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Original Article

New insights on the seasonal trend of *Goniodoridella picoensis* (Paz-Sedano, Ortigosa & Pola, 2017) along the central-eastern coast of Sicily: A possible warning of its expansion and establishment in the Mediterranean Sea?

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Abstract: *Goniodoridella picoensis* is a nudibranch species originally described from Pico Island (Azores, Atlantic Ocean). After its description, the reports of this species suddenly increased in a short timeframe, leading to two main hypotheses: one that it is native to the entire Atlanto-Mediterranean region, the other that it is a non-indigenous species that entered the Mediterranean from the Atlantic Ocean. The present study explored the seasonal trend of *G. picoensis* along the central-eastern coast of Sicily to understand whether this species is non-indigenous and its possible pattern of spread and establishment in that area. To carry out this research, a photographic visual census activity through scuba dives was performed in four sites with different anthropogenic pressures (Ognina, Santa Maria La Scala, Scalo Pennisi, and Acque Fredde) throughout three years (from 2021 to 2023). A similar seasonal trend was found in all the sites. The number of individuals of this species started to increase in the autumn months, maintained or rose during the spring, and throughout the summer months underwent a sharp decline. The negative correlation between the temperature and the number of specimens also evidenced this seasonal trend. Indeed, even if this species can tolerate a wide temperature range, it would seem to prefer lower temperatures, like those of the winter-spring months, when there is a higher availability of food supply. The sites with the highest number of specimens were Scalo Pennisi and Acque Fredde, probably both because of the higher natural conditions compared to the other sites and because of the influence of tidal currents of the Strait of Messina and the upwelling currents of the Ionian Sea, which might act as a veliger conveyor belt. Overall, from 2021 to 2023, an increase in assemblages' size was observed in all the sites. Moreover, the breeding activity was documented between 2022 and 2023. Considering the above, *G. picoensis* is most likely a non-indigenous species coming from the Atlantic Ocean. The collected data reflect that this species has settled in these study areas in the last years and indicate that *G. picoensis* is establishing stable populations there. Indeed, the probability that this species is Atlanto-Mediterranean and has been so far overlooked seems unlikely, not only due to the intense research in the Mediterranean area but also for the conspicuousness of this species.

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Introduction

The genus *Okenia* Menke, 1830 is a taxon of nudibranchs belonging to the family Goniodorididae H. Adams & A. Adams, 1854 (Paz-Sedano et al., 2017), which includes species of 5-50 mm in size that are mainly distributed from cold, temperate, and tropical waters of the Pacific Ocean to the north and south Atlantic Ocean, including the Mediterranean Sea (Pola et al., 2019). In this last Basin, there are three endemic species: *Okenia hispanica* Á. Valdés & Ortea, 1995, *O. longiductis* Pola, Paz-Sedano, Macali, Minchin, Marchini, Vitale, Licchelli & Crocetta, 2019, and *O. problematica* Pola, Paz-Sedano, Macali, Minchin, Marchini, Vitale, Licchelli & Crocetta, 2019 (Pola et al., 2019); four Atlantic-Mediterranean species: *O. aspersa* (Alder & Hancock, 1845), *O. elegans* (Leuckart, 1828), O. *mediterranea*, (Ihering, 1886) and *O. picoensis* Paz-Sedano, Ortigosa & Pola, 2017; and one non-indigenous species *O. pellucida* Burn, 1967, recently reported in Libya and Malta (Rizgalla et al., 2023).

Recently, the family Goniodorididae underwent a

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taxonomic and phylogenetic revision and many *Okenia* species were transferred to other genera, such as *Cargoa problematica* (Pola, Paz-Sedano, Macali, Minchin, Marchini, Vitale, Licchelli & Crocetta, 2019) (= *O. problematica*), *Goniodoridella picoensis* (Paz-Sedano, Ortigosa & Pola, 2017) (= *O. picoensis*) and *Bermudella pellucida* (Burn, 1967) (= *O. pellucida*) (Paz-Sedano et al., 2024).

Among the abovementioned species, *G. picoensis* showed a complex and intricate history. This species was originally described from Pico Island (Azores, Portugal, Atlantic Ocean) by Paz-Sedano et al. (2017). Subsequently, it was recorded for the first time in the Mediterranean Sea in 2020 (Orfanidis et al., 2021) and then suddenly expanded its range, being found in several areas in a short timeframe (Crocetta et al., 2021; Lombardo and Marletta, 2021a; Trainito et al., 2022; Fantin et al., 2023). This species was first found in Malta and Granada between 2020 and 2021 (Orfanidis et al., 2021), and then in several sites of Italy and Croatia between 2021 and 2023 (Table 1), with several sightings in other Mediterranean areas, published to date on specialist portals (DORIS; OPK-Opistobranquis).

Paz-Sedano et al. (2017) described two morphotypes of *G. picoensis*: one with a bright yellow body colouration and with the ends of rhinophores, gill branches, and tail coloured in orange, and one with a white body colouration and with the same ends coloured in yellow. In the Mediterranean Sea, only this latter morphotype has been found.

As concerns, the prey preference of this species, there is still no information (Paz-Sedano et al., 2024). *Goniodoridella picoensis* shows a wide thermal adaptability, being found from 13 to 21°C (Orfanidis et al., 2021; Crocetta et al., 2021; Lombardo and Marletta, 2021a), which could represent the principal reason for its expansion success in the Mediterranean Sea (Lombardo and Marletta, 2021a). Moreover, it was found in a wide depth range both in the Atlantic (8-30 m of depth) (Paz-Sedano et al., 2017) and in the Mediterranean (7-34 m of depth) (Orfanidis et al., 2021; Crocetta et al., 2021; Lombardo and Marletta, 2021a; Trainito et al., 2022; Fantin et al., 2023).

To date, several hypotheses are rotating around the origin and distribution of *G. picoensis*. According to Trainito et al. (2022), it is impossible to establish if this species is native to the Mediterranean or Atlantic. Thus, they considered *G. picoensis* an endemic species of the entire Atlanto-Mediterranean region, which has only recently increased in consideration after its population explosion. On the other hand, according to Lombardo and Marletta (2021a), the occurrence of this species in multiple records held more or less contemporarily in several localities of the Mediterranean Sea might suggest a non-indigenous species, which has spread in this Basin from the Atlantic Ocean. Indeed, considering that the Mediterranean malacofauna is the best-known in the world (Orfanidis et al., 2021), the possibility that this species could be overlooked so far seems unlikely. Nevertheless, insufficient information exists to determine this species' origin and distribution pattern in the Mediterranean Sea and the Atlantic Ocean (Trainito et al., 2022). This study aims to provide new insights into the seasonal trend of *G. picoensis* along the central-eastern coast of Sicily, where this species has been previously documented (Crocetta et al., 2021; Lombardo and Marletta, 2021a). It also aims to understand whether this species is non-indigenous and its possible pattern of spread and establishment in that area.

Materials and Methods

The seasonal trend of *Goniodoridella picoensis* was studied from 2021 to 2023 in different sites located along the central-eastern coast of Sicily: Ognina (37°31'50.4"N, 15°07'10.8"E), Santa Maria La Scala (37°36'46.5"N, 15°10'31.4"E), Acque Fredde (37°38'15.7"N, 15°10'52.1"E) and Scalo Pennisi (37°38'23.2"N, 15°11'04.6"E) (Fig. 1). The sea-bottom of these areas consists of basaltic rocky outcrops of variable sizes (Sciuto et al., 2017). All the sites have a sloping topography, and the seabed becomes progressively muddier at major depths with rocky outcrops locally exposed (Sciuto et al., 2017). The study areas were selected based on different anthropogenic pressures: Ognina is located in the city

Table 1. Reports of *Gonodoridiella picoensis* in the published papers.

Site	Coordinates	Date	Number of specimens	Temperature (°C)	Depth (m)	Reference
Pico island, Azores, Portugal	38.4867 °N, 28.5400° E	Jun, Nov 2013		_	8-30	Paz-Sedano et al. (2017)
Rozi wreck, Ċirkewwa, Malta	35.9878° N, 14.3286° E	Nov 2020	_	21	29	Orfanidis et al. (2021)
Um El Faroud wreck, Wied iż-Żurrieq, Malta	35.8191° N, 14.4498° E	Nov 2020	_	21	23	Orfanidis et al. (2021)
Ċirkewwa arch, Malta	35.9878° N, 14.3286° E	Nov 2020	_	21	17	Orfanidis et al. (2021)
Um El Faroud wreck, Wied iż-Żurrieq, Malta	35.8191° N, 14.4498° E	Jan 2021	_	16	27	Orfanidis et al. (2021)
"La Piedra del Hombre", Granada, Spain	36.7231º N, 3.7375º W	Mar 2021	_	15	16	Orfanidis et al. (2021)
Acque Fredde, Sicily, Italy	37.6375° N, 15.1811° E	Mar 2021	1	14	21.9	Orfanidis et al. (2021)
Ognina, Sicily, Italy	37.5306° N, 15.1196° E	Mar 2021	1	14	21.2	Lombardo and Marletta (2021a)
Scalo Pennisi, Sicily, Italy	37.6397° N, 15.1264° E	Mar 2021	2	14	14.9-21.4	Lombardo and Marletta (2021a)
Santa Maria La Scala, Sicily, Italy	37.6129° N, 15.1753° E	Mar 2021	1	14	20	Lombardo and Marletta (2021a)
Bellatrix, Sicily, Italy	37.5342° N, 15.1264° E	May 2021	1	16	15.9	Lombardo and Marletta (2021a)
Scalo Pennisi, Sicily, Italy	37.6397° N, 15.1264° E	May 2021	3	15	15-22.3	Lombardo and Marletta (2021a)
Ustica, Sicily, Italy	38.6951° N, 13.1539° E	May 2021		_	_	Trainito et al. (2022)
Ustica, Sicily, Italy	38.7243° N, 13.1831° E	Jun 2021		_	_	Trainito et al. (2022)
Lampedusa, Sicily, Italy	35.5133 °N, 12.6312° E	Jul 2021		_	_	Trainito et al. (2022)
Franata dei Mufloni, Sardinia, Italy	40.9918° N, 9.6587° E	Apr 2022	1	14	34	Trainito et al. (2022)
AMP Tavolara, Sardinia, Italy	40.8965° N, 9.7072° E	May 2022	1	16	25	Trainito et al. (2022)
Kostrena, Rijeka, Croatia	45.3032° N, 14.48866° E	Dec 2022	1	_	11	Fantin et al. (2023)
Pantelleria, Sicily, Italy	36.8245° N, 12.0140° E	Jul 2022	8	18 – 23	6.8-29.1	Lombardo and Marletta (2023a)
Pantelleria, Sicily, Italy	36.8002°N, 12.0490°E	Jul 2022	1	17	29	Lombardo and Marletta (2023a)
Girandella diving, Croatia	45.0794°N, 14.1751°E	Jun 2023	1	_	27	Fantin et al. (2023)
Marco Polo Diving, Croatia	45.3408°N, 14.3723°E	Jun 2023	1	_	22	Fantin et al. (2023)
Girandella diving, Croatia	45.0794°N, 14.1751°E	Jun 2023	1	_	17.5	Fantin et al. (2023)
Lipari, Aeolian Islands, Sicily, Italy	38.5221°N, 14.9636°E	Jul 2023	1	16	33	Lombardo and Marletta (2023b)
Lipari, Aeolian Islands, Sicily, Italy	38.4391°N, 14.9416°E	Jul 2023	3	17 – 18	20.9-22	Lombardo and Marletta (2023b)
Lipari, Aeolian Islands, Sicily, Italy	38.4410°N, 14.9390° E	Jul 2023	2	19	20	Lombardo and Marletta (2023b)
Vulcano, Aeolian Islands, Sicily, Italy	38.4095°N 14.9742°E	Jul 2023	2	17	27.7-27.8	Lombardo and Marletta (2023b)
Kostrena, Rijeka, Croatia	45.3032°N, 14.48866°E	Aug 2023	1	_	23	Fantin et al. (2023)

Figure 1. Geographical location of the study area in the Mediterranean: (A) Sicily, and (B) Study sites along the eastern coast of Sicily.

of Catania and is the most anthropogenically-affected site, presenting a harbour, a manifold, and several apartment buildings and bathing establishments along the coast. Acque Fredde and Scalo Pennisi are two neighboring sites that show the most natural conditions, hosting healthy populations of the bioindicator brown seaweed *Ericaria zosteroides* (C. Agardh) Molinari & Guiry (Marletta and Lombardo, 2023). Finally, the site of Santa Maria La Scala is located halfway between the other study areas. It is affected by a lower anthropogenic pressure than Ognina, mainly due to the presence of a marina and a drainage pump releasing fresh water into the sea.

Data on *G. picoensis* were collected through underwater visual census by scuba dives. Data was collected during daylight for three years (from 2021 to 2023). Each dive, of a duration of 90 minutes, was carried out once a month for each site, and the same underwater pathway was always followed according to the seabed morphology of each site. All specimens of *G. picoensis* encountered during the scuba dives were photographed through an Olympus TG-4 underwater camera. The depth and temperature data were taken through a Suunto D6i dive computer. By analysing the photographic material, it was possible to determine the substrates where the specimens were found and their breeding activities.

A two-way ANOVA (Analysis of Variance) design was applied to test the effect of the month and year and site and year on the number of individuals. The assumptions of normality (Shapiro-Wilk) and homoscedasticity (Levene) were verified prior to conducting the analyses. When the ANOVA detected significant differences ($P<0.05$), post-hoc comparisons with Tukey's test were used for differences between means. A Principal Component Analysis (PCA) was used to analyse the differences among the assemblages of *G. picoensis* in the four study sites based on Bray-Curtis dissimilarity.

A correlation matrix was constructed to check the relationship between the number of individuals and the average temperature (℃). Finally, the Chi-Square ($\chi 2$) test of independence was applied to assess whether there was a significant association between the average depth (m) of findings and the sites. The line charts depicting the seasonal trends in the four sites were created using Excel. Statistical analyses were performed using the software Jamovi version 2.3.

Results

Morphology of the observed specimens: All the specimens of *G. picoensis* observed during this study presented the white-coloured morphotype. The body parts (i.e., rhinophores, gills, and papillae) were white-coloured proximally and yellow distally. The specimens presented more frequently a stubby body (Fig. 2A-B), but sometimes individuals with more elongated and slender bodies were found as well (Fig. 2C). Rarely, some observed specimens presented

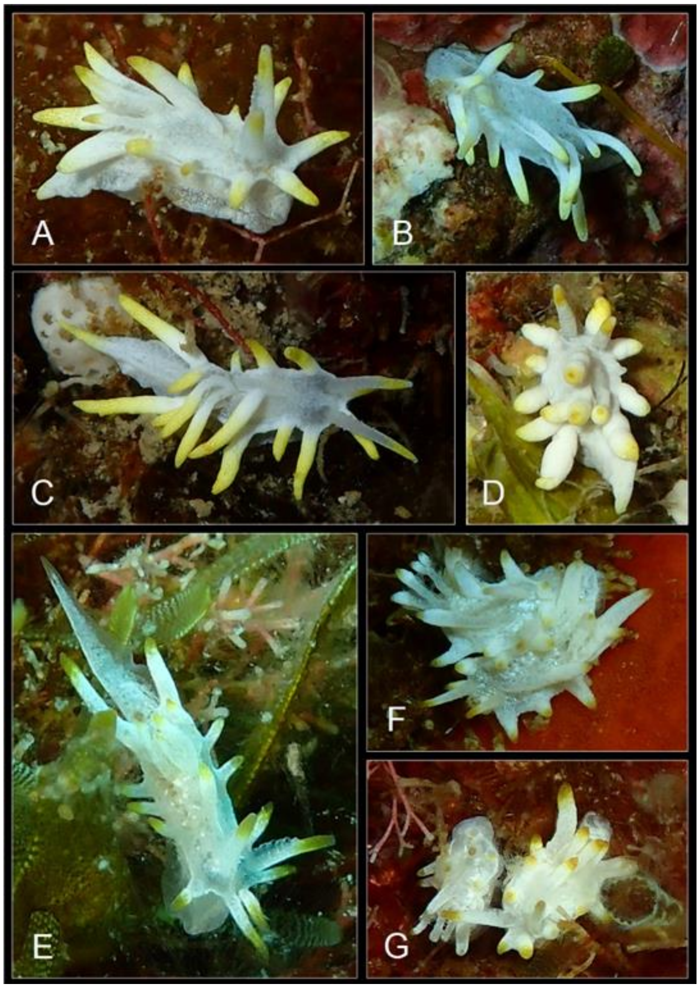

Figure 2. Some specimens of *Goniodoridella picoensis* observed in this study: (A) white morphotype of *G. picoenis*; (B) specimen with a stubby body; (C) specimen with a slender body; (D) specimen with swollen body eversions; (E-F) specimens with a complex of dorsally scattered small blotches; (G) specimen with distally orange-coloured body eversions.

swollen body eversions (Fig. 2D). Sometimes, a few individuals showed slight differences in the body colouration, such as a complex of dorsally scattered small blotches (some grey-coloured and other opalescent white), and distally orange-coloured body eversions (Fig. 2E–G).

Many specimens differed in the notal-papillae arrangement documented for this species (i.e., two anterior, three on each side of the notum, and two posterior). Indeed, it was not uncommon to see specimens with four lateral papillae per side and rarely even five or six on one side of the body (Fig. 3A-E). In some cases, there were specimens with less than three papillae on one side of the body. Moreover, in most of these specimens, not all the lateral papillae were fully formed, and there were even remarkable size differences among them (Fig. 3A-E). Indeed, some of these "extra" papillae were only small buds.

During this study, we detected several specimens with different teratologies. These latter involved almost always the papillae of the individuals, such as bifid lateral papillae (Fig. 3F), small emergences on the distal part of the papillae (Fig. 3G), trifid anterior papillae (Fig. 3H), a row of small orange button-like spots along lateral papillae (Fig. 3I), a bifid rhinophore's apex (Fig. 3J). Finally, a total white-coloured specimen was documented (Fig. 3K).

Seasonal trend in the study areas: The results showed that the month, year, and the crossed factors year × month significantly affected the number of individuals ($P<0.05$). Moreover, the number of individuals was significantly affected by the site and year but not by the two crossed factorsyear × site (Table 2).

Tukey's post hoc test showed the most significant differences between 2021 and the other two years. Regarding the month, the most evident differences were between May and August, and September. Finally, regarding the site, the most significant differences were observed between Ognina and Acque Fredde and Scalo Pennisi and Santa Maria La Scala and Acque Fredde and Scalo Pennisi (Table 3). The sites with the highest number of individuals were Acque Fredde and Scalo Pennisi, while Ognina and Santa Maria La Scala showed fewer specimens (Fig. 4).

The PCA of the assemblages of the four sites showed two separate clusters: one for Acque Fredde and another for the other sites (Fig. 5). Indeed, as it is possible to note from the line charts of *G. picoensis*, the assemblage from Acque Fredde had a different seasonal pattern during the years of study than the other sites (Fig. 6). Despite this, in all the sites *G. picoensis* assemblages showed similar seasonal trend during the years of study. Generally, the number of specimens of *G. picoensis* started to increase at the beginning of autumn and then grew during spring. During the summer months, there was a sharp decline

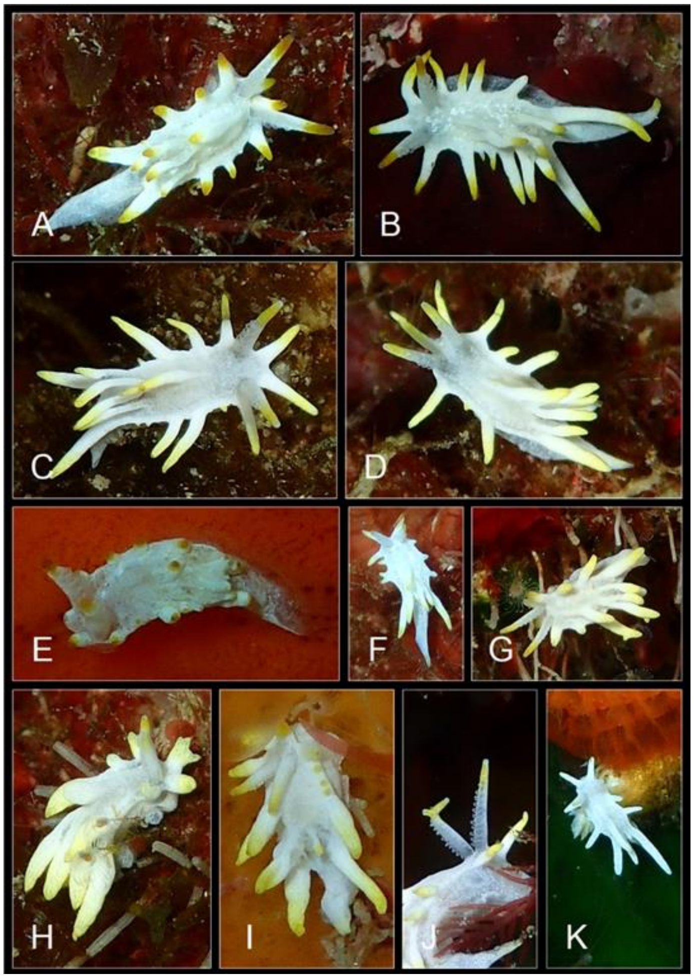

Figure 3. Specimens of *Goniodoridella picoensis* with a different pattern of papillae and teratological ones: (A) specimen with four lateral papillae on each side of the body (some are small buds); (B) specimen with six papillae on the left side and four on the right one (some are small buds); (C) specimen with four on the left side and six on the right one (some are small buds); (D) specimen with three lateral papillae on each side of the body (some are small buds); (E) specimen with six short and swollen lateral papillae on the left side of the body and three on the right one (some are small buds); (F) specimen with bifid lateral papillae on the left side of the body; (G) specimen with small emergences on the distal part of the papillae; (H) specimen with trifid anterior papillae; (I) specimen with a row of small orange button-like spots along lateral papillae; (J) specimen with a bifid rhinophore's apex; (K) a total white coloured specimen.

in the number of specimens throughout the years. Overall, it was noted that at Scalo Pennisi and Acque Fredde, *G. picoenis* managed to maintain a more stable trend during the years, while at Ognina and S. Maria La Scala spikes and drops were frequently observed throughout the years (Fig. 6).

The breeding activity was documented at Acque Fredde in February and June 2022 and Scalo Pennisi

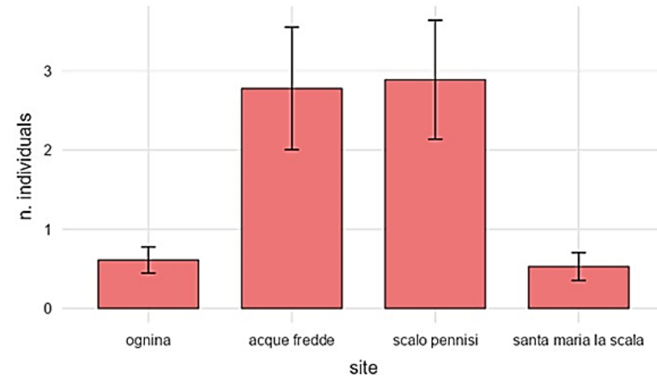

Figure 4. Number of individuals of *Goniodoridella picoensis* in the four study sites from 2021 to 2023.

in March 2022 and May 2023. The breeding activities were followed by a subsequent peak, indicating a rise in the number of individuals. The egg masses were never observed during this study. Overall, in all the sites, it was observed an overall increase in the number of specimens of *G. picoensis* from 2021 to 2023 (Fig. 7).

The correlation matrix highlighted a negative correlation between the number of individuals and the temperature of findings: as temperature decreased, the number of individuals increased (Fig. 8). This relationship fits with this species' seasonal trend. Finally, the Chi-Square ($\chi 2$) test of independence found a significant association between the sites and the average depth of findings ($\chi 2 = 216$; $P<0.001$). In Ognina and Scalo Pennisi, most of the specimens were observed from 10 to 30 m of depth, at Santa Maria La Scala from the surface to 40 m, with a higher proportion of findings in the first 10 m, and finally at Acque Fredde from 20 to 30 m (Fig. 9).

Substrates and habitats: In the study areas, *G. picoensis* was mainly observed on the bryozoan *Nolella* sp. with detritus and other invertebrates such as small-sized tunicates, arborescent and encrusting bryozoans, and hydrozoans. Moreover, some specimens were reported on other invertebrates, such as *Myriapora truncata* (Pallas, 1766), *Crambe crambe* (Schmidt, 1862), or at the base of *Eudendrium* sp. strongly colonised by bryozoans, algae, and detritus. Specimens of *G. picoensis* were also documented on different algal substrates, such as turf of red and/or brown filamentous algae, with *Feldmannophycus*

Table 2. Output of p values resulting from ANOVA analyses, testing for the effect of year, site, month, year × site and year × month on the number of individuals. * Indicates the level of significance and ns indicates not significant.

Factors	Sum of Squares	df	Mean Square	F	p
year	171	2	85.42	8.96	< .001***
site	185	3	61.62	6.47	< .001***
month	279	11	25.33	2.97	0.002**
year × site	110	6	18.40	1.93	ns (0.08)
year × month	352	22	16.02	1.88	0.02*

Table 3. Tukey's post hoc comparisons with their level of significance (*).

Comparison	Mean Difference	SE	df	t	pTukey
2021 – 2022	-2.42	0.63	132	-3.84	< .001***
2021 – 2023	-2.19	0.63	132	-3.47	0.002**
May – August	3.92	1.19	108	3.28	0.05*
May – September	3.92	1.19	108	3.28	0.05*
Ognina – Acque Fredde	-2.17	0.73	132	-2.98	0.02*
Ognina – Scalo Pennisi	-2.17	0.73	132	-3.13	0.01**
Santa Maria La Scala – Acque Fredde	-2.25	0.73	132	-3.09	0.01**
Santa Maria La Scala – Scalo Pennisi	-2.36	0.73	132	-3.25	0.01**

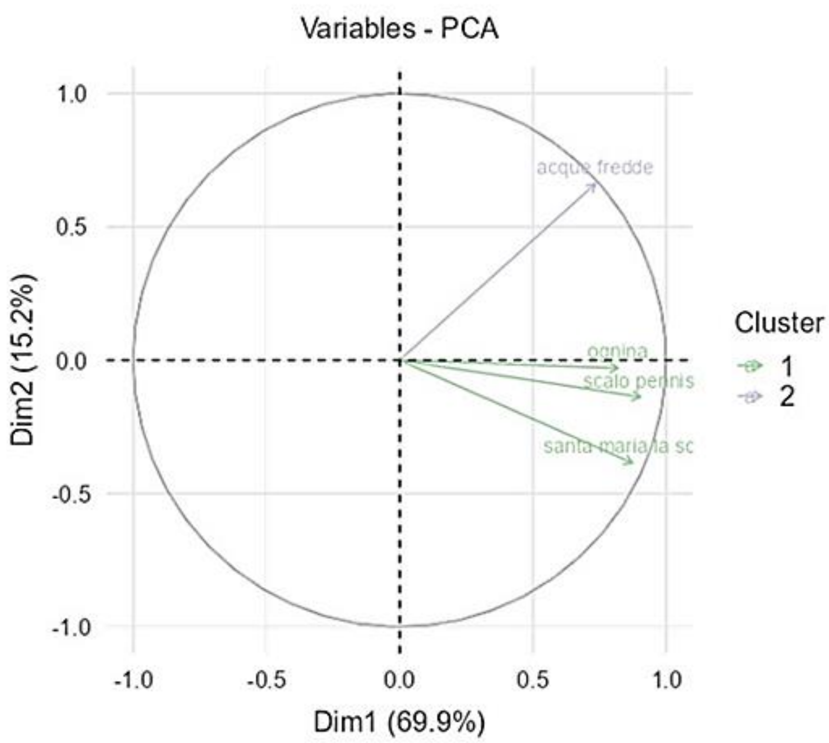

Figure 5. Principal Component Analysis (PCA) of the assemblages of *Goniodoridella picoensis* in the four sites during the years of study, showing two separate clusters.

rayssiae (Feldmann & G. Feldmann) H. Augier & Boudouresque or detritus or gravel; on the red algae *Peyssonellia* spp. and encrusting calcareous algae; on brown algae such as *Dictyota dichotoma* (Hudson) J.V. Lamouroux, *Dictyota implexa* (Desfontaines) J.V. Lamouroux, *Halopteris filicina* (Grateloup)

Kützing, *Halopteris scoparia* (Linnaeus) Sauvageau,

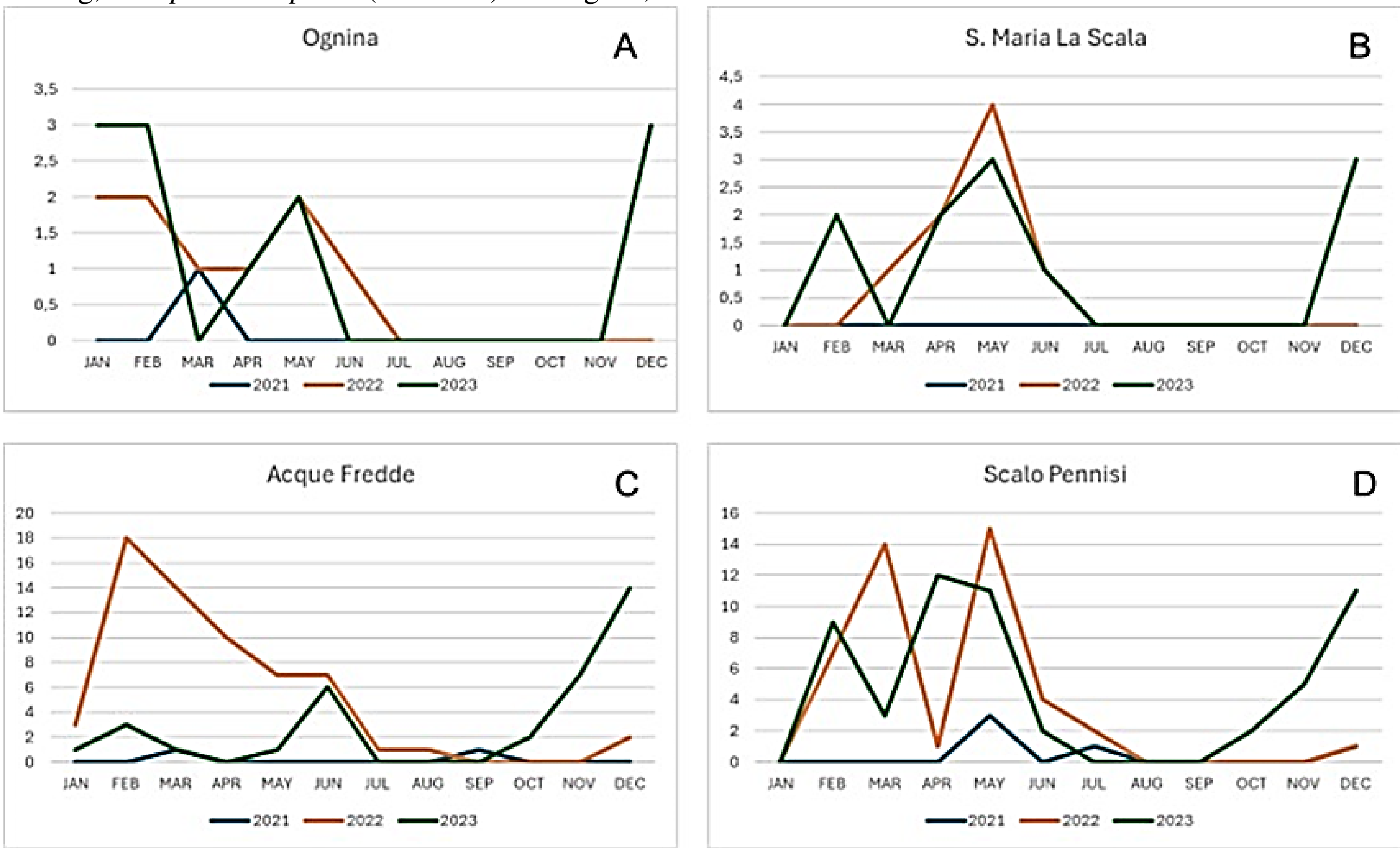

Figure 6. Line charts depicting the seasonal trend of *Goniodoridella picoensis* in the four sites from 2021 to 2023: (A) Ognina; (B) Santa Maria La Scala; (C) Acque Fredde; (D) Scalo Pennisi.

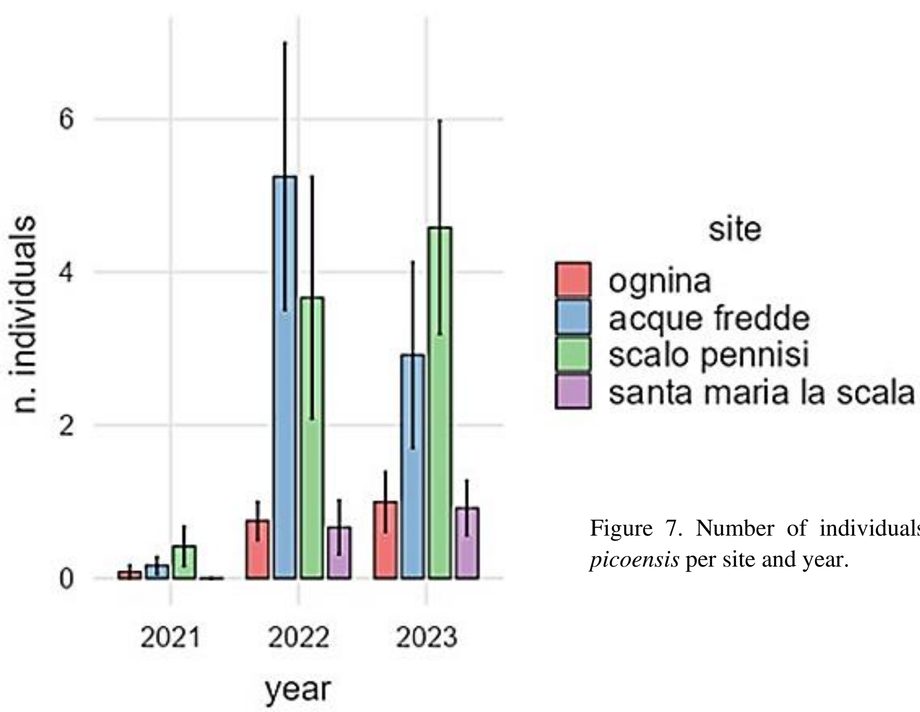

Figure 7. Number of individuals of *Goniodoridella picoensis* per site and year.

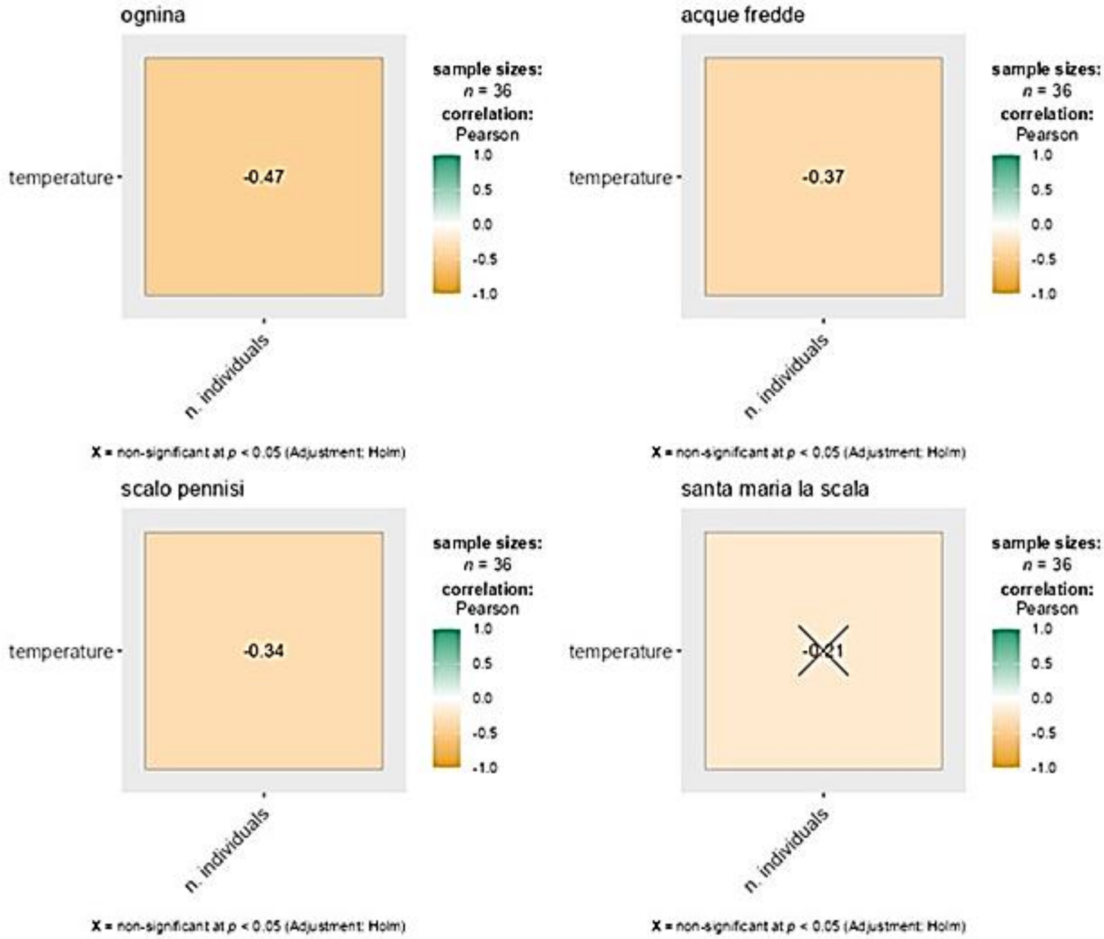

Figure 8. Correlation matrix showing a negative correlation between the temperature of findings and the number of individuals of *Goniodoridella picoensis*.

Lobophora variegata (J.V. Lamouroux) Womersley ex E.C. Oliveira and *Zonaria tournefortii* (J.V. Lamouroux) Montagne. Finally, on the green alga *Palmophyllum crassum* (Naccari) Rabenhorst.

Discussions

In the present study, the seasonal trend of the nudibranch *G. picoensis* along the central-eastern coast of Sicily was explored, and some information regarding the preferential substrates and breeding activity in the study areas was reported. This study observed that the populations of *G. picoensis* have numerically grown from 2021 to 2023. In all the study sites, the number of individuals of this species started to increase in the autumn months, maintained or rose during the spring, and throughout the summer months underwent a sharp decline. The negative correlation between the temperature and the number of specimens also evidenced this seasonal trend. Therefore, as previously documented, *G. picoensis* can tolerate a wide temperature range (Lombardo and Marletta, 2021a), even if it would seem to prefer lower temperatures, like those of the winter-spring months. This is in accordance with the general seasonal trend of the nudibranch species along the central-eastern coast of Sicily, which is related to the seasonality of the sessile benthic suspension feeders, the principal nudibranchs' preys (Lombardo and Marletta, 2023c). Even if the preferential prey of *G. picoensis* is unknown (Paz-Sedano et al., 2024), this species was frequently observed in the study areas on *Nolella* sp.. Therefore, like other members of the family Goniodorididae, this species might feed mostly on bryozoans, which, as sessile benthic suspension

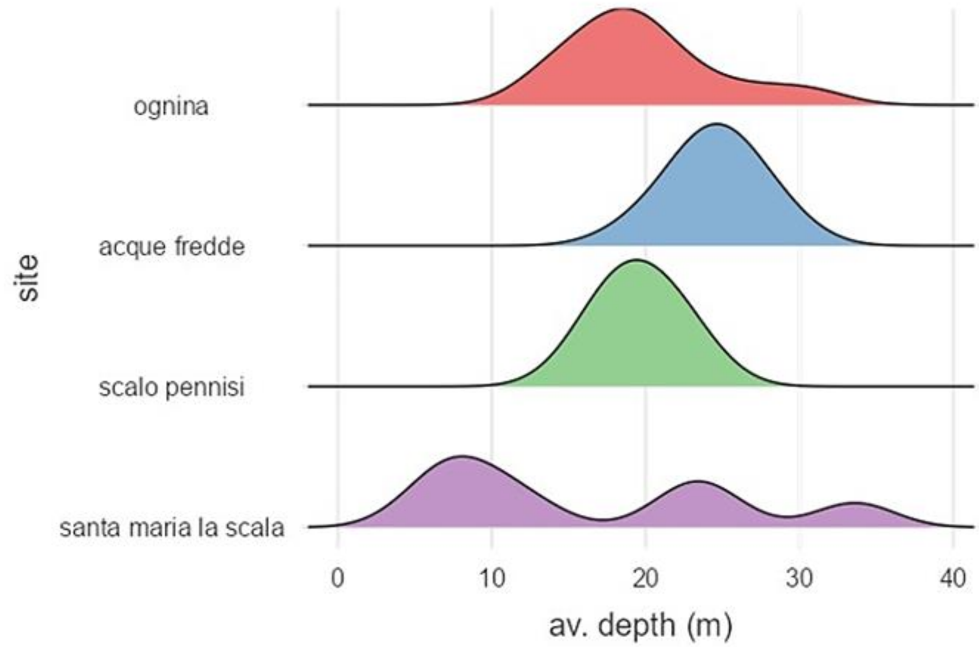

Figure 9. Average of depth (m) of finding of *Goniodoridella picoensis* in the four sites.

feeders, show a marked summer inactivity in the Mediterranean (Coma et al., 2000). Consequently, the decline in the number of individuals of *G. picoensis* in the summer months could also depend on the low availability of prey at that time of year.

As concerns the depth range, in both Atlantic and Mediterranean *G. picoensis* was found either in shallow or deeper waters (Paz-Sedano et al., 2017; Orfanidis et al., 2021; Crocetta et al., 2021; Lombardo and Marletta, 2021a; Trainito et al., 2022; Fantin et al., 2023). Also in this study, we observed this species from the first meters below the sea level to about 40 m of depth, even if the most frequent depth range was 10-30 m.

During this study, it was observed that the sites with the highest number of specimens of *G. picoensis* were Acque Fredde and Scalo Pennisi, where, differently from the other study areas, this species maintained a more stable trend during the years. Moreover, breeding activity was documented in these two sites in 2022 (Acque Fredde, Scalo Pennisi) and 2023 (Scalo Pennisi). The irregular seasonal trend observed at Ognina and Santa Maria La Scala could be linked to the higher anthropogenic pressure in these sites than the other two. Indeed, also for the nudibranch *Edmundsella pedata* (Montagu, 1816), the most unstable seasonal trend was observed in the most polluted and anthropized site (Lombardo et al., 2020). Moreover, another reason for this numerical difference among the assemblages of *G. picoensis* in the study areas is most likely related to the fact that the coastal area of Scalo Pennisi and Acque Fredde is affected by the tidal currents of the Strait of Messina and the upwelling currents of the Ionian Sea (Catra et al., 2006), which might act as a veliger conveyor belt. Moreover, the area of the Strait of Messina, and especially the Faro Lake, is known to be a reservoir and a starting point for the expansion of many non-indigenous species along the eastern coast of Sicily (Furfaro et al., 2018; Lombardo and Marletta, 2019a). Indeed, many non-indigenous species from the Atlantic Ocean, cross the Gibraltar Strait and move eastward through the surface Atlantic Water (AW) (Ben Rais Lasram et al., 2008). In the central Mediterranean, the Algerian Current (AC), a coastal boundary current belonging to AW, splits into two branches due to a topographic effect. The first branch directly flows into the Tyrrhenian Sea along the northern coast of Sicily as the Bifurcation Tyrrhenian Current (BTC) (Sorgente et al., 2011). As many other marine heterobranch species recently entered the Mediterranean, such as *Aporodoris millegrana* (Alder

& Hancock, 1854), *Pleurobranchus wirtzi* Ortea, Moro & Caballer, 2014 and *Taringa tritorquis* Ortea, Perez & Llera (Lombardo and Marletta, 2019b, 2020a, b; Gerovasileiou et al., 2020), this may have been the spreading pattern of *G. picoensis* in this Basin. Indeed, even from the map of the distribution records of *G. picoensis*, reported by Trainito et al. (2022), it can be deduced that the spreading pattern of this species reflects the Mediterranean currents' outline.

The present study is the first on *G. picoensis* conducted over a long period. Overall, from 2021 to 2023, an increase in assemblages' size was observed in all the sites. Moreover, the breeding activity was documented between 2022 and 2023. Considering the above, *G. picoensis* is most likely a non-indigenous species coming from the Atlantic Ocean. The collected data reflect that this species has settled in these study areas in the last years and indicate that *G. picoensis* is establishing stable populations there. Indeed, contrary to what hypothesised by Trainito et al. (2022), the probability that this species has been overlooked until now seems unlikely, not only due to the intense research in the whole Mediterranean area (Orfanidis et al., 2021) but also to the conspicuousness of this species, which can be easily found from both amateurs and researchers.

The establishment success of *G. picoensis* in the Mediterranean could be related to its chemical/mechanical defences and aposematism. Indeed, many goniodoridid species have been considered aposematic because of their bright colour as well as the size of the specimens, being larger than their prey (Rudman, 2004; Paz-Sedano et al., 2024). Because of this, during this study, many specimens were found with detached or missing papillae. This could indicate that this species presents toxic substances that defend it against possible predators. Therefore, given its coloration, predators could easily recognize this species, thus performing an aposematic function. An example of this has already been seen for the haminoeid cephalaspidean *Lamprohaminoea ovalis* (Pease, 1868) in the same area (Lombardo and Marletta, 2021b). Indeed, the colonization success of *L. ovalis* in the Mediterranean has been associated with its aposematic colouration combined with its toxicity (Mollo et al., 2008). Moreover, *G. picoensis* could pose a serious threat to the autochthonous heterobranch fauna by substituting the ecological niche of other marine heterobranch species with the same prey preferences [i.e., as the genus *Diaphorodoris* Iredale & O'Donoghue, 1923 (Picton and Morrow, 2023)] due to its higher competitiveness.

In conclusion, this study represents a standpoint for the research of *G. picoensis* in the Mediterranean, but to have a complete overview of the seasonal trend of this species, further research in other Mediterranean areas is needed. Moreover, in the future, it would be critical to clarify this nudibranch's functional traits and ecological niche to better understand its pattern of spread and establishment in the Mediterranean and its possible impact on native nudibranch populations.

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