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LEPADOGASTER PURPUREA (ACTINOPTERYGII: GOBIESOCIFORMES: GOBIESOCIDAE) FROM THE EASTERN MEDITERRANEAN SEA: SIGNIFICANTLY EXTENDED DISTRIBUTION RANGE

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Abstract. The Cornish sucker, *Lepadogaster purpurea* (Bonnaterre, 1788), a clingfish species thus far known from the north-eastern Atlantic south to western Africa, the Canary Islands and Madeira, and the western Mediterranean basin, was recently collected in Sicily (Italy), Croatia and Greece. Species identification was based on morphological and/or molecular data. These new Mediterranean records of *L. purpurea* are the first evidence of the species' occurrence in the eastern Mediterranean basin and significantly extend its known distribution range, which likely mirrors that of its sister species *Lepadogaster lepadogaster* (Bonnaterre, 1788).

Keywords: clingfish, biogeography, overlooked diversity, sister species

Clingfishes (Gobiesocidae) are small cryptobenthic fishes that inhabit crevices in rocks or sea grass rhizomes or are found under large boulders and in pebble interstices. Among the eight species reported from the Mediterranean Sea, the species of the genus *Lepadogaster* are certainly the best known. Restricted to temperate waters, members of this genus inhabit mainly shore reefs of the intertidal zone (Hofrichter unpublished[**]). Although being quite abundant in suitable habitat (e.g., 23 individuals of *L. lepadogaster* per m^2; Hofrichter and Patzner 2000) the taxonomy of the genus *Lepadogaster* has long been unclear. Briggs (1955) recognized three species of this genus: *Lepadogaster candolii* (Risso 1810), *Lepadogaster zebrina* Lowe 1839, as well as two subspecies of *Lepadogaster lepadogaster* (Bonnaterre 1788)—*L. lepadogaster lepadogaster* and *L. lepadogaster purpurea*. However, Henriques et al. (2002) invalidated the species-status of *L. zebrina*, which is currently regarded as a *L. lepadogaster* population from Madeira. Moreover, Henriques et al. (2002) showed that *L. lepadogaster* and *Lepadogaster purpurea* (Bonnaterre, 1788) are two closely related, but clearly distinct species living sympatrically in the interstices of boulder beaches. Subsequent molecular phylogenetic analyses showed that the genus *Lepadogaster* is polyphyletic, with the genus *Gouania* being the sister taxon of the species pair *L. lepadogaster* and *L. purpurea* (see Almada et al. 2008).

Even though *L. purpurea* and *L. lepadogaster* are very similar in overall appearance, the species can be distinguished by a number of morphological traits, such as size and number of papillae on the sucking disc, size of head-marks and eyespots, different body coloration, snout length and interorbital distance (Henriques et al.

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2002). Furthermore, they show clear differences in larval development and behaviour (Faria and Gonçalves 2010, Tojeira et al. 2012). The two species further differ in their microhabitat preferences and breeding seasons (Patzner 1999, Henriques et al. 2002). Both species are broadly sympatric along the European and north-western African Atlantic coasts and islands, whereas only *L. lepadogaster* is thought to be present and common throughout the Mediterranean and the Black Sea. Only a few positive records of *L. purpurea* are available for the Mediterranean Sea, with the easternmost reported occurrence from Genova, Italy (Henriques et al. 2002).

Here, based on morphological and molecular data, we provide evidence for the occurrence of *L. purpurea* also in the eastern Mediterranean basin.

In August 2016, specimens of the genus *Lepadogaster* were caught in Greek waters south of Athens at Chamolia (37.916250°N, 24.035750°E), Thimari (37.685111°N, 23.937944°E), and at three locations on Crete (Plakias: 35.194667°N, 24.380806°E; Petres: 35.357833°N,24.368972°E;Myrtos:34.994722°N,25.554806°E)in around one meter water depth, underneath boulders and pebbles with a diameter of about 5–20 cm. Following euthanization with MS-222, standardized photographs were taken and fishes were preserved in ethanol (>95%, small individuals) or formalin (7%, large individuals; prior to fixation in formalin a finclip was taken from the right pectoral fin and preserved in ethanol) for subsequent genetic and morphological analysis. In addition, two ethanol-preserved tissue samples of putative *L. purpurea* from Messina, Italy (38.219137°N, 15.567788°E), collected in 2014 were included in the genetic analysis. For one putative individual of *L. purpurea* collected on the island of Ilovik, Croatia (44.445389°N, 14.57625°E) in October 2014 only morphological data were taken, as DNA quality proved to be insufficient for PCR and sequencing.

Morphometric and meristic measurements followed Hofrichter (unpublished*). Total length (TL), standard length (SL), head length (Hl), body depth (Bd), body width (Bw), sucking disc length (SDl), sucking disc width (SDw), interorbital distance (iO) and number of papillae in sucking disc regions A, B, C (papA, papB, papC) was measured/counted in two individuals of putative *L. purpurea* and *L. lepadogaster* from Greece, and one putative *L. purpurea* individual from Croatia. Voucher specimens of all individuals used for morphological analysis were deposited at the Natural History Museum Rijeka, Croatia (*L. purpurea* voucher IDs: PMR VP3580, Prisliga, island of Ilovik, 10 October 2014; PMR VP4054 LG2, Chamolia, south to Athens, Greece, 6 August 2016; PMR VP4055 LG3, Chamolia, south to Athens, Greece, 6 August 2016; *L. lepadogaster* voucher IDs: PMR VP4053 LG1, Chamolia, south to Athens, Greece, 6 August 2016; PMR VP4056 LG7, Petres, Crete, Greece, 10 August 2016).

DNA was extracted from fin tissue using a rapid Chelex protocol (Richlen and Barber 2005). A 390 bp long fragment of the third domain of the mitochondrial 12S rDNA and a 577 bp long part of the mitochondrial COI gene were amplified and sequenced according to the protocols described in Henriques et al. (2002) and Duftner et al. (2005), respectively. The primer pairs used for PCR and chain termination sequencing were 12sFor/12sRev (Henriques et al. 2002) and FishF1/FishR1 (Ward et al. 2005) for 12S and COI respectively. DNA fragments were purified with Sephadex™ G-50 (GE Healthcare) and visualized on an ABI 3130xl capillary sequencer (Applied Biosystems). In addition, following sequences were downloaded from GenBank and added to the dataset: for 12S rDNA AY036587 and AY036589–AY036605 (Henriques et al. 2002), and for COI KF369136, KJ616457 and KJ768244–KJ768246 (Lobo et al. 2013, Conway et al. 2014, Landi et al. 2014). Sequences were aligned in MEGA 6.0 (Tamura et al. 2013) using MUSCLE (Edgar 2004). All newly generated sequences are deposited in GenBank under the accession numbers MF425769–MF425781 and MF544114–MF544120. Some sequences from Greece (of the individuals also used for morphological analysis) are also available from BOLD (project MEDLP, Mediterranean *Lepadogaster purpurea*). For phylogenetic tree inference, sequences were collapsed into haplotypes. Unrooted maximum likelihood (ML) trees were inferred in PhyML 3.0 (Guindon et al. 2010), employing the best fitting substitution models selected based on the Bayesian Information Criterion (BIC) in MEGA. Statistical support was assessed from 1000 bootstrap replicates.

Based on morphology, two specimens from Chamolia (Greece) and one individual from Ilovik (Croatia) were identified as *L. purpurea*, whereas all the other eight Greek specimens were identified as *L. lepadogaster*. The morphological characteristics of the collected specimens usually fell within the ranges provided earlier by other researchers for the two focal species (Henriques et al. 2002). Interestingly, one of the *L. purpurea* specimens (PMR VP4054 LG2) had fewer rows of papillae in sucking disc region B than is typical for this species (see Henriques et al. 2002). The morphological species identification was further supported by DNA sequence data, which clustered the individuals into two distinct groups corresponding to *L. lepadogaster* and *L. purpurea* (Fig. 1; only the 12S rDNA tree is shown as COI shows the same pattern). 12S and COI net divergence (uncorrected p-distance) between *L. purpurea* and *L. lepadogaster* was 2.7% and 8.5%, respectively.

Our new *Lepadogaster purpurea* records from Messina (Italy, molecular), the island of Ilovik (Croatia, morphological) and Chamolia (Greece, molecular and morphological) are the easternmost records of this species so far and include the first definitive records from the eastern Mediterranean basin. A possible occurrence of *L. purpurea* in the Black Sea was documented by Briggs (1986), who refers to a record by Murgoci (1964). Taking our findings into account, it seems very likely that Murgoci's (1964) individuals of putative *L. purpurea* from the Black Sea are indeed *L. purpurea*. Consequently, the actual distribution

* See footnote on page 409

range of *L. purpurea* in the Mediterranean (and possibly also Black) Sea is much larger than previously assumed and mirrors that of its sister species *L. lepadogaster*.

Considering its widespread distribution in the Mediterranean Sea and its occurrence in shallow water, it is quite astonishing that *L. purpurea* has been overlooked for such a long time. A likely reason therefore might be its cryptobenthic life style (Henriques et al. 2002). *Lepadogaster purpurea* preferentially occurs under large boulders (Henriques et al. 2002) and thus could be very easily missed in ichthyofaunal surveys. However, all individuals of *L. purpurea* collected in the present study were found in pebble or cobble sized substrates (~5–20 cm diameter). As only juveniles (<4 cm SL) were caught in the presently reported study, this indicates that juvenile *L. purpurea* are not restricted to large boulders and do also occur in regular pebble fields. Whether this is a general pattern or only true for *L. purpurea* from the eastern Mediterranean basin remains to be confirmed through further studies.

It is very likely that *Lepadogaster purpurea* has been erroneously recorded as *L. lepadogaster* in the past. This is not at all surprising considering that the two species were regarded as two sub-species of *L. lepadogaster*. On the other hand, the two species are easy to tell apart based on a number of characters (Henriques et al. 2002). Size (smaller in *L. purpurea*) and number of papillae on the sucking disc region A (5–6 rows in *L. purpurea*, 3–4 rows in *L. lepadogaster*) and B (5–6 rows in *L. purpurea*, 3–4 rows in *L. lepadogaster*), as well as the eyespots on the back (see Fig. 2) are reliable characters for species identification. Whereas the papillae of the sucking disc stay intact even after preservation of fishes, the size of the eyespots (head markings) becomes useless in lab identification.

Our study shows that even though the importance of small cryptobenthic fishes for coastal marine ecosystems has been assessed in a variety of studies (e.g., Allen et al. 1992, Ackermann and Bellwood 2000, Depczynski and Bellwood 2003, Smith-Vaniz et al. 2006), the level of knowledge about these fishes is still very poor. The general presumption that *L. lepadogaster* has a much larger distribution range in the Mediterranean Sea than its sister species *L. purpurea* appears to be due to a lack of taxonomic expertise combined with the fact that *L. lepadogaster* and *L. purpurea* were elevated to species rank just a few years ago. We assume that the distribution of *L. purpurea* has been underestimated and expect this species to have a pan-Mediterranean distribution. Probably, species assignment of many specimens deposited in museums is incorrect and needs to be updated, which should be straightforward employing the characters listed by Henriques et al. (2002).

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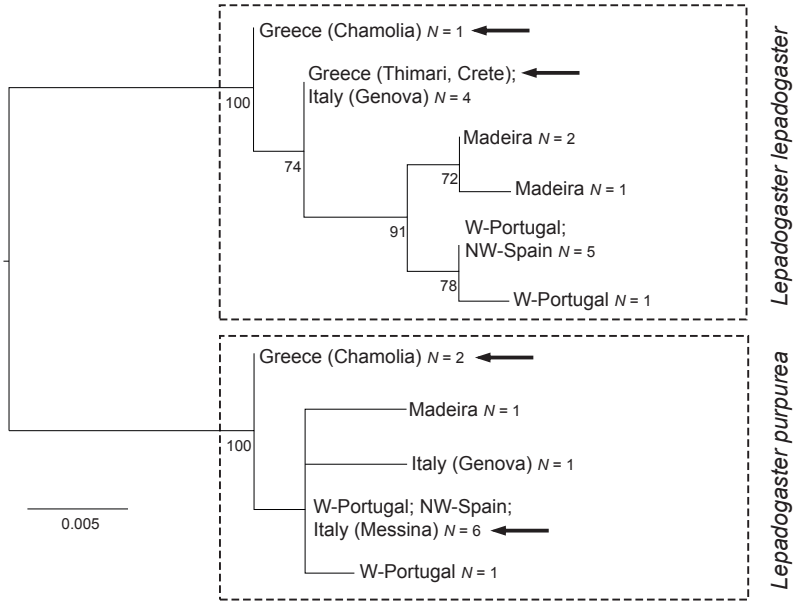

Fig. 1. Maximum likelihood tree based on 12s rDNA data (only bootstrap values > 50 are shown) showing the phylogenetic relations between *Lepadogaster lepadogaster* and *L. purpurea*; the tree was rooted using the midpoint rooting criterion; arrows indicate the haplotypes found in newly sequenced *Lepadogaster* specimens from the Mediterranean Sea

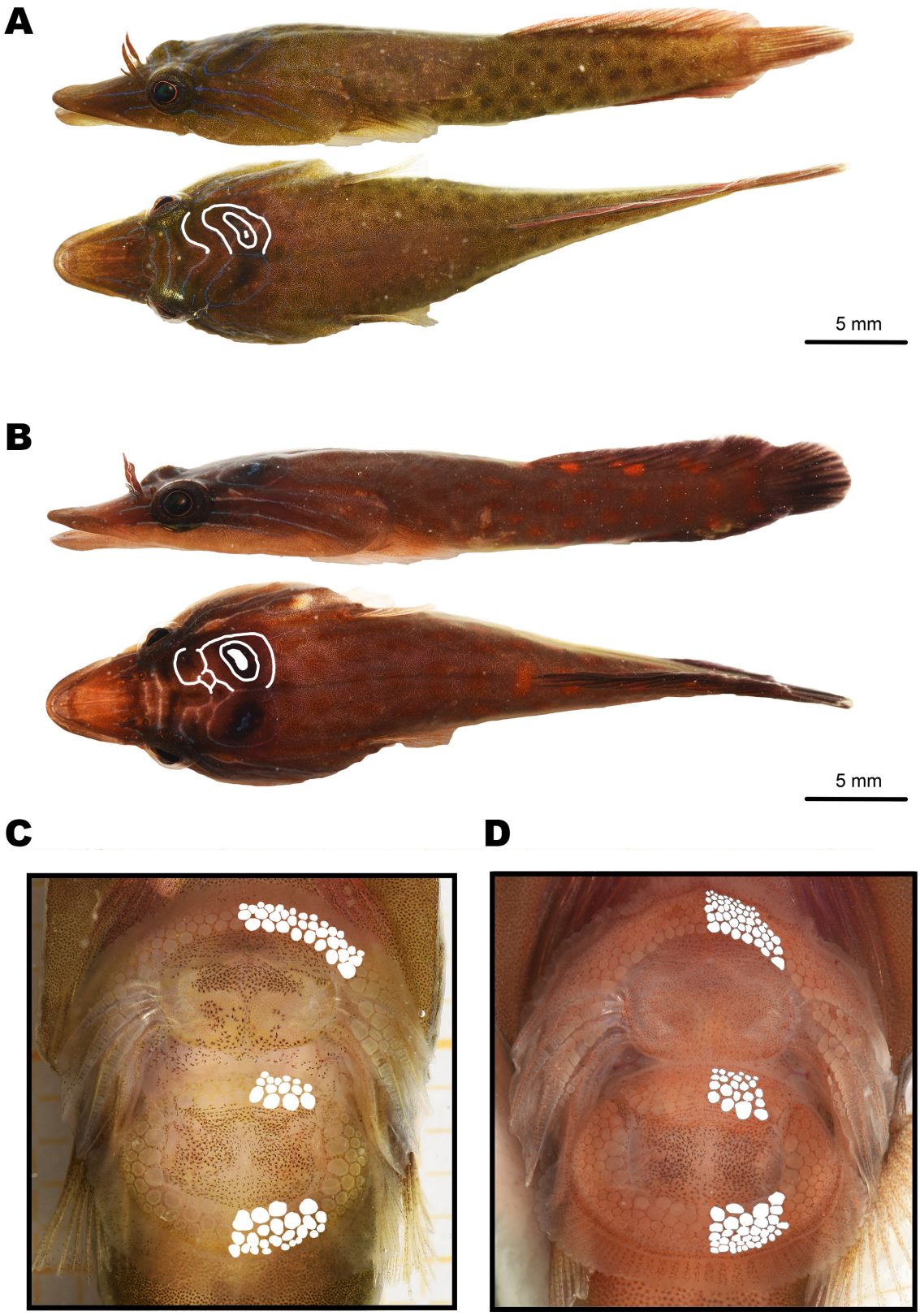

Fig. 2. Photographs and overlaid drawings highlighting the distinctive phenotypic characters that distinguish the two *Lepadogaster* species: (**A**) *L. lepadogaster* has smaller eyespots on the head than (**B**) *L. purpurea*; sucking-disc papillae differ in size and number of rows between (**C**) *L. lepadogaster* and (**D**) *L. purpurea*; the specimens shown are: *L. lepadogaster*, PMR VP4053 LG1, and *L. purpurea*, PMR VP4055 LG3, both from Chamolia, Greece

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