Taxonomic status of the Mediterranean-endemic goby

*Pomatoschistus adriaticus* Miller, 1973 inferred with both morphological and genetic data

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Minor morphological differences between the Atlantic and Mediterranean populations of *Pomatoschistus pictus* (Malm, 1865) resulted in the description of two subspecies of this species by Miller (1973): *Pomatoschistus pictus pictus* and *P. p. adriaticus*. However, the similarity of morphometric and meristic characteristics led to an ambiguity about their status. Despite high morphological similarities between the Atlantic and Mediterranean populations, we found that the population in the Sea of Marmara (Erdek) differs from the Atlantic population in terms of the frequency of papillae in the occipital rows g and h, the position of the suborbital row b and the colouration of the second dorsal fin. Examination of the DNA sequence of the COI gene showed that the K2P genetic distance between the population in the Atlantic and the Sea of Marmara (Erdek) was 7.9%, while the minimum interspecific distance between any other *Pomatoschistus* species was determined as 4.3%. We concluded based on the combined genetic and morphological results that the population in the Sea of Marmara represents a species that is distinct from the Atlantic species *P. pictus*. It is likely that it belongs to *Pomatoschistus pictus adriaticus* described in the Adriatic Sea and should be given a species rank.

**Keywords:** Sand goby; endemic; Mediterranean; Sea of Marmara; *Pomatoschistus adriaticus*; *Pomatoschistus pictus*

**Introduction**

Gobiids are the most speciose group of fish, in the Mediterranean Sea (Miller, 1986), they are extremely adaptable to different environments and show remarkable morphological and ecological variability (Zander, 2011). Within the European gobiid fauna, the intensively studied sand gobies in the genus of *Pomatoschistus* comprise 13 species (Eschmeyer, Fricke, van der Laan, 2018). Several new gobiid species including sand gobies have been described in the Mediterranean in the past decade (Miller & Šanda, 2008; Kovačić & Šanda, 2016; Kovačić, Ordines, & Schliewen, 2016, 2017, 2018; Engin & Seyhan, 2017; Engin & İnal, 2017).

The Painted Goby, *Pomatoschistus pictus* (Malm, 1865), is a small inshore sandy goby that lives in habitats with gravel or sandy bottom and is abundant in the temperate north-eastern Atlantic from Norway to Spain including the Madeira and Canary Islands (Bañón, Villegas-Rios, Serrano, Mucientes, & Arronte, 2010; Knebelsberger & Thiel, 2014; Miller, 1990). There are historical records about the occurrence of *P. pictus* in the Mediterranean Sea (Iljin, 1927; Ninni, 1938; Sözer, 1941; Erazı, 1942). Miller (1973) described the subspecies *P. pictus adriaticus* in the Mediterranean Sea which differs from the eastern Atlantic population. He did not assign all historical Mediterranean

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records to *P. pictus* but assigned them to this subspecies or regarded them as misidentifications (see discussion for details). Following Miller’s study, there was no other study confirming the validity of this subspecies, so, ambiguities arose. While Fricke, Bilecenoğlu, and Sari (2007) and Bilecenoğlu et al. (2014) regarded *P. adriaticus* as a valid species, Miller (1986), Quignard and Tomassini (2000), Bilecenoğlu et al. (2002), Bouchereau, Houder, Marques, and Rebelo (2003) regarded this taxon as a subspecies of *P. pictus*. Furthermore, it was considered as a synonym of *P. pictus* by Kovačić (2005), Kovačić et al. (2012), Psomadakis, Giustino, and Vacchi (2012), Parin (2014) and Froese and Pauly (2018).

In order to contribute to the clarification of the taxonomic status of *P. adriaticus*, we applied DNA barcoding in combination with a detailed morphological examination including the sensory papillae pattern.

**Material and Methods**

**Sampling.** The surveys were conducted at Turkish coasts of Black Sea, Aegean Sea and Sea of Marmara. Totally eight specimens (four adults and four juveniles) of *Pomatoschistus adriaticus* were collected from the Sea of Marmara, Erdek (40°31′25″N, 27°42′32″E) and five specimens of *P. quagga* (Heckel, 1840) from the Aegean Sea near Dikili (38°56′04″N, 26°50′46″E). Both species were caught at 5 m depth and euthanized by an overdose anaesthetics. Pectoral fin clips of *P. quagga* specimens and juvenile *P. adriaticus* samples were fixed in 96% ethanol for genetic analysis whereas all specimens were fixed in 4% formalin for morphological examination. All specimens were deposited in the Fish Collection Centre of Izmir Katip Celebi University (*P. adriaticus*: IKC.PIS.1092–1099; *P. quagga*: IKC.PIS.1100-1104).

**Morphological analysis.** Morphological methods follow Engin and Seyhan (2017). Some additional characters (% SL of body depth at anal fin and pelvic fin origins, pelvic and pectoral fin lengths, pelvic fin origin to anus, snout to origin of D2, D1, D2 and A bases; % HL of postorbital length) were added according to Miller (1973). Specimens length is given as standard length + caudal-fin length.
Meristic abbreviations. A, anal fin; C, caudal fin; D1, first dorsal fin; D2, second dorsal fin; P, pectoral fin; V, pelvic disc; LL, scales in lateral series; TR, scales in transverse series (TR was counted from the anterior origin of the anal fin obliquely upwards and rearwards to the base of D2); LL was counted from the axilla along the lateral midline, including scales over the origin of C; in D2 and A counts the last bifid ray was counted as a single element. Orbit diameter was measured horizontally; body depth was measured at the anal-fin origin and pelvic fin origin; caudal-peduncle length from the end of the anal-fin base to the posterior end of the caudal peduncle; head length from snout to midline opposite upper origin of opercle; head width between upper origin of post opercle; head depth between upper origin of posterior part of eyes. Terminology of the LL system follows Miller (1986) based on Sanzo (1911).

Genetic analysis. Total genomic DNA was isolated from the fin clips using the PureLink Genomic DNA mini kit (Invitrogen). Approximately 655 bp were amplified from the 5′ region of the COI gene using primers described in Ward, Zemlak, Innes, Last, and Hebert (2005) by polymerase chain reaction (PCR) using the 5x FIREPol® Master Mix (Solis Biodyne; www.sbd.ee) according to the manufacturer’s instructions. The thermocycler profile consisted of an initial step of 1 min at 94°C, five cycles of 94°C for 30 s, 50°C for 40 s, and 72°C for 1 min, followed by 35 cycles of 94°C for 30 s, 54°C for 40 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were checked by 2% agarose gel. Sequencing was carried out by Macrogen Inc., Seoul, South Korea.

Nucleotide sequences were aligned using ClustalW (Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997) implemented in MEGA 6.0 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) and edited with BioEdit (Hall, 1999). Reference sequences belonging to members of the genus Pomatoschistus and other sand gobies were downloaded from GenBank and BOLD to investigate genetic relationships of closely related species (Supplementary Table S1). Aligned sequences were compared with existing data and submitted to GenBank with accession numbers: MH729194-MH729197 and MK302484-MK302488. Maximum Likelihood (ML) and Neighbour Joining analysis were performed with the software package MEGA6 (Tamura et al., 2013) using the Kimura two Parameter (K2P) distance model. The Kimura two Parameter model was chosen after running the ‘Model Selection’ tool in MEGA6 and was used to calculate sequence divergence values within and between species. A bootstrap test with 1000 replicates was performed to verify the robustness of the tree.

Results

Pomatoschistus adriaticus (Figures 1–2)

Material examined: 2♀, 2♂ and 4 juveniles with unidentified sex. ♀ 38.6 + 6.8 mm (IKC.PIS. 1092); ♀ 35.2 + 6 mm (IKC.PIS. 1093); ♂ 40.2 + 6.8 mm (IKC.PIS.1094); ♂ 37.5 + 6.9 mm (IKC.PIS. 1095); 4 juveniles IKC.PIS. 1096-1099), 19–22.4 mm SL, TL not determinable due to damages. All samples collected at Erdek, Sea of Marmara (40°31′25″N, 27°41′10″E), at 5 m depth, June–August 2015, S. Engin leg.

Diagnosis. Suborbital papilla of longitudinal row a anteriorly passing the pupil and with one transverse row atp. Six transverse suborbital rows. Anterior point of suborbital row b passing the rear edge of eyes with 2 or 3 papillae but, not reaching level of posterior point of the pupil. Anterior and posterior oculoscapular canals and preopercular canals present but β pore missing in all specimens. Anterodorsal occipital rows g and h with (6–10) and (9–10) papillae respectively. Branchiostegal membrane attached to entire lateral margin of isthmus. Predorsal area and breast naked. First dorsal fin with two or three diagonal rows of black spots and the tip of fin dark shaded. Second dorsal fin with three conspicuous diagonal rows of black spots but the third one is more inconspicuous. Caudal fin rounded and P 17.

Description. Morphometric and meristic characters are given in Supplementary Table S3. Body scales ctenoid but, predorsal area and breast naked. D1 spines almost equal.
Narrow interdorsal space without membrane between D1 and D2. Last ray of D2 aligned with last ray of anal-fin. Anal fin origin aligned with the second ray of D2. Depressed D1 does not reach D2. Uppermost and lowermost pectoral fins rays lacking segments and having a membrane. Pelvic fin rounded in females but become narrow to posterior in males and reaching to urogenital papilla in both sexes. Free edge of anterior membrane of pelvic fin slightly crenate.

*Figure 2. Colouration of preserved Pomatoschistus adriaticus from Erdek/Sea of Marmara IKC.PIS.1092.*

*Lateral line system* (Figure 3). *Oculoscapular and opercular canal pores*: Head with anterior oculoscapular canal with pores: \(\sigma, \lambda, \alpha, \rho, \omega\) (\(\beta\) and \(\kappa\) missing in all specimens); posterior oculoscapular canal with pores: \(\rho_1, \rho_2\) and preopercular canal with pores: \(\varepsilon, \gamma\), and \(\delta\). Anterior oculoscapular head canal ends at the rear margin of the anterior edge of the eye with paired \(\sigma\) pores. – *Preorbital rows of sensory papilla*: Row \(r\) in two vertical parallel sections with 2 papillae and begins just near \(\sigma\) pores. \(s_1\) parallel to nostril (2-3), \(s_2\) not visible. Horizontal row \(s_3\) stated above the upper lip with 1-2 papillae. \(c^2\) row series V shape around AN with 7–8 papillae, \(c^1\) row series (6–7) below \(c^2\) and reaching to \(c_1\), \(c_1\) (7–8) and \(c_2\) (3–4) rows vertical and extending along the lip. – *Suborbital rows of sensory papilla*: Suborbital row \(a\) (16–17) and transverse row \(atp\) (3–4) present. Suborbital row \(a\) anteriorly passing the pupil. Anterior section of row \(atp\) sometimes proliferated with 2–3 papillae. Six transverse suborbital rows of sensory papilla. \(c_1\) (3–4), \(c_2\) (3–4) and \(c_3\) (4–5) rows before the level of row \(b\). \(c_4\) (4–5), \(c_5\) (4–5) and \(cp\) (8–10) below level of row \(b\). Anterior point of suborbital row \(b\) (12–14) passing the rear edge of eyes with 2 or 3 papillae but, not reaching level of posterior point of the pupil. Longitudinal row \(d\) discontinuous in two sections and not reaching \(cp\). Row \(d_1\) (10–16) above the upper lip and \(d_2\) (11–12) under the second to fifth transverse row \(c\). Row \(cp\) starting below longitudinal suborbital row \(b\) and extending to level of longitudinal row \(d\) but not interrupted by row \(d\). – *Preoperculo-mandibular rows of sensory papilla*: Row \(e\) and \(i\) in two sections. Row \(e^1\) (22–23) and \(e^2\) (37–38); \(i^1\) (18-23) and \(i^2\) (21–25). Mental row \(f\) (2–3). – *Oculoscapular rows of sensory papilla*: Anterior transverse series \(tra\) in upper (3–5) and lower (3–4) sections. Transversal row \(z\) (4–5) located behind pore \(\gamma\). Transversal rows \(trp\) (8–9) between pores \(\rho\) and \(\rho_1\) and interrupts anterior longitudinal row \(x\) in three parts. \(x\) (9–10), \(x^1\) (4–5) and \(x^2\) (4–5). Row \(q\) (1–2) before \(p_1\) and below \(x^1\). Row \(y\) with one papilla next to pore \(p_2\). Axillary rows \(as^1\) (9–10), \(as^2\) (5–6) and \(la\) (5–6) present. – *Opercular rows of sensory papilla*: Transversal row \(ot\) (29-33) with superior longitudinal row \(os\) (15–16) and inferior \(oi\) (10–13). – *Anterodor sal occipital rows of sensory papilla*: anterior transversal row \(n\) (6–8), longitudinal rows
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Figure 3. Sensory papillae pattern of Pomatoschistus adriaticus from Erdek/Sea of Marmara.

g (6–10), m (4–5) and row h (9–10) present. Transversal row o with a single papilla above the x.

Colouration in life (Figure 1). Pale yellowish or greenish-yellow body with coarse dark reticulation on the scale edges along the lateral midline. Four conspicuous saddles along the dorsal side. Saddles beginning at origin of first dorsal fin. The second and the third saddle located at origin and end of second dorsal fin, the fourth on the middle of caudal peduncle. Another but smaller saddle at superior origin of caudal fin. Doubled spots below the saddles. Dark triangular blotch at origin of caudal fin. Lower part of the trunk and cheeks silvery. First dorsal fin with four oblique band-like spots, the superior one at the tip of the fin and paler. Second dorsal fin with four conspicuous oblique band-like and ensuing spots but as in D1 the superior one paler. Anal, pectoral and caudal fins greyish transparent. No conspicuous differences were observed between male and female specimens.

Colouration of preserved specimens (Figure 2). Tawny body with scattered melanophores on the upper part of the trunk. An oblique narrow preorbital bar meets with the lips and wider triangular dark suborbital mark from orbit to posterior part of mouth. Scattered dots on the cheek. Post-opercle and belly without melanophores. Saddles paler and doubled spots below the saddles. A black horizontal T shaped bar at the origin of the caudal fin. Four oblique band-like spots on the first dorsal fin. Inferior two more conspicuous but the superior third and the fourth paler. The fourth one at the tip of the fin. Second dorsal fin with three conspicuous rows of oblique bands and traces of the fourth present. Anal fin with irregular pigmentation but pectoral and caudal fins whitish. No conspicuous differences were observed between male and female specimens.

Genetic relationships. Publicly available barcode sequences of ten Pomatoschistus species were analysed. Mean intraspecific between these species was 0.5±0.003%, and mean K2P distance 17.2±0.04%. The smallest genetic distance (4.3% K2P) was observed between P. norvegicus (Collett, 1902) and P. lozanoi (de Buen, 1923). The genetically closest taxon is P. pictus in both Maximum Likelihood (Supplementary Annex, Figure S1) and Neighbour Joining analyses (Figure 4). The genetic distance between P. pictus and our material from the Sea of Marmara was determined as 7.9%, hereby supporting specific distinctiveness.
Ecology. Our material was collected in the Sea of Marmara within a shallow depth range of 0–5 m that is above the halocline layer. This layer was observed between 18–25 m depths around Kapıdağ Peninsula in Erdek (the salinity was measured above the halocline 23%0 and below the halocline 39.4%0). The sea surface temperature in the region may decrease to around 10°C in winter and exceeds 25°C in summer. The substratum on which the specimens were collected was a coarse-grained sand containing biogenic calcareous fragments and shell gravel with coralline deposits. The most common syntopic gobiid species were *Pomatoschistus bathi* Miller, 1982, *Pomatoschistus quagga* and *Gobius niger* Linnaeus, 1758. Juvenile specimens were hyperbenthic hovering 10–50 cm above the substrate but adult specimens were on the ground.

Discussion

We conclude based on the combined genetic and morphological results that the population in the Sea of Marmara represents a species that is distinct from the Atlantic species *P. pictus*. It is likely that it belongs to *Pomatoschistus pictus adriaticus* described by Miller (1973) in the Adriatic Sea and should be given species rank. In line with the
genetic data, the consistency between Miller’s description (1973) and our morphological results, and information in literature (Miller 1973; 1986; Quignard and Tomasini, 2000; Bilecenoğlu et al. 2002; 2014; Bouchereau, Houder, Marques, and Rebelo, 2003 and Fricke, Bilecenoğlu, and Sarı, 2007), we treated the Turkish population as *P. adriaticus* Miller, 1973.

The first occasion of recognising of *Pomatoschistus adriaticus* dates back to Miller’s (1973) study in which he had examined the syntypes of *G. affinis* that were described by Kolombatovic (1891) from the Dalmatian coast. Based on this study, 15 syntypes of *G. affinis* from Split and Vranjic Port were determined not to be *G. affinis* but rather *P. pictus* (a detailed chronological history was provided by Miller 1973). The author also recognised that there are minor differences between Atlantic and Adriatic specimens of *P. pictus* and he consequently described a new subspecies.

After Miller’s study, no detailed study has been made for testing the differences between these populations and a nomenclatural dilemma was seen in the studies that referred to the Mediterranean populations. In addition to this nomenclatural problem, the distribution of *P. adriaticus* in the Aegean Sea has also remained uncertain. Fage (1918) partitioned European Gobiidae into four groups and considered *G. affinis* as a subgroup within the Group II (Type: *G. quadrimaculatus*) while he placed *G. pictus* in Group III (Type: *G. minutus*). Apparently, the ambiguity about *G. affinis* which is mentioned above and the relegation of some syntypes of *G. affinis* to *P. pictus adriaticus* by Miller (1973) led to some confusions about the presence of *P. pictus* in the Aegean Sea, as, for example, stated by Bilecenoğlu et al. (2014) who followed Fage (1918). Notably, Fage (1918) remarked that the distribution of *P. pictus* is restricted to the North Atlantic. In our surveys *P. adriaticus* was only observed in the Sea of Marmara, which could indicate that the species also has a restricted distribution.

Miller (1973) remarked that both the lateral line system and other morphologic characteristics of *P. adriaticus* are similar to *P. pictus* in general, but unavailability of well-preserved material made it impossible to count the numbers of papillae in the particular rows. However, the occipital rows *g* and *h* of *P. pictus* from Atlantic coasts have 3–6 and 10–13 papillae respectively according to Fage (1918) and Miller (1973), whereas the row *g* has 6–10 and *h* has 9–10 papillae in *P. adriaticus* in this study. In addition to these, Miller (1973) remarked that *G. pictus* figured by Ninni (1938) from Lagoon of Venice has different *g* and *h* papillae patterns although an exact comparison could not be made.

Miller (1973) remarked that the most important criterion for the definition of these taxa was the differentiation of the second dorsal fin colouration pattern. Furthermore, more distant pelvic and second dorsal fins (to anus and to snout respectively), a longer caudal peduncles and caudal fin, a deeper body and a narrower cheek in both sexes would differentiate *P. adriaticus* from *P. pictus*. However, Miller (1973) also reported that morphometric characteristics are not distinctive for these populations, which was also the case in this study. Minor differences were observed for the mean values, and ranges of all metric characters overlapped (Table S3).

The lowest genetic distance among the *Pomatoschistus* species was observed between *P. norvegicus* and *P. lozanoi* (4.3% K2P distance). Similar results were documented well in other sand goby studies (Knebelsberger & Thiel, 2014; Engin & Innal, 2017; Engin & Seyhan, 2017). The genetic analysis also confirmed that, with a 7.9% K2P distance, the haplotypes of *P. pictus* are closer to the haplotypes in the Sea of Marmara than of any other species. The genetic distance between these two samples was higher than the minimum interspecific distance observed between *P. norvegicus* and *P. lozanoi*, thus providing additional evidence that they are distinct species. The
results of the DNA barcoding analysis showed that the Erdek haplotypes are clearly distinct from the all analysed *Pomatoschistus* species.

**Supplementary Material**

Supplementary Material is available via the “Supplementary” tab on the article’s online page (http://dx.doi.org/10.1080/09397140.2019.1580932).

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No potential conflict of interest was reported by the authors.

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