Short communication

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First record of *Ruppia maritima* in West Africa supported by morphological description and phylogenetic classification

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Abstract: *Ruppia maritima* (widgeon grass) has been identified phylogenetically for the first time from West Africa (Santiago Island, Cape Verde). Genetic markers distinguished this species from the tetraploid *Ruppia cf. maritima* previously known from West Africa. Taxonomic description, photographs and molecular phylogenetic classification are provided here. The results show that, in Cape Verde, the species is phylogenetically closer to European and northeast American than to Indo-Pacific populations. This study extends the known distribution of *R. maritima*, an ecosystem structuring species that provides essential habitat for threatened animals.

Keywords: molecular phylogenetic; morphological traits; *Ruppia* genus; seagrass; waterbirds.

The Cape Verde archipelago is situated off the West Africa coast in the southernmost part of the Macaronesian region. In contrast to other tropical and subtropical coastlines where seagrass species are frequent, only a single seagrass species, *Halodule wrightii* Ascherson, has been recorded recently in the archipelago (Creed et al. 2016).

*Ruppia* (Ruppiaceae) is the only genus included in the “World Atlas of Seagrasses” (Green and Short 2003), for which distribution range maps were not presented because of the lack of existing data. It occurs in shallow habitats such as coastal lagoons, wetlands and salt-marshes (Verhoeven 1979). Meadows formed by *Ruppia* play a key ecological role in the trophic network of aquatic ecosystems. They also provide shelter and food for invertebrates, fishes, aquatic birds, marine mammals and reptiles such as the threatened green turtle *Chelonia mydas* Linnaeus (Kantrud 1991, Hemminga and Duarte 2000, Lopez-Calderon et al. 2010).

Phylogenetic and morphological studies have provided new insights into the complex evolutionary history of the genus *Ruppia* with polyploidization, hybridization and introgression events (Ito et al. 2010, 2013, Triest and Sierens 2014, Martínez-Garrido et al. 2016). These evolutionary phenomena have resulted in different criteria being used to determine which clades are considered lineages or species. Ito et al. (2013) considered the cosmopolitan *Ruppia maritima* complex to contain lineages rather than species. However, some of these species had previously been well supported by morphological and ecological studies (Verhoeven 1979, Talavera and García-Murillo 2010), for example, *Ruppia drepanensis* Tineo ex Guss (i.e. the “Drepanensis” clade of Ito et al. 2013) and *R. maritima* (i.e. the “Diploid” clade of Ito et al. 2013). In contrast to Ito et al. (2013), other authors consider the diploid *R. maritima* complex to contain lineages rather than species. However, some of these species had previously been well supported by morphological and ecological studies (Verhoeven 1979, Talavera and García-Murillo 2010), for example, *Ruppia drepanensis* Tineo ex Guss (i.e. the “Drepanensis” clade of Ito et al. 2013) and *R. maritima* (i.e. the “Diploid” clade of Ito et al. 2013). In contrast to Ito et al. (2013), other authors consider the diploid *R. maritima* (i.e. “Diploid” clade of Ito et al. 2013) to be a species distinct from several others in this complex, clearly supported by both nuclear (internal transcribed spacer) and chloroplast (*psbA-trnH*) markers (Triest and Sierens 2014, Martínez-Garrido et al. 2016). Hybridization processes are one of the causes of this controversy. Although *R. maritima* presents a distinctive and unique chloroplast haplotype, its nuclear haplotype is present in other species that are putative hybrids, such as *Ruppia cf. maritima*. Although
morphologically similar to diploid *R. maritima*, *R. cf. maritima* differs in the chloroplast haplotype and chromosome number (Ito et al. 2013, Triest and Sierens 2014, Martínez-Garrido et al. 2016). Despite the different taxonomic approaches to the clades, all these studies have shown a great diversity in the *Ruppia* genus and clearly distinct genetic entities, some of which have a hybrid origin.

According to the bioregional model of seagrass distribution of Short et al. (2007), *R. maritima* Linnaeus is expected to occur in all biogeographical regions. However, the difficulty in properly identifying species and the traditional use of *R. maritima* as a catch-all taxon may have resulted in an exaggerated distributional range of *R. maritima* and a underestimation of *Ruppia* species diversity (den Hartog and Kuo 2006, Triest and Sierens 2014). The type of *R. maritima* was collected by Linnaeus in the Baltic Sea and corresponds to the “Diploid” clade of Ito et al. (2013) and to the *R. maritima* of Triest and Sierens (2014) and of Martínez-Garrido et al. (2016). The distribution range of this *R. maritima*, sensu stricto needs to be accurately determined based on morphological and phylogenetic analyses (Short et al. 2010, Triest and Sierens 2015). This will enable the biogeographical ranges and putative hybridization zones with other *Ruppia* species to be described, thereby helping to define species distributions and evolutionary history better.

In 2015, *R. maritima* was found at Lagoinha, Santiago Island, Cape Verde (15°7’40.66″ N, 23°31’15.15″ W) growing in one of two lagoons of the Pedra Badejo wetland (Ramsar Site nº 1577; Figure 1). These lagoons are fed mainly by seawater that reaches them during high tides. They are an important habitat for migratory birds, and the adjacent beach is one of the most important nesting beaches for the endangered loggerhead seaturtle (*Caretta caretta* Linnaeus) on the island (Veiga et al. 2015).

To obtain photographs and morphological data, 10 specimens were haphazardly sampled along a 100-m linear transect. The morphological description is based on minimum, mean and maximum measures of specimens, based on four replicate measurements per specimen of a suite of taxonomic characters as defined according to descriptions in Oliveira et al. (1983), Kantrud (1991) and Flora Ibérica (Talavera and García-Murillo 2010). The total

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**Figure 1:** Map of the Cape Verde Islands, and detail of the *Ruppia maritima* site at Santiago Island (black square).
number of measurements for each taxonomic character were as follows: 40 for internode length, leaf length, leaf width and leaf sheath length; 39 for rhizome diameter and root length; 36 for inflorescence peduncle length, fruitlet length, podogyn length and number of mature fruitlets per inflorescence; 35 for fruitlet width; 34 for fruitlet beak length; 33 for peduncle diameter; 10 for plant height; 9 for number of fruitlets per plant; 8 for carpel length; and 2 for anther length.

A morphological description of the sampled population is provided here: submerged herbaceous plant (Figure 2A–C). Plants 27–109 cm in height: Rhizome

![Figure 2: Ruppia maritima: photographs of specimens from Santiago Island.](image)

(A) Sampled population of R. maritima; (B) detail of the biomass accumulation; (C) R. maritima with flowers and fruits; (D) acute leaf tip; (E) leaf cross-section showing the central nerve; (F) inflorescence showing anthers and gynoeicum; (G) detail of the four carpels of each flower; (H) detail of a mature fruitlet; (I) infructescence showing elongated podogynes and six mature fruitlets. Photographs are by the authors, except (C) by Peter Wirtz, used with permission.
Figure 3: *Ruppia maritima* samples from Santiago Island.

Phylogenetic classification of the specimens when compared to the samples used in Martínez-Garrido et al. (2016). (A) Phylogenetic correspondence based on chloroplast *psbA-trnH*. (B) Phylogenetic correspondence based on nuclear internal transcribed spacer (ITS) regions. 

All accessions show the sample's origins, the species names used by the authors and, in parentheses, the authors that deposited the sequences (I, Ito et al.; T, Triest and Sierens; MG, Martínez-Garrido et al.). New accessions deposited in the present study are shown in bold type and emphasized in large font, with the species names identified by morphology according to the taxonomic criteria of the Flora Ibérica (Talavera and García-Murillo 2010) and descriptions in Oliveira et al. (1983) and Kantrud (1991). Bootstrap support is indicated as numbers at the branches. The names listed between *psbA-trnH* and ITS trees are the taxonomic conclusions proposed by Martínez-Garrido et al. (2016), Ito et al. (2013) and Triest and Sierens (2014). The two groups supported by *psbA-trnH* and ITS markers in the *R. maritima* clade are boxed and marked with vertical labels (IPG, Indo Pacific Group; ENAG, European and Northeast America Group). Accession numbers of sequences 0–32 are listed in Martínez-Garrido et al. (2016). Samples labeled #1 and #2 show heterogeneous ITS sequences. New accession numbers from the present study (No. 33) are KY002069-KY002070. DNA extraction, molecular markers amplification and phylogenetic analyses were conducted as described in Martínez-Garrido et al. (2016).
India, Vanuatu; Figure 3). The source of Ruppia maritima has also been listed for the Azores (Morton et al. 1995), the Canaries (Santos and Fernández 1984), Cape Verde (Sánchez-Pinto et al. 2005), the Oranjemund wetland in Namibia (Williamson 1997), the Keta coastal lagoon in Ghana (Lamptey and Arnah 2008) and at different sites in South Africa (Cook 2004, see Ito et al. 2015). However, these studies did not present supporting morphological descriptions and phylogenetic analyses, and therefore a precise species cannot be assigned.

The complete taxonomic description included in this study can be used as a guide to help researchers identify R. maritima based on morphological traits. Additionally, the genetic analyses have allowed us to circumscribe the Cape Verde samples in the phylogenetic clade of R. maritima, discriminating this species from the morphologically similar Ruppia cf. maritima detected in other African, Mediterranean and Iberian locations (“Tetraploid α” of Ito et al. 2013; “haplogroup E” of Triest and Sierens 2014, Ruppia cf. maritima of Martínez-Garrido et al. 2016). Although both species present similar nuclear ITS haplotypes, their organelles (studied using chloroplast DNA markers) are phylogenetically distant. This is due to past hybridization in the likely allopolyplody specification origin of tetraploid R. cf. maritima (Triest and Sierens 2014, Martínez-Garrido and Martínez-Garrido et al. 2016) referred as “haplogroup E”).

The source of R. maritima in Cape Verde is unknown, and it has been previously described as a “probable native” in Sal and Boa Vista Islands (Sánchez-Pinto et al. 2005). However, as R. maritima likely did not evolve on the volcanic islands of Cape Verde, it probably colonized them from unknown nearby populations in West Africa, with similar haplotypes to the European and northeast American populations. Species of Ruppia are known to be dispersed by seabirds and ocean currents (Figuerola et al. 2002, Triest and Sierens 2013). As the main ocean currents on the West African coast near Cape Verde run from north to south, it seems probable that aquatic birds could be the main dispersal vector. The location where R. maritima was sampled is visited by a great number of waterbirds, the main migratory bird group in the archipelago (Veiga et al. 2015). Previous studies in South Africa and the Iberian Peninsula have detected Ruppia seeds in the diet of several ducks and coots, and these seeds maintained their capacity to germinate after being defecated (Figuerola et al. 2002, Reynolds and Cumming 2016). Studies using microsatellites in R. maritima (Triest and Sierens 2015) and in Ruppia cirrhosa (Petagna) Grande (Martínez-Garrido et al. 2017) have also proposed waterbirds as the dispersal vector, so this
hypothesis should be further investigated with more genetic markers and samples from more locations.

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References


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Samir Martins is a conservation biologist from Cabo Verde Islands, with a special interest in animal ecology. His research interest is focused on sea turtle response to environmental changes and the effects on offspring survival. He has participated actively in all aspects and phases of important projects regarding Cape Verde sea turtle conservation. Currently, he is involved in conservation activities for all endangered species (sea turtles, whales and birds) and a Marine Protection Area, the definition of ecotourism guidelines and information, education and awareness activities addressed to the local people in as well as visitors of Boa Vista Island, Cabo Verde.

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Carmen H. Almada has a PhD in Coastal Zone Management, an MSc in Ecology and a BA in Marine Biology. Her PhD thesis deals with the flora and ecology of the benthic algae of the Cape Verde Islands. She is a biologist specializing in marine botany, with experience in phycology. She has also conducted research at University of Gran Canaria (Spain) and Federal University of Rio de Janeiro (Brazil). At present, her research is centered on the study of macroalgae and seagrasses of the Cape Verde Islands, with a focus on taxonomy, ecology and conservation.

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Ester A. Serrão (lecturer at the University of Algarve) coordinates a research team at CCMAR-Centre for Marine Sciences, which aims to understand patterns and processes mediating marine genetic diversity, function and evolution, from ecological to deep evolutionary scales. Her research topics include causes and consequences of genetic biodiversity, population dispersal/connectivity, environmental genomics and adaptive evolution, in the context of climate and environmental change. These are studied across the diversity of marine systems ranging from microorganisms to large marine forests.