Evolutionary Trends in *Hydrocharitaceae* Seagrasses

Alice Benzecry\(^1\) and Sheila Brack-Hanes\(^2\)

\(^1\)Fairleigh Dickinson University, School of Natural Sciences, H-DH4-03, 1000 River Road, Teaneck, New Jersey 07666, USA.

\(^2\)Eckerd College, Collegium of Natural Sciences, 4200 54th Ave. South, St. Petersburg, Florida 33711, USA.

**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.

**Article Information**

DOI: 10.9734/ARRB/2016/24354

**Editor(s):**

(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.

**Reviewers:**

(1) Flavio de Almeida Alves Junior, Universidade Federal de Pernambuco, Brazil.

(2) Manuel Mendoza Carranza, El Colegio de la Frontera Sur (ECOSUR), Mexico.

(3) Romulo Diego de Lima Behrend, Unicesumar, Brazil.

Complete Peer review History: [http://sciencedomain.org/review-history/13642](http://sciencedomain.org/review-history/13642)

**Received 16th January 2016**

**Accepted 2nd March 2016**

**Published 11th March 2016**

**Mini-review Article**

**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 *Hydrocharitoidae*.

*Corresponding author: E-mail: benzecry@fdu.edu*;
Keywords: Hydrocharitaceae; seagrasses; marine monocots; evolutionary trends.

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 (alismatids). 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 lines. 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 Rupiaceae). 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; witch 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, Rupiaceae 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.
Futher 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](image-url)

(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 µm x 12 µm each, with prominent poral thickenings surrounded by two large (43µm x 17µm ) reniform subsidiary cells (SC). Scale bar = 10µm

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.
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 phylogetic 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 Hydrazil 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 Hydroilloideae.

ACKNOWLEDGEMENT

This investigation was supported by a Fairleigh Dickinson University Research Grant to Alice Benzecry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Les DH, Tippery NP. In time and with water the systematics of alismatid monocotyledons. In Wilki, P. & Mayo SJ. editors. Early events in monocot evolution. Published by Cambridge University Press. The Systemic Association. 2013;118-164.
2. Barrett SCH, Eckert CG, Husband BC. Evolutionary processes in aquatic plant populations. Aquatic Botany. 1993; 44(2):105-145.
3. Les DH. Breading systems, population structure and evolution in hydrophylus angiosperms. Annals of the Missouri Botanical Gardens. 1988;75:819-835.
4. Friis EM, Pedersen KR, Crane PR. Araceae from the early cretaceous of Portugal: Evidence on the emergence of monocotyledons. Procedures of the National Academy of Sciences (USA). 2004;101:16565-1570.
5. Smith S. The fossil record of noncommelinid monocots, In Wilki P, Mayo SJ. editors. Early events in monocot evolution. Published by Cambridge University Press. The Systemic Association. 2013;29-59.
6. Bremer K. Early cretaceous lineages of monocot flowering plants. Proceedings of the National Academy of Sciences (USA). 2000;97:4707-4711.
7. Wikström M, Savolainen V, Chase MW. Evolution of the angiosperms: Calibrating the family tree. Proceedings of the Royal Society of London, Biological Sciences. 2001;268:2211–2220.
8. Chase MW. Monocot relationships: An overview of the best characterized major angiosperm clade. American Journal of Botany. 2004;91:1645–1665.
9. Sanderson MJ, Thorne JL, Wilk N, Bremer KG. Molecular evidence on plant
divergence times. American Journal of Botany. 2004;91:1656–1665.

10. Janssen T, Bremer KG. The age of major monocot groups inferred from 800 + rbcL sequences. Botanical Journal of the Linnean Society. 2004;146:385–398. DOI: 10.1111/j.1095-8339.2004.00345.x

11. Bell CD, Soltis DE, Soltis PS. The age of the angiosperms: A molecular time-scale without a clock. Evolution. 2005;59:1245–1258.

12. Arber A. Water plants – a study of aquatic angiosperms. Cambridge at the University Press. 1920;436.

13. Cronquist A. The evolution and classification of flowering plants. 2nd edition. New York Botanical Garden, Bronx, New York. 1988:555.

14. Den Hartog C. The sea-grasses of the World. Amsterdam: North Holland Publishing Co. 1970;275.

15. Les DH, Moody ML, Soros CL. A reappraisal of phylogenetic relationships in the monocotyledon family Hydrocharitaceae (Alismatidae). Aliso. 2006;22:211-230.

16. Wissler L, Codoner FM, Gu J, Reusch TB, Olsen JL, Proccacini G, Bomberg-Bauer E. Back to the sea twice: Identifying candidate plant genes for molecular evolution to marine life. BMC Evolutionary Biology. 2011;11:8. DOI: 10.1186/1471-2148-11-8

17. Chen LY, Chen JM, Gituru RW, Wang QF. Genetic phylogeny, historical biogeography and character evolution of the cosmopolitan aquatic plant family Hydrocharitaceae. BMC Evolutionary Biology. 2012;12. DOI: 10.1186/1471-2148-12-30

18. Ross TG, Barrett CF, Gomez MS, Lama VKY, Henriquez CL, Lese DH, Davsf JI, Cuenca A, Peterseng G, Sebergg O, Thadeoh M, Givnishi TJ, Conranj J, Stevensonh DW, Grahama SW. Plastid phylogenomics and molecular evolution of Alismatales. Cladistics. 2015;1:19.

19. Benzecry A. Field notes on Thalassia testudinum growing under stress conditions. European Journal of Environment. 2013;1:7-10.

20. Les DH, Garvin DK, Wimpee CF. Phylogenetic studies in the monocot subclass Alismatidae: Evidence for a reappraisal of the aquatic order Najadales. Molecular Phylogenetics and Evolution. 1993;2(4):304-14.

21. Les DH, Haynes RR. Systematics of alismatiflorae: A synthesis of molecular and non-molecular approaches. In Monocotyledons: Systematics and evolution, Rudell P, et al. eds. Royal Botanic Gardens, Kew, Great Britain. 1995;353-377.

22. Les DH, Cleland MA, Waycott M. Phylogenetic studies in alismatidae, II: evolution of marine angiosperms (Seagrasses) and Hydrophy. Systematic Botany. 1997;22(3):443-463.

23. Chen JM, Gituru RW, Wang QF. Evolution of aquatic life forms in Alismatidae: Phylogenetic estimation from chloroplast rbcL sequence data. Israel Journal of Plant Sciences. 2004;52:323-329.

24. Petersen G, Seberg O, Davis JI, Stevenson DW. RNA editing and phylogenetic reconstruction in two monocot mitochondrial genes. Taxon. 2006:55:871-886.

25. Kato Y, Aoji K, Omori Y, Takahata N, Satta, Y. Phylogenetic analyses of Zostera species based on rbcL and matK nucleotide sequences: Implications for the origin and diversification of seagrasses in Japanese waters. Genes Genetic Systematics. 2003:8:329-342.

26. Castillo MS. An angiosperm diversification through time. American Journal of Botany. 2009;96(1):349-65.

27. Waycott M, Freshwater DW, York RA, Calladine A, Kenworthy WJ. Evolutionary trends in the seagrass genus Halophila (Thouars) insights from molecular phylogeny. Bulletin of Marine Sciences. 2002;71(3):1299-1308.

28. Tanaka N, Uehara K, Murata J. Evolution of floral traits in relation to pollination mechanisms in Hydrocharitaceae. In Wilki P, Mayo SJ. editors. Early events in monocot evolution. Published by Cambridge University Press. The Systemic Association. 2013:185-207.

29. Cronquist A. An integrated system of classification of flowering plants. Columbia University Press, New York. 1981:1288.

30. Dahlgreen RMT, Clifford HT, Yeo PF. The families of the monocotyledons. Springer-Verlag, Berlin. 1985:520.

31. Edwards D, Kerp H, Hass H. Stomata in early land plants: An anatomical and ecophysiological approach. Journal of Experimental Botany. 1998;49:255-278.
32. Gray JE, Holroyd GH, Van Der Lee FM, Bahrami AR, Sijmons PC, Woodward FI, Schuch W, Hetherington AM. The HIC signalling pathway links CO$_2$ perception to stomatal development. Nature. 2000;408: 713–716.

33. Wellsa CL, Pigliuccia M. Adaptive phenotypic plasticity: The case of heterophylly in aquatic plants. Perspectives in Plant Ecology, Evolution and Systematics. 2000;3:1-18.

34. Bird SM, Gray JE. Signals from the cuticle affect epidermal cell differentiation. New Phytologist. 2003;157:9-23.

35. Bergmann DC. Integrating signals in stomatal development. Current Opinion in Plant Biology. 2004;7:26-32.

36. Prabhakar M. Structure, delimitation, nomenclature and classification of stomata. Acta Botanica Sinica. 2004;46(2):242-252.

37. Benzecry A, Brack-Hanes SD. A new hydrocharitacean seagrass from the eocene of Florida. Botanical Journal of the Linnean Society. 2008;57:19–30.

38. Swarzenski PW, Reich CD, Spechler RM, Kidinger JL, Moore WS. Using multiple geochemical tracers to characterize the hydrogeology of the submarine spring of Crescent Beach, Florida. Chemical Geology. 2001;179:187–202.

39. Swarzenski PW, Martin JB, Cable JC. Submarine ground-water discharge in upper Indian River Lagoon, Florida. U.S. Geological Survey Karst Interest Group Proceedings. St. Petersburg, Florida. 2001; 194-197.

40. Vesque JJ. Epharmosis, sive, materiae ad instruendan anatomiam: Systematis naturatis, Folia capparearum. Vincennes: Delapierre; 1889.

41. Metcalf CR, Chalk L. Anatomy of dicotyledons. Oxford at the Clarendon Press; 1950.

42. Dilcher DL. Approaches to the identification of angiosperm leaf remains. Botanical Review. 1974;40:1-157.

43. Schlichting CD. The evolution of phenotypic plasticity in plants. Annual Review of Ecology and Systematics. 1986; 17:667–693.

44. Sultan SE. Phenotypic plasticity for plant development, function and life history. Trends in Plant Science. 2000;5:537–542.

45. Santamaria L. Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. Acta Oecologica, International Journal of Ecology. 2002; 23:137–154.

46. Riis T, Lambertini C, Olesen B, Clayton JS, Bri x H, Sorrell BK. Invasion strategies in clonal aquatic plants: Are phenotypic differences caused by phenotypic plasticity or local adaptation? Annals of Botany. 2010;106:813–822.

47. Vernon RO. Geology of citrus and Levy counties, Florida. State of Florida Board of Conservation, Florida Geological Survey Bulletin. 1951;33:95-111.

48. Stringfield VT, LeGrand HE. Relation of sea water to fresh water in carbonate rocks in coastal areas, with special reference to Florida, USA, and Cephalonia (Kephallinia), Greece. Journal of Hydrology. 1969;9:387–404.

49. Stringfield VT, LeGrand HE. Effects of karst features on circulation of water in carbonate rocks in coastal areas. Journal of Hydrology. 1971;14:139-157.

50. Müller RD, Sdrolias M, Gaina C, Steinberger B, Heine C. Long-term sea-level fluctuations driven by Ocean basin dynamics. Science. 2008;319(5868):1357-1362.

51. Carruthers TJB, Van-Tussenbroek BI, Dennison WC. Influence of submarine springs and wastewater on nutrient dynamics of Caribbean seagrass meadows. Estuarine, Coastal and Shelf Science. 2005;64(2-3):191-199.

52. Allsopp A. Land and water forms: physiological aspects. Handbuch Pflanzenphysiol. 1965;15:1236–1255.

53. Sculthorpe CD. The biology of aquatic vascular plants. Edward Arnold, London; 1967.

54. Ueno O, Samejima M, Muto S, Miyachi S. Photosynthetic characteristics of an amphibious plant, Eleocharis vivipara: Expression of C4 and C3 modes in contrasting environments. Proceedings of the National Academy of Sciences (USA). 1988;85:6733–6737.

55. Bowes G, Salvucci MA. Plasticity in the photosynthetic carbon metabolism of submersed aquatic macrophytes. Aquatic Botany. 1989;34:233–266.

56. Reiskind JB, Madsen TV, Van Ginkel LC, Bowes G. Evidence that inducible C4-type photosynthesis is a chloroplastic CO$_2$-concentrating mechanism in Hydriilla, a submersed monocot. Plant, Cell and Environment. 1997;20:211–220.
57. Sultan SE. Promising directions in plant phenotypic plasticity. Perspectives in Plant Ecology, Evolution and Systematics. 2004; 6:227–233.

58. Ueno O. Induction of Kranz anatomy and C4-like biochemical characteristics in a submerged amphibious plant by abscisic acid. Plant Cell. 1998;10:571–583.

59. Kraemer GP, Chamberlain RH, Doering PH, Steinman AD, Hanisak MD. Physiological responses of transplants of the freshwater angiosperm Vallisneria americana along a salinity gradient in the Caloosahatchee Estuary (Southwestern Florida). Estuaries. 1999;22:38–148.

60. Doering PH, Chamberland RH, McMunigal, JM. Effects of simulated salt water intrusions on the growth and survival of wild celery, Vallisneria americana, from the Caloosahatchee Estuary (South Florida). Estuaries. 2001;24:894–903.

61. French GT, Moore KA. Interactive effects of light and salinity stress on the growth, reproduction and photosynthetic capabilities of Vallisneria americana (wild celery). Estuaries. 2003;26:1255–1268.

62. Boustany RG, Michot TC, Moss RF. Effects of salinity and light on biomass and growth of Vallisneria americana from Lower St. Johns River, FL, USA. Wetland Ecological Management. 2010;18:203–217.

63. Lauer N, Yeagera M, Kahna A, Dobberfuhlb DR, Ross C. The effects of short term salinity exposure on the sublethal stress response of Vallisneria americana Michx. (Hydrocharitaceae). Aquatic Botany. 2011;95:207–213.

64. Murphy LR, Kinsey ST, Durako MJ. Physiological effects of short-term salinity changes on Ruppia maritima. Aquatic Botany. 2003;75:293–309.

65. Jarvis JC, Moore KA. Influence of environmental factors on Vallisneria americana seed germination. Aquatic Botany. 2008;88(4):283–294.

66. Larking P, Quevedo E, Salinas S, Parker J, Storey K, Hardegree B. Genetic structure of two Thalassia testudinum populations from the south Texas Gulf coast. Aquatic Botany. 2006;85:198–202.

67. Hackney JW, Durako MJ. Size–frequency patterns in morphometric characteristics of the seagrass Thalassia testudinum reflect environmental variability. Ecological Indicators. 2004;4:55–71.

68. Kahn AE, Durako MJ. Thalassia testudinum seedling responses to changes in salinity and nitrogen levels. Journal of Experimental Marine Ecology. 2006; 335:1–12.

69. Koch MS, Schopmeyer SA, Kyhn-Hansen C, Madden CJ. Synergistic effects of high temperature and sulfide on tropical seagrass. Journal of Experimental Marine Biology and Ecology. 2007;341:91–101.

70. Koch MS, Schopmeyer SA, Holmer M, Madden CJ, Kyhn-Hansen C. Thalassia testudinum response to the interactive stressors hypersalinity, sulfide and hypoxia. Aquatic Botany. 2007;87:104–110.

71. Koch MS, Kletou DC, Tursi R. Alkaline phosphatase activity of water column fractions and seagrass in a tropical carbonate estuary, Florida Bay. Estuarine, Coastal and Shelf Science. 2009;1–11 DOI: 10.1016/j.ecss.2009.04.007

72. Garrote-Moreno A, McDonald A, Sherman, TD, Sanchez-Lizaso JL, Heck KL, Cebrian J. Short-term impacts of salinity pulses on ionic ratios of the seagrasses Thalassia testudinum and Halodule wrightii. Aquatic Botany. DOI: org/10.1016/j.aquabot.2014.09.011

73. Mai DH, Walter H. Die obereozänen floren des Weisselster-Beckens und seiner Randgebiete. Abhandlungen des Staatlichen Museums für Minerologie und Geologie zu Dresden. 1985;33:1–260. (German)

74. Kvacek Z. The Hydrocharitaceae foliage from the North Bohemian early miocene. Vestník Českého Geologického Ústavu. 1995;70:21–28.