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Ecological preference of the diving bell spider *Argyroneta aquatica* in a resurgence of the Po plain (Northern Italy) (Araneae: Cybaeidae)

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Abstract
The diving bell spider *Argyroneta aquatica* is the only known spider to conduct a wholly aquatic life. For this reason, it has been the object of an array of studies concerning different aspects of its peculiar biology such as reproductive behavior and sexual dimorphism, physiology, genetic and silk. On the other hand, besides some empirical observations, the autoecology of this spider is widely understudied. We conducted an ecological study in a resurgence located in the Po Plain (Northern Italy, Province of Vercelli) hosting a relatively rich population of *Argyroneta aquatica*, aiming at identifying the ecological factors driving its presence at the micro-habitat level. By means of a specific sampling methodology, we acquired distributional data of the spiders in the study area and monitored physical-chemical and habitat structure parameters at each plot. We analyzed the data through Bernoulli Generalized Linear Models (GLM). Results pointed out a significant positive effect of the presence of aquatic vegetation in the plot. In addition, the presence of *A. aquatica* was significantly associated with areas of the resurgence characterized at the same time by high prey availability and low density of predators. Considering the ecological importance and rarity of this species, we update and revise the data on the distribution of *A. aquatica* in Italy.

Key words: Water spider, autoecology, wetlands, binomial regression.

Introduction

Spiders are considered one of the most successful group of organism in terms of evolutionary radiation and ecological plasticity (Foelix 1996), as they have conquered all the possible terrestrial ecological niches on our planet (Turnbull 1973). On the other hand, spiders are virtually absent in underwater marine and freshwater ecosystems. The diving bell spider *Argyroneta aquatica* (Clerck, 1757) (Araneae, Cybaeidae) represents an exception in this regard, being the only known species that has a wholly aquatic life (Seymour & Hetz 2011; Figs 1-2). *A. aquatica* is a large-sized spider that dwells in clean, densely overgrown still waters (Nentwig et al. 2016). Thanks to specific adaptations enabling the spider to breathe in immersion, such as the presence of hydrophobic hairs on the opisthosoma and legs (Levi 1967; Schütz et al. 2007), this species is able to hunt, to consume the prey, to moult, to deposit eggs, and to copulate underwater.

The peculiar life of this spider has been intriguing scientists for long time. Among other topics, studies on *A. aquatica* focused on circadian activity (Masumoto et al. 1998a), unusual sexual dimorphism and reproductive behavior (Schütz & Taborsky 2003, 2005), feeding activities (Perevozkin et al. 2004; Nyffler & Pusey 2014), physiology (Schütz et al. 2007; Pedersen & Colmer 2011; Neumann & Woermann 2013), genetic (Liu et al. 2015) and silk properties (Bakker et al. 2006; Neumann & Kureck 2013).

Considering ecological studies, the impact of future climate and land use changes on the bioclimatic range of *A. aquatica* has been recently pointed out by Leroy et al. (2014). On the other hand, when considering the local scale, besides empirical observations about habitat preferences and a common consensus on its sensitiveness to environmental pollution (e.g. Dolmen 1977; Masumoto et al. 1998b; Aakra & Dolmen 2003; Seyyar & Demir 2009; Seyyar et al. 2010; Kim & Lim 2011; Komnenov et al. 2011), a general lack of knowledge emerges. Considering its ecological peculiarity and its wide distribution in the Palearctic region (World Spider Catalog 2016), this is rather surprising.

Italian records of *A. aquatica* are scattered in less than twenty scientific papers, most of them dating back to the XIX century (Table 1). In Piedmont (NW-Italy), the occurrence of the species has been rarely documented (Isaia

Table 1 – List of the Italian records of *Argyroneta aquatica* (Clerck, 1757) in chronological order according to the year of collection cited in the publication (column "Year").

Author(s)	Year	Region	Municipality	Locality	Map index (Fig. 7)
Contarini 1843	1843	Veneto	-	Padova and Venezia (unspecified locality)	-
Contarini 1847	1847	Veneto	-	In the vicinity of the lagoon of Venezia (unspecified locality)	1
Ambrosi 1851	1851	Trentino Alto Adige	-	Generic citation for Trentino	-
Sordelli 1868	1868	Lombardia	Calvairate (MI)	Senavra	2
	1868	Lombardia	Segrate (MI)	Redecesio	3
Canestrini & Pavesi 1868	1868	Lombardia, Piemonte, Veneto	-	Generic citations for Lombardia, Piemonte and Veneto	-
Canestrini & Pavesi 1870	1870	Lombardia, Piemonte, Veneto	-	Generic citations for Lombardia, Piemonte and Veneto	-
Pavesi 1873	1873	Lombardia	Pavia (PV)	Campeggi and Lanca San Lanfranco	4-5
di Caporiacco 1940	1896	Veneto	Gazzo (PD)	Gazzo	6
	1897	Veneto	Gazzo (PD)	Taone stream	6
	1898	Veneto	Valeggio (VR)	Valeggio	7
Garbini 1898	1898	Veneto	-	Generic citation for Veronese (Verona province)	-
Stammer 1932	1932	Friuli Venezia Giulia	Duino-Aurisina (TS) and Monfalcone (GO)	Shallow waters and springs at the mouth of the river Timavo	8
Zangheri 1966	1966	Emilia-Romagna	Ravenna (RA)	San Alberto	10
Brignoli 1977	1966	Veneto	Torreselle (PD)	River Sile	9
	1968	Lombardia	Iseo (BG)	Torbiere del Sebino	11
Isaia et al. 2007	1970	Lombardia	Monasterolo del Castello (BG)	Lake of Endine	12
	1989	Lombardia	Casirate d'Adda (BG)	Fontanile Roccolo	13
Groppali 2002	2002	Lombardia	Pavia (PV)	Surroundings of Pavia	14
Rossi & Bosio 2012	2003	Val d'Aosta	Saint-Rhémy-en-Bosses (AO)	In a small stream near Saint-Rhémy-en-Bosses	15
This work	2014	Lombardia	Somma Lombardo (VA)	Swamps of Arsago	16
	2014	Piemonte	Livorno Ferraris (VC)	Cascina Spinola	17
	2015	Piemonte	Tricerro (VC)	Fontana Gigante	18
	2015	Piemonte	Lachelle (VC)	Prato Lungo	19

et al. 2007) and the last available records are generic regional references dating back to 1868-1873. After more than 140 years, we discovered a relatively abundant population of *A. aquatica* in Piedmont, in several small resurgences in the rice-growing area of Vercelli. Given its accessibility and its general absence of direct disturbance, we choose one of these area (Prato Lungo, Lachelle) for an ecological investigation on the habitat preference of this species.

Specifically, we carried out a field survey aiming to identify the ecological factors driving the presence of this species at the micro-habitat level in our study site. As a complement of the ecological analysis, we also report new original records of *A. aquatica* in Piedmont, updating the distribution of this species in Italy.

Material and methods

Study site

The study was performed in the resurgence of Prato Lungo, located in Lachelle, municipality of Ronsecco, province of Vercelli, region of Piedmont (plain of the Po River), NW-Italy [8.2488 E, 45.2824 N (WGS84 reference

Figs 1-4 – **1-2**. Our model organism, *Argyroneta aquatica* (Clerck, 1757) [photo credits: Riccardo Cavalcante]; **3**. collection of submerged debris; **4**. drying and sieving of the debris on a fine mesh, in order to convey the aquatic invertebrates into the plastic bowl.

system); 150 m a.s.l.]. There, a relative abundance population of *Argyroneta aquatica* occurs in the vegetated shallow marsh (Figs 3, 5), confined in the middle of two slow-streaming water channels. Vegetation in the marsh mainly consists of reeds (*Thypha* and *Phragmites*), forming spotted rushes, and aquatic plants such as watercresses (*Nasturtium officinale*), water forget-me-not (*Myosotis scorpioides*) and common duckweeds (*Lemna minor*). The resurgence is entirely surrounded by rice fields.

Sampling design

We conducted the field survey on June 24, 2015. We evenly distributed 33 sampling plots of 1x1 m over the marsh and utilized them as basic sampling units for data acquisition. The spatial position of the plots is reported in Fig. 5. In each sampling plot, we evaluated two sets of metrics (i.e. explanatory variables): physical-chemical and habitat structure parameters. Physical-chemical parameters [water dissolved oxygen (DO), temperature, pH, conductivity and turbidity] were measured through a standard multiparametric probe (Hydrolab mod. Quanta). *Proxies* for habitat structure [water depth at the middle of the plot (cm), mean vegetation eight (cm); percentage of submerged, free-floating, and emerging plants in the sampling plot (%); shaded area of the plot (%)] were measured using a measuring tape or assessed by visual inspection.

After the measurements, at each sampling plot we collected a standard quantity of submerged debris with a dip-net (Fig. 3). We placed the debris on a fine mesh, which, in turn, was allocated on a plastic bowl for drying and sieving (Fig. 4). After a time-interval of 30 minutes, we recorded the presence of *Argyroneta aquatica* and other species in the bowl, identifying the latter up to the family level. In light of the general lack of information about the diet of *A. aquatica* (Crome 1951; Perevozkin et al. 2004; Nyffler & Pusey 2014), the by-catch species were classified on the

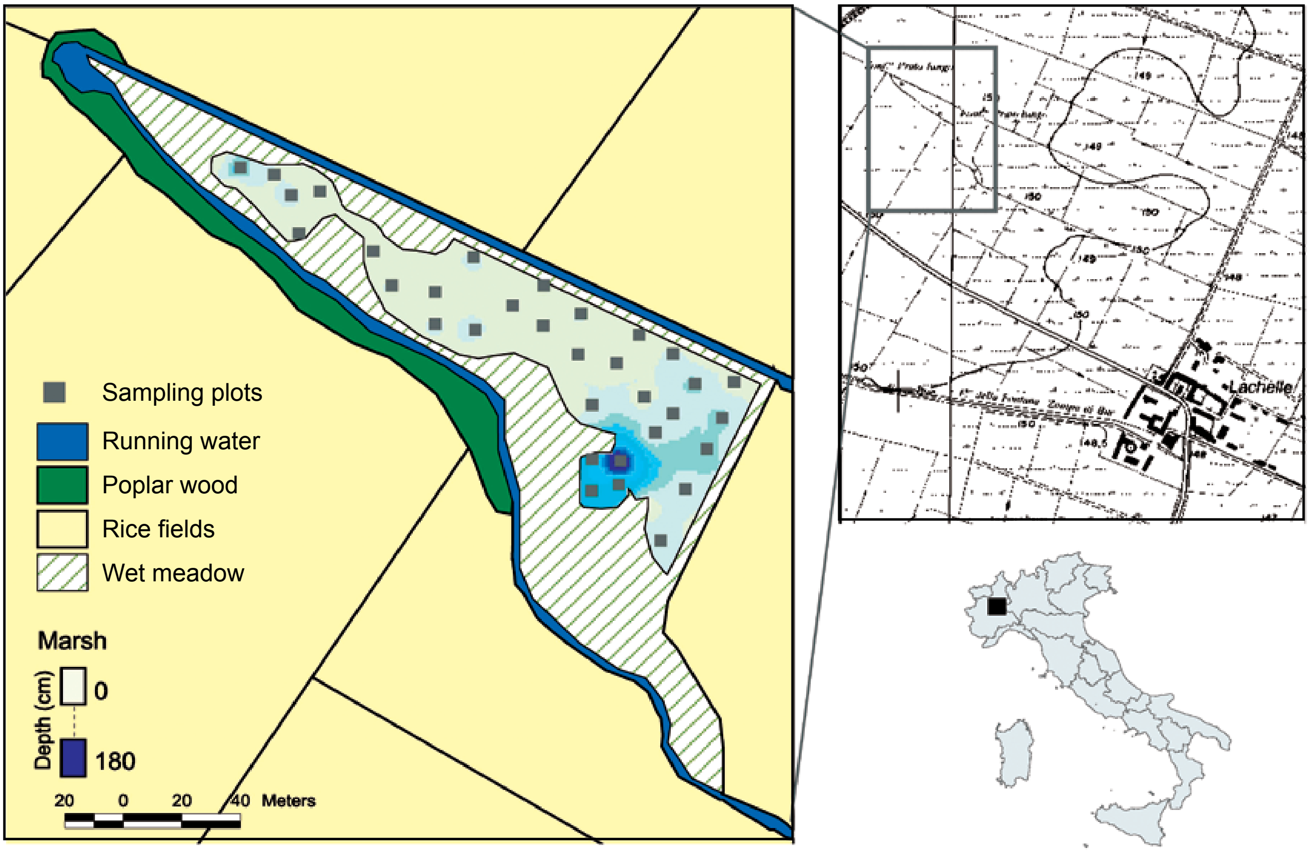

Fig. 5 – Map of the study area. Shades of blue denote water depth and were obtained in GIS environment by interpolating the depth values at each sampling plot.

basis of their body size and general biology in three categories: i) potential prey items for *A. aquatica*, ii) potential predators of *A. aquatica,* and iii) other species. We considered the following as potential prey items (hereinafter *Prey*): larvae of Dipterans, small-sized beetles (Dytiscidae, gen. *Hydroglyphus*), blackswimmer (Notonectidae), and small-sized leeches (Hirudinea). We considered the following as potential predators (hereinafter *Predators*): true loaches (Cobitidae), water scorpions (Nepidae) and larvae of dragonflies (Anisoptera and Zygoptera). The other species censused in this study – three morpho-species of gastropods – were not further considered in the statistical models.

Data analysis

The ecological factors driving the presence of *Argyroneta aquatica* were tested via a Generalized Linear Model (GLM) in R (R Development Team 2015). Given the low density of individuals per sampling plot (1 individual in over 90% of the presence points), we expressed counts as presence/absence (i.e., Bernoulli distribution, 0-1).

We selected the following continuous covariates as potential variables explaining the presence of *A. aquatica* in the plots: *DO*, *pH*, *Temperature*, *Water depth*, *Conductivity*, *Turbidity*, *Vegetation height*, *Submerged plants (%)*, *Free-floating plants (%)*, *Emerging plants (%)*, *Shadow (%)*, *Prey*, *Predators* (see variables explanation in the previous paragraph).

Prior to model fitting, we explored the dataset following the standard protocol of Zuur et al. (2010), ensuring that there were no outlying observations in the independent variables and no collinearity between predictors. Specifically we employed Cleveland's dotplots to assess the presence of outliers. We investigated multi-collinearity via Pearson correlation tests (r) and variance inflation factors values (VIFs) - threshold for collinearity: $r > \pm 0.7$ and VIF > 3 (Zuur et al. 2009, 2010). We fitted the Bernoulli GLM via the *glm* command in the stats R package (R Development Team 2015). Once we fitted the initial model, we applied model selection (Johnson & Omland 2004), whereby model reduction was carried out on the full model by sequentially deleting terms under an Akaike Information Criteria (Hurvich & Tsai 1989; Burnham & Anderson 2002) corrected for small samples (AICc).

Results

At the day of the survey, the sampling plots were characterized by water temperature ranging from 19 to 31 °C

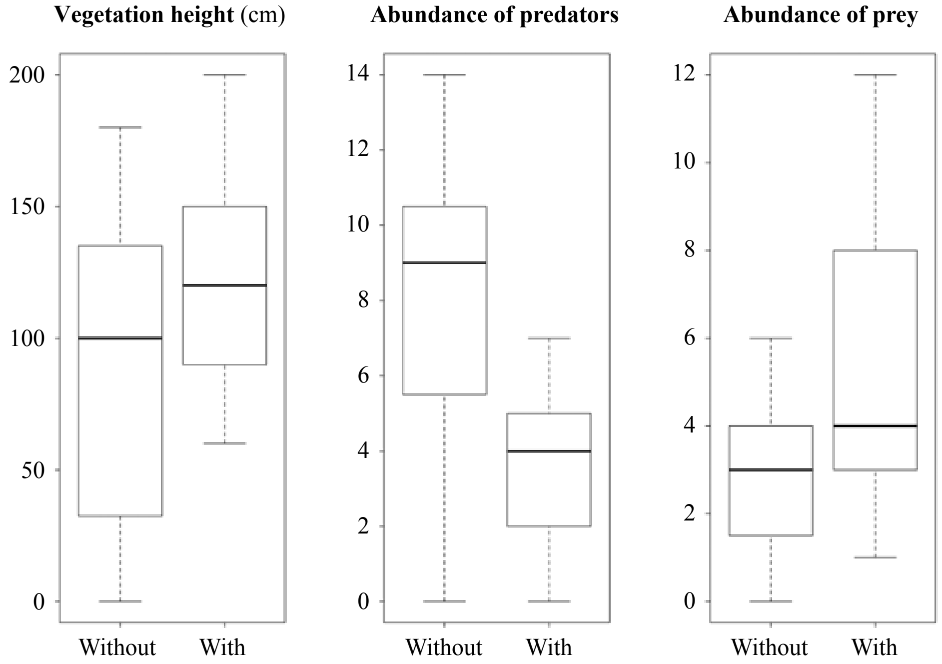

Fig. 6 – Boxplots showing the difference between plots with or without *Argyroneta aquatica* in terms of vegetation height, abundance of predators and abundance of prey.

(mean = 24.2), DO from 0.11 to 10.39 mg/l (mean = 2.6), pH from 6 to 8 (mean = 6.9), conductivity from 0.01 to 0.17 µS/cm (mean = 0.11) and turbidity from 0 to 0.36 NTU (mean = 0.24). Overall, we recorded 16 individuals of *Argyroneta aquatica* (in 14 out of 33 plots), the majority of which were juveniles.

In light of data exploration (*sensu* Zuur et al. 2010), the variables *Conductivity* and *Turbidity* were collinear with *Temperature* (Pearson $r > 0.7$). The variables *Free-floating plants (%), Emerging plants (%),* and *Shadow (%)* were correlated (Pearson $r > 0.7$) with *Vegetation height,* while the variable *Water depth* was anticorrelated (Pearson $r > -0.7$) with *Vegetation height*. According to VIF analysis, we dropped from the initial model all collinear variables with *Temperature* and *Vegetation height.* As a result, we included *Temperature*, *DO*, *pH, Vegetation height*, *Submerged plants (%), Prey*, *Predators* in the structure of the initial regression model. The backward model selection process based on comparative AICc values indicated that the only important covariates in the Bernoulli GLM were *Vegetation height*, *Predators* and *Prey* (Fig. 6). In particular, the probability of presence of *A. aquatica* was found to increase at increasing abundance of potential prey items and with increasing values of vegetation height. The probability of presence of *A. aquatica* was also found to decrease at increasing abundance of potential predators (Table 2).

Table 2 – Estimated regression parameters according to Bernoulli GLM.

	Estimated β	SE	z	p-value
Intercept	-2.1529	1.665	-	-
Vegetation height	2.4518	1.1133	2.202	0.02
Prey	0.6495	0.2972	2.186	0.02
Predators	-0.5716	0.2253	-2.573	0.01

Discussion

In this contribution, we investigated the ecological requirements of the diving bell spider *Argyroneta aquatica* in a shallow marsh in the Po plain (Piedmont, Italy). As far as we are aware, this is the first such an attempt in the scientific literature.

Although the study site is relatively small and shallow, it represents one of the typical biotope inhabited by this species. Ordinarily, *A. aquatica* is found in oligotrophic/dystrophic bog ponds, marshes and swamps, resurgences, small lakes and slow-moving streams (e.g. Brignoli 1977; Dolmen 1977; Masumoto et al. 1998b; Aakra & Dolmen 2003; Isaia et al. 2007; Seyyar & Demir 2009; Komnenov et al. 2011). Only occasionally, the species has been reported in running waters (Seyyar & Demir 2009). Aakra & Dolmen (2003) observed that in the Scandinavian peninsula, this species exploits almost any kind of standing and vegetated water bodies, showing therefore a relative high ecological plasticity.

Our results suggest that the height of the emerging plants, which could be interpreted, at least in our case, as

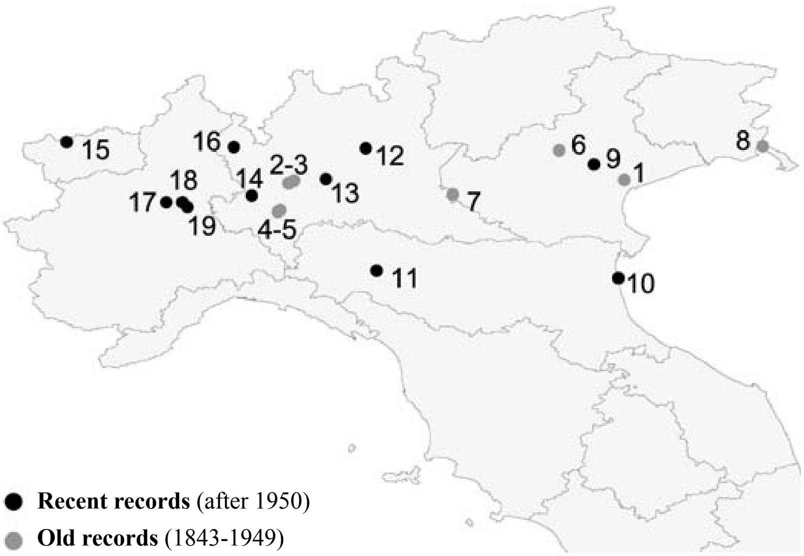

Fig. 7 – Distribution of *Argyroneta aquatica* (Clerck 1757) in Italy. Numeric codes refer to Table 2.

a *proxy* for the complexity of the vegetation structure, is the most important factor conditioning the presence of *A. aquatica* in the study area. Our results somehow align the empirical observations by Bristowe (1971) and Masumoto et al. (1998b), pointing out that *A. aquatica* needs water plants to anchor webs and use them as resting sites.

In light of the collinearity analysis, the variable *Vegetation height* can be interpreted as surrogate for several other variables having a likely effect on the general environmental and microclimatic conditions of the marsh. Indeed, plots with taller vegetation are, in turn, more shaded, with more floating vegetation and shallower. According to Bristowe (1971), shallow areas of the ponds are generally preferred by *A. aquatica,* also for lying the cocoons. Although the species is able to exchange enough oxygen from the water even at high temperature (Seymour & Hetz 2011), shaded areas may be preferred during warm days, such as in our case.

Our analysis also reports a significant effect for prey availability (positive) and abundance of predators (negative). In this regard, the choice of the spider for specific area of the marsh represents a trade-off between the need to maximize the energy intake from one side, and to lower the predatory pressure from the other (e.g. Simpson et al., 2004). This compromise represents a classic dilemma in ecology, and has been previously assessed in other spider species (e.g. Blamires et al. 2007). In the case of our model species, it has been observed that it can survive in moderate acid and unoxygenated waters, where other predators cannot survive (Masumoto et al. 1998a,b). It has been pointed out the reduced diurnal activity in juveniles and adult females may reduce the risk of predation as well (Schütz & Taborsky 2003, 2005).

Although the analysis here discussed may serve as a baseline for the study of the autoecology of *Argyroneta aquatica*, a number of caveats needs to be made when interpreting the results. First, the collection of the data was carried out during a single survey. The ecological inferences here reported must, therefore, be considered as relevant to the – early – summer period and should be mostly related to juveniles. Second, if on one side, the general low sample size and the low density of spiders do not allow to make predictions from our data (Zuur et al. 2009), the low density of spiders per sampling plot can be explained in light of the general behavior of *A. aquatica*, which is reported to be solitary and territorial (Schütz & Taborsky 2005, and reference therein).

New records and remarks on the Italian distribution of *Argyroneta aquatica*

New records

Italy: Piemonte, Vercelli Province, Riserva Naturale Speciale di Salvaguardia Fontana Gigante (S.C.I. IT1120008), Tricerro, 21 Jul 2015, Cavalcante R. leg., 1 ♀; Piemonte, Vercelli Province, Prato Lungo, Lachelle, Ronsecco, 8 Jun 2015, Cavalcante R. & Seglie D. leg., 1 ♀; 10 May 2015, Cavalcante R. & Seglie D. leg., 2 ♂♂; same locality, 6 Sep 2015, Cavalcante R. leg., 1 juv; Piemonte, Vercelli Province, Cascina Spinola, Livorno Ferraris, 10 May 2013, Cavalcante R. & Seglie D. leg., 1 ♀, 2 juv; same locality, 12 May 2013, Cavalcante R. & Seglie D. leg., 3 ♂♂; Lombardia, Varese Province: Palude di Arsago, Somma Lombardo, 8 May 2012, Soldato G. & Bergò P. leg., 1 ♀.

According to literature records (1843-present), in Italy *Argyroneta aquatica* is mostly distributed along the Po Plain. Most of the records are older than 50 years (Table 1, Fig. 7) and therefore in need of confirmation. On the other hand, the presence of this species is verified by earlier records (>1950) in Piemonte (this work), Lombardia (Brignoli 1977; Groppali 2002; Isaia et al. 2007; this work), Veneto (Brignoli 1977) and Emilia Romagna (Zangheri 1966). The record of *A. aquatica* in Val d'Aosta (Rossi & Bosio 2012) needs verification, being based on alive specimen identified in the field and released (see also Paschetta et al. 2016).

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