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Short communication

Diploid *Ruppia cirrhosa* populations from a southern Mediterranean shallow system

Anna M. Mannino*, A. Geraci

Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, Section of Botany and Plant Ecology, University of Palermo, Via Archirafi 38, 90123 Palermo, Italy

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ABSTRACT

This paper focuses on the morphology and karyology of representative *Ruppia* populations from a southern Mediterranean shallow system. The cosmopolitan genus *Ruppia* L. generally inhabits shallow waters such as coastal lagoons and brackish habitats. *Ruppia* species are characterised by a simplified morphology and high intraspecific phenotypic plasticity. The chromosome number of Sicilian *Ruppia* populations is reported here for the first time. The analysed populations showed morphological and reproductive characters of *Ruppia cirrhosa* (Petagna) Grande but a diploid cytotype (2n = 20). A low fruit production was also observed, suggesting that vegetative reproduction is the main reproductive strategy adopted by the plant. This study did not resolve conclusively the taxonomic identity of these *Ruppia* populations and it also raises some open questions, needing further investigations.

1. Introduction

The cosmopolitan genus *Ruppia* L. (Ruppiaceae), an annual/perennial aquatic plant complex, is generally restricted to shallow systems (i.e. brackish habitats and coastal lagoons) where it plays a key role in the functioning of the ecosystem (Verhoeven, 1979; Obrador and Pretus, 2012; Mannino et al., 2015). The simplified morphology, the high phenotypic plasticity as well as the existence of polyploidy and hybrids made it difficult to name *Ruppia* species (Mannino et al., 2015).

In the Mediterranean region three species were traditionally recognized, *Ruppia maritima* L., *Ruppia cirrhosa* (Petagna) Grande and *Ruppia drepanensis* Tineo in G. Gussone, the latter occurring only in the south-western Mediterranean **(**Mannino et al., 2015**).**

However, recent cpDNA (chloroplast DNA) analyses revealed the presence in the Mediterranean area of a greater number of entities, precisely a diploid *R. maritima* taxon and a tetraploid *R. cirrhosa* complex, including *R. drepanensis* (or *R. cirrhosa* (Petagna) Grande var. *drepanensis* (Tineo) Symoens) and traces of hybrid origin (Triest and Sierens, 2009a,b, 2010, 2013, 2014). Subsequently, Ito et al. (2013) included the taxa and the hybrids found by Triest and Sierens (2010, 2013) in a single world-wide *R. maritima* complex.

Differences in chromosome numbers have also been observed (Aedo and Fernández Casado, 1988; Triest and Symoens, 1991; Talavera et al., 1993; Mannino et al., 2015). Diploid ($2n = 20$) and tetraploid ($2n = 40$) races have been generally reported, even though triploids ($2n = 30$) and hexaploids ($2n = 60$) races have been occasionally found (Talavera et al., 1993). Reese (1962) considered *R. maritima* and *R. cirrhosa* as diploid and polyploid, respectively. Instead, *R. maritima* was considered as both diploid and tetraploid and *R. cirrhosa* as tetraploid by Van Vierssen et al. (1981). Moreover, Cirujano (1986) considered *R. maritima* as tetraploid and *R. cirrhosa* as diploid, whereas Triest and Symoens (1991) recognized diploid and tetraploid entities in both morphological species. For western Mediterranean populations of *Ruppia*, diploid and tetraploid *R. maritima*, tetraploid *R. cirrhosa* (except for old records from Spain; Cirujano, 1982; Castroviejo, 1983) and diploid *R. drepanensis* (from Spain and Sardinia; Cirujano, 1982; Marchioni Ortu, 1982) were recorded. The data on chromosome numbers, that must be reevaluated in the context of the cpDNA haplotypic and nuclear variation (Mannino et al., 2015), also reflect the ambiguity of a classification for this genus based on morphology alone (Ito et al., 2010).

From literature data, in the Sicilian shallow systems the three Mediterranean species, *R. maritima*, *R. cirrhosa* and *R. drepanensis*, were reported (Pasta and Lo Cascio, 2002; Raimondo et al., 2004; Mannino and Sarà, 2006). Since the taxonomic identity of these Sicilian populations was based exclusively on morphological analyses, and have not been confirmed by either genetic (except for “Trapani Saline ” in: Triest and Sierens, 2010) or karyological

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* Corresponding author.
E-mail address: annamaria.mannino@unipa.it (A.M. Mannino).

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analyses, it is very likely that some of these records should be reevaluated (Triest and Sierens, 2009a, 2010).

The aim of this paper is to contribute to clarify the taxonomic identity of the Sicilian *Ruppia* populations through morphological and karyological analyses of representative populations inhabiting the Natural Reserve "Saline di Trapani e Paceco" where *R. cirrhosa* and *R. drepanensis* were both reported (even if this area represents the *locus classicus* of *R. drepanensis*, it was no longer found; e.g. Mannino and Sarà, 2006; Mannino, 2010; Mannino and Graziano, 2016).

2. Materials and methods

2.1. Study area and samples collection

The Natural Reserve "Saline di Trapani e Paceco" (Western Sicily, 37°52′N; 12°28′E) hosts the most important saltworks system of the Western Mediterranean (about 1000 ha), considered the main ecological corridor in the central-western Mediterranean for avifauna migrating from Africa to Europe (Sorci et al., 1991). The ponds, permanently flooded, are characterised by a muddy-sand bottom and a water depth of 0.4–0.5 m, which showed little variation during the year. Mean salinity ranged from 20.7 psu in winter to 40.1 psu in summer and mean temperature ranged from 12.1 °C in winter to 26.4 °C in summer.

In total, 5 ponds belonging to 3 saltworks: Galia (populations G_1, G_2), Bella (population B) and Salinella (populations S_1, S_2), which is further away from the other two, were studied.

2.2. Morphological analysis and chromosome counts

Ponds were visited on two occasions (spring and summer) in order to check for the presence of plants with both flowers and fruits. In summer, at each pond *Ruppia* samples were collected using a quadrat sampler of 400 cm^2 (in triplicate). In the laboratory, flowers and fruits of all collected plants were separated and counted in order to assess the mean number of flowers and fruits per m^2. Moreover, for each pond twenty individual shoots, considered representative of the studied populations (Malea et al., 2004; Triest and Sierens, 2009a), were randomly chosen within each replica. The twenty shoots were examined both by light and scanning electron microscopy and rigorously identified on the base of morphological and reproductive characters (see Triest and Symoens, 1991; Sfriso, 2008; Mannino et al., 2015). Specimens of each population are deposited at the *Herbarium Mediterraneum* of the University of Palermo (PAL).

For karyological analyses, at each pond 15 fruits (for each replica) collected from the same shoots used for the morphological analysis, were used. The seeds obtained by these fruits were germinated at 25 °C on moist filter paper in petri dishes (the germination percentage was approximately 75%). Actively growing root tips (5–10 mm long) were excised from the germinating seeds and pre-treated in 0.5% colchicine in saturated paradichlorobenzene solution for 1 h at room temperature. Then, they were fixed with a solution of ethanol and glacial acetic acid (3:1) for 12–18 h. The root tips were rinsed with distilled water, hydrolysed in 1 N HCl at 60 °C for 6–8 min, rinsed with distilled water for at least 3–5 min, and squashed in 1 % aceto-orcein.

Squashed preparations were stained with Feulgen's method for 1–2 h (Marino et al., 2012). Chromosome numbers were determined counting at least 20 somatic metaphase plates for each germinated seed.

The nomenclature used to describe the chromosome morphology is that proposed by Levan et al. (1964). The used abbreviations M, m and sm designate the centromere position within the chromosome (median point, median region and submedian region, respectively).

3. Results and discussion

3.1. Morphological analysis

From a morphological point of view no significant inter- and intra-population variations were observed. The morphological and reproductive features of the analysed specimens, matching those of *R. cirrhosa* (see Triest and Symoens, 1991; Sfriso, 2008; Mannino et al., 2015; see Table 1 for the detailed data), are summarized as follows: horizontal rhizomes (on average 0.99 ± 0.04 mm wide) with simple roots occurring at the nodes; narrow linear leaves (on average 0.98 ± 0.03 mm wide and 18.2 ± 1.04 cm long) with a central vein and obtuse irregularly toothed apices (Fig. 1a); inflorescences, consisting of two flowers without perianth and inserted opposite on the top of a spirally coiling peduncle (on average 12.5 ± 0.64 cm long); boomerang-shaped pollen grains with a reticulate exine in the central portion of the grain and smooth knob-like extremities (Fig. 1b); asymmetric fruits, in groups of 6–8 per inflorescence (Fig. 1c). It should be noted that all examined fruits were asymmetric even though they are both reported as symmetric (Sfriso, 2008) and asymmetric (Gamerro, 1968; Zhao and Wu, 2008). It should be also highlighted that for *R. cirrhosa* the apex is both reported as obtuse and acute (Verhoeven, 1979; Triest and Symoens, 1991; Mannino et al., 2015) and even highly variable in relation to ecological conditions (Reese, 1962; Gamerro, 1968).

A low percentage of fruits were found, precisely a mean number of flowers per m^2 of 511.98 ± 19.06 and a mean number of fruits per m^2 of 46.24 ± 2.22 (see Table 1 for the detailed data). Triest and Sierens (2009a) already observed that *R. cirrhosa* usually produces less fruits than *R. maritima* and *R. drepanensis*. It is noteworthy that in the past three years, flowering and fructifying individuals were not observed in the studied ponds. This would suggest that, even though both sexual and vegetative reproduction are possible in *Ruppia* plants, the second one is used more frequently than the sexual reproduction. In small populations, the predominance of the vegetative reproduction, responsible for the decrease of the gene flow and consequently for the increase of clones, could lead to a genetic drift with consequent reduction of genetic variability.

3.2. Chromosome counts

The chromosome number of Sicilian *Ruppia* populations is reported here for the first time. Surprisingly, the studied populations resulted diploid ($2n = 20$), while for western Mediterranean populations of *Ruppia*, tetraploid populations of *R. cirrhosa* (except for old records from Spain; Cirujano, 1982; Castroviejo, 1983) were recorded (Triest and Sierens, 2009a,b, 2010, 2013, 2014). The studied specimen presented nine small (0.8–1.3 μm long) and one large (about 2.1 μm long) pair of chromosomes (Fig. 1d). As a whole, the karyotype of the analysed specimens resulted 2 M, 7m, 1sm, showing a predominance of m chromosomes. Moreover, the formula and the size of the chromosomes resulted very similar to those reported for diploid *R. drepanensis* from Sardinia (Marchioni Ortu, 1982), displaying as well one large pair of chromosomes and nine small ones. They are also comparable to those reported by Talavera et al. (1993) for Spanish populations of *R. drepanensis* and by Castroviejo (1983) for populations of *R. cirrhosa*, even though these authors found a pair of satellite chromosomes (the largest pair) that we did not observe in our plates.

Our data make it difficult to draw any definite conclusions about the studied *Ruppia* populations, but let us to argue a hypothesis. Since this area represents the main ecological corridor in the