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Gymnocoronis spilanthoides (*Asteraceae*, *Eupatorieae*), a new naturalized and potentially invasive aquatic alien in S Europe

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Abstract: *Gymnocoronis spilanthoides*, a naturalized South American neophyte introduced as an aquarium and aquatic ornamental plant, is recorded for the first time from southern Europe. Two populations were found in irrigation canals and in a rice field in northern Italy. Distribution, invasion status, ecology, and pathways of introduction of the species are presented. Additionally, the modes of dispersal, never investigated before in the European continent, are discussed and the potential invasive behaviour and impacts in the new growing sites are assessed.

Key words: *Asteraceae*, *Compositae*, *Eupatorieae*, *Gymnocoronis*, *Gymnocoronis spilanthoides*, Italy, biological invasion, aquarium trade, rice fields flora, weeds, seed germination

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Introduction

The genus *Gymnocoronis* DC. (*Asteraceae*, *Eupatorieae*, *Adenostemmatinae*) includes two aquatic species, *G. latifolia* Hook. & Arn. and *G. spilanthoides* (D. Don ex Hook. & Arn.) DC., native to Mesoamerica and South America, respectively (Turner 1997; Tippery & al. 2014). *Gymnocoronis spilanthoides* (Senegal tea plant, water snowball) has been traded worldwide as an aquarium and aquatic ornamental plant since the second half of the 20th century and has been recorded as an invasive alien in E Asia, Australia, New Zealand, and C Europe, which has a single site in Hungary (Lukács & al. 2014; Tippery & al. 2014). The species represents a serious threat in Australia, where it is listed on the "National Environmental Alert List" (Australian Government 2012), and is rapidly expanding across China and Japan (Kadono 2004; Gao & Liu 2007; Gao & Chen 2011). In 2012, the European and Mediterranean Plant Protection Organization (EPPO) listed *G. spilanthoides* on the "EPPO Observation List of invasive alien plants" (EPPO 2016), which contains plant species (absent or present in the EPPO region) featuring a medium risk or for which information currently available is not sufficient to produce an accurate assessment.

In July 2015, an extended population of *Gymnocoronis spilanthoides* was discovered in an irrigation canal in Zerbolò (province of Pavia, Lombardia, Italy); further field surveys conducted in the following months revealed a wider distribution of the species across the local irrigation network. *Gymnocoronis spilanthoides* is a novelty to the flora of Italy and S Europe, but the presence of this aquarium plant in the study area was not to-

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tally unexpected: the aquarium and aquatic ornamental species trade is emerging as a major pathway for the introduction of new alien taxa (Padilla & Williams 2004; Hussner 2012), enhanced in recent times by online commerce, which is poorly regulated and accessible to a large number of users (Padilla & Williams 2004; Derraik & Philipps 2010; Mazza & al. 2015). Species like *Egeria densa* Planch., *Elodea nuttallii* (Planch.) H. St. John, *Lagarosiphon major* (Ridl.) Moss and *Myriophyllum aquaticum* (Vell.) Verdc., invasive or potentially invasive in Italy (Lastrucci & al. 2006; Celesti-Grapow & al. 2009; Banfi & Galasso 2010; Mazza & al. 2015), were originally introduced for ornamental purposes and, like *G. spilanthoides*, found rice fields and related irrigation infrastructures to be a suitable artificial freshwater ecosystem to establish and spread.

The potential risk of *Gymnocoronis spilanthoides* is regarded as "medium" in the EPPO region (Brunel 2009; EPPO 2016), and the species is declared invasive in every country where it has been introduced. Therefore, its mode of dispersal and spread potential in the Italian growing sites have been investigated with the aid of germination tests, in order to evaluate the role of sexual reproduction. Additionally, the potential impacts and invasive behaviour have been assessed by means of the EPPO prioritization process scheme for invasive alien plants (EPPO/OEPP 2012).

Material and methods

The present paper is based on observations and material collected on field trips conducted by the authors in Zerbolò (province of Pavia, Lombardia, Italy) and surrounding localities between July 2015 and July 2016. Voucher specimens are stored at BR, FI, MSNM and PAV (herbarium codes according to Index Herbariorum: Thiers 2016+).

Germination tests were conducted on achenes collected from the population located along the provincial road 185 in Zerbolò, in December 2015. Achenes were sown on 1 % distilled water-agar held in 90 mm diameter Petri dishes, using five replicates of 20 achenes for each temperature treatment; they were placed in temperature and light-controlled incubators (LMS Ltd, Sevenoaks, United Kingdom) using a 12-h daily photoperiod (photosynthetically active radiation 40–50 µmol $m^{-2}s^{-1}$). Germination was tested at five temperature treatments, two constant (15 °C, 25 °C) and three alternating (15/5 °C, 20/10 °C, 25/15 °C) regimes (in alternating temperatures, light was provided during the warm phase). The experiment lasted six weeks, during which germinated achenes were counted every five days. Germination was defined as visible radicle emergence. Achenes were not removed once germinated but kept on the Petri dishes to check the rate of seedling establishment of germinated achenes. We considered a seedling established when the cotyledons were fully unfolded. Seedling establishment rate was calculated as the percentage of germinated achenes that established seedlings. Data were analysed in SPSS 21.0. Final germination and seedling establishment responses to temperature treatments were tested using univariate ANOVA test. Homogeneity of variances was tested by means of Levene's test. The Bonferroni post-hoc test was applied to find out the differences between temperature treatments. Achene samples are stored at the University of Pavia Seed Bank.

Mean temperatures in the growing sites were acquired from WorldClim (2016+).

Potential impacts and invasive behaviour of the Italian populations were assessed following the "EPPO prioritization process for invasive alien plants" introduced by the European and Mediterranean Plant Protection Organization (EPPO/OEPP 2012).

Results and Discussion

Nomenclature

Gymnocoronis spilanthoides (D. Don ex Hook. & Arn.) DC., Prodr. 7(1): 266. 1838 ≡ *Alomia spilanthoides* D. Don ex Hook. & Arn. in Compan. Bot. Mag. 1(8): 238. 1835. – Lectotype (designated by Freire & Ariza Espinar 2014: 351): [Argentina, Buenos Aires], "Buenos Ayres", s.d., *J. Tweedie s.n.* (K K000486489 [image!]). – Image of the lectotype available at http://apps.kew.org/herbcat/getImage.do?imageBarcode=K000486489 [accessed on 20 Feb 2016].

= *Gymnocoronis attenuata* DC., Prodr. 5: 106. 1836 ≡ *Piqueria attenuata* (DC.) Gardner in London J. Bot. 6: 430. 1847 ≡ *Gymnocoronis spilanthoides* var. *attenuata* (DC.) Baker in Martius, Fl. Bras. 6(2): 183. 1876. – Holotype: [Brazil, Rio Grande do Sul], "Brésil. Province de Rio-Grande", 1833, *C. Gaudichaud 782* (P P02458159 [image!]; isotype: G-DC G00465443). – Image of the holotype available at http://science.mnhn.fr/institution/mnhn/collection/p/item/p02458159 [accessed on 12 Jun 2016].

= *Gymnocoronis subcordata* DC., Prodr. 5: 106. 1836 ≡ *Piqueria subcordata* (DC.) Gardner in London J. Bot. 6: 430. 1847 ≡ *Gymnocoronis spilanthoides* var. *subcordata* (DC.) Baker in Martius, Fl. Bras. 6(2): 184. 1876. – Holotype: [Brazil, Rio Grande do Sul], "Brésil. Province de Rio-Grande", 1833, *C. Gaudichaud 886* (P P02458162 [image!]; isotype: G-DC G00465429). – Image of the holotype available at http://science.mnhn.fr/institution/mnhn/collection/p/item/p02458162 [accessed on 12 Jun 2016].

Description of the specimens

Helophyte, rhizomatous, 1–1.5 m tall. *Stems* erect or ascending, to 1.5 cm in diam., branched, fistulose, glabrous. *Leaves* opposite; *petiole* 1–7 cm long; *leaf blade* dark

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Fig. 1. *Gymnocoronis spilanthoides* morphological traits (Zerbolò, Italy, August 2015) – A: inflorescence; B: rooting stem nodes; C: young individual. – Photographs by C. Ballerini.

green, glossy, 7.5–18 cm long, 3.5–7.5 cm wide, thick, coriaceous, pinnately veined, glabrous, base attenuate, occasionally truncate to slightly cordate, margin irregularly obtusely serrate, apex acute. *Synflorescence* terminal, cymose; axis and branches with short glandular hairs. *Capitula* pedunculate, homogamous and discoid, >100-flowered, 9–14 mm long, 14–17 mm in diam., peduncle with short glandular hairs; *involucre* hemispherical, 5–7 mm long, 9–11 mm in diam.; *phyllaries* 2-seriate, narrowly lanceolate, with subsessile to sessile glands; *receptacle* epaleaceous, glabrous and with discrete oval scars. *Corollas* yellowish to white, narrowly funnelform, 3.5–4 mm long, with shortly stalked glands on outer surface; *lobes* green, as wide as long; *style branches* white, oar-shaped, mamillose except at apex. *Achenes* pale brown, ellipsoid, slightly curved, 1.7–2 mm long, 0.8–0.9 mm wide, 5-ribbed, gland-dotted; *carpopodium* white, from conical to cylindrical, very short; *pappus* absent. – Fig. 1A–C.

Worldwide distribution, ecology, and pathways of introduction

Gymnocoronis spilanthoides is a neotropical species, native to Peru, N Argentina, Bolivia, Paraguay, Uruguay and S Brazil (King & Robinson 1974; Cabrera & Freira 1997; Dillon & Sagastegui Alva 2002; Hind 2011). Since the early 1980s it has been recorded as an invasive al-

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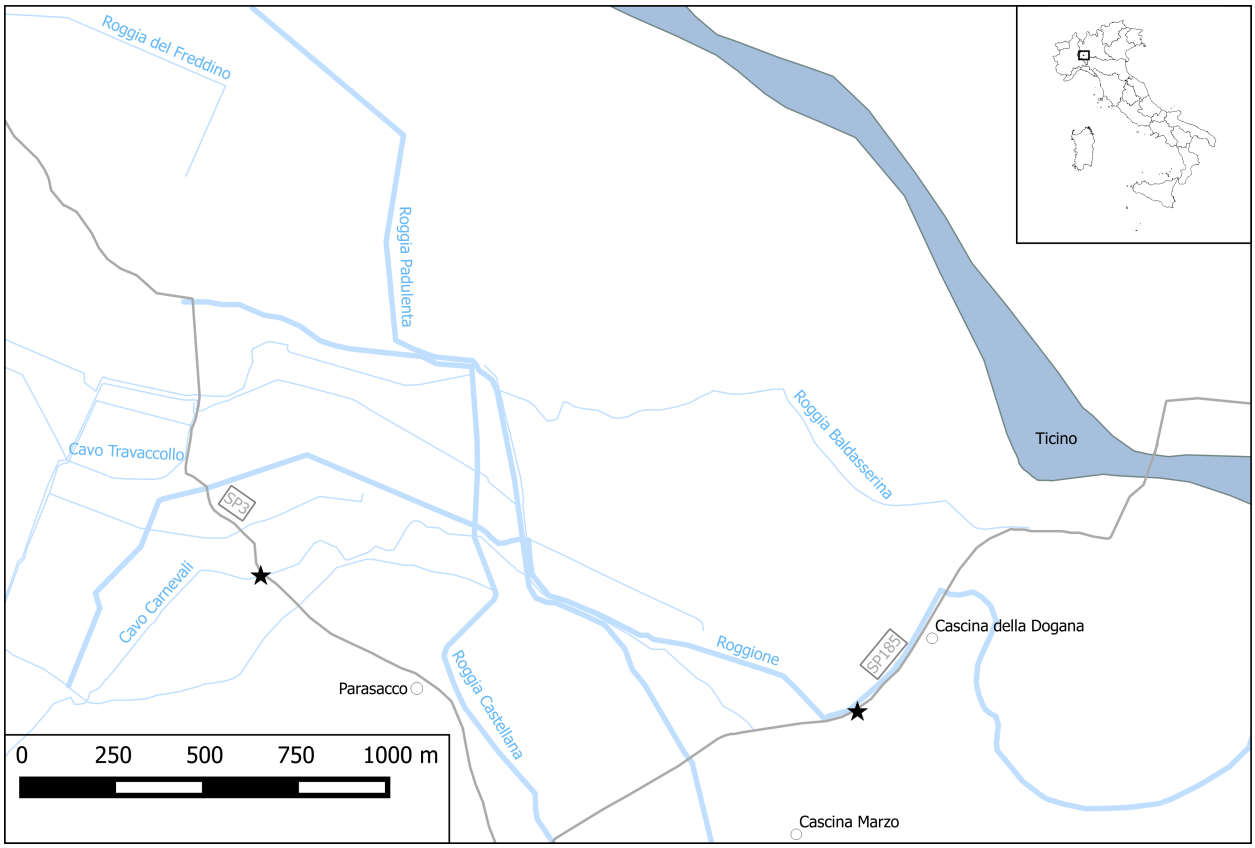


Fig. 2. Location of the Italian populations of *Gymnocoronis spilanthoides* (★). – Map by C. Ballerini.

ien in Australia (Cook 1990; Porteners 1992; Parsons & Cuthbertson 2001; Gunasekera & al. 2002), New Zealand (Timmins & Mackenzie 1995; Howell 2008), Japan (Suyama 2001; Kadono 2004), China and Taiwan (Gao & Liu 2007; Chen & al. 2011; Gao & Chen 2011). In Europe, it has been known since 1988 from the thermal lake of Hévíz, Hungary (Szabó 1998, 2002; Hussner 2012; Lukács & al. 2014), the northernmost growing site within its range.

In its primary and secondary ranges, *Gymnocoronis spilanthoides* grows in freshwater habitats, mainly in marshes and waterbodies with slow-running to standing water, such as ponds, creeks and drains (Cook 1990; Cabrera & Freira 1997; Porteners 1992; Parsons & Cuthbertson 2001; Chen & al. 2011). It is resistant to frost and is able to survive periods of submersion (Timmins & Mackenzie 1995).

Gymnocoronis spilanthoides (including cultivars featuring reddish stem and foliage or variegated leaves) has been traded worldwide as an aquarium plant since the 1960s; it is also cultivated outdoors in water gardens and occasionally as a water purifier and for its butterfly-attracting flowers. The species, popular for its hardiness, is commonly propagated from cuttings (Cook 1990; Parsons & Cuthbertson 2001; Kadono 2004; Allgayer & al. 2007; Brunel 2009; Panetta 2009; Champion & al. 2010; Lowe 2013). A survey of the online aquarium shops conducted in February 2016 revealed that the species is currently sold in Italy on at least seven different websites, at relatively low prices (€ 3.50 to € 5.82).

The new Italian populations: location and invasion status

Gymnocoronis spilanthoides was discovered in the S part of Lombardia (NW Italy), within the area of the regional natural park "Parco Lombardo della Valle del Ticino", in two different localities of the town of Zerbolò (province of Pavia): provincial road 185 between Cascina Marzo and Cascina della Dogana, both canals running along the sides of the road (45.22872°N, 08.99742°E); and canal Cavo Carnevali in Parasacco (45.23191°N, 08.97683°E). The two populations are located at a distance of 1.9 km from each other and grow in two canals belonging to the same irrigation network (Fig. 2). The growing sites are characterized by hot summers, in which the monthly mean maximum temperatures approaches 30 °C, and relatively cold winters with the mean minimum temperature of January slightly below –1 °C (Fig. 3A). The plant was first detected in July, and its presence was confirmed the following year at the end of and after the winter season in early March and mid-July, respectively.

In these waterbodies, about 1 m deep, the species occupies extended stretches up to 519 m long, forming isolated but dense monospecific mats with a 90–100 % cover, usually occupying the whole canal width (1–4 m)

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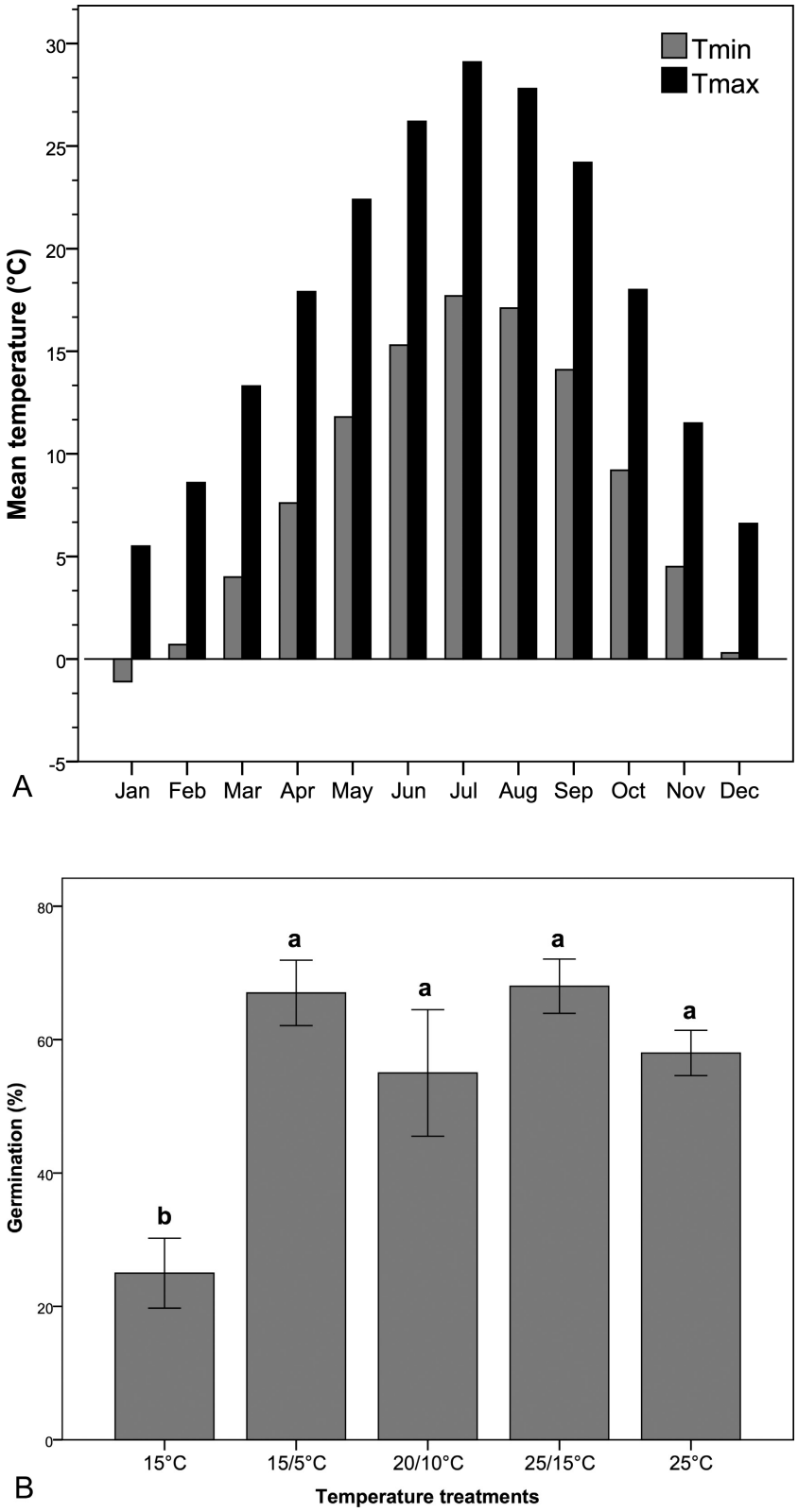


Fig. 3. *Gymnocoronis spilanthoides*, temperatures in the growing sites and achene germination – A: monthly mean maximum temperatures (Tmax) and monthly mean minimum temperatures (Tmin) in the location of achene collection (45.22872°N, 08.99742°E). Data acquired from WorldClim (2016+); B: mean final germination percentages at the five temperature treatments tested. Error bars represent mean final germination percentage ± st.err. Lowercase letters indicate statistically significant differences among temperature treatments; same letter indicates non-significant differences; different letters indicate differences significant at $P<0.05$.

(Fig. 4A). The plant grows both in the bed and on the banks, surrounded by submerged rooted (*Potametalia pectinati* Koch 1926) and amphibious (*Phragmito-Magnocaricetea* Klika in Klika & V. Novák 1941) vegetation. A large stand, covering an area of 12 m^2, was also detected on the edge and within a rice field, in proximity of the southern canal running along the provincial road 185 (Fig. 4B).

Similarly to other alien macrophytes recently discovered in the surroundings (e.g. *Myriophyllum aquaticum*, naturalized in a drain near a farmhouse in Vigevano, see Ardenghi & Cauzzi 2014), *Gymnocoronis spilanthoides* was probably introduced from private aquaria deliberately or unintentionally after cleaning.

Taking into account the observed distributional pattern and the occurrence of several young individuals without flowers (Fig. 1C), it is likely that the presence of the species in Zerbolò dates back to more than a year ago. The introduction probably occurred near the bridge on the Cavo Carnevali canal, whose banks are close to private dwellings. On the west side of the bridge, where the plant is still present, a dense roundish mat, resembling in shape those of *Gymnocoronis spilanthoides*, can be spotted within a panoramic picture taken in October 2011 for the web mapping service "Google Street View", about four years before our discovery; unfortunately, the considerable distance between the mat and the camera prevents an accurate identification of the plant. From here, in the course of one or more vegetative seasons, it is likely that achenes or vegetative fragments reached the drains and the rice fields between Cascina Marzo and Cascina della Dogana, transported by the flow of water, which runs from the first site southwards to the second one.

In view of the of extension of the populations, the establishment of the species and its competition with

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the surrounding vegetation, we consider *Gymnocoronis spilanthoides* naturalized in Italy, in line with the definition provided by Celesti-Grapow & al. (2009).

Dispersal and achene germination

Spread by vegetative fragmentation represents the primary mode of dispersal in the secondary range of the species. It occurs when parts of the stem or the rhizome containing nodes break off from the parent plant and are transported away by the streamflow; these fragments quickly develop adventitious roots and stems, generating new colonies. New plants have been observed to arise also from leaf fragments provided with the midrib (Timmins & Mackenzie 1995; Parsons & Cuthbertson 2001; Panetta 2009). The growth rate of adult individuals is c. 15 cm a week in optimal conditions; it is slower when the plant is completely under water (Timmins & Mackenzie 1995).

In Zerbolò, dispersal of vegetative parts may have been enhanced by mechanical cleaning of the rice fields canals, whose vegetation (weedy or not) is regularly mown at least once a year. The population located between Cascina Marzo and Cascina della Dogana was still observed in July 2016, eight months after the last mowing operations, and new growth was noticed emerging from mown stems (Fig. 4C).

Spread by means of achenes is reported to be less significant than vegetative dispersal, at least in the areas of introduction: being relatively heavy and without



Fig. 4. *Gymnocoronis spilanthoides* naturalized population in Zerbolò (provincial road 185 between Cascina Marzo and Cascina della Dogana), Italy, August 2015 (A–B) and March 2016 (C) – A: monospecific stand in irrigation canal; B: dense mat in a rice field; C: emergence of new growths from mowed stems. – Photographs by C. Ballerini.

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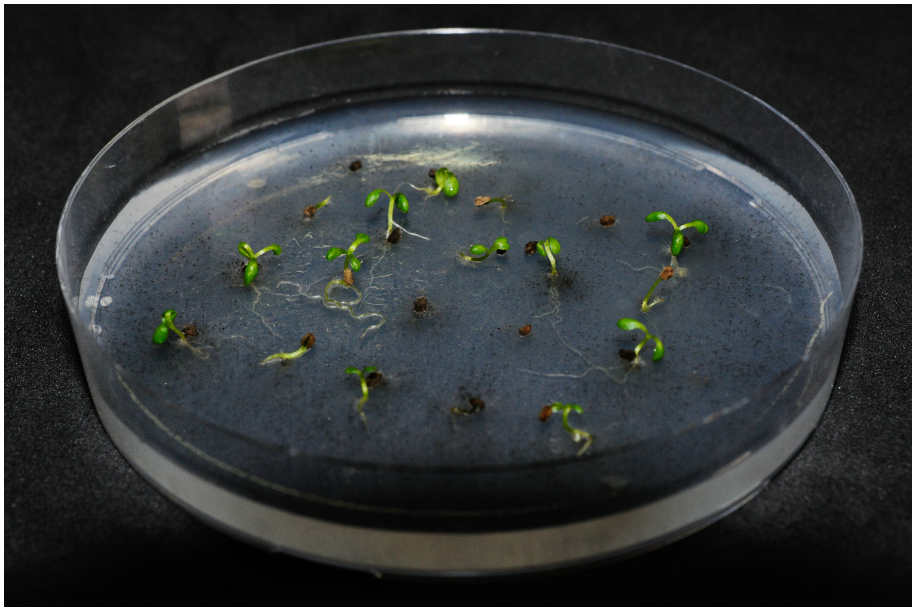


Fig. 5. *Gymnocoronis spilanthoides*, seedling emergence during germination tests. – Photograph by C. Ballerini.

pappus, they are not dispersed by wind but only by water and mud sticking to animals or machinery (Timmins & Mackenzie 1995; Parsons & Cuthbertson 2001; Panetta 2009). Achenes are very persistent, remaining viable for about 3 years when half-buried or located on the soil surface, and up to 16 years if completely buried (Panetta 2009).

In the Italian growing sites considered here, flowering has been observed from July until November, while achene production has been recorded from August. Germination tests conducted on the collected achenes confirmed their high viability (Fig. 5). Additionally, the effect of the different temperature treatments proved to be significant on final germination percentages (F=9,006; P<0,001). The final germination percentage at 15 °C (25 %) was significantly lower than at the other temperatures, while there were no significant differences among the other temperatures (25 °C, 15/5 °C, 20/10 °C, 25/15 °C). The best germination results were achieved at the two alternating temperatures, 15/5 °C (67 %) and 25/15 °C (68 %) (Fig. 3B). The mean seedling establishment rate was high for all the treatments, with the percentage of germinated achenes that reached the seedling establishment always higher than 60 %, without differences between the temperature treatments (F=2,429; P=0,081).

The germination requirements of *Gymnocoronis spilanthoides* are typical of wetland species: lack of germination in darkness (Vivian-Smith & al. 2005) and better performances under alternating temperatures. This can be explained by the need to enhance the germination near the soil surface and prevent its occurrence under the water table (Fenner 2000). On the basis of our results, germination and seedling establishment can occur, to a large extent, at a temperature regime comparable to that at the time and place in which the achene dispersal has been recorded (Fig. 3A). *Gymnocoronis spilanthoides* achenes are persistent in the soil (Panetta 2009) and are reported to be capable of surviving winter low temperatures and germinating when the temperatures increase after the cold season (Department of Agriculture, Fisheries and Forestry. Biosecurity Queensland 2014). This, linked to the wide range of temperatures in which the germination occurs, makes it likely that the achenes of *G. spilanthoides* can germinate throughout most of the year in the study area. Thus, as already noticed in Australia (see Panetta 2009), achenes can be safely regarded as a vector for the dispersal and establishment of *G. spilanthoides* across the study area.

Impact and potential invasive behaviour

In view of the establishment of *Gymnocoronis spilanthoides* discussed in the previous paragraphs, positive responses are given to questions A.1 and A.2 of the prioritization process scheme for evaluating the invasive behaviour of alien plants (EPPO/OEPP 2012), the species being a naturalized alien (neophyte) in the area under assessment.

The spread potential (question A.5) is “high”, since both vegetative and sexual reproduction take place in the study area; moreover, the plant is able to spread over distances longer than 500 m, either by water or unintentionally by human activities (mechanical cleaning of irrigation canals). Uncertainty rating is therefore ranked as “low”.

The potential negative impact on native species, habitats, and ecosystems in the area under assessment (question A.6) is regarded as “medium”: *Gymnocoronis spilanthoides* forms large and dense populations but only in habitats modified by human activities (irrigation canals and rice fields); its negative impact on agriculture (question A.7) is ranked as “low”, since the presence of the species in rice fields seems to be currently accidental, with no evidence of yield- or economic losses. The occurrence of additional impacts, especially on infrastructures (question A.8), is “medium”: in other countries outside its native range (e.g. Australia, see Gunasekera & al. 2002), *G. spilanthoides* produces dense mats, similar to those recorded from the study area, capable of obstructing irrigation canals. Uncertainty ratings for these three questions are ranked as “low”.

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The combination of the spread potential ranking ("high") with the maximum rating obtained from the questions on the adverse impacts ("medium"), suggest that *Gymnocoronis spilanthoides* be included in the "observation list of the invasive alien plants"; thus, the prioritization process for the identification of invasive aliens worthy of pest risk analysis (section B) is not required. Overall uncertainty is regarded as "medium", since the species, although present in the area under assessment, is a newcomer.

The results of the EPPO prioritization process confirm that *Gymnocoronis spilanthoides* is a potential invasive alien in Italy and agree with the listing of this species on the "EPPO Lists of Invasive Alien Plants" (EPPO 2016). The absence of *G. spilanthoides* from the list of the invasive and potentially invasive plants for Italy sold online (Mazza & al. 2015: Table 1) suggests an increase in its trade in our country during the last four years (online aquarium stores were surveyed in 2012 by Mazza & al. 2015). Pending further research across the study area, the high spread-potential of this macrophyte, along with the considerable extension of suitable habitats surrounding the discovered growing sites and the possibility of further introductions linked to its growing commerce, are likely to cause a shift in invasive behaviour, as has already occurred in Hungary, Australia and E Asia.

Specimens examined

Italy: Lombardia: Zerbolò (Pavia), SP185 tra Cascina Marzo e Cascina della Dogana, 45.22872°N, 08.99742°E, 68 m, corsi d'acqua, Jul 2015, leg. *G. Barcheri s.n.*, det. *N. Ardenghi* (PAV); Zerbolò (Pavia), SP185 tra Cascina Marzo e Cascina della Dogana, canale sul lato S della strada, 45.22829°N, 08.99596°E, 68 m, sponda di canale con *Glyceria maxima*, *Lolium arundinaceum*, *Galium palustre* subsp. *elongatum*, *Filipendula ulmaria*, *Urtica dioica*, 5 Aug 2015, *N. Ardenghi*, *C. Ballerini* & *P. Cauzzi s.n.* (FI); Zerbolò (Pavia), SP185 tra Cascina Marzo e Cascina della Dogana, lato S della strada, 45.22880°N, 08.99788°E, 68 m, margine inondato di risaia, con *Setaria pumila*, *Echinochloa crus-galli*, *Veronica anagallis-aquatica*, *Digitaria sanguinalis*, *Spirodela polyrhiza*, *Heteranthera reniformis*, 5–6 m dentro la risaia, 5 Aug 2015, *N. Ardenghi*, *C. Ballerini* & *P. Cauzzi s.n.* (BR, MSNM); Zerbolò (Pavia), Parasacco, Cavo Carnevali, su entrambi i lati del ponte di via Borgo San Siro (SP3), a N della chiesa, 45.23191°N, 08.97683°E, 69 m, sponda con *Urtica dioica*, *Persicaria* cfr. *hydropiper*, *Rubus* sect. *Corylifolii*, 5 Aug 2015, *N. Ardenghi*, *C. Ballerini* & *P. Cauzzi s.n.* (FI).

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