First record of Nymphaea × marliacea Lat.-Marl. ‘Rosea’ in the Iberian Peninsula: identification based on morphological features and molecular techniques

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**ABSTRACT:** This work reports the first population of Nymphaea × marliacea ‘Rosea’ in the wild in the Iberian Peninsula. The population is the southernmost known so far in Europe and is located under the warmest climate type of all European populations of N. × marliacea. Nymphaea × marliacea is an artificial hybrid raised by J. B. Latour-Marliac at the end of the 19th century. Identification of the species was faced by molecular techniques and based on morphological characters. Molecular techniques did not allow to conclude about its identity, surely due to errors in the assignation of sequences recorded in GenBank. Nomenclatural and taxonomic issues are discussed and information about ecological features is reviewed.

Key words: Alien plants, Iberian Peninsula, Nymphaea × marliacea ‘Rosea’.

**RESUMEN:** En este trabajo se notifica la primera cita de Nymphaea × marliacea ‘Rosea’ en la península ibérica. Se trata de la población más sureña de Europa, que está bajo el clima más cálido de todas las poblaciones encontradas hasta fechas recientes. Se trata de un híbrido artificial obtenido por J. B. Latour-Marliac a final del siglo XIX. La determinación se abordó, tanto mediante análisis molecular como por los métodos basados en caracteres morfológicos. El enfoque basado en técnicas moleculares no permitió determinar el taxón, posiblemente debido a la existencia de errores en asignación de bases en GenBank. Se revisan diversos aspectos nomenclaturales, taxonómicos y ecológicos.

Palabras clave: Nymphaea × marliacea ‘Rosea’, península ibérica, plantas alóctonas.

**INTRODUCTION**

Nymphaea L., the most diverse genus of the order Nymphaeales, comprises a group of aquatic perennial herbs with exceptionally beautiful flowers and floating leaves. This genus is considered to be of evolutionary significance as it represents a group of early evolving flowering plants that are widely distributed globally (Dkhar & al., 2012).

Europe and the Mediterranean Basin harbour six native taxa within the genus Nymphaea (Uotila, 2009): Nymphaea alba L., N. caerulea Savigny, N. candida J. Presl & C.Presl, N. lotus L., N. rubra Salisb. and N. tetragona Georgi. In the Iberian Peninsula and the Balearic Islands, only N. alba is considered to be native (Sánchez, 1986). The invasive alien N. mexicana Zucc., native to the southern USA, is also present in Badajoz in the southwest of Spain (García-Murillo, 1993). In addition, a high number of taxa (including cultivars) are nowadays being cultivated in Europe in water gardens.
**Nymphaea × marliacea: nomenclature, parentage and taxonomy**

The name *Nymphaea × marliacea* has a complicated history with regard to its nomenclature, parentage and taxonomy. It has been associated with three different authors: J. B. Latour-Marliac, W. Watson and W. Wildsmith. The French horticulturist Joseph Bory Latour-Marliac (1830-1911), reputed worldwide as a breeder of hybrid water lilies, raised this hybrid in Le Temple-sur-Lot in France. He gave it pre-Linnean names such as “Nymphaea Marliacea-chromatella” and “Nymphaea Marliacea-chromatella foliis hepatico-marmoratis”. According to Watson (1888), who received seedlings from Latour-Marliac and raised them at Kew Gardens, there were not any differences between all these plants and he introduced the binomial *Nymphaea marliacea* (pro species), in honor of the raiser. Many databases, including Tropicos (http://www.tropicos.org) and The Plant List (http://www.theplantlist.org) erroneously assign this name to William Wildsmith. The text of the protologue, published in *The Garden* on 31 March 1888, was signed as “W.W.”. These initials could refer to both William Watson and William Wildsmith, two regular contributors to the journal. However, the latter always signed his contributions with “W. Wildsmith”, while the abbreviation “W.W.” referred to William Watson (e.g. Jstor database, note from “W.W.” [William Watson] to the Royal Botanic Gardens; accessed 8 January 2016 from http://plants.jstor.org/stable/10.5555/al.ap.visual.kd cas7018). However, in his note Watson exactly copied (in smaller print, on p. 292) the information about the plant sent to him by Latour-Marliac. This paragraph not only includes characteristic features of *N. × marliacea*, but also his name. Thus, this binomial should be assigned to Latour-Marliac, as correctly cited in the International Plant Names Index (http://www.ipni.org).

Likewise, the taxonomy of *N. × marliacea* is not fully clear. The parentage in the original description (Watson, 1888) was not clearly indicated. *Nymphaea × marliacea* ‘Chromatella’ was assigned to a yellow-flowered cultivar. As putative parental species, a species similar and related to *N. tuberosa* Paine [now accepted as *N. odorata* Aiton subsp. *tuberosa* (Paine) Wiersema & Hellq.] and *N. flava* Leitner (a synonym of *N. mexicana*) were suggested by Watson (1888). *Nymphaea × marliacea* closely resembled the former in all aspects except for the yellowish flowers and marbled leaves that apparently were obtained from the latter. In the course of time numerous cultivars were bread and these mainly differed in flower colour, depending on the parental species involved. For some cultivars *N. alba* was also suggested as a probable parent species [see for instance cultivar names listed by Knotts (2010)]. Based on molecular data, Dkhar et al. (2012) suggest that the lack of sequence data on *N. alba* var. *rubra* Lönroths prevents the demonstration of a genetic contribution of Indian accessions of *N. × marliacea* from *N. mexicana* and *N. alba* var. *rubra*. At present, the binomial *N. × marliacea* is accepted in a broad sense and encompasses a complex assemblage of multiple hybrids involving *N. alba*, *N. mexicana*, *N. odorata* (s. l.) and possibly still others.

The name *Nymphaea × marliacea* has not yet been properly typified. The colour illustration (Plate 642) that accompanies Watson’s article in *The Garden* could hardly serve as the lectotype as suggested by Gardeners’ Chronicle (1888): “(...) the illustration, unfortunately, affords no means whatever of ascertaining what the affinities of the plant really are. It is a yellow Water Lily, and it is nothing more. Not even the disposition of the air-canals in the leaf and flower-stalks respectively is indicated, though a momentary inspection of these tubes with the unassisted eye would be sufficient to show to what section of the genus the plant belongs”. The seeking and designation of a lectotype, however, is beyond the scope of this article.

In this paper, we report on the first population of *N. × marliacea* Lat.-Marl. in the Iberian Peninsula, review its nomenclature and existing knowledge on taxonomic and ecological aspects. Finally, we review the climate types from naturalised records worldwide as a preliminary basis to assess its ecological amplitude.

**MATERIALS AND METHODS**

**Invaded locality**

On 4 October 2012, a population of an unknown *Nymphaea* species was detected in the Campanario reservoir (Valverde del Camino and Beas municipalities, Huelva, southwestern Spain; 127 m a.s.l.; 37.53° N, 6.83° W). This reservoir was constructed in 1911, with a capacity of 1 hm³ and forms a permanent pond of 4.0 ha. In this area, two patches of 10 and 45 m² were found (Fig. 1). The reservoir is used for fishing black bass ([Micropterus salmoides](http://www.ipni.org) (Lacepède, 1802) and carp...
Species identification

Given the taxonomic complexity of the genus, we followed a two-way procedure for the identification of the species: DNA-based molecular analyses and the diagnosis of morphological characters.

Molecular analyses for the identification of *Nymphaea* spp. were based on the amplification of DNA by simple polymerase chain reaction (PCR). A portion from the inner part of the stem was macerated in sterile bi-distilled water (1:3 weight/volume). DNA was extracted following the automatic method provided in the Maxwell 16 Viral Total Nucleic Acid Purification Kit (Promega Ibérica) and the solution was adjusted to 50 ng/μL. PCR amplification of ITS1 and ITS2 regions were conducted using the primers described by Dkhar & al. (2012). Therefore, ITS1 region was amplified with universal primers ITS2 and ITS5, while the ITS2 region was amplified with universal primers ITS3 and ITS4 (Dkhar & al., 2012). Primers employed were synthesised by Life Technologies.

The amplification reaction was carried out over a total reaction volume of 50 μL containing 150 ng of ADN, 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100, 2.0 mM MgCl2, 0.5 mM for each dNTPs (Promega, Madison), 4 U Taq Polymerase (GenScript Taq, Bionova, Pisgataway, USA) and 0.5 μM for each primer (in both directions). The amplification phase was performed using a Sensroquest Labcycler termocycler, as follows; initial denaturation phase during 5 min at 94°C, followed by 40 amplifying cycles during 30 s at 94°C, base pairing (30 s at 50°C), extension step with 40 s at 72°C and, finally, an elongation step 72°C during 5 min. Amplicons for 380 pb for ITS1 and 430 pb for ITS2 regions were detected running an agarose 3% gel electrophoresis. PCR products were purified using the Purelink column (Invitrogen, Life Technologies, Löhne), following the instructions provided by the manufacturer. Sanger sequencing method was applied to purified amplified DNA using BDT v.3.1 (Applied Biosystems, Life Technologies, Austin) on a genetic analyzer ABI PRISM 310 DNA sequencer (Applied Biosystems, Life Technologies) following the manufacturer’s recommendations. All amplicons were sequenced in both directions. Material used for analyses and DNA extracted are kept frozen at −80°C and preserved at the Instituto Valenciano de Microbiología. BLAST (Basic Local Aligned Search Tool), was used to compare similarity between the obtained sequences with those for the genus Nymphaea registered in the GenBank of the National Center for Biotechnology Information (NCBI).

Taxonomical identification based on morphological characters followed the criteria established by Cullen & al. (2011) and by Latour-Marliac (1899). In addition, the nomenclature of *N. × marliacea* was critically revised. For this purpose, bibliographic citations in databases, such as IPNI (The International Plant Names Index; http://www.ipni.org), Tropicos (http://www.tropicos.org) and The Plant List (http://www.theplantlist.org) were examined and its protologue was studied.

Once the taxon was identified, we assessed its invasion history. We reviewed published records that considered *N. × marliacea* as a naturalised or invasive species in the wild. Climate type of the locations where the species is recorded as naturalised or invasive was assigned according to Köppen-Geiger’s climate classification system (Kottek & al., 2006). Xenotype was assigned following Kornás (1990) and Sanz-Elorza & al. (2004), which take into account the habitat features and population state.

RESULTS AND DISCUSSION

Species identification

Results of BLAST analysis showed 100% homology with GenBank accessions corresponding to three different taxa (Table 2): *N. × marliacea*, *N. alba* and *N. candida* (sequence cover = 100%, E-value = 2x10⁻³³⁰, 100% of homology in all cases). Consequently, molecular approach, did not allowed a foolproof identification of the analysed specimens. Especially for taxonomically complex groups public DNA repositories like GenBank often have compromised taxonomic annotations (Nilsson & al., 2006).

Sequences obtained also matched some of the samples assigned to *N. alba* by Dkhar & al. (2012) for India. However, all samples assigned to *N. alba*
were classified very closely to *N. × marliacea*.

The information provided by published references [Table 2, and Dkhar & al. (2012)], did not allow evaluating whether our taxonomical assignment was correct. Recently, it has been found that a high number of populations assigned to *N. alba* in Germany correspond to cultivated specimens or even hybrids involving *N. alba* (Nierbauer & al., 2014).

Morphological features such as petal colour (not yellow), flowers opening during the day, sepal venation (with obscure veins) and lower leaf surface (greenish-red) were among the diagnostic features that exclude most of the congeners that are cultivated as ornamentals in Europe (Cullen & al., 2011). The combination of these characters leads to a group of closely similar and genetically related species like *N. alba*, *N. candida*, *N. tuberosa* and (often complex) hybrids of these and other species. The more or less fragrant flowers and leaves with non-overlapping lobes further exclude *N. candida* and *N. tuberosa*. The similar hybrid *N. × laydekeri* Lat.-Marl., probably derived from *N. alba*, *N. tetragona* (and possibly *N. mexicana* as well) (Cullen & al., 2011), has almost circular leaves that are usually mottled with brown (while leaves in the *Nymphaea* species analysed are longer than wide). Finally, the plants also differed from *N. alba* and its cultivars in having rose-pink sepals (not green flushed with red or brown) and in being more fragrant.

The *Nymphaea* specimens analysed had pink petals, a feature that clearly differs from the majority of cultivated species cited in Cullen & al. (2011). Flower colour is similar to *N. alba* ‘Candidissima Rosea’, *N. stellata* Willd. var. versicolor Hook.f. & Thompson, *N. candida*, *N. odorata* var. rosea Pursh, *N. × marliacea* ‘Carnea’ and *N. × marliacea* ‘Rosea’. However, like *N. alba*, *N. candida* and *N. odorata* our specimens showed radial walls in the ovary without two distinct layers, contrary to *N. stellata* var. versicolor, a taxon with radial walls separating in two distinct layers.

Leaf characters in *Nymphaea* specimens analysed differed from those of *N. alba*, *N. alba* ‘Candidissima Rosea’ and *N. candida* (dark green above, yellowish to reddish green beneath) and from those of *N. odorata* and *N. odorata* var. rosea (dark green and smooth above, rough and usually purplish red beneath).

To conclude, the specimens of the population found matched the description of the complex hybrid *N. × marliacea* ‘Rosea’ (Fig. 1).

**New records**

Spain, Huelva: 29S 691583 m E, 4155841 m N (coordinates ETRS89), Campanario reservoir (Valverde del Camino), 127 m a.s.l., reservoir, with *Typha domingensis* Pers., plus Mediterranean shrubland in the riverside, size 45 m², mean cover density = 100%, hemiagriophyte, 19-9-2015, José Luis Rodríguez Marzal (Legit.), D. Guillot & E. D. Dana (Det.) (MGC 83477); 29S 691355 m E, 4155840 m N (coordinates ETRS89), Campanario reservoir (Beas), 127 m a.s.l., reservoir, with *Typha domingensis*, plus Mediterranean shrubland in the riverside, size 10 m², mean cover density > 75%, hemiagriophyte, 19-9-2015, José Luis Rodríguez Marzal (Legit.), D. Guillot & E. D. Dana (Det.) (MGC 83479); 29S 691583 m E, 4155841 m N (coordinates ETRS89), Campanario reservoir (Valverde del Camino), 127 m a.s.l., reservoir, with *Typha domingensis*, plus Mediterranean shrubland in the riverside, size 45 m², mean cover density > 75%, hemiagriophyte, 1-10-2012, Francisco Paredes Carretero & José Luis Rodríguez Marzal (Legit.), D. Guillot & E. D. Dana (Det.) (MGC8 3478).

In Europe, so far, *N. × marliacea* has been found as alien in Hungary (Soós, 1966). It has naturalised also in Italy (Gariboldi & Beretta, 2008; Lazzari & al., 2012). It is also known from the center and south of England (NBN, 2015). Therefore, the population found in Spain seems to be the first within the Iberian Peninsula and the south westernmost population detected in Europe. Also, this location represents the warmest climate conditions so far recorded in Europe for a population of *N. × marliacea* in the wild.

The Iberian locality is far away from any inhabited area, while no other population has been recorded upstream (Pajarón stream, a tributary of Odíel river). This leads us to suggest that it could have been introduced on purpose into the reservoir. As other *Nymphaea* species, it produces flowers during the warmest months. The population of *N. × marliacea* starts flowering in July, and flowers can be found until mid-October. Local inhabitants indicated that the plants could have first appeared around the year 2000. During our field visits in the summers of 2012 and 2015, we did not detect any change in extent of surface invaded. It is unclear whether or not common carps (*Cyprinus carpio*) in the reservoir feed on *N. × marliacea*, hindering a potential invasion. A number of studies report that common carps have a deep impact on macrophytes and ecosystem functioning. Singh & al. (2010) found that *Nymphaea* represented 8.5% of the gut
content of carps in the Ganga river in India. Smits & al. (1989) even found that carps completely destroyed the seeds of the nympheaid waterplants, suggesting that this species hardly contributes to seed dispersal of water lilies. In contrast, other studies suggest that Nymphaea spp. are not affected by common carps (Pipalová, 2006). Bajer & al. (2009) found that white water lily (Nymphaea odorata) and American lotus (Nelumbo lutea Willd.) remained relatively unaffected by the increase in carp biomass in a shallow lake. In an invasion by N. odorata, Eichmiller & al. (2014) stated that “due to high carp density, the lake lacks aquatic vegetation except for white water lily (Nymphaea odorata), which covers less than 10% of the lake area”. Similarly, Bonar & al. (2002) did not find triploid grass carp to control N. odorata. In sum, additional specific studies are required in search for the potential interaction of common carps with N. × marliacea.

Climatic affinities

According to Köppen-Geiger’s climate classification system, the invaded area in Spain is under a ‘Csa’ climate type, with January and August as the coolest and warmest months, respectively (Kottek & al., 2006). Hungarian populations of N. × marliacea are located under ‘Dfb’ climate type. Italian and English records are subjected to ‘Cfa’ and ‘Cfb’ climate type, respectively (Kottek & al., 2006). The Italian population of Piemonte is invasive and often managed (Selvaggi & al., 2011). However, information available for English populations did not allow evaluating whether or not the populations are invasive. Outside Europe it has been reported as “established invader” in South Africa (Henderson, 2007), south-western Australia and the United States (GCW, 2007). In Australia, there are some records corresponding to N. × marliacea ‘Chromatella’ (vouchers MEL 2096385A and MEL 2103434A, Australia’s Virtual Herbarium, available at http://avh.chah.org.au). The information of their labels suggests a high invasion potential. The referred localities are subjected to a ‘Cfa’ and ‘Cfb’ climate type, respectively. This taxon has clearly naturalised in these Australian localities (Walsh & Stajsic, 2007).

In sum, all records found in the present work were reported from permanent ponds. So far N. × marliacea has naturalised at least under ‘Csa’, ‘Cfa’, ‘Cfb’, ‘Dfb’ climate types (Kottek & al., 2006). We believe it would be of interest to conduct niche-modelling studies in order to elucidate which areas are more susceptible to be invaded by this taxon.

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REFERENCES


BORSCH, T., J.H. WIERSEMA, B.H. HELQUIST, C. LOEHNE & K. GOVERS (2014) Speciation in...


**Nymphaea × marliacea** Lat.-Marl. ‘Rosea’: first record in the Iberian Peninsula


Table 1. Main climate data of the studied site, according to [http://www.climate-data.org](http://www.climate-data.org).

<table>
<thead>
<tr>
<th>Climatic parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average temperature (°C)</td>
<td>16.7</td>
</tr>
<tr>
<td>Annual rainfall (mm)</td>
<td>553</td>
</tr>
<tr>
<td>Mean temperature of coldest month (°C)</td>
<td>9.2</td>
</tr>
<tr>
<td>Mean of minimum temperature in coldest month (°C)</td>
<td>5.4</td>
</tr>
<tr>
<td>Mean temperature of warmest month (°C)</td>
<td>24.9</td>
</tr>
<tr>
<td>Mean of maximum temperature in warmest month (°C)</td>
<td>31.1</td>
</tr>
<tr>
<td>Variation of precipitation between driest and the wettest month (mm)</td>
<td>80</td>
</tr>
<tr>
<td>Within year variation of average temperature (°C)</td>
<td>15.7</td>
</tr>
</tbody>
</table>

Table 2. Accessions and species assignment resulting from BLAST at 100% homology level (sequence cover = 100%, E-value = $2 \times 10^{-330}$ of 100% identification in all cases).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Place of collection</th>
<th>Accession number</th>
<th>Author(-s) and Reference</th>
</tr>
</thead>
</table>
| *N. alba*      | Germany: Bavaria, Luttensee              | HG518071.1             | Borsch, T. [Unpublished]
| *N. alba*      | Ukraine: Kiev region, rivers Dnep and river Kozinka | EU428032.1, EU428033.1, EU428035.1 | Volkova & al. (2010)
| *N. candida*   | Russia: Chelyabinsk region, lake Maloe, Miassovoe | EU428041.1             | Volkova & al. (2010)

1 Accession was later published by Borsch & al. (2014).
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Fig. 1. General aspect of one of the patches and detail of a flower (photographs: J.L. Rodríguez-Marzal).