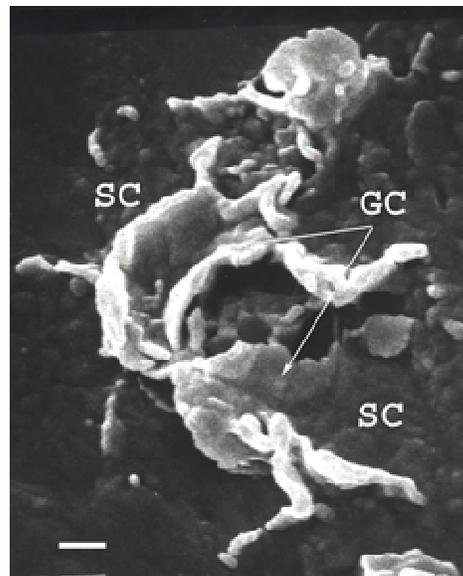


Fig. 1. *Thalassites parkavonensis* Benzecry & Brack-Hanes

(Fossil Leaf sample # 267Ac, Fairleigh Dickinson University Paleobotanical Collection) SEM micrograph of leaf epidermis and paracytic stomata. Stomatal apparatus comprised of two small guard cells (GC) $32\ \mu\text{m} \times 12\ \mu\text{m}$ each, with prominent poral thickenings surrounded by two large ($43\ \mu\text{m} \times 17\ \mu\text{m}$) reniform subsidiary cells (SC). Scale bar = $10\ \mu\text{m}$

Vallisneria and *Hydrilla* species are vital components of many freshwater habitats that can tolerate moderately short-term exposure to mesohaline conditions have become the main subject of numerous salt tolerance studies

[59-63]. Experiments dealing with chronic or sub-lethal salinity exposure of submerged aquatic vegetation have resulted in physiological changes [64] and population level changes [65]. Larkin et al. [66] studies of *Thalassia testudinum* revealed low levels of genetic diversity and differentiation among *Thalassia* populations. However, Hackney and Durako [67] and Kahn and Durako [68] reported that regional environmental differences in Florida Bay have significantly affected trends in the morphology of *Thalassia testudinum*. A series of temperature and salinity stress tolerance experiments in *Thalassia testudinum* [68-72] have confirmed the ability of this species to adapt to environmental changes leading to the full modification necessary for a harsh marine environment. The occurrence of stomata in some marine hydrocharitaceans (*Thalassia testudinum* and *Thalassites parkavonensis*) growing in "leaky coastal margins" provides evidence for phylogenetic pathways in that family, but does not address the subject of the evolution of seagrasses within other families. The fact that stomata were found in only those seagrasses living in close proximity to freshwater intrusions of coastal environments with SGD, implies that freshwater influx is now and possibly was a recurring stressful condition for the plants. Since only *Hydrocharitacean* seagrasses collected from coastal environments having freshwater intrusions have been described with stomata-bearing leaves, it becomes credible that the predisposition (genetic makeup) for stomata is present in the plant, regardless of expression. Furthermore, the occurrence of stomata in an Eocene hydrocharitacean freshwater *Hydrilla* species [73,74] and the marine *Thalassites parkavonensis* [37] from a coastal area with SGD demonstrates that it was a character expressed by hydrocharid in similar environments millions of years ago (about 38 MYA) just as it is today.

3. CONCLUSION

Records indicate that aquatic monocot ancestors were able to adapt to a continuously changing environment caused by the widespread continental flooding during the Cretaceous Period where seagrasses first occurred [4,5] to a gradual regression of inland seas during the Eocene, leading to the subsequent adaptation into a complete submerged marine environment. *Hydrocharitaceae* seagrasses' aquatic ancestry, as confirmed by molecular studies [1,17,18] and the facts outlined in this paper, further support the idea of a complete adaptation from a fresh

water environment to a submerged marine environment within the Subfamily Hydrilloideae.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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