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Article

The Delimitation of Geographic Distributions of *Gobius bucchichi* and *Gobius incognitus* (Teleostei: Gobiidae)

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Abstract: After the description of *Gobius incognitus* Kovačić & Šanda, 2016, all previous knowledge about the geographic distribution of *Gobius bucchichi* Steindachner, 1870, as well as its ecology and biology, became obsolete, since it represented the data from the mixture of two species. The known geographic distribution of *G. bucchichi* and *G. incognitus* is revisited by validating previously published records, but also and foremost by integrating many new photographic records posted by anglers and divers on social media and on citizen science databases. The present research uses only positively identified records with exact data on locality, coordinates and date of collecting. A total of 1024 confirmed records were collected and retained for inferring distribution maps: 805 records of *G. incognitus* and 219 records of *G. bucchichi*. *Gobius incognitus* is a widespread Mediterranean Sea species with limited presence in the Lusitanian province of the Eastern Atlantic Ocean. It is absent from the Sea of Marmara and the Black Sea. *Gobius bucchichi* is recorded only in the eastern half of the Mediterranean Sea, from the Adriatic to the Aegean Sea, and in the Black Sea and Sea of Marmara.

Keywords: *Gobius*; benthic fishes; geographic distribution; Mediterranean; identification; photographs

1. Introduction

With 78 species, the family Gobiidae is the species-richest fish family in the Mediterranean [1,2]. A significant part of Mediterranean gobies is rarely collected, and most species are poorly studied. Hence, little is known about their biological traits, ecology and geographic distribution [3]. The records of elusive Mediterranean gobiid species or of recently described species, both generally known from only a very limited number of published records, are often scattered in the Mediterranean Sea, reflecting more a gap in our knowledge than their actual geographic distribution [1]. However, a high level of uncertainty

persists even for the more common species [3]. In ichthyological surveys, by means of fishing gears or visual censuses, gobies are generally not reported, not identified and rarely collected, except for a few larger species [1,4]. Consequently, despite the deficiency of exact published records, the geographic distribution of these common species is often assumed to be widespread and continuous. Since most of the goby species live in the infralittoral zone, or range from the infralittoral belt to the circalittoral belt [1], their distribution is usually displayed as continuous bands along the coastline on Mediterranean minimaps (e.g., see [3]). Not only does this approach misrepresent the distribution of species, but it also precludes any biogeographic study to understand the historical and ecological factors explaining the distributions.

Gobius incognitus Kovačić & Šanda, 2016 is one of about a dozen Mediterranean gobiid species described during the last decade [1,5,6]. This species was discovered through phylogenetic analysis, which revealed a genetically distinct lineage among specimens tentatively identified as *Gobius bucchichi* Steindachner, 1870. Subsequently, the distinction between *G. incognitus* and *G. bucchichi* was further supported by clear morphological differences [5]. Therefore, all previous knowledge about the geographic distribution of *G. bucchichi*, as well as its ecology and biology, became obsolete, since it could have been derived from two different species, leaving the material examined by Kovačić and Šanda [5] as the only source of reliable data. Records published since Kovačić and Šanda [5] are still rare, and suggest a distribution of *G. bucchichi* restricted to the Adriatic, Ionian and Aegean Seas and to the Sea of Marmara [5,7,8]. The records of *G. bucchichi* elsewhere in the Mediterranean and in the Black Sea and from south-west Portugal and Morocco on the Atlantic coast [3], published before Kovačić and Šanda [5], were considered doubtful [5]. Knowledge on the geographic distribution of *G. incognitus* has almost not progressed since the species description: north, middle and south Adriatic Sea, Sicily, Malta, in the north-western Mediterranean in France, and in the eastern Mediterranean along the Turkish coast, at Crete Island and in Israel [4,8–10].

Over the last few years, significant progress has been made in the identification of the different Mediterranean species of pale gobies with longitudinal dotted lines from the fish coloration pattern [1,7,11], enabling us to exploit the rich source of collected photographic material. The number of available exact records has therefore increased tremendously, compared to earlier positive records restricted to scientific publications or to material stored in natural history collections. In this study, we revisit the known geographic distribution of *G. bucchichi* and *G. incognitus* by validating previously published records, but also and foremost by integrating many new photographic records posted by anglers and divers on social media and on citizen science databases.

2. Materials and Methods

The geographic distribution of *G. bucchichi* and *G. incognitus* is based on exact data. The exact data are confirmed records of these species with exact locality, coordinates and date of collection, and with known collector. Records with different locality, or records with the same locality but a different date, are considered as separate records. Confirmed records of *G. bucchichi* and *G. incognitus* were searched in the following source categories (Supplementary Material Table S1): original observations, scientific articles, books, other publications, citizen science databases and social media. The records from scientific articles and books were accepted if they fullfilled Bello et al.'s [12] recommendations for the confirmed records of fishes. Photographs from other publications, citizen science databases and social media were accepted after verification of the species identity following the diagnostic characters presented in Kovačić et al. [1]. Because the photographs varied in quality (resolution, blurriness) or in usefulness for identification (orientation of the fish), they were classifed with an identification confidence level ranging from 0 to 2, where 0—no positive identification, 1—one reliable character discriminating *G. bucchichi* and *G. incognitus* can be clearly seen in the photo, 2—two or more reliable characters discriminating *G. bucchichi* and *G. incognitus* can be clearly seen in the photo. Only photographs of identification quality

"1" and "2" were used in this work (Supplementary Material Table S1). The original observations were also checked and validated by the diagnostic characters of Kovačić et al. [1] for photographs, the diagnostic characters of Kovačić [6] for stored specimens or by genetic data from the sampled tissue [5]. The following data were associated with each record: species, date, country, sea area, locality, site, identification method, latitude, longitude, observer, source category, source, source link and photographer (Supplementary Material Table S1). The sea areas of the Mediterranean subareas division were simplified from FAO General Fisheries Commission for the Mediterranean Geographical Subareas (FAO GFCM GSAs) (https://www.fao.org/gfcm/data/maps/gsas/es/ (accessed on 20 January 2023)), by merging of areas with low records, but bearing in mind that the two studied *Gobius* species are shelf-distributed (Supplementary Material Table S2, Supplementary Material Figure S1). The maps were created using the Free and Open Source QGIS [13]. The WGS84 Geographic Coordinate System is used as the default Coordinate Reference System in the QGIS. The distances between records were calculated by the Distance Matrix analysis tool in QGIS [13].

In order to identify the hierarchical similarity between the defined sites (Supplementary Material Table S2, Supplementary Material Figure S1) with respect to occurrences of *G. incognitus* and *G. bucchichi* (number of records given per GFCM GSA), a cluster analysis was performed by the group-average sorting method based on the Bray–Curtis similarity index using fourth-root transformed data to reduce the relative influence of extreme observations [14]. The obtained similarity matrix was then subjected to a non-metric multidimensional scaling (nMDS) ordination to analyze the heterogeneity of goby composition between GFCM GSAs and to graphically display the two-dimensional plot of the existing interrelationships. The strength (goodness of fit) of the nMDS analysis was measured by the stress coefficient, where coefficients <0.05, 0.1 and 0.2 correspond to excellent, good and potentially useful ordination, respectively [15]. A one-way analysis of similarity (ANOSIM) was also carried out to detect the significance of differences in groupings separated in the nMDS ordination. The value of the ANOSIM statistic R, ranging from -1 to +1, is an absolute measure of how well-separated the groups are, in which a value around zero indicates complete randomness, and R = 1 represents maximal separation of the groups. Groupings identified in the cluster and nMDS ordinations were further explored by applying the similarity percentages (SIMPER routine) to determine the contribution of each goby species to the average dissimilarity between groups. In order to assess if the groups obtained could be characterized by indicator species on the basis of their relative abundance and frequencies of occurrence, we used the indicator index (IndVal) as proposed by Dufrêne and Legendre [16]. The IndVal uses only untransformed abundances and is maximum (100%) when the individuals of species i are observed in all sites of only one site group. IndVal > 25 implies that the species is present in at least 50% of the sites of the cluster, and that this cluster contains at least 50% of the total data of the species (for full account see [16]). Cluster analysis, nMDS, ANOSIM and SIMPER were performed using the PRIMER v. 5 package [14], while the IndVal index was calculated with the PAST v. 4.12 software [17].

3. Results

A total of 1024 confirmed records were collected and retained for inferring distribution maps: 805 records of *G. incognitus* and 219 records of *G. bucchichi* (Supplementary Material Table S1). Additional 51 photographic records were rejected because of the identification confidence level "0". The oldest record dates from August 9, 1965, and the most recent record is from 31 October 2022. The used identification methods for the single record ranged from one to all three (i.e., genetic, morphological and coloration) methods. The diagnostic characters on photos were applied to 955 positive identifications (230 with identification confidence level "1"; 725 with identification confidence level "2"), the morphological diagnostic characters on stored material of 98 specimens, and genetic data from sampled tissues further allowed 14 identifications (Supplementary Material Table S1). The records with confidence level "2" were enough to cover presently established geographic

distribution of *G. incognitus* and *G. bucchichi* (Figures 1–5, Supplementary Material Table S1). The citizen science databases, with 813 records, were the richest source of verified records, followed by 185 original observations, 17 records from scientific articles, 9 records from social media and no verified records from books or from other publications. The most important source of records was the iNaturalist database (https://www.inaturalist.org/ (accessed on 20 January 2023)), totaling 812 records alone.

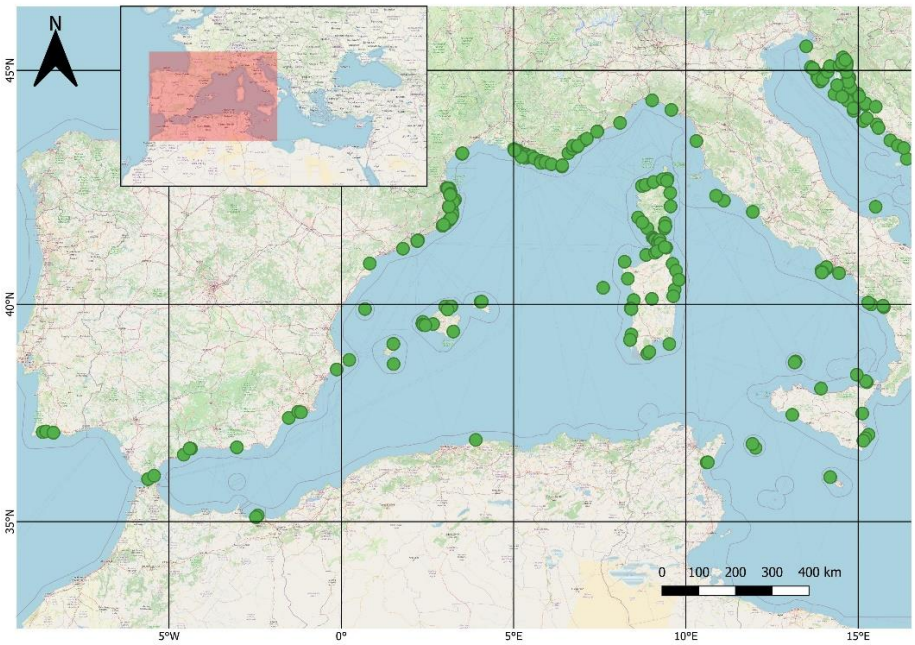

Figure 1. Records of *Gobius incognitus* in the western Mediterranean.

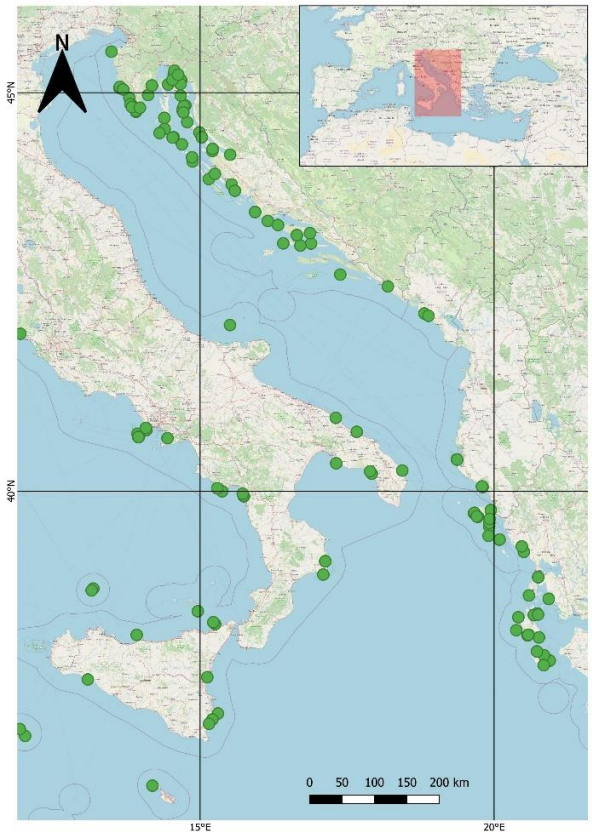

Figure 2. Records of *Gobius incognitus* in the Adriatic Sea and Ionian Sea.

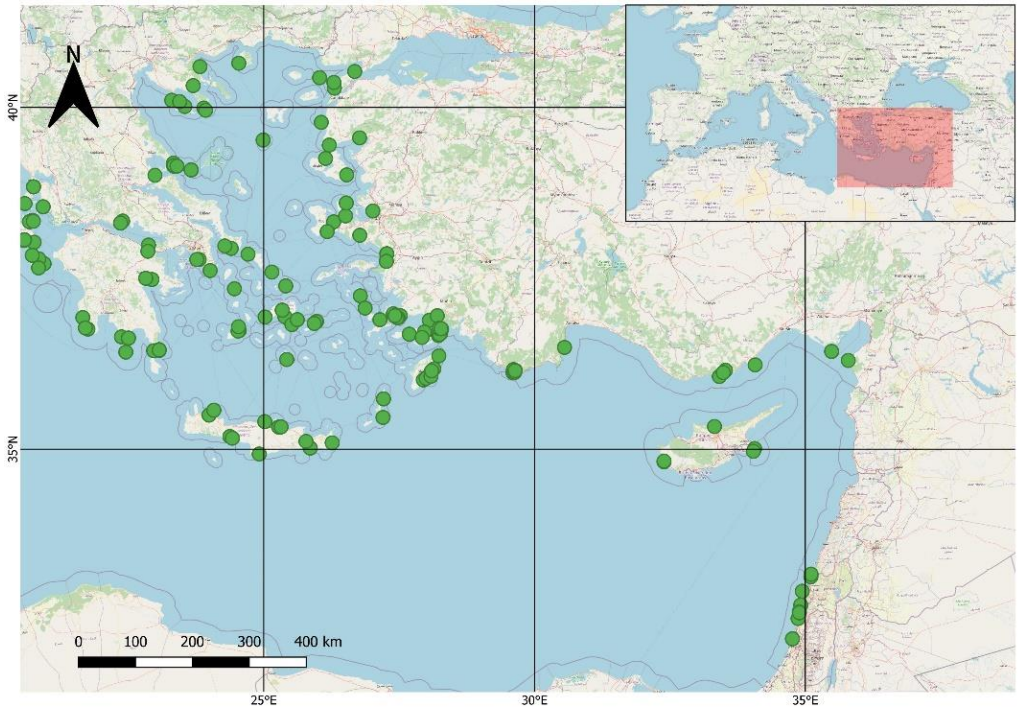

Figure 3. Records of *Gobius incognitus* in the eastern Mediterranean.

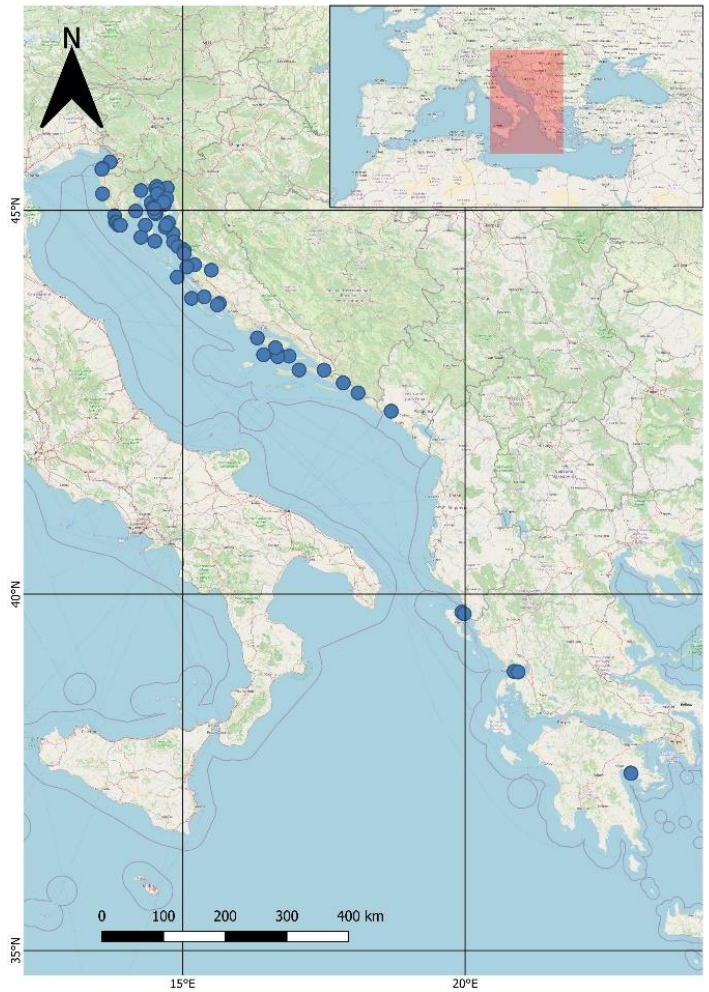

Figure 4. Records of *Gobius bucchichi* in the Adriatic Sea and Ionian Sea.

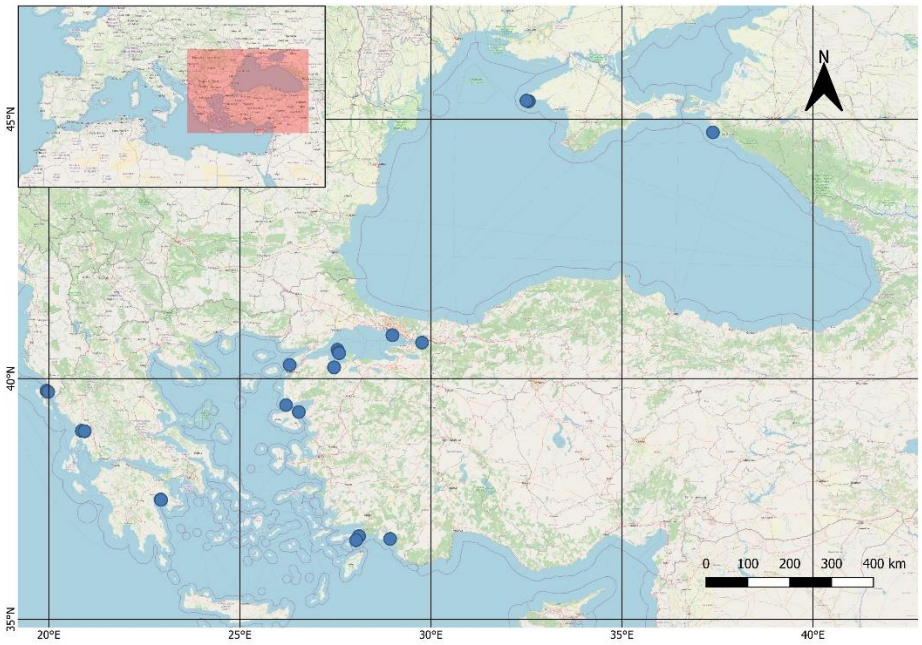

Figure 5. Records of *Gobius bucchichi* in the Aegean Sea, Sea of Marmara and Black Sea.

Regarding the geographic distribution, *G. incognitus* was recorded in all 16 Mediterranean Sea areas and in the North Eastern Atlantic (Table 1). It is absent from the Sea of Marmara and the Black Sea. Most records come from the Aegean Sea, followed by the Adriatic Sea, Gulf of Lion, Ionian Sea and the Spanish Western Mediterranean (Table 1, Figures 1–3, Supplementary Material Table S1). *Gobius incognitus* (Figures 1–3) was recorded in 15 out of 22 Mediterranean countries and in Portugal, having >100 records within each of the following countries: France, Greece, Spain and Croatia (Table 1, Figures 1–3, Supplementary Material Table S1).

Table 1. Number of records of *G. incognitus* per country and per sea area.

Country	Number of Records	Sea	Number of Records
France	165	Aegean Sea	138
Greece	163	Adriatic Sea	128
Spain	144	Gulf of Lion	119
Croatia	118	Ionian Sea	94
Italy	94	Spanish Western Mediterranean	87
Turkey	69	Corsican shelf	47
Israel	15	Levantine Sea	45
Albania	8	Balearic Sea	45
Portugal	7	Tyrrhenian Sea	36
Cyprus	5	Sardinian shelf	28
Algeria	4	Gibraltar and Alboran Sea	16
Monaco	3	North Eastern Atlantic	7
Montenegro	3	African Western Mediterranean	4
Morocco	3	Ligurian Sea	4
Tunisia	2	Southern Sicily	3
Malta	2	African Central Mediterranean	2
		Maltese shelf	2

The distribution of *G. incognitus* ranges from the westernmost record in Faro, Portugal, in the North Eastern Atlantic, to the easternmost record in Yumurtalik, Adana, Turkey, in the Levantine Sea (Figures 1 and 3, Supplementary Material Table S1). Except for the limited extension to the North Eastern Atlantic, the species is distributed in the Mediterranean Sea, present along the shelf of the continents, as well as at the largest Mediterranean islands and at archipelagos (Figures 1–3). Along the Mediterranean coasts, the largest gap in known distribution is in the south Mediterranean from Tunisian to Israeli records (2270.0 km).

The other largest distances between known records are almost all from the southern coast: Algerian to Moroccan records (603.0 km), Tunisian to Algerian records (604.7 km) and Israeli to Turkish records (365.2 km) (Figures 1 and 3). In the north-western Mediterranean, the records are quite dense, with the largest distance between records along the west Italian coast (218.8 km) (Figure 1). The most puzzling absence of this species occurs along the Adriatic north and west coasts, with distances of 406.8 km between Umag, Istria, Croatia, and Tremiti Islands, Italy, and 199.5 km between Tremiti Islands, Italy, and Monopoli, Bari, Italy, and again on the eastern Adriatic coast between Budva, Montenegro, and Vlorë, Vlorës, Albania (208.3 km) (Figure 2). The records are dense across the Ionian Sea and Aegean Sea, with the largest distance between records of 141.5 km, and fairly dense in the Levantine Sea, with a single larger gap between records of 261.2 km (Figure 3).

Gobius bucchichi was recorded in only four eastern Mediterranean Sea areas and in the Black Sea and Sea of Marmara. Most records come from the Adriatic Sea (154), followed by the Aegean and Black Seas (18), Ionian Sea (16), Sea of Marmara (12) and a single Levantine record (Figures 4 and 5, Supplementary Material Table S1). *Gobius bucchichi* is known from 9 countries, with the vast majority of records from Croatia (145), followed by Turkey (27), Greece (19), Ukraine (17), Italy (4), Slovenia (3), Montenegro (2), Russia and Albania (1) (Figures 4 and 5, Supplementary Material Table S1).

The record of *G. bucchichi* in Muggia, Gulf of Trieste, Italy, northeastern Adriatic Sea, is the most northwestern record of this species following the Mediterranean coast (Figure 4, Supplementary Material Table S1). The nearly triangular area of occurrence of *G. bucchichi* is shaped by this record, the northern Black Sea records and the single record at the northwest edge of the Levantine Sea (Figures 4 and 5, Supplementary Material Table S1). The species shows dense records along the eastern Adriatic Sea from Muggia, Gulf of Trieste, Italy, to Tivat, Montenegro, southeastern Adriatic Sea, with the largest distance between known records of 73.3 km (Figure 4). Records across the Ionian Sea, Aegean Sea and Levantine Sea are scarce. The distance between the closest Adriatic Sea and Ionian Sea records was 374.4 km; Ionian Sea and western Aegean Sea records were 234.0 km apart, and the distances from western Aegean Sea to northeastern and southeastern Aegean Sea records were 357.6 km and 464.1 km, respectively (Figures 4 and 5). Another cluster of records is in the northeastern Aegean Sea and Marmara Sea, with the largest gap between the records of 124.4 km (Figure 5). The Marmara Sea records are again very distant from the Black Sea records, 574.6 and 810.3 km.

Comparing both species, we collected 3.7 times more records of *G. incognitus* than *G. bucchichi*. In terms of geographic distribution, *G. incognitus* was present in nearly twice as many countries and in nearly three times as many sea areas as *G. bucchichi* (Table 1, Supplementary Material Table S1).

The hierarchical cluster analysis, performed on the Bray-Curtis similarity matrix derived from the distribution of two goby species among nineteen sub-areas (Supplementary Material Table S2, Supplementary Material Figure S1), revealed the existence of four main assemblages (A, B, C and D) at a similarity level of 67.6% (Figure 6A). Group A, which includes the Black Sea and the Sea of Marmara, comprises 13.7% of the total *G. bucchichi* records and is characterized by the absence of *G. incognitus*. Group B (Adriatic to Levantine Sea) is the only cluster in which the two gobies co-exist, further representing the highest number of observations for both species throughout the study area. Group C is completely formed of sub-areas within the western Mediterranean Sea basin, comprising 46.1% of the total *G. incognitus* records, while only very rare occurrences of the same species were found in Group D. The differences denoted in the cluster analysis are also clearly identifiable visually on the nMDS ordination plot (Figure 6B), and the overall stress value (0.02) gives an excellent representation with no prospect of misinterpretation [14].

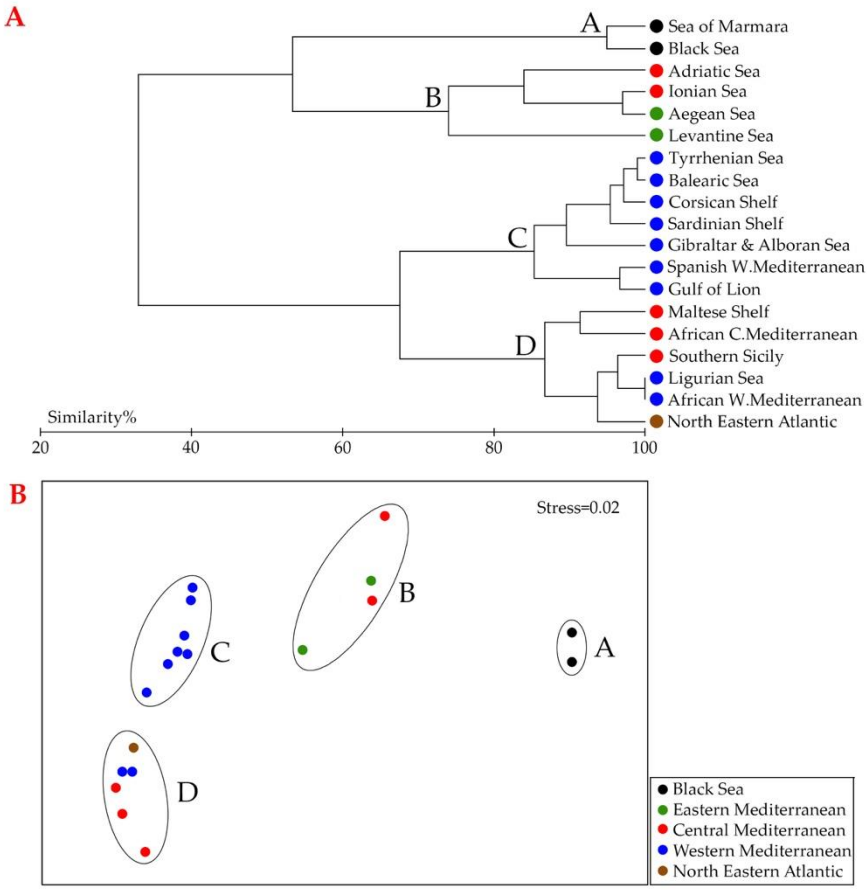

Figure 6. Grouping of records of *G. incognitus* and *G. bucchichi* based on their geographical coordinates. Dendrogram (**A**) and non-metric multidimensional scaling (nMDS) ordination (**B**), indicating the groupings obtained from the cluster analysis.

The ANOSIM test revealed that the overall difference between groupings was statistically significant (Global R = 0.967, $p < 0.05$), demonstrating that the achieved division is robust, despite no difference observed between groups A and B ($p = 0.067$). SIMPER analysis showed that the average similarities within groups A, B, C and D were 94.94%, 81.17%, 89.93% and 90.63%, respectively. Results of pairwise comparisons of the groups, discriminating species and their respective contribution rates, are given in Table 2. Concerning the IndVal index, *G. incognitus* and *G. bucchichi* displayed a significant relation to group B (index value > 25% and $p < 0.05$ for both species), revealing them to be indicator species for that cluster (Table 3); any further links to other groups are not statistically supported.

Table 2. Results of similarity percentage analysis (SIMPER) to analyze dissimilarity between groups (Av. Diss.: average dissimilarity; Contrib.: contribution; SD: standard deviation). ** - not available.

Groups	*G. incognitus*			*G. bucchichi*		
	Av. Diss.	**Contrib.%**	**Diss./SD**	**Av. Diss.**	**Contrib.%**	**Diss./SD**
B & C	7.22	18.07	1.56	32.72	81.93	3.83
B & D	24.65	39.18	6.36	38.27	60.82	4.37
C & D	32.40	100.00	3.42	**	**	**
B & A	35.97	77.13	11.98	10.66	22.87	1.09
C & A	48.69	48.69	11.51	51.31	51.31	12.13
D & A	32.56	32.56	9.44	67.44	67.44	19.56

Table 3. Indicator species (IndVal) index values of *G. incognitus* and *G. bucchichi* (statistically significant values at $p < 0.05$ indicated in bold). ** - not available.

Groups	*G. incognitus*		*G. bucchichi*	
	IndVal%	**Significance (*p*)**	**IndVal%**	**Significance (*p*)**
A	**	**	24.1	0.1377
B	64.75	**0.0005**	75.90	**0.0031**
C	33.16	0.1807	**	**
D	2.09	0.9985	**	**

4. Discussion

The present data show that *G. bucchichi* is an eastern Mediterranean Sea and Black Sea species. The records of *G. bucchichi* elsewhere in the Mediterranean and along the Atlantic coast prior to Kovačić and Šanda [5] can be rejected [3]. The present data also expand the confirmed geographic distribution of *G. bucchichi* established after the description of *G. incognitus* [5,7,8]. According to present knowledge, geographic distribution is limited in the west to the eastern Adriatic Sea and in the south to the edge of the Levant Sea. This pattern of geographic distribution is unique among Mediterranean marine gobies [3]. From the density of the present records, the eastern Adriatic Sea seems to concentrate most *G. bucchichi* populations (Figures 4 and 5). In other gobies, a similar distribution that ranges from the eastern Adriatic Sea to the edge of the Levant Sea can be found only in *Knipowitschia* Iljin, 1927, when considering the genus as a whole [18], with its 17 currently recognized species [19]. *Knipowitschia* species are freshwater or euryhaline fishes, contrary to *G. bucchichi,* and some also occur in the Caspian Sea [18]. Despite the existing phylogenetic and biogeographic studies on the genus [20], no hypothesis has been proposed for the geographic distribution and origin of the entire genus. However, the Adriatic and Ionian Seas are expected to be the center of diversity for sand gobies in general, with some lineages of *Knipowitschia* also restricted to these areas [20]. Among fishes in general, a geographic distribution similar to that of the genus *Knipowitschia*, i.e., a spillover from the Black Sea to the Aegean Sea and to the Adriatic Sea, is visible only in some anadromous Acipenseridae species [21]. Again, these species are strongly associated with freshwater and are present in the Caspian Sea. Only one marine fish species, *Microlipophrys adriaticus* (Steindachner & Kolombatović, 1883), resembles *G. bucchichi* in its geographic distribution [21–23]. This small, marine, shallow benthic blenny, first described in the Adriatic Sea, is also present in the Ionian, Aegean and Black Seas, like *G. bucchichi* [24]. For both species, *G. bucchichi* and *M. adriaticus,* their closely related species show different and much more widespread geographic distributions [5,25].

Based on the present data, *Gobius incognitus* is a Mediterranean species with limited expansion to the Atlantic Ocean and absence from the Black Sea. Some other gobiid species (*Deltentosteus collonianus* (Risso, 1820), *Deltentosteus quadrimaculatus* (Valenciennes, 1837), *Gobius roulei* de Buen 1928, *Lesueurigobius suerii* (Risso, 1810), *Vanneaugobius dollfusi* Brownell, 1978)) have a similar endemic Mediterranean distribution in the broad sense, i.e., widespread in the Mediterranean with an extension to the warm temperate Lusitanian province of the Eastern Atlantic Ocean, and absent from the Black Sea [3,26]. However, those species have different depth and bottom composition preferences compared to the shallow-water *G. incognitus*.

Multivariate analyses showed that *G. bucchichi* and *G. incognitus* occupy statistically distinct geographic sub-areas. The four distinct groupings revealed by the cluster and nMDS analyses (Figure 6) express evident discrimination latitudinally based on abundance heterogeneity of the species, with an increasing abundance pattern for *G. incognitus* from south to north throughout the Mediterranean Sea, while *G. bucchichi* decreases from the central to the eastern basin (including the Black Sea). This result may reflect real differences in alpha diversity, but differences related to sampling effort among certain sites should also not be overlooked; for example, the Corsican shelf (group C) holds almost ten times

more records of *G. incognitus* than the adjacent Ligurian Sea (group D). In terms of relative abundance, both gobies are indicator species for group B (comprising the Adriatic, Ionian, Aegean and Levantine Seas), supporting previous observations of Tiralongo and Pillon [7] that the locally distributed *G. bucchichi* is generally found sympatric, and sometimes even syntopic [5], with the ubiquitous *G. incognitus* in the central-eastern Mediterranean Sea. Yet it remains unclear at present whether or not the two species co-occur in other regions.

The presently established distributions of *G. bucchichi* and *G. incognitus*, based on exact data records, are only the minimum known ranges of the species; their real distribution is likely broader. However, the density of records in certain areas of the Mediterranean Sea and the comparison of this density between *G. bucchichi* and *G. incognitus* allow for some well-supported conclusions. The two species have very similar habitus and habitat and depth preferences [5,7,10]. The method used for recording one species should thus be similarly suitable for recording the second species. The lack of *G. bucchichi* records from southern Italy, Sicily and the western Mediterranean, where *G. incognitus* is densely recorded, therefore indicates that the absence of *G. bucchichi* from these regions is real (Figures 1, 2 and 4). Moreover, the scarce records of *G. bucchichi* in the east Ionian Sea and the Aegean Sea, compared to the Adriatic, and dense records of *G. incognitus*, indicate the relative rareness of *G. bucchichi* in this area (Figures 2–5). The scarcity of records of *G. bucchichi* in the Black Sea and of *G. incognitus* along the south Mediterranean coast could represent their real rareness or punctual presence in these areas, but could just be the result of undersampling and lack of research on these species in the areas (Figures 1, 3 and 5). On the other hand, the absence of records of both species along the western Adriatic coast, which contrasts with the high density of records on the opposite side of the Adriatic, strongly suggests that both species are indeed absent or very rare on the Italian Adriatic coast (Figures 2 and 4). In addition, *G. incognitus* is present along the rest of the Italian coast, and the entire Italian coast can be considered a well-studied area based on the high research and diving efforts (Figure 2).

The data presented in this article will serve as a reference for further investigation on the ecological separation between these two closely related species. The data also constitue a benchmark for monitoring future changes in the geographic distribution or in the relative density of both species. Together, these types of studies should shed light on the effects of global changes and on the strategies that both species have developed to respond to these changes. Moreover, the present delimitation of the geographic distribution of two gobiid species shows the need for updated and reliable data on the geographic distribution of other species of Gobiidae, the species-richest fish family in the Mediterranean. The published summaries on distribution are either short descriptions [1] or vague minimaps [3]. A contemporary review of confirmed records based on exact data is needed both for species assumed to be widespread and continuous and for those with a very limited number of published records [1,3]. For species for which positive identification from photos is possible [1], the use of citizen science databases or even photos with exact data from social media can increase the spatial density of records and even expand the species' known geographic distributions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse11030516/s1, Table S1. The confirmed records of *G. bucchichi* and *G. incognitus*., Table S2, The sea areas. Figure S1. The sea areas, the division explained in the Supplementary Material Table S2.

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