New eastern limit of the geographic distribution of *Orsinigobius punctatissimus* (Canestrini, 1864) (Teleostei: Gobiiformes: Gobiidae) in northeastern Italy, with biological notes on the species

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ABSTRACT

A record of the gobiid *Orsinigobius punctatissimus* (Canestrini, 1864) from the springs of the Gorizia Karst (Italy, Friuli-Venezia Giulia) is reported, extending the eastern limit of the geographic distribution of the species. This goby lives in threatened spring habitats, and has recently become rarer. However, although *O. punctatissimus* is listed in the Italian Red List of threatened species as “Critically Endangered” (CR), the International Union for Conservation of Nature Red List of threatened species classifies it as “Near Threatened” (NT). Despite its risk of extinction, the species is not included in the annexes of the Habitat Directive (EU Directive 92/43/EEC) or other international wildlife protection conventions. Information is given on the taxonomy, distribution, biology and conservation of the species.

INTRODUCTION

There are three strictly freshwater Italian gobids: *Padogobius bonelli* (Bonaparte, 1846), *Padogobius nigricans* (Canestrini, 1867) and *Orsinigobius punctatissimus* (Canestrini, 1864). *P. bonelli* is a subendemic species, mainly distributed in the Padano-Veneto ichthyogeographic district and in Dalmatia (Zerunian 2004, Kottelat & Freyhof 2007). This species was also introduced by fish restocking activities in various localities in western and
central Italy. *P. nigricans* is an Italian endemic species, in the Tuscan-Latial ichthyogeographic district (Gandolfi et al. 1991; Zerunian 2004). Another gobiid, *Knipowitschia panizzae* (Verga, 1841), considered by various authors as a congener of *O. punctatissimus* (Economidis & Miller 1990 and references listed below in the taxonomic history of the Italian spring goby), is an Italian subendemic euryhaline species and is present along the Italian coasts (Gandolfi et al. 1991, Zerunian 2004, Nocita et al. 2010).

**Taxonomic history of Orsinigobius punctatissimus**

The Italian spring goby (in Italian: “panzarolo”, “ghiozzetto punteggiato” or “ghiozzetto striato”) was described by Giovanni Canestrini (1864) as *Gobius punctatissimus*. The ten specimens on which the author based his description came from Mantova, Modena and Castelfranco Emilia (Modena). A few years later the species was reported by Ninni (1868) from some rivers in the Veneto region (Piave, Sile, Livenza and other minor rivers). Later on, Canestrini (1866, 1872) added more information on its morphology and colouration pattern. Giglioli (1880) reported it from the surroundings of Bologna and the Veneto region; Torossi (1888) from the Tesina River (province of Vicenza); Tellini (1895) from Sevegliano (Udine); and Pavesi (1898) from the Sarca-Mincio river basin.

Due to the poor original description, Berg (1932) and Tortonese (1935) proposed *G. punctatissimus* as junior synonym of *Padogobius bonelli*. Ninni (1938) provided a concise account of the species, highlighting the differences from *Gobius fluviatilis* Bonelli, 1837 (currently *P. bonelli*), and *Gobius panizzae* (currently *Knipowitschia panizzae*). Subsequent authors (e.g. Miller 1972) informally confirmed the synonymy with *P. bonelli*. Both Gandolfi and Tongiorgi (1974), and Tortonese (1975) considered *G. punctatissimus* as a young of *P. bonelli*, based on a poorly preserved syntype (deposited in the Museo Civico di Storia Naturale di Genova "Giacomo Doria" (MSNG), collection number MSNG 126547).

Gandolfi and Tongiorgi (1978) later considered *G. punctatissimus* as a valid species, based on collections made in several rivers of the Veneto region. Gandolfi et al. (1986) examined more specimens from the Livenza River (La Salute, Venezia), Fosso Cordano (Penarolo, Vicenza), Roggia Miliana (Ariis di Rivignano, Udine) and Tesina River (Lupia, Vicenza), and redescribed the species, including it in the new genus *Orsinigobius* (masculine gender; *O. punctatissimus*). *Orsinigobius* is characterized by the absence of a cephalic canal system, diagnostic distribution of genipores, and presence of scales only in a subtriangular area below the pectoral fins.

Economidis and Miller (1990) synonymized the genus *Orsinigobius* with *Knipowitschia* Iljin, 1927. Subsequently, several authors used the name *K. punctatissima* (Miller 1990, Bianco 1995, Zerunian & Taddei 1996, Kottelat 1997, Zerunian 1992, 1997, 1998, 2002a, 2002b, 2003, 2004, 2005, 2006, 2007, 2013, Porcellotti 2001, 2005, Razzetti et al. 2002, Crivelli 2006, Kottelat & Freyhot, 2007, Zanella et al. 2010, Bianco et al. 2013, Razzetti et al. 2013, Rondini et al. 2013, Accorsi et al. 2014 (sub *K. punctatissimus*), Fortini 2016, Froese & Pauly 2018). Other authors continued to use the name *O. punctatissimus* (Gandolfi & Zerunian 1987, Grimaldi & Manzoni 1990, Gandolfi et al. 1991, Bruno & Maugeri 1992, Groppali 1992 (sub *Orsinogobius*), Nocita & Vanni 1998 (sub *Orsinogobius*), Grimaldi et al. 1999, Geiger et al. 2014, Fricke et al. 2019, Thacker et al. 2019). Mrakovčič et al. (1996) described a subspecies from a restricted area in Croatia, *K. p. croatica*, which was later considered a valid species: *K. croatica* Mrakovčič, Kerovec, Mišetić and Schneider, 1996 (Kottelat 1997). Based on Geiger et al. (2014), Crivelli (2018) updated the attribution of the Italian spring goby to the genus *Orsinigobius*, but
erroneously considered it feminine in gender (*O. punctatissima*).

Based on a molecular study, Thacker et al. (2019) recently supported the monophyly of the genus *Orsinigobius*, including the species *O. punctatissimus* and *O. croaticus*, within a clade including *Economidichthys* and *Knipowitschia* species. The sand gobies of Europe and of the Ponto-Caspian region are presently all included in the family Gobionellidae Bleeker, 1874 (Thacker 2013).

**Distribution**

*Orsinigobius punctatissimus* is an endemic Italian species, whose distribution is limited to the Po River valley in northern Italy, from south-eastern Piemonte to eastern Friuli-Venezia Giulia (Fig. 1a). There is no consensus about the details of this distribution.

Several authors report this species from the Po River valley (Gandolfi et al. 1991, Zerunian & Taddei 1996, Zerunian 1997, Razzetti et al. 2002, Bobbio & Sala 2004, Bianco et al. 2013, Fortini 2016). Marconato et al. (1996) reported it from the two branches of the Po River in the Alessandria province in the Piemonte region (north-western Italy), i.e. the Cesarina Channel near Valmacca, and the Storto Channel near Bozzole (Parco Fluviale del Po e Orba, southeast Piemonte). It was recorded in the Cesarina Channel in 2018 by M. Pascale (pers. comm.). Grimaldi et al. (1999) recorded it in the Lanca (=oxbow lake) Badiola of the Ticino River (Cerano, Novara, Southeast Piemonte), in the Delizia tributary near La Fagiana (Magenta, Milano, Southwest Lombardia), and in the Nasino Channel near Cascina Carenna (Vigevano, Pavia, Southwest Lombardia). Razzetti et al. (2002), based on research carried out in 1998-1999, recorded it also in the Ticino River basin in Pavia (southwest Lombardia), in Ramo dei Prati near Villa Reale (Vigevano), and in Rabica Roggia, at the junction of the Nasino Channel (Vigevano) with the Vecchio Channel, near Cascina Torricella (Zerbolò). However, several authors do not report *O. punctatissimus* from Piemonte, considering Lombardia as its westernmost distribution limit (Maio et al. 2005, Forneris et al. 2011, 2012, Bianco et al. 2013, Macchio 2017).

Some authors report *O. punctatissimus* from the entire Padano-Veneto district, being more common and uniformly distributed in the north-eastern portion of this area, from Vicenza to Udine (Grimaldi & Manzoni 1990, Bruno & Maugeri 1992, Zerunian 2002a, 2002b, 2003, 2004). Other authors report *O. punctatissimus* only from north-eastern Italy (Bianco 1995, Zerunian 1998, Porcellotti 2001, 2005, Kottelat & Freyhof 2007, Crivelli 2006, 2018, Bianco et
al. 2013), excluding it from Piemonte and Lombardia.

Recent studies record the species in various localities in Lombardia, in the provinces of Milano, Lodi, Pavia and Cremona (Puzzi et al. 2007, Rossi et al. 2009, Provincia di Pavia 2010, Lombardi et al. 2012, Razzetti et al. 2013). In Emilia-Romagna, the species was recorded in few sites in Reggio Emilia (Forneris et al. 1990, Castaldelli & Rossi 2008). Sala (2009) reported it as extinct in the province of Modena. Some recent fish maps also report the species in Veneto (Padova: Turin et al. 2010; Rovigo: Bioprogram & Aquaprogram 2010; Venezia: Marconato et al. 2012, Accorsi et al. 2014; Verona: Turin et al. 2003, Confortini et al. 2008).

In Friuli-Venezia Giulia (Fig. 1b), Tellini (1895) recorded the species in Sevegliano (Udine). Stoch et al. (1992, 1995) reported it in the Sile River (Fiume Veneto, Pordenone), Lin River (San Vito al Tagliamento, Pordenone), Roial River (Cordenons, Pordenone), Paisetta River (Sacile, Pordenone), Rognia dei Molini (Castions di Strada, Udine), Aussa River (Cervignano del Friuli, Udine), Natissa River (Aquileia, Udine), Rognia Cincessa and Rognia Puroia (Bertiolo, Udine), Canale Morto and Tiel River (Fiumicello, Udine), Ragnia Revonchio (Pocenia, Udine), Canale Novacco (Aiello del Friuli, Udine), Corno Stream (Maiano, Udine) and Rognia Barbariga (Rivignano, Udine). These records were also used by Bobbio and Sala (2004), and updated by Pizzul et al. (2005). The species was not recorded by Pizzul in Friuli-Venezia Giulia probably because the waterways in which the new samplings were carried out did not include the previous records (E. Pizzul, pers. comm.; unpublished data 2009-2012, ARPA Friuli-Venezia Giulia). Based on the data from Stoch et al. (1995), the easternmost record was the Novacco Channel (Novacco, Aiello del Friuli, Udine); while the easternmost record inferred from Pizzul et al. (2005) was the Versa stream, near Moraro (Gorizia).

Ecology and biology

*Orsinigobius punctatissimus* is found in karst springs, streams and branches of rivers that are characterized by clear, well-oxygenated, cool waters (11–16°C), with moderate currents and thermal fluctuations, rich in macrophytic vegetation, and with sandy, silty or fine gravel bottoms. It is a stenotypic species, which requires specific habitat conditions (Gandolfi et al. 1991, Bruno & Maugeri 1992, Zerunian 1998, 2002a, 2002b, 2003, 2004, Kottelat & Freyhof 2007, Porcellotti 2005, Fortini 2016).

The lifespan is typically of 2 years, rarely 3, and sexual maturity is reached the second year. Reproduction usually takes place between early spring (February) and early summer (May-June). The male digs a nest under stones, pieces of wood and reeds, dead leaves and debris, and then attracts the female inside, using visual and acoustic signals. The female lays a few hundred elliptical eggs of about 2 mm in diameter on the ceiling of the nest. The nest can receive the egg batches from several females, and females can spawn several times per breeding season. The eggs are guarded by the male during the 10–15 days of incubation. Newly hatched larvae are planktonic (3–4 mm in total length, TL), and after 1–2 months, become benthonic. Subadults and adults feed on benthic invertebrates (Gandolfi et al. 1991, Bruno & Maugeri 1992, Zerunian 1998, 2002a, 2002b, 2003, 2004, Kottelat & Freyhof 2007, Porcellotti 2005, Fortini 2016). The reproductive biology of *O. punctatissimus* is similar to that of *O. croaticus*, studied by Zanella et al. (2011). *O. punctatissimus* reaches a maximum length of 55 mm TL (mostly 20–40 mm TL when mature).

MATERIALS AND METHODS

The specimens were collected with nets that have a mesh size of 0.5–10mm, during a field survey on the crenobiont and stygobiont malacofauna of the Gorizia Karst (Fig. 2).
Data on the collecting site are listed as follow: locality and collecting site, municipality and province (in parenthesis), altitude, coordinates in decimal degrees (Datum WGS84), collectors and dates, number of specimens and collection numbers (in parenthesis). Geographical names of the localities were taken from the official Italian maps of the Italian Military Geographic Institute (IGM), at the scale 1:25,000. The collected material is preserved in the ichthyological collection of the Sistema Museale di Ateneo, Natural History Museum, Zoological Section “La Specola” (MZUF), Via Romana 17, Florence, Italy. Fish size is reported as standard length (SL) and total length (TL); the terminology for the fin ray count is the one used and illustrated by Miller (1986). Fish were sexed observing the shape of the urogenital papilla (Miller 1984) and sexually dimorphic colouration patterns.

RESULTS
Specimens were collected in a site within the Gorizia Karst area, at Stagni di Sablici, northnortheast environs of Monfalcone (Doberdò del Lago, Gorizia), about 9m a.s.l., 45.806°N, 13.575°E, by S. Cianfanelli and M. Calcagno, on 22 June 2014 (1 young and 1 subadult, MZUF Nos 17638-17639, respectively), and on 08 April 2015 (2 females, MZUF Nos 17640-17641) (Fig. 1b, Fig. 2). In order to safeguard the site, the coordinates are provided with a precision of three decimals. Specimens were sampled in a karst spring, up to 5-7m wide and about 80cm deep (Fig. 2a-d). The bottom was composed by sand, silt and fine gravel, and was
covered by abundant aquatic macrophytic vegetation. The specimens were collected at a depth of 40cm. A mixed deciduous forest was present around the spring. The geological substrate was formed by skeletal limestone of neritic and carbonate platform facies from the Upper Cretaceous. The various small streams that originate from this and similar springs in the area flow into the Moschenizza River, which then flows into the Locavaz Channel; the latter then joins the Timavo River, which flows into the Panzano Gulf (northern Adriatic Sea). The area of the mentioned springs is included between the route of the A4 Torino-Trieste motorway and the north-eastern suburb of Monfalcone. The territory is part of the Special Area of Conservation (SAC) “IT3340006 Carso Triestino e Goriziano”.

Of the four collected specimens, MZUF 17638-17639 (10.5 and 17.5mm TL, respectively) are in poor conservation state, and fin-rays could not be reliably counted. Meristic counts of both MZUF 17640 (35.5mm TL, 29.9mm SL) and MZUF 17641 (28.2mm TL, 23.8mm SL) are: first-dorsal-fin spines (D1) VII, second-dorsal-fin rays (D2) I/7, caudal-fin rays (C) 13, anal fin rays (A) I/7, pelvic-fin elements (V) I/5, pectoral-fin rays (P) 16 (Fig. 3a-b).

DISCUSSION

Orsinigobius punctatissimus is closely associated to highly specific and isolated habitats, such as springs. In addition to habitat destruction, degradation and agricultural pollution, this species is also threatened by declines of the groundwater level, caused by both excessive anthropogenic extraction and climate change (Gandolfi et al. 1991, Bianco 1995, Zerunian & Taddei 1996, Zerunian 1998, 2002a, 2002b, 2003, 2004, 2007, Turin et al. 2003, 2008, Porcellotti 2005, Crivelli 2006, 2018, Kottelat & Freyhof 2007, Rossi et al. 2009, Bianco et al. 2013, Froese & Pauly 2018). These factors can determine local extinctions, as it already happened in several locations where the species was common until the early or mid-20th century. The more frequent isolation of small populations also leads to genetic isolation and erosion (Bianco et al. 2013, Fortini 2016), with a reduction in population viability and adaptability. The Italian spring goby is also an endemic species with a small distribution range. For these reasons, O. punctatissimus should be considered a highly threatened species.

Zerunian (1997, 1998, 2006, 2007) includes it among the “Endangered” (EN) Italian fishes, according to the threat criteria of the International Union for Conservation of Nature (IUCN) A2 (alteration of habitats and artificial barriers of riverbeds) and A3 (water pollution). In the IUCN “Red List of Threatened Species”, it is considered “Near
Threatened” (NT) (Crivelli 2006, 2018). In the “Red List” of the Italian species, by the Italian Committee of IUCN, it is more correctly assigned to the category “Critically Endangered” (CR) (Bianco et al. 2013), based on the B2ab (iii) criteria, i.e. estimated extent of occurrence <100km²; estimated area of occupancy <10km²; severely fragmented or known to exist at only a single location; continuing decline for area, extent and/or quality of habitat. Rondinini et al. (2013) also consider the species as “Critically Endangered”. Regarding Veneto region, Turin et al. (2008) assigned it to the “Vulnerable” category (VU) according to the threat criteria A2, A3 and B8 (competition and predation by alien species). However, *O. punctatissimus* is not included in any of the annexes of the “Habitat Directive” (EU Directive 92/43/EEC) nor in any other international wildlife protection convention.

Even the future of the newly described locality appears to be uncertain. Despite being included in a Special Area of Conservation in the frame of the “Natura 2000” network, it is located near a motorway track, equipped with a large entrance/exit tollgate, not far from Monfalcone suburbs. This could lead to the degradation of the site’s environmental conditions, and to the extinction of this small population (Gandolfi et al. 1991).

This new record extends the eastward limits of the Italian spring goby’s distribution. The Sablici population is the second record for Gorizia province, being the other the Versa Stream one near Moraro (Pizzul et al. 2005). From a geographical point of view, compared to the easternmost sites of Friuli-Venezia Giulia, the new site is at about 18 km from the Novacco Channel (Aiello del Friuli) (Stoch et al. 1992, 1995) and 14 km from the Versa Stream. In the event that the “Natura 2000” sheet of the Special Area of Conservation IT3340006 is updated, it will be possible to add this rare goby to the list of species, as its future depends on appropriate management and conservation criteria (see also Bressi 2009). Prompt and effective safeguard actions are required, to guarantee the survival of this species in this and other sites.

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