

ORIGINAL ARTICLE

Integrative taxonomy raises doubts on the identity of the non-indigenous *Ianiropsis* occurring in the Adriatic Sea

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Abstract: In the present paper we report the results of taxonomic analysis through morphological and molecular tools of *Ianiropsis* (Isopoda) specimens collected in the lagoon of Venice in Italy within Marine Strategy Framework Directive monitoring campaigns. Six specimens previously identified as *Ianiropsis serricaudis* by morphological analysis were assigned with high confidence to *Ianiropsis epilittoralis* through barcode sequence analysis of the partial COI gene. This would be the first evidence, based on molecular tools, of the presence of the non-indigenous species (NIS) *I. epilittoralis* in the Mediterranean Sea. However, the absence of clear morphological data associated to the deposited sequences in BOLD raises doubts on the identity of these *Ianiropsis* specimens. Diagnostic morphological characters and molecular analysis results are discussed.

Keywords: barcoding; cytochrome oxidase I; Isopoda; non-indigenous species; molecular taxonomy

Sažetak: INTEGRATIVNA TAKSONOMIJA DOVODI U PITANJE IDENTITET NEZAVIČAJNIH JEDNAKONOŽACA RODA *IANIROPSIS* U JADRANSKOM MORU. U ovom radu prikazujemo rezultate taksonomske analize primjeraka roda *Ianiropsis* (Isopoda), prikupljenih u Venecijanskoj laguni u Italiji tijekom monitoringa unutar Okvirne direktive o morskoj strategiji. Analiza je provedena morfološkim i molekularnim metodama. Šest primjeraka, prethodno morfološkom analizom identificiranih kao *Ianiropsis serricaudis*, analizom DNA barkod-sekvencije dijela gena COI s visokim je stupnjem pouzdanosti pripisano vrsti *Ianiropsis epilittoralis*. Taj bi nalaz predstavljao prvi molekularni dokaz prisutnosti nezavičajne vrste *I. epilittoralis* u Sredozemnom moru. Međutim, nedostatak jasnih morfoloških podataka povezanih sa sekvencama pohranjenima u bazi BOLD dovodi u pitanje pouzdanost identifikacije tih primjeraka roda *Ianiropsis*. U radu se raspravlja o dijagnostičkim morfološkim obilježjima i rezultatima molekularne analize.

Ključne riječi: barkodiranje; citokrom oksidaza I; Isopoda; nezavičajne vrste; molekularna taksonomija

INTRODUCTION

The spread of invasive alien species (IAS) may cause several impacts at ecosystem and economic levels (Secretariat of the Convention on Biological Diversity, 1992; Diagne *et al.*, 2021). In the past decades, the intensification of anthropogenic activities such as maritime traffic and aquaculture has favoured an increasing number of newly introduced species in the marine environment. In particular, the Mediterranean Sea is considered to be a hot spot for non-indigenous species (NIS) introductions with a positive trend in the number of new introductions and a large number of established and invasive NIS species (Galanidi *et al.*, 2023).

As far as the marine environment is concerned, NIS are considered within the Marine Strategy Framework Directive 2008/56/EC (MSFD) as an anthropogenic pressure within the Descriptor 2 (European Commission, 2017). The difficulty to successfully manage NIS with invasive characters, especially in a marine environment, has strengthened the strategy of a precautionary ap-

proach based on the reduction of newly introduced species through the appropriate management of vectors as conveyed in the first criterion of the Descriptor 2 of the MSFD (European Commission, 2017). To this purpose, the correct monitoring of NIS is crucial for the evaluation of their pressure and for their effective management.

Monitoring of NIS is quite demanding as it usually relies on a wide spectrum of taxonomic expertise. Moreover, some taxonomic groups are very challenging when classification at species level is required. In these cases, the molecular barcoding approach may represent a valid complementary tool to reduce doubts and misidentifications in line with a more integrative taxonomic approach (Goulding and Dayrat, 2016).

In this work, we report the cases of the Crustacea Isopoda *Ianiropsis epilittoralis* Menzies, 1952, and *Ianiropsis serricaudis* Gurjanova, 1936, two species belonging to a genus that is broadly distributed mostly in temperate and cold temperate coastal waters and that includes 23 described species (WoRMS Editorial Board, 2026). Although *Ianiropsis breviremis* (G.O. Sars, 1883)

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has been reported from the French Mediterranean coast in the Ocean Biodiversity Information System (OBIS), *I. serricaudis* remains, to date, the only species of the genus *Ianiropsis* documented in the scientific literature from the Mediterranean Sea. In particular, this species was reported in 2016 in the lagoon of Venice (Marchini et al., 2016a, b), in the south coast of France (Ulman et al., 2017) and in the Balearic Islands (Martínez-Laiz et al., 2018). *Ianiropsis serricaudis* was originally described from the North-West Pacific Ocean in 1936 (Gurjanova, 1936). Subsequently, it was reported as an invasive alien species in several regions: the Eastern Pacific region in California (Carlton, 1979) and Washington (Cordell et al., 2013), the Western Atlantic region in Connecticut (Pederson et al., 2005), New-Jersey (Hobbs et al., 2015) and Maryland (Morales-Núñez and Chigbu, 2018), in the Netherlands (Faasse, 2007), in England (Hobbs et al., 2015) and along the French Atlantic coast (Gouillieux, 2018; Raoux et al., 2020). On the other hand, specimens of *I. epilittoralis* have only been described along the Californian coast (Menzies, 1952; Wetzler, 2001) and in Korea by Jang and Kwon (1990).

In the present paper we discuss the discordant results obtained from taxonomic analysis conducted with morphological and molecular approaches on *Ianiropsis* specimens sampled within the MSFD monitoring campaigns in the Adriatic Sea. Different hypotheses are formulated to explain incongruencies of the obtained results.

MATERIAL AND METHODS

The sampling area is located in the Venice Lagoon, northern Adriatic Sea, Italy, and more precisely on the “Luigi Rizzo” dam at the central inlet that connects the Venetian Lagoon to the Adriatic Sea, also named “port of Malamocco” (Fig. 1). The macrozoobenthic community associated with hard substrates was collected through the scraping of 0.1 m² hard surface at depths of 2 and 6 m along 3 vertical transects spaced 15 m from each other. The *Ianiropsis* specimens were collected in October 2021 at a depth of 2 m at the following spatial coordinates (Latitude and Longitude WGS84): 45°19'42.924"N, 12°20'36.852"E; 45°19'43.104"N, 12°20'37.464"E.

Morphological analysis

The individuals were identified using 80X Zeiss Discovery stereomicroscope and referring to Hobbs et al. (2015). These authors proposed four key diagnostic characters for the identification of *Ianiropsis* species: maxilliped palp size, antennal peduncle segments 6 and 7, pleotelson spination and pereopod dactyl claws. Some of these features are typical of the terminal males: the size of the maxilliped palp is pronounced enough to be seen easily from a dorsal view of the cephalon and the peduncle segments 6 and 7 of the antennae are particularly elongate relative to the overall length of the half the body length.

Morphometric measurements were made on all specimens using a camera mounted on a Leica S8 APO stereomicroscope and a compound microscope Leica DM2500 to take digital photographs, and ImageJ 1.53 software was used for measurements. Photographs of relevant morphological features were taken to illustrate the descriptions.

Molecular analysis

DNA extraction from 6 individuals of *Ianiropsis* was performed using E.Z.N.A. Mollusc DNA kit (Omega Bio-tek Inc., Norcross, GA, USA), following the protocol provided by the manufacturer. The target COI region was amplified using two primer sets, LCO1490/HCO2198 (Folmer et al., 1994) and jgLCO1490/jgHCO2198 (Geller et al., 2013). PCR reactions were carried out in a 25 µl reaction volume containing 11.05 µl ddH₂O, 5 µl PCR buffer (5X), 0.25 µl BSA, 1.5 µl MgCl₂ (25mM), 0.5 µl dNTP mix (10mMx4) 1.25 µl of each primer (10 µM), 0.2 µl Taq polymerase (5 U/µl), 4 µl DNA template (10–50 ng). The thermocycling profile for both primer sets consisted of one cycle of 1 min at 94 °C and 35 cycles of 30 s at 94 °C, 90 s at 45 °C, and 90 s at 72 °C, with a final extension for 5 min at 72 °C. The amplification product obtained with primers LCO1490/HCO2198 showed the highest quantity of DNA with neat bands on agarose gel, it was therefore used for the following sequencing reactions on an ABI 3730 automatic sequencer (Applied Biosystems Foster City, CA, USA) using the same PCR primers for forward and reverse sequences. The obtained nucleotide sequences were aligned with ClustalW. Basic sequence analyses, amino acid composition, the estimates of evolutionary divergence between sequences were carried out with MEGA 12; the selection of best-fit substitution model and tree construction through Maximum Likelihood (ML) was performed in IQ-TREE 2. The taxonomic identification of sequences was conducted through both the BOLD Identification System (<https://v3.boldsystems.org/>) and the Basic Local Alignment Search Tool (<https://www.ncbi.nlm.nih.gov>). Sequences of *Ianiropsis* spp. and a sequence of *Chauliodoniscus* sp. Lincoln, 1985, used as outgroup, were downloaded from Bold for further analysis: *I. kincaidi*_BHAK3341; *Ianiropsis*_sp._BHAK689; *I. epilittoralis*_FCFC048; *I. epilittoralis*_GBCMI0037; *I. epilittoralis*_ZPC1231; *I. epilittoralis*_GBCMI705; *I. breviremis*_HABFA445; *I. breviremis*_HABFA446; *I. serricaudis*_NJCGS731; *I. serricaudis*_NJCGS724; *I. serricaudis*_NJCGS774; *I. serricaudis*_NJCGS811; *I. serricaudis*_BHAK809; *I. serricaudis*_BHAK2638; *I. derjugini*_BHAK3096; *Chauliodoniscus*_sp._MW066950.

RESULTS

Following morphological analysis (Fig. 2), the six specimens of *Ianiropsis* (three males and three females)

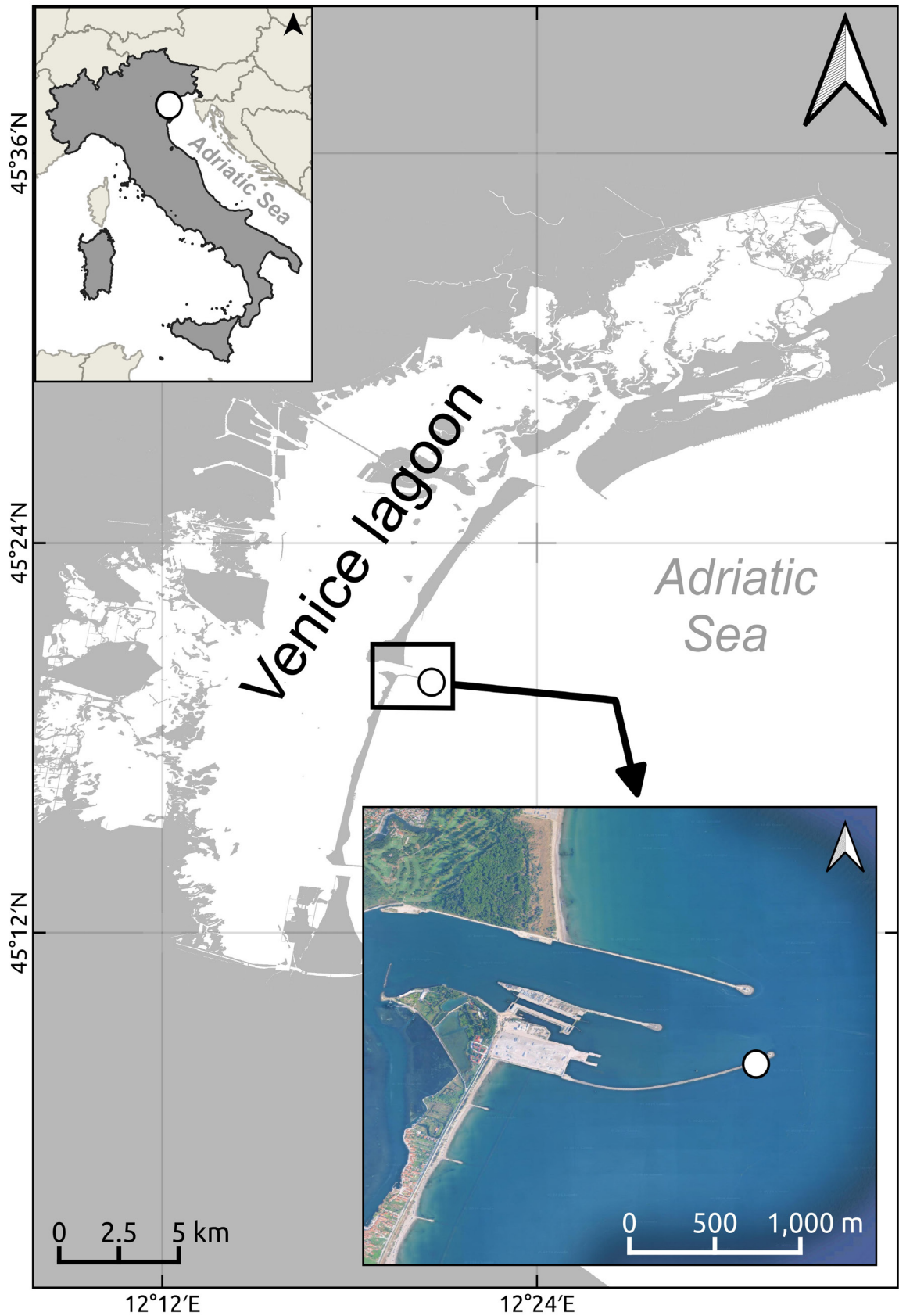


Fig. 1. Sampling area indicated by the white circle at the central inlet that connects the Venetian Lagoon to the Adriatic Sea, also named “port of Malamocco”.

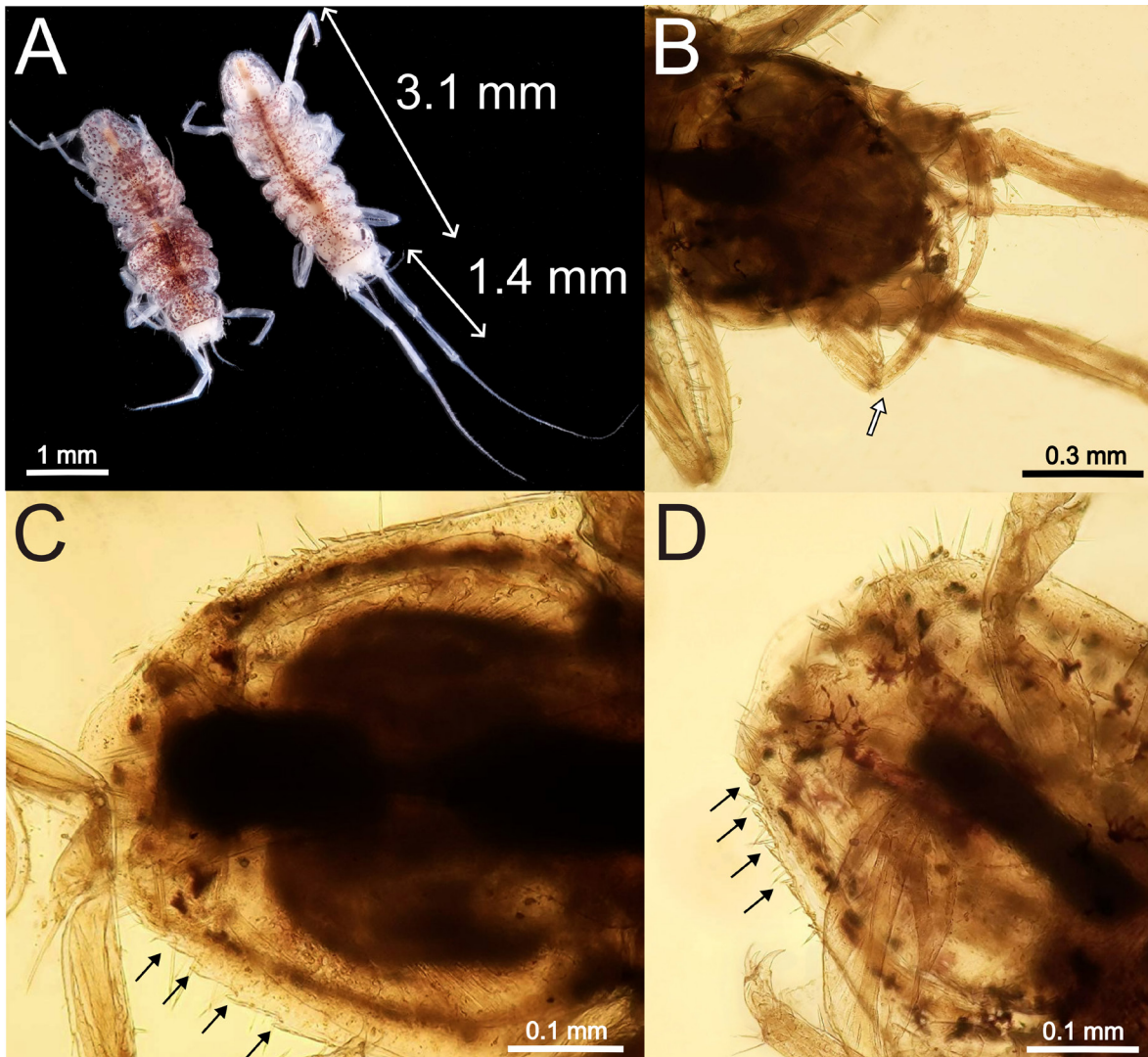


Fig. 2. *Ianiropsis* G.O. Sars, 1897 specimens from Venice Lagoon: female (left) and male (right) individuals (**A**); head dorsal view showing maxillipedal palp of male (**B**); lateral borders of telson with spines, male (**C**); lateral borders of telson with spines, female (**D**). White arrow indicates the maxillipedal palp. Black arrows indicate the spines on the lateral side of telson.

collected in the Lagoon of Venice displayed the following characters: elongate distal segments of antennal peduncle indicated by some authors as segments 6 and 7 and by some others as segments 5 and 6 depending on whether the first tiny segment, partially fused with the head's shell, is counted (Fig. 2A); two claws on dactyli of pereopod 1 and three claws on pereopod 7; the male exhibits elongated maxillipedal palps visible in dorsal view (Fig. 2B). They also have three or four denticles on the lateral margin of the pleotelson, along its posterior half (Fig. 2C, 2D).

For all the specimens, partial COI sequences were obtained. After direct and reverse fragment alignment, six sequences of nucleotide length ranging from 467bp to 597bp were obtained. All sequences were identical except one, which showed an unambiguous single nucleotide difference, the two sequences are hereafter nominated haplotype A (hA) (carried by five individuals) and

haplotype B (hB) (carried by a single individual). Both haplotypes can be transcribed, showing a single amino acid difference. All downloaded public sequences were aligned with hA and hB and trimmed to the length of the shortest sequence, obtaining a 472 bp alignment.

Following the run in the BOLD Identification System, the sequences are assigned to the species *I. epilittoralis* with the highest confidence – 100% similarity for hA and 99.78% similarity for hB. A similar result was obtained using BLAST.

The BOLD TaxonID Tree (data not shown) obtained through the BOLD Identification System showed two well separated clusters of *I. serricaudis* and *I. epilittoralis*. The first one includes six sequences of *I. serricaudis* and one of *Ianiropsis derjugini* Gurjanova 1933, all collected in Canada, British Columbia. The second includes the hA and hB obtained in this study and seven sequences of *I. epilittoralis*, three public sequences

sampled in California, one private sequence sampled in Portugal and three private sequences with unspecified sampling country. In addition, there is one public sequence of *I. epilittoralis*, with Barcode Index Number (BIN) AAE6127, which falls outside the main *I. epilittoralis* cluster with BIN AAE6126. The BIN system clusters DNA barcode sequences into operational taxonomic units that often correspond to species.

The phylogenetic tree constructed using all publicly available *Ianiropsis* sequences, with *Chauliodoniscus* sp. as the outgroup (Fig. 3), revealed three well-defined clusters. Two of these clusters were strongly supported by bootstrap values greater than 99. The first cluster included the three reference sequences of *I. epilittoralis* together with haplotypes hA and hB obtained in the present study, whereas the second cluster comprised the two available sequences of *Ianiropsis breviremis*. A third cluster, supported by a bootstrap value of 88, included all public sequences of *I. serricaudis* as well as a sequence deposited in BOLD as *I. derjugini*. The single sequence of *I. kincaidi* Richardson, 1904, appears well separated from all the others. A very similar tree (data not shown) with a high bootstrap value (> 99) also for *I. serricaudis* cluster was obtained using only first and

second codon positions to avoid third codon position saturation (Wetzer, 2001). Estimates of genetic p -distances between and within groups are reported in Table 1. The intraspecific and interspecific distance values are coherent with genetic distances reported in the literature for marine isopoda (Brix *et al.*, 2018) and marine crustaceans (Raupach *et al.*, 2015). Based on pairwise distances (data not shown) the p -distance value between hA and hB is equal to 0.2%, well below the threshold level of 3% assessed by Brix *et al.* (2018), as the lowest interspecific distance among 72 isopoda species.

DISCUSSION

According to our results, the samples initially assigned to *Ianiropsis serricaudis* on morphological bases were afterwards assigned, with high confidence, to *I. epilittoralis* on molecular bases. Our morphological assessment was based on Hobbs *et al.* (2015), who carried out an extensive analysis on seven *Ianiropsis* species. The authors identified four diagnostic characters for the analysed species, however, the only discriminant character between *I. epilittoralis* and *I. serricaudis* resulted to be the combined length of antennal peduncle seg-

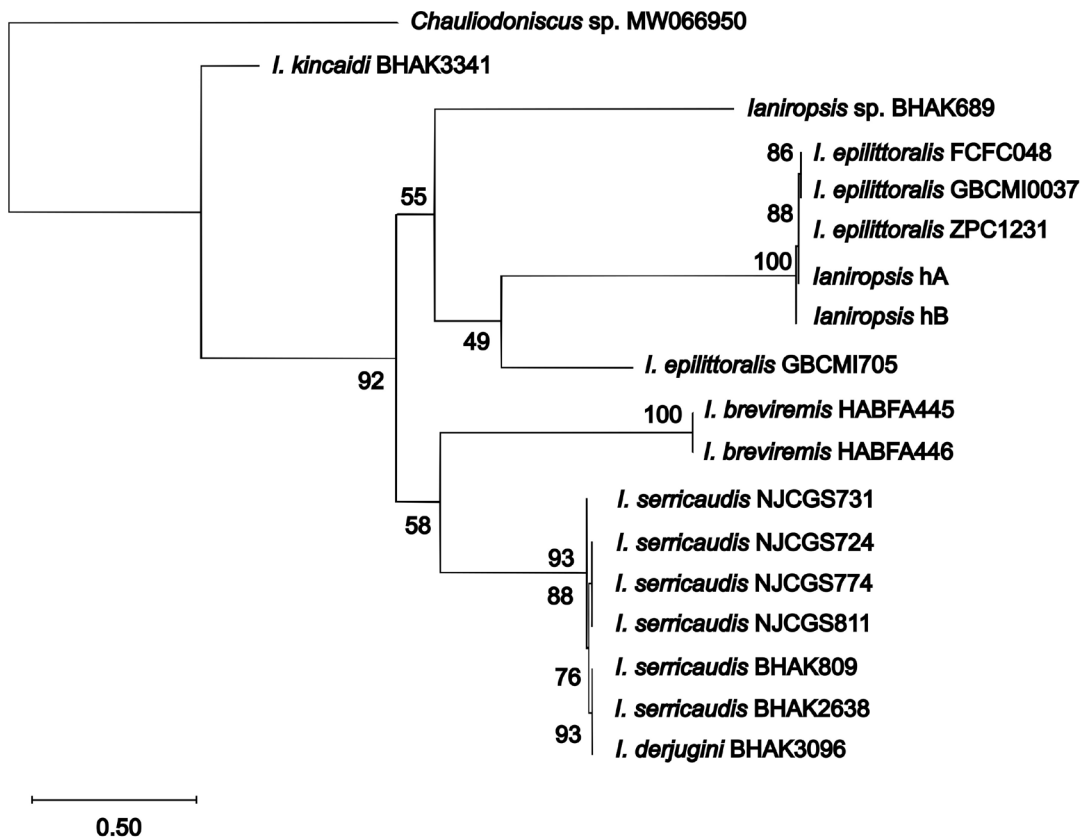


Fig. 3. Maximum Likelihood (ML) tree of *Ianiropsis* G.O. Sars, 1897 species sequences deposited in BOLD and obtained in the present study (hA and hB) and *Chauliodoniscus* sp. used as outgroup. Best-fit model according to Bayesian information criterion (BIC): TN+I+G4.

Table 1. Estimates of average evolutionary divergence between and within groups. Analyses were conducted using nucleotide *p*-distance model in MEGA 12: **Gp_1:** *Chauliodoniscus* sp._MW066950; **Gp_2:** *I. kincaidi*_BHAK3341; **Gp_3:** *I. epilittoralis*_FCFC048; *I. epilittoralis*_GBCMI0037; *I. epilittoralis*_ZPC1231; *Ianiropsis*_hA; *Ianiropsis*_hB. **Gp_4:** *I. breviremis*_HABFA445; *I. breviremis*_HABFA446; **Gp_5:** *I. serricaudis*_NJCGS731; *I. serricaudis*_NJCGS724; *I. serricaudis*_NJCGS774; *I. serricaudis*_NJCGS811; *I. serricaudis*_BHAK809; *I. serricaudis*_BHAK2638; *I. derjugini*_BHAK3096.

	Gp_1	Gp_2	Gp_3	Gp_4	Gp_5
Gp_1	n/c				
Gp_2	0.28889	n/c			
Gp_3	0.30857	0.29778	0.00466		
Gp_4	0.29841	0.30159	0.30032	0.00000	
Gp_5	0.31020	0.27574	0.29760	0.26939	0.01362

n/c denotes cases in which it was not possible to estimate evolutionary distances

ments 6 and 7 in terminal males, which is elongate (~½ main body length) in *I. serricaudis* and short (~ ¼ main body length) in *I. epilittoralis*. As shown in Fig. 2A the ratio length of this character in our samples would confirm their assignment to *I. serricaudis*.

Ianiropsis serricaudis was originally described from the Sea of Japan by Gurjanova (1936) and reported on the undersurface of rocks, and on sponges, ascidians, and the coralline alga *Amphiroa* Quoy & Gaimard, 1827 from the shore to 2 m. Kussakin (1988) reported that it occurs also from the intertidal zone to 7 m, in water temperatures ranging from 1.8 °C in the winter to 24 °C in the summer, adding the coralline algae, the rhizoids of the kelp, and brown algae as additional habitats. Conversely, *I. epilittoralis* was originally found in California, in elevated, spray-filled tide pools, on top of *Mytilus*-covered rocks, often with specimens of *I. kincaidi* (Menzies, 1952).

Although the species *I. serricaudis* has been reported in many marine regions of the northern hemisphere as a spreading invasive species, and has been the object of several morphological investigations, very few barcode sequences are reported in the reference databases. On the other hand, the few barcode sequences deposited in BOLD as *I. serricaudis* are not attributable to specimens morphologically described in any publication. This is a gap that may occur in several cases and that may lead to erroneous assignment of the sequences. For this reason, a request was made to obtain on loan samples of specimens assigned to *I. serricaudis* from the Institute that deposited the sequences, but unfortunately samples were not available. As far as *I. epilittoralis* is concerned, the morphological descriptions are quite scarce in the literature. The public COI sequences deposited in the databases are all sampled in California, two reported in Wetzer (2001), one in Osborn (2009) and one deposited by the Centre for Biodiversity Genomics. In addition, a private sequence is indicated by the BOLD identification system belonging to a Portuguese Institution. Following contacts taken with the Portuguese Institute, we

found out that the assignment of their sequence to *I. epilittoralis* was based only on molecular evidence.

According to our results, two hypotheses can be formulated: (i) the species found in the Lagoon of Venice is *I. serricaudis*: in this case the BOLD sequences of *I. serricaudis* and *I. epilittoralis* are wrongly assigned. The presence of both species along the Californian coast may have contributed to the misidentification of these species, besides, there is one sequence deposited in GenBank as *I. epilittoralis* (GBCMI705) which does not cluster with the *I. epilittoralis* group (*p*-distances > 20%) leading to the hypothesis that two different species were morphologically classified as *I. epilittoralis*. The specimen was sampled in California and used in a phylogeny study by Osborn (2009). Furthermore, the sequence deposited in BOLD as *I. derjugini* and clustering in the group of *I. serricaudis* is further evidence of possible mistakes and errors associated to morphological classification of these taxa; (ii) the species found in the Lagoon of Venice is *I. epilittoralis*: in this case, further morphological investigations should be made to review diagnostic characters of these *Ianiropsis* species to avoid any kind of misidentification by using morphological diagnosis. Indeed, the species of the genus *Ianiropsis* are very difficult to distinguish because only a limited number of reliable diagnostic characters are available. For example, in Doti and Wilson, 2010, the discrimination between *I. serricaudis* and *I. epilittoralis* is primarily based on the number of denticles on the pleotelson margin, however, this character was subsequently shown to be unreliable due to its high variability among individuals. In conclusion, to disentangle the issue of *Ianiropsis* identity in the Adriatic Sea, barcode sequences should be made on type material of *I. serricaudis*, or at least from specimens from the precise type locality (topotypes). Furthermore, a broader effort to generate barcode sequences from morphologically documented specimens representing different *Ianiropsis* species would greatly improve our understanding of species boundaries and help resolve the current uncertainties surrounding *Ianiropsis* identification.

The evidence provided in this study should be an inducement to strengthen the collaboration between taxonomists and molecular biologists. The acceleration of sequencing innovations and technological facilities improvement may contribute to a better and more thorough comprehension of biodiversity as long as it is supported by taxonomic knowledge.

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Collected samples are deposited at the Marine Molecular Biology Laboratory in ISPRA, Italy, and are available upon request. The partial COI sequences of *Ianiropsis* sp. have been deposited in NCBI (PV261153–PV261158).

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