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# Ecological notes of the alien species *Godiva quadricolor* (Gastropoda: Nudibranchia) occurring in Faro Lake (Italy)

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#### ABSTRACT

The first record from Sicily of the introduced facelinid nudibranch Godiva quadricolor allowed the detection of trophic relationships with the polycerid Polycera hedgpethi, another non-native nudibranch, and with two bryozoan species, namely the naturalized Cheilostomatida Bugula neritina and the cryptogenic Ctenostomatida Amathia verticillata. The settlement of both nudibranchs was presumably promoted by a trophic shift of *P. hedapethi* from the natural prey *B. neritina* towards the largely available and not exploited A. verticillata. This short food web, without evident links with native fauna and having G. quadricolor as the top predator, is described. A DNA barcoding approach was used to confirm the identity of this facelinid species and to explore the possible genetic divergence occurring among the samples analysed.

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#### **KEYWORDS**

Alien species; DNA barcoding; first record; food web; *Godiva quadricolor* 

#### Introduction

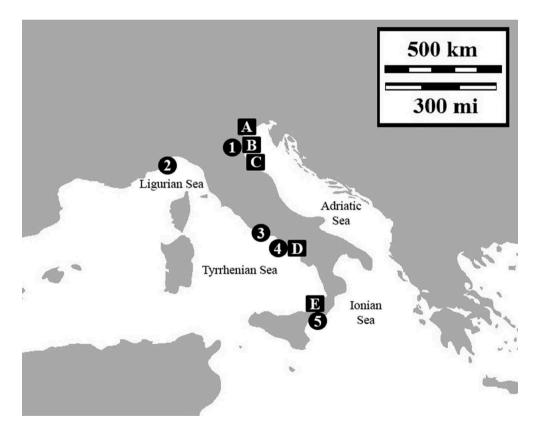
The Mediterranean Sea is notoriously the marine area most affected by allochthonous organism invasions, due to a high concentration of human activities (Zenetos et al. 2012), the presence of numerous habitats susceptible to invasions such as lagoons, estuaries and marinas (Galil 2000), and favourable geographic/climatic conditions (Bianchi et al. 2012). Molluscs, according to recent literature (Sabelli and Taviani 2014), include the greatest number of invading taxa, with more than 200 species introduced in the Mediterranean Sea. In the literature, records of single introduced species are frequently reported (Crocetta and Vazzana 2008; Barbieri et al. 2011), as well as grouped per area and/or taxonomic level (Lipej et al. 2012; Zenetos et al. 2012; Crocetta et al. 2013), but frequently, the same vector can introduce whole clusters of associated organisms, as widely demonstrated for algal species (Verlague 2001). In this paper, the first record for Sicily (Italy) of the invasive nudibranch Godiva quadricolor (Barnard 1927) is reported and the trophic relationships with the non-native nudibranch Polycera hedgpethi Er. Marcus 1964 and bryozoans Amathia verticillata (Delle Chiaje, 1844) and Bugula neritina (Linnaeus, 1758) are described as an example of a rapidly realized food web of invasive organisms. This trophic web, described for Faro Lake (Vitale et al. 2016),

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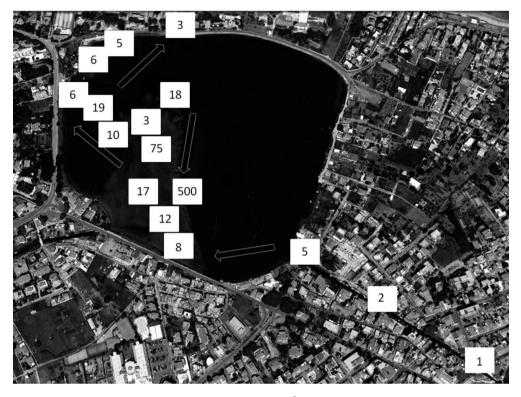
a protected transitional brackish area, might represent a case study to be considered in the context of appropriate conservation strategies. Furthermore, the mitochondrial cytochrome oxidase subunit I (COI) barcoding marker has been used to confirm the identity of the *G. quadricolor* specimens. The possible occurrence of genetic divergence among individuals of this species was also investigated to elucidate if the observed morphological variability was correlated with intra- or inter-specific diversity.

# **Materials and methods**

Field work has been carried out since February 2010 in Faro Lake (Sicily, Italy) (38.2658333N, 15.630555555556E) (Figures 1 and 2), in the framework of a research project aimed at monitoring the introduction of non-indigenous species introduction. Sixteen stations, each representative of a peculiar substratum typology, have been examined monthly by means of a 100 m<sup>2</sup> grid (Figure 2). From the 16 stations, all 'opisthobranch' specimens were identified and counted *in situ*, and species density was



**Figure 1.** Italian distribution of *Polycera hedgpethi* (letters, square) and *Godiva quadricolor* (numbers, circles). 1: north Adriatic lagoon, Zenetos et al. (2016); 2: Noli, Ligurian Sea, Betti et al. (2015); 3: Sabaudia Lake, Macali et al. (2013); 4: Fusaro Lake, Gulf of Naples, Cervera et al. (2010); 5: Faro Lake, Sicily, present study. A: Sacca Sessola Island, Venice Lagoon, Italy (Keppel et al. 2012); B: Marina di Ravenna docks, Italy (Rudman 2005; Trainito 2005); C: Rimini coast, Italy (Ioni, 2011); D: Fusaro Lake, Gulf of Naples, Cervera et al. 1991 (1988)); E: Faro Lake, Sicily, Giacobbe and De Matteo (2013).



**Figure 2.** Diachronic peaks of density (individuals/100m<sup>2</sup>) of *Godiva quadricolor* at the 16 sampling stations of Faro Lake. The arrows indicate the supposed spread.

evaluated as average number of individuals/100 m<sup>2</sup>; their association with *A. verticillata* and other biotic/abiotic substrates was recorded *in situ*. Aquarium behavioural observations have also been carried out on individuals of *G. quadricolor* and *P. hedgpethi*. Specimens collected were preserved in 95% ethanol and deposited at the Department of Chemical, Biological, Pharmaceutical and Environmental Sciences – Benthic Ecology Laboratory, Messina University (vouchers: BEL118 LF2017G001-4).

The identity of three specimens of G. quadricolor from Faro Lake, and three other individuals collected from Sabaudia Lake (Latium, Italy) (41.4233333N, 13.07611111111112E), was tested using partial COI sequences. The last three samples were stored at the Department of Science of the Roma Tre University (vouchers: RM3\_117, RM3\_153, RM3\_154). Collection data, voucher IDs and accession numbers are listed in Table 1. Total genomic DNA was extracted according to Oliverio and Mariottini (2001). Partial COI sequences were amplified by polymerase chain reaction using the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). Amplicons were sequenced by European Division of Macrogen Inc. (Amsterdam, The Netherlands), using the same polymerase chain reaction primers. Sequences obtained were edited with Staden Package 2.0.0b9 (Staden et al. 2000). A BLASTN (Altschul et al. 1990) search was conducted to rule out sample contamination. Sequences were aligned with the use of the program MEGA 6.0 (Tamura et al. 2013). The Mediterranean COI sequences of G. quadricolor were compared with one sequence present in GenBank (citation) from a

			Accession numbers	
Species	Code/Voucher	Locality	COI	References
Godiva quadricolor	RM3_117	Sabaudia Lake, Latium, Italy	MG546001	Present study
Godiva quadricolor	RM3_153	Sabaudia Lake, Latium, Italy	MG546002	Present study
Godiva quadricolor	RM3_154	Sabaudia Lake, Latium, Italy	MG546003	Present study
Godiva quadricolor	BEL118 LF2017G001	Faro Lake, Sicily, Italy	LT839025	Present study
Godiva quadricolor	BEL118 LF2017G002	Faro Lake, Sicily, Italy	LT839026	Present study
Godiva quadricolor	BEL118 LF2017G003	Faro Lake, Sicily, Italy	LT839027	Present study
Godiva quadricolor	CASIZ176385	Knysna Lagoon, Western Cape Province, South Africa	HM162756	Pola and Gosliner (2010)
Babakina anadona	MNRJ10893	Brazil	HQ616746	Pola and Gosliner (2010)
Babakina anadona	MNCN15.05/46,704	Galicia, Spain	HQ616767	Pola and Gosliner (2010)
Babakina indica	CASIZ177458	Calumpan Mainit Bubbles, Luzon, Batangas, Philippines	HM162754	Pola and Gosliner (2010)
Dondice banyulensis		Spain, Mediterranean Sea	AF249782	Wollscheid-Lengeling et al. (2001)
Phyllodesmium hyalinum	Phy.orig.	. 1	GQ403778	Wägele et al. (2010)
Phyllodesmium jakobsenae	CASIZ177576	Batangas, Philippines	HQ010489	Moore and Gosliner (2011)
Out-group				
Tritonia striata	BAU2695	Giannutri Is., Tuscany, Italy	LT596540	Furfaro et al. (2016)
Tritonia striata	BAU2696	Le Formiche Is., Grosseto, Italy	LT596541	Furfaro et al. (2016)

Table 1. Species names, vouchers, localities, accession numbers and references of the Facelinidae used for the analyses.

South African specimen of *G. quadricolor*. A haplotype network (Templeton et al. 1992) was produced using PopART (Population Analysis with Reticulate Trees) open source population genetics software (http://popart.otago.ac.nz). Uncorrected intra- and interspecific *p*-distances were calculated using MEGA 6.0.

For phylogeny generation, the best evolutionary model for sequence evolution was selected in JModel Test 0.1 (Posada 2008) according to the Bayesian Information Criterion. A single gene data set (COI) was analysed using Bayesian Inference and Maximum likelihood methods implementing the model selected by JModel Test. Bayesian Inference analysis was carried out with MrBayes 3.2.6 (Ronquist et al. 2012) with four Markov chains of five million generations each, sampled every 1000 generations. Consensus trees were calculated on trees sampled after a burn-in of 25%. Maximum likelihood analyses were performed using MEGA 6.0 with a starting tree topology generated by a neighbour joining algorithm (Zwickl 2006). Nodal support was assessed by means of 1000 bootstrap replicates. *Tritonia striata* Haefelfinger, 1963 was used as the out-group because of its basal placement within Cladobranchia (following Pola and Gosliner 2010) (Table 1).

# Results

Along the Italian coasts, ephemeral or occasional introductions of G. quadricolor have been reported from the Ligurian Sea and from a north Adriatic lagoon (Betti et al. 2015; Zenetos et al. 2016), and stable populations occurring in transitional waters have been documented from the Fusaro Lake since 1985/86 (Cervera et al. 2010; Crocetta 2012) and from Sabaudia Lake (Macali et al. 2013), both localities north of Faro Lake (Figure 1). A large number of specimens of this taxon have been reported in France (Étang de Thau) since November 2015 (Gerovasileiou et al. 2017). We report the presence of this species from another locality in Italy, namely Faro Lake (Sicily). In particular, two specimens were first found in June 2015 in the canal connecting Faro Lake with the Strait of Messina. Subsequently, in October 2015, G. quadricolor was recorded with an occurrence frequency of 5 individuals (ind.)/100 m<sup>2</sup>, concentrated in the central area of Faro Lake. The density of G. quadricolor rapidly increased, reaching 50 ind./100 m<sup>2</sup> in January 2016. In late winter of the same year, the population slowly declined, numbering only 5 ind./100  $m^2$  in February 2016. To summarize, the seasonal occurrence of G. quadricolor can be described as follows: in autumn the average population density grows rapidly, reaching a maximum of approximately 100 ind./100 m<sup>2</sup> in winter, then it decreases in spring. This trend (Figure 3) fits that showed by the contextually observed P. hedgpethi, but slightly delayed and with lower densities. Fluctuations in P. hedgpethi densities, in turn, reflected seasonal changes in surface water temperatures (Figure 3). The two non-native bryozoan species B. neritina and A. verticillata, both present in Faro Lake before their sea slug predators, showed different frequency and abundance. Bugula neritina, was almost regularly recorded in all the seasons, which differed from A. verticillata, whose impressive blooms have been recorded from April to January, except for during the warmest period, in August. The colonization of Faro Lake by G. quadricolor started from the canal and quickly spread to the centre of the basin, with a progressive shift of the density peaks (Figure 2). Since the earlier records (2015), specimens of G. quadricolor showed high colour variability (Figure 4 (a,b)). All the specimens observed during fieldwork were adults laying egg masses

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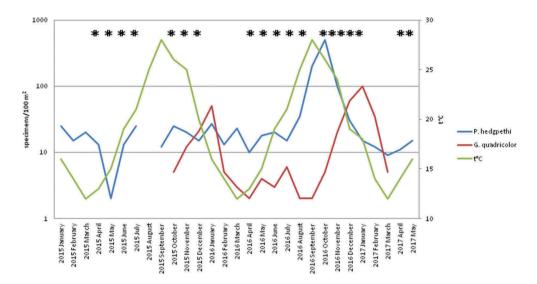
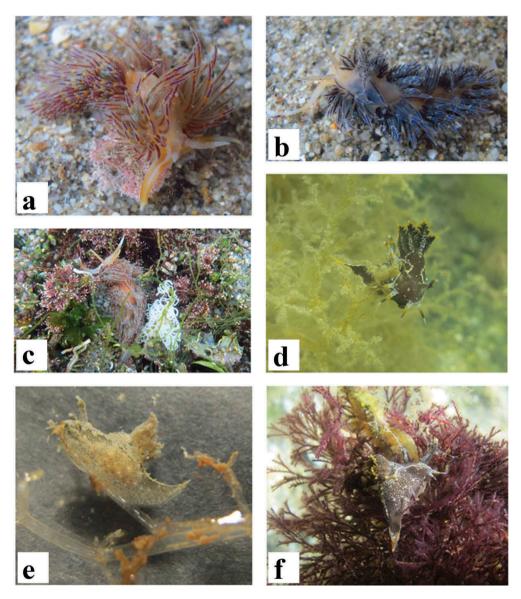


Figure 3. Monthly average densities of *Godiva quadricolor* and *Polycera hedgpethi* in comparison with surface water temperatures. Asterisks indicate *Amathia verticillata* blooms.

(Figure 4(c)), but mating has never been witnessed. Individuals of *G. quadricolor* have been frequently recorded on *A. verticillata* colonies (formerly known as *Zoobotryon verticillatum* Della Chiaje, 1822), preying upon *P. hedgpethi*, and bryozoan polyps. When these two nudibranch species are near each other, *G. quadricolor* quickly attacks *P. hedgpethi*, devouring first the gills, probably the most vulnerable part, while abandoning the rest of the prey. In contrast, no upper trophic level of consumers preying upon *G. quadricolor* has been identified. First evidence of the prey–predator relationship between the two nudibranch species occurred 5 months after the first *G. quadricolor* record. Their co-occurrence rapidly increased, reaching 80% in January 2016 and 100% in April 2016. The association between *A. verticillata/P. hedgpethi/G. quadricolor* was evaluated as a percentage of co-occurrence of the three species with respect to the *A. verticillata* presence, ranging from 0%, at the beginning of the *G. quadricolor* colonization, to 100% in October 2017 (average 75  $\pm$  5% the whole period).

*Polycera hedgpethi* has a worldwide distribution. First described from the Gulf of California (Wilson 2006), it spread into the Mediterranean Sea through the same steps as *G. quadricolor* with an initial report from Fusaro Lake (Cervera et al. 1991, 1988)), then from other transitional waters including the Venice Lagoon (Keppel et al. 2012) and Faro Lake (Giacobbe and De Matteo 2013). It was initially recorded from this Sicilian Lake in 2012 with a low number of individuals, followed by increased population density of slugs with a more variable chromatic pattern (Figure 4(d and e)). *Polycera hedgpethi* occurs in Faro Lake during all seasons, decreasing in number in the coldest periods (January–February). Despite mating never being observed, egg deposition is almost continuous so that the frequency of adult specimens and egg masses appears in accordance, both reaching an estimated maximum density of almost 100 individuals of *P. hedgpethi* in several stations. The species preferentially colonizes the central area of Faro Lake, but has also been reported from the canal that connects the Lake to the Sicilian Channel (Figure 1). *Polycera hedgpethi* was observed during field work preying



**Figure 4.** (a,b) *Godiva quadricolor*, different colour patterns; (c) *G. quadricolor* in deposition; (d) *Polycera hedgpethi*, typical colour pattern, feeding *Amathia verticillata*; (e) *P. hedgpethi*, 'pale' colour pattern, feeding *A. verticillata*; (f) *P. hedgpethi*, typical colour pattern, feeding *Bugula neritina*.

upon bryozoans, namely the cheilo-ctenostoma species *B. neritina* (Figure 4f) and the ctenostoma species *A. verticillata* (Figure 4d and e). *Bugula neritina* was regularly present on all hard substrates, and especially abundant in farmed mussel beds; *A. verticillata* showed wide seasonal fluctuations, being absent in the coldest (January–March) and warmest (July–August) periods. During mild seasons, *A. verticillata* colonies grow rapidly in size and number, spreading widely over the lake-floor surfaces and then declining. The frequency of *P. hedgpethi* tracks such fluctuations, reaching a maximum in spring with a secondary peak in the autumn, declining in summer, and disappearing in winter.

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In general, blooms of *A. verticillata* are followed by reproductive peaks of *P. hedgpethi*, which uses the bryozoan colonies as a substrate for egg deposition.

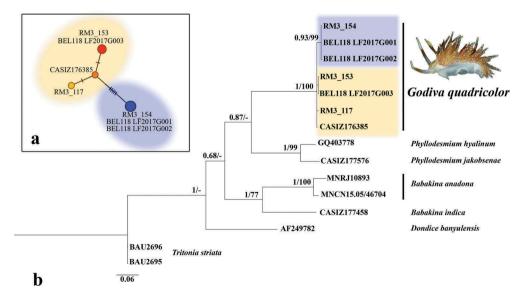
### **Molecular analyses**

Partial COI sequences, from six *G. quadricolor* specimens collected in the two different Italian Lakes, were obtained and deposited at the European Molecular Biology Laboratory databank and the NCBI GenBank database (Table 1). The maximum intraclade COI *p*-distance (0.5%) was found within group 1 (see Table 2, in bold), which resulted in three different haplotypes (Figure 5a). The minimum inter-clade COI *p*-distance was 1.1% (see Table 2, in italic bold), whereas the mean *p*-distance between

**Table 2.** Pairwise uncorrected *p*-distances of cytochrome oxidase subunit I (COI) for the specimens of *Godiva quadricolor* analysed.

	RM3_117	RM3_153	RM3_794	G_qua_GB1	RM3_154	RM3_792	RM3_793
	{group 1}	{group 1}	{group 1}	{group 1}	{group 2}	{group 2}	{group 2}
RM3_117 {group 1}	-						
RM3_153 {group 1}	0.005	-					
RM3_794 {group 1}	0.005	0.000	-				
G_qua_GB1 {group 1}	0.002	0.002	0.002	-			
RM3_154 {group 2}	0.014	0.014	0.014	0.011	-		
RM3_792 {group 2}	0.014	0.014	0.014	0.011	0.000	-	
RM3_793 {group 2}	0.014	0.014	0.014	0.011	0.000	0.000	-

Maximum intra-specific distances shown in bold. Minimum inter-specific distances shown in italic bold.



**Figure 5.** (a) Cytochrome oxidase subunit I (COI) haplotype TCS network showing genetic mutations occurring within *Godiva quadricolor* specimens; (b) Bayesian topology based on the COI data set (TrN +G model of evolution). Numbers at nodes indicate the support by Bayesian Inference ( $5 \times 10^6$  generations and 25% burn-in, left half) and Maximum Likelihood (1000 bootstrap replicates, right half).

	G.	G.						
	quadricolor	quadricolor	В.	В.	D.	Р.	Р.	Т.
	group 1	group 2	anadoni	indica	banyulensis	hyalinum	jakobsenae	striata
Godiva quadricolor	_							
group 1								
G. quadricolor	0.013	_						
group 2								
Babakina anadoni	0.188	0.194	-					
Babakina indica	0.188	0.187	0.155	-				
Dondice banyulensis	0.184	0.181	0.209	0.202	_			
Pyllodesmium hyalinum	0.159	0.170	0.176	0.181	0.174	-		
Pjyllodesmium jakobsenae	0.178	0.181	0.177	0.208	0.174	0.078	_	
T. striata	0.214	0.219	0.212	0.190	0.199	0.184	0.196	-

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groups 1 and 2 was 1.3% (Table 3). The sequence evolution model TrN+G was the best fit for the COI data set. Results from the Bayesian Inference and Maximum Likelihood analyses (Figure 5b) were congruent both to each other and to the results based on genetic distances. Specimens analysed were clustered in one monophyletic group congruent to a haplogroup recovered in the haplotype network analysis (shown in blue in Figure 5).

#### **Discussion and conclusions**

The molecular identity of Mediterranean specimens of *G. quadricolor* (from Faro and Sabaudia Lakes) was confirmed, for the first time, by comparison with GenBank sequence (Voucher: CASIZ176385). A haplotype analysis of COI data from these slugs recovered two haplogroups that have an uncorrected *p*-distance of 1.1% for COI (Table 2).

Two non-native nudibranch species, *G. quadricolor* and *P. hedgpethi*, today inhabit the Mediterranean basin with stable populations, albeit with a scattered distribution pattern primarily due to mussel farming in coastal lagoons. Only in one case (Fusaro Lake: Figure 1) has the co-occurrence of these two alien species been reported, but without evidence of trophic associations between them and other organisms (F. Crocetta, personal communication).

Two bryozoan species, *B. neritina* and *A. verticillata*, both non-native in Faro Lake but occurring there before their sea slug predators, allowed the development of a simple food web, regardless of the presence of native species. In this scenario, the ctenostoma bryozoan *A. verticillata* might play a key role. In fact, in agreement with the respective pluriannual trends (Figure 3), the trophic shift of *P. hedgpethi* from *B. neritina* towards the abundant *A. verticillata* allowed a rapid increase in the polycerid population, which in turn might favour *G. quadricolor* settlement as a consequence of food richness. Consumption by *P. hedgpethi* of *A. verticillata*, on which this sea slug has never fed, may has caused the appearance of 'pale' polycerid individuals, as body colour in nudibranchs is often related to the colour of their diet. Non-native prey may increase indigenous predator populations by providing an additional food source and simultaneously decreasing native prey populations by outcompeting them for a limited resource (Dijkstra et al. 2013). In this case, no evidence exists that native predators take advantage of the introduced prey *A. verticillata*; nevertheless, we may suppose that

a high amount of primary production has been depleted by the 'impressive' suspensionfeeder bryozoan (Amat and Tempera 2009), to the detriment of native species, as the reported rarefaction of sponge fauna might suggest (Marra et al. 2016). Futhermore, the role of *A. verticillata* in the introduction of other alien species through transport on vessel hulls, has been demonstrated by Marchini et al. (2015) and Dailianis et al. (2016). We suggest that repeated introduction of *A. verticillata* through vessel fouling and mussel imports, and acting as food substrate, may be responsible for the introduction of both nudibranch species, as well as other taxa introduced throughout the Mediterranean basin through mussel culture. The concomitant presence of both nudibranchs was first reported from the Mediterranean Sea (Fusaro Lake) and from the Atlantic Ocean by Cervera et al. (2010), and now with this note also from Faro Lake.

The observed pattern of colonization agrees with current literature, which reports that non-native species may take advantage of the availability of 'empty' ecological niches, but emphasizes how in most cases they arrange a new realized niche as a response to the new habitat features (Olenin and Leppäkoski 1999). In the present case, the niche adjustment by the new colonizers primarily responds to the availability of a new trophic resource that is in turn extraneous to the native ecosystem. As regards conservation, the possible interference of the two introduced nudibranch species with the indigenous biota could be considered minimal as they do not compete with autochthonous species.

In conclusion, *B. neritina* and *A. verticillata* can be considered in different ways accountable for *P. hedgpethi* and *G. quadricolor* colonization. The establishment of both nudibranchs, promoted by a trophic shift of *P. hedgpethi* towards the largely available and not exploited *A. verticillata*, was further favoured by the lack of native predators and competitors, creating a short food web with no native fauna involved, and with *G. quadricolor* as the top predator. Furthermore, for the first time the molecular identity of Mediterranean specimens of *G. quadricolor* was confirmed by comparison with those already present in GenBank.

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No potential conflict of interest was reported by the authors.

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