

Article

Crustaceans (Malacostraca and Thecostraca) from the International Minho River, Iberian Peninsula

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Abstract: Crustaceans (Arthropoda) are a diverse and abundant group with chitinous exoskeleton, living on coastal/estuarine environments, at community invertebrate fauna. A survey on the species of some crustacean groups found on the Minho River estuary (international section) in the Iberian Peninsula, is presented with specimens collected through glass eel fishing bycatch, grab sampler, trammel net, beam trawl and fyke net sampling methods. A total of 98 specimens were examined belonging to 7 orders, 21 genera and 23 species (17 new records from Minho River, including one for Portugal). Brief diagnosis, ecological notes, species distributions and figures are provided intending to present taxonomic support on future projects in this region.

Keywords: Atlantic Ocean; distribution; estuary; invertebrates; taxonomy



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1. Introduction

The subphylum crustacea Brünnich, 1772 is one of the most numerous and diverse groups with more than 60,000 known species [1], with significant importance on estuarine ecosystems for providing a major source in fish and bird diets [2–5]. Additionally, these marine and brackish arthropods play various ecological roles, like predation [2], parasitism [6] and mesograzing [7]. They are widely used commercially (e.g., *Crangon* and *Palaemon* spp.) [2], for the food industry, pharmacological products, aquaculture and indirectly with research involving biotechnology for the reuse of waste from aquaculture [8]. Although crustaceans are a well-studied group, taxonomic problems arise from the lack of knowledge about certain groups, especially species with high sexual dimorphism or differences in juvenile-adult morphology [2,9,10] compounded by information fragmentation. Estuarine ecosystems are of utmost biological importance, being productive and valuable for biodiversity maintenance [11]. Macroinvertebrate surveys on the Minho River estuary are still insufficient to access its biodiversity with few works on macrobenthic ecology or specimens collected through glass eel fishing bycatch [12–15] and a survey on crustaceans of the order Isopoda [16]. In this study we provide an overview of some groups of the Crustacean fauna (brief diagnosis, ecological notes, global and Portuguese distributions) collected on the Minho River estuary, with a total of 23 species, in which 17 are new records for the Minho River, including one new record for Portugal—*Nebalia strausi* Risso, 1826. In addition, an update to the list of isopods from Minho River is provided here.

2. Materials and Methods

2.1. Study Area

Located northwest of the Iberian Peninsula, the Minho River originates in the Meira mountains (Spain), with an extension of around 300 km [17]. The last section of 70 km represents the northwest Spanish/Portuguese border, which is part of the protected areas of the Natura 2000 network as a site of ecological importance and a special area of conservation, marked as an Important Bird Area [18,19]. The estuarine area has a total area of 23 km² and

a length of approximately 40 km [13], characterized by a mesotidal partially mixed system tending towards a salt wedge during the high floods [20].

2.2. Sampling, Identification and Preservation of Specimens

Specimens examined were collected during previous sampling campaign (including ecological researches from [15,21] at the estuary of the international Minho River (Figure 1), with following methods: (1) glass eel fishing bycatch, in Caminha, Portugal ($41^{\circ}52'59.00''$ N/ $8^{\circ}50'14.00''$ W), on April, 2020, on a new moon night during flood tides, using stow net (length of float lines 10 m, bottom anchored lead line of 15 m, height 8 m, mesh size 1–2 mm, covering an area of 50 m²); (2) glass eel fishing bycatch, in Caminha, Portugal ($41^{\circ}52'44.80''$ N/ $8^{\circ}50'26.25''$ W), on March 2021; (3) fyke nets (length 7 m, mesh size 10 mm, with two funnel shaped openings), in Vila Nova de Cerveira, Portugal ($41^{\circ}57'1.69''$ N/ $8^{\circ}44'42.74''$ W); (4) on sediment sampling on saltmarsh with a Van Veen grab sampler in Caminha, Portugal ($41^{\circ}52'30.42''$ N/ $8^{\circ}49'52.97''$ W) on March, 2006; (5) by hand on rock in Caminha, Portugal ($41^{\circ}52'00''$ N/ $8^{\circ}51'15.90''$ W) on March 2021; (6) beam trawl in Caminha ($41^{\circ}52'04.8''$ N $8^{\circ}51'18.8''$ W) on June 2021; (7) in buccal cavity of *Alosa alosa*, captured with trammel fishing in Vila Nova de Cerveira, Portugal ($41^{\circ}55'57.67''$ N/ $8^{\circ}45'33.85''$ W).

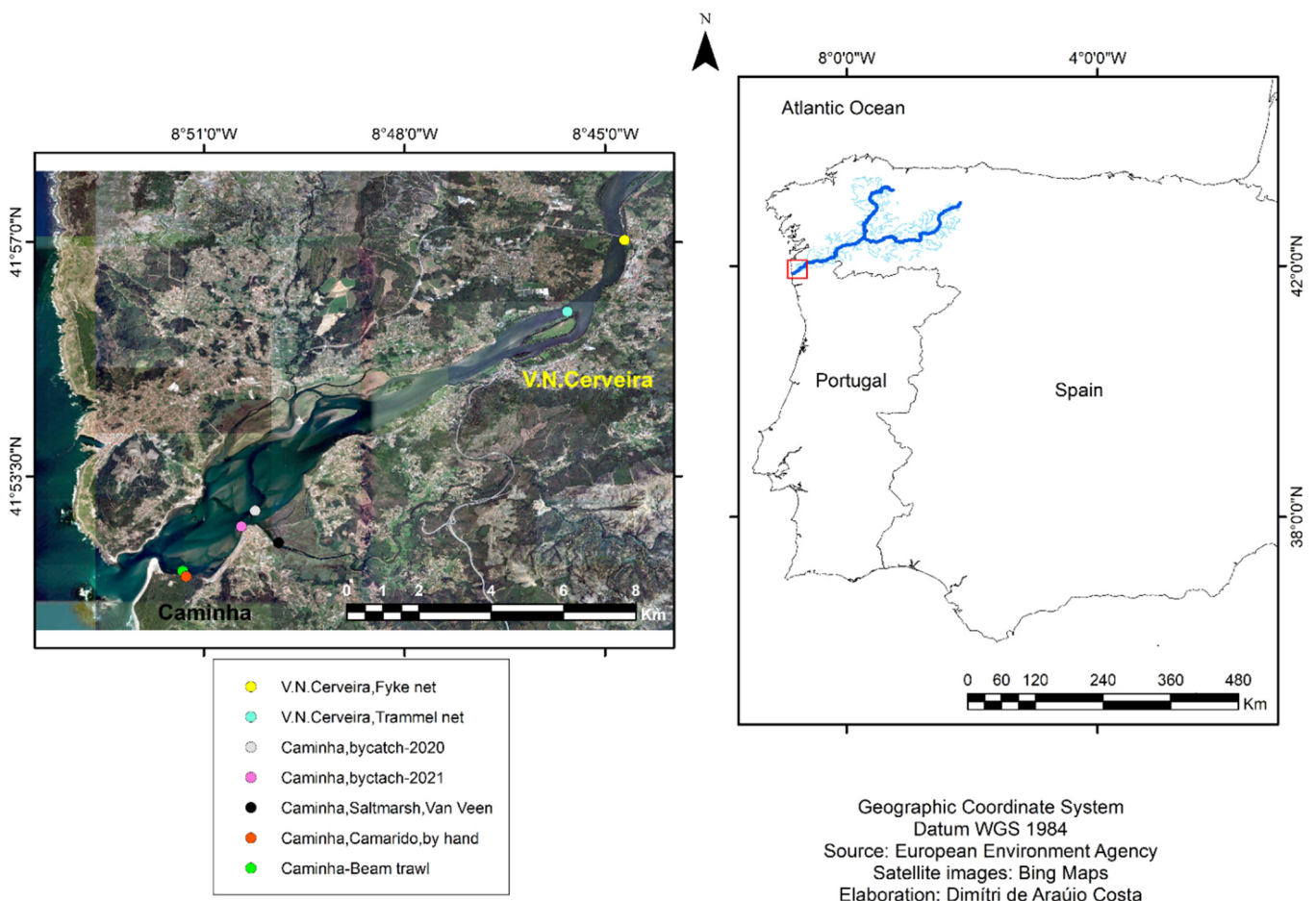


Figure 1. Study area of the international Minho River (northwest Iberian Peninsula), displaying the sampling points and methods. Satellite images adapted from Bing Maps.

Taxonomic identification was performed using specialized literature, e.g., [2,20–31]. Synonym compilation was based on WoRMS (World Register of Marine Species) database [32] and on species descriptions manuscripts. Original information data (material and locality type) data were compiled from GBIF (Global Biodiversity Information Facility) [33] or from

first species descriptions, whenever available. Specimens were analyzed and photographed with a Nikon Digital Sight D5-L1 camera using a Nikon SMZ800 stereomicroscope and a Nikon ECLIPSE 50i microscope. All specimens were deposited at Arthropod Collection from Natural History Museum of the Iberian Peninsula (NatMIP—“Museu de História Natural da Península Ibérica”), at Aquamuseu do Rio Minho, Vila Nova de Cerveira, Portugal.

2.3. DNA Extraction, Amplification and Sequencing

DNA samples were extracted using E.Z.N.A. Mollusc DNA Kit (Omega Bio-tek). Amplification of the mitochondrial gene cytochrome c oxidase I (COI-5P), was performed using the primer pairs LoboF1 and LoboR1 [34]. PCR (Polymerase Chain Reaction) reactions contained 21 µL of VWR company (Monroeville, PA, USA) Red Taq DNA Polymerase Master Mix, 1 µL of each primer (10 mM) and 2 µL of DNA template. The PCR cycling conditions used were: 1 min at 94 °C; 5 cycles of 30 s at 94 °C, 1.5 min at 45 °C, 1 min at 72 °C; 35 cycles of 30 s at 94 °C, 1.5 min at 52 °C, and 1 min at 72 °C; 5 min at 72 °C. PCR products were visualized in a 1% agarose gel and purified using ExoSap at 37 °C for 15 min, followed by 15 min at 80 °C. Bidirectional sequencing provided by service supplier (STAB Vida Ltd., Lisboa, Portugal). Obtained sequences were submitted to GenBank with the following accession numbers *Ceratothoa* aff. *oestroides* OK642789 and *Cymodoce* sp. OK646549.

2.4. Data Analysis

COI-5P sequences were edited and manually aligned using MEGA software (Kumar et al., 2018) and verified for the presence of stop codons, insertions or deletions. Homology searches were performed in BOLD database [35] and with BLASTn [36] in GenBank database [37]. Maximum Likelihood (ML) trees for the genus *Cymodoce* (dataset of 36 sequences (Table S1)) and *Ceratothoa* (dataset of 73 sequences (Table S1)) were constructed with MEGA software using HKY model with Gamma distribution with invariant sites (HKY + G + I), chosen as the best-fit model of nucleotide substitutions by MEGA software. Bootstraps were performed using 10,000 replicates. Mined *Ceratothoa oestroides* sequences GQ240268 to GQ240280, from GenBank, were trimmed on the final 18 base pairs due to the presence of a stop codon followed by a shift on the reading frame. On the mined sequences from *Ceratothoa italica* JN604342 to JN604371 the same pattern was observed, and the final 118 base pairs were also trimmed. As no trace files are available for these public sequences, it is not possible to confirm these as pseudogenes or just errors on sequence editing or during submission to GenBank. Intra and interspecific divergence were calculated with the Kimura-2-Parameter (K2P) model as the standard genetic distance used in DNA barcode studies.

3. Results

A total of 98 specimens were examined belonging to 23 species, six from the order Decapoda, four from Cumacea, three from Mysida, two from Leptostraca, one from Tanaidacea and one from Balanomorpha. An update to the list of isopods from the Minho River [16] was also performed with the addition of six new records.

Subphyllum Crustacea Brünnich, 1772

Superclass Multicrustacea Regier, Shultz, Zwick, Hussey, Ball, Wetzer, Martin & Cunningham, 2010

Class Malacostraca Latreille, 1802

Subclass Eumalacostraca Grobben, 1892

Superorder Eucarida Calman, 1904

Order Decapoda Latreille, 1802

Suborder Pleocyemata Burkenroad, 1963

Infraorder Astacidea Latreille, 1802

Superfamily Astacoidea Latreille, 1802

Family Cambaridae Hobbs, 1942

Genus *Procambarus* Ortmann, 1905

Procambarus clarkii (Girard, 1852, [38]) (Figure 2A,B).

Synonym: *Cambarus clarkii* Girard, 1852: 91 [38]; Hagen, 1870: 39–41, Pl. I–IV [39]; Faxon, 1885: 26–27 [40].

Type material.

Holotype: Two male specimens collected in Monterrey, Mexico, deposited at Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, catalogue number UANL-FCB-C3103984 [33].

Type locality: Texas, USA [38].

Material examined: Six megalopes size range 6 to 6.6 mm, collected at the international Minho River, Vila Nova de Cerveira, 41°57'1.69" N/8°44'42.74" W, 21 December 2020 on fyke nets; deposited as NatMIP-CMDe-0039; 1 adult size 150 mm, collected at the international Minho River, Vila Nova de Cerveira, 41°57'1.69" N/8°44'42.74" W, 6 April 2021 on fyke nets; deposited as NatMIP-CMDe-0032.

Diagnosis: Antenna almost as long as body; rostrum triangular gradually tapering from the base, with two connected tubercles on the basis; anterior pair of pleopods with two rounded tubercles [38,39].

Global distribution: Native to North America and Mexico [41]; introduced on Europe in Portugal, Spain, France, Austria, Cyprus, England, Germany, Italy, Netherlands and Switzerland [42]; on the Middle East in Israel [43]; on Africa in Kenya [44] and Uganda [45]; on South America in Brazil [46]; on Asia in China [47] and Japan [48].

Distribution in Portugal: Species recorded in the rivers Minho [49], Maçãs [50], Douro, Mondego, Sado, Caia [51], Tejo [52], Degebe [53], Alamos [54] and on São Miguel, Azores archipelago [42].

Ecological notes: Freshwater species, burrower, feeds on macrophytes [55].

Infraorder Brachyura Latreille, 1802

Superfamily Portunoidea Rafinesque, 1815

Family Carcinidae MacLeay, 1838

Genus *Carcinus* Leach, 1814

Carcinus maenas (Linnaeus, 1758) (Figure 2C–E).

Carcinus maenas Zariquiey-Alvarez, 1968: 354, Fig. 115A–C [25]; Hayward & Ryland, 2017: 434, Fig. 8.64 [22].

Synonyms: *Cancer maenas* Linnaeus, 1758: 627 [56].

Cancer granarius Herbst, 1790: 107–108, Tab. II, Fig. 28a.A. [57].

Cancer viridis Herbst, 1790: 148, Tab. VII, Fig. 47. [57].

Cancer pygmeus Fabricius, 1787: 320 [58].

Cancer rhomboidalis Montagu, 1804: 65, Tab. VI, Fig. 1 [59].

Cancer granulatus Say, 1817: 61 [60].

Megalopa montagui Leach, 1815: Tab. XVI, Figs. 1–6 [61].

Portunus carcinoides Kinahan, 1857: 13 [62].

Type material: Unknown.

Type locality: Unknown.

Material examined: Six megalopes (size 3.2 mm), collected at the international Minho River, Caminha 41°52'59.00" N/8°50'14.00" W, 6 April 2020 on glass eel fishing bycatch; deposited as NatMIP-CMDe-0036; 4 juveniles, carapace length 12 mm, same data as preceding, deposited as NatMIP-CMDe-0037; 1 adult, carapace length 58 mm collected at the international Minho River, Caminha 41°52'76.00" N/8°50'41.70" W, 14 March 2021 on glass eel fishing bycatch deposited as NatMIP-CMDe-0038.

Diagnosis: Carapace frontal margin with three rounded lobes dactyl of pereopod 5 slightly broader than pereopods 2–4 [22,25].

Global distribution: Native to Northeast Atlantic from Norway to North Africa [22]; introduced in Australia [63], Canada, USA, Japan, South Africa [64] and Argentina [65].

Distribution in Portugal: Common species along the Portuguese coast and estuaries [13,66–68].

Ecological notes: Intertidal species on estuaries, salt marshes and shallow coastal areas [22].

Infraorder Caridea Dana, 1852

Superfamily Atyoidea De Haan, 1849

Family Atyidae De Haan, 1849

Genus *Atyaephyra* de Brito Capello, 1866

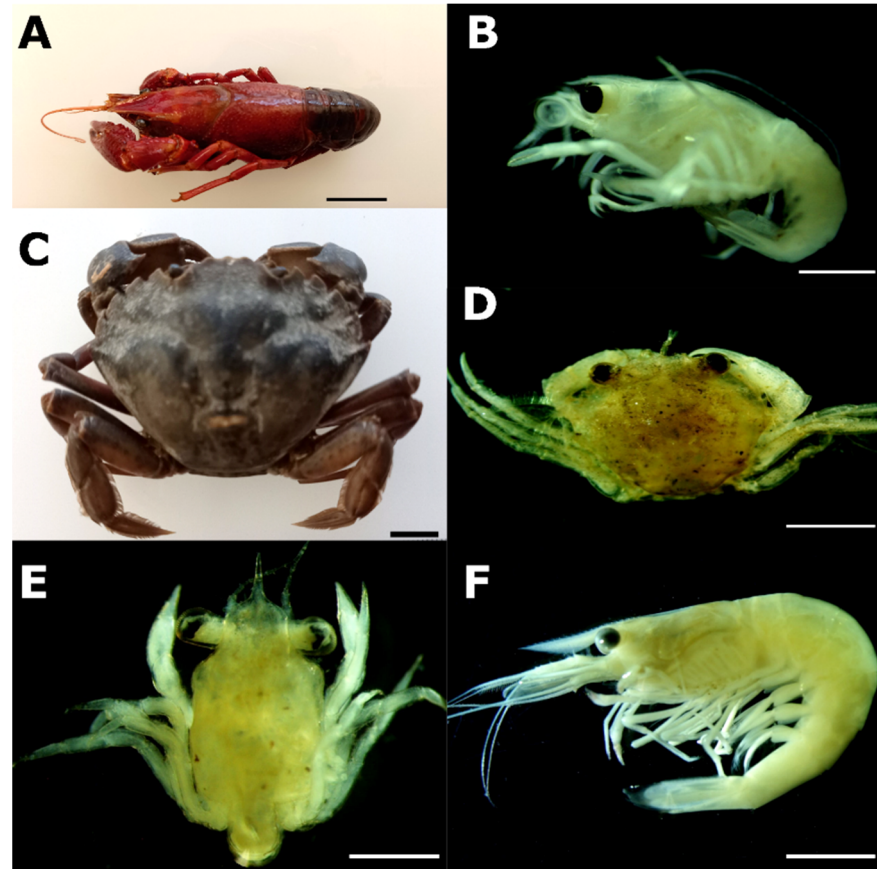


Figure 2. *Procambarus clarkii* (Girard, 1852): adult dorsal view (A), megalope side view (B); *Carcinus maenas* (Linnaeus, 1758): adult dorsal view (C), juvenile dorsal view (D), megalope dorsal view (E); and *Atyaephyra desmarestii* (Millet, 1831): adult side view (F). Scale bars: (A) = 2 cm; (B,E) = 1 mm; (C) = 1 cm; (D,F) = 5 mm.

Atyaephyra desmarestii (Millet, 1831) (Figure 2F).

Atyaephyra desmarestii Zariquiey-Alvarez, 1968: 79–81, Figs. 13C,D and 32A–D [25]; Anastasiadou et al., 2004: 6–13 [69]; Anastasiadou et al., 2006: 1196–1207, Figs. 1–5 [26]; Christodoulou et al., 2012: 66–71 [70].

Synonyms: *Symethus fluviatilis* Rafinesque, 1814: 23–24 [71].

Hippolyte desmarestii Millet, 1831: 56–57, Pl. 1, Fig. 1A,B [72].

Caridina desmarestii Joly, 1843: 46–82, Pl. 3 Figs. 1–78 [73].

Atyaephyra rosiana De Brito Capello, 1866: 6–7, Pl. 1, Fig. 1 [74].

Atyaephyra desmarestii var. *occidentalis* Bouvier, 1913: 65–74, Figs. 1–3 [75].

Type material.

Neotype: One ovigerous female collected in Maine-et-Loire, France, deposited at Muséum National d’Histoire Naturelle, Paris, France (MNHN), catalogue number MNHN-IU-2009-2270 [69].

Type locality: France [71].

Material examined: Six specimens, size range 17 to 27 mm collected at the international Minho River, Vila Nova de Cerveira, 41°57'1.69" N/8°44'42.74" W on fyke nets, deposited as NatMIP-CMDe-0034.

Diagnosis: Rostrum long and slender, slightly curved upwards with 23–28 teeth (2–4 behind eye orbit) on upper margin and 5–10 in the lower margin [26,69].

Global distribution: West Europe and North Africa [69].

Distribution in Portugal: Present on the rivers Minho [13,14], Lima, Cávado, Ave, Douro, Mondego, Tejo and Guadiana [76].

Ecological notes: Omnivorous decapod present in freshwater [69,75].

Superfamily Crangonoidea Haworth, 1825

Family Crangonidae Haworth, 1825

Genus *Crangon* Fabricius, 1798

Crangon (Linnaeus, 1758) (Figure 3A).

Crangon Zariquiey-Alvarez, 1968: 184–187, Figs. 77, and 78A,B [25]; Smaldon, 1993: 114, Fig. 42 [2]; Hayward & Ryland, 2017: 408, Fig. 8.51 [22].

Synonyms: *Cancer crangon* Linnaeus, 1758: 632 [56].

Crangon vulgaris Fabricius, 1798: 410 [77].

Crangon rubro punctatus Risso, 1816: 85–86 [78].

Crangon maculosus Rathke, 1837: 366–368 [79]; Czerniavsky, 1884: 71 [80].

Cancer schillinus Nardo, 1847.

Steiracrangon orientalis Czerniavsky, 1884: 73–75 [80].

Type material: Unknown.

Type locality: Unknown.

Material examined: Five specimens, size range 20 mm to 50 mm, collected at the international Minho River, Caminha 41°52'59.00" N/8°50'14.00" W, 6 April 2020 on glass eel fishing bycatch; deposited as NatMIP-CMDe-0035.

Diagnosis: Rostrum unarmed; carapace with a spine on the anterior median line; pereopod 2 with 0.75 times the length of pereopod 1 propodus; pereopod 2 dactylus with half or more the length of propodus; telson with two pairs of lateral spines [2].

Global distribution: Northeast Atlantic from the Baltic Sea to Northwest Africa and Mediterranean Sea [22].

Distribution in Portugal: Species recorded on the estuaries of the rivers Minho [13,14,81], Lima [66], Cávado [67], Douro, Aveiro, Mira [82], Mondego [83] and Tejo [84], and at Fuzeta beach [85].

Ecological notes: Common on sandy bays and beaches and lower estuarine areas; species with commercial value [2].

Superfamily Palaemonoidea Rafinesque, 1815

Family Palaemonidae Rafinesque, 1815

Genus *Palaemon* Weber, 1795

Palaemon longirostris Milne Edwards, 1837 (Figure 3B,C).

Palaemon longirostris Milne Edwards, 1837: 394–395 [86]; Zariquiey-Alvarez, 1968: 168, Fig. 69B [25]; Smaldon, 1993: 36, Fig. 10 [2]; Hayward & Ryland, 2017: 394, Fig. 8.44 [22].

Synonyms: *Astacus albescens* Pennant, 1812: 25–26 [87].

Palaemon edwardsii Heller, 1863: 265 [88].

Palaemon longipes Fischer, 1872: 421 [89].

Leander longirostris De Man, 1915: 149, Pl. 12, Fig. 3.

Palaemon garciacidi Zariquiey-Alvarez, 1968: 167, Figs. 69C, and 70 [25].

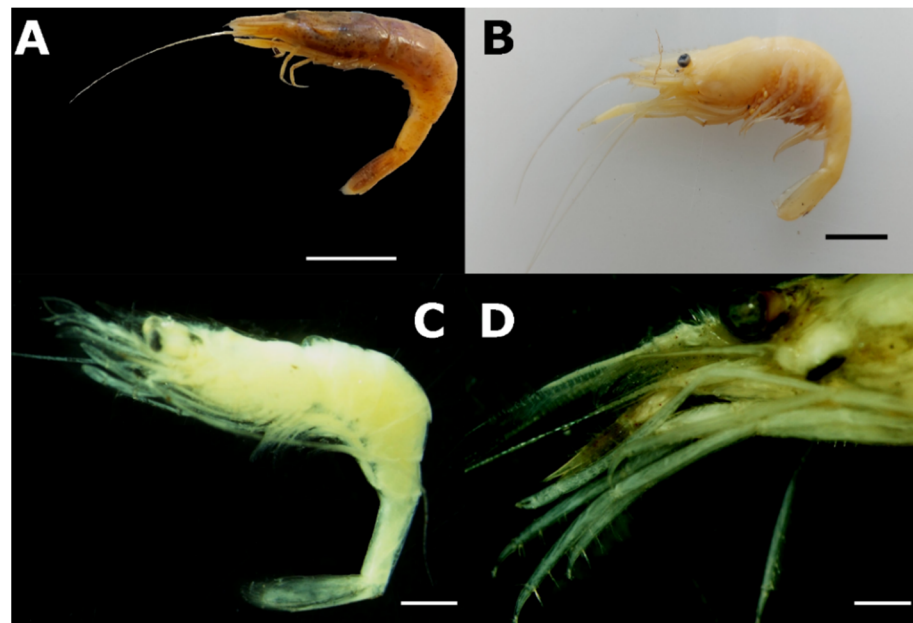


Figure 3. *Crangon crangon* (Linnaeus, 1758): adult side view (A); *Palaemon longirostris* Milne Edwards, 1837: adult side view (B), megalope side view (C); and *Processa modica* Williamson, 1979: adult pereopod 2 detail (D). Scale bars: (A,B) = 1 cm; (C,D) = 1 mm.

Type material.

Syntypes: Six specimens collected on Hollands Diep, Netherlands, deposited at Naturalis Biodiversity Center, catalogue number RMNH.CRUS.D.29725 [33].

Type locality: Unknown.

Material examined: Six megalopes, size range 9.9 to 12.2 mm, collected at the international Minho River, Caminha, 41°52′59.00″ N/8°50′14.00″ W, 6 April 2020; deposited as NatMIP-CMDe-0040 on glass eel fishing bycatch; two adults, one ovigerous female, size range 50 to 60 mm, collected at the International Minho River, Caminha, 41°52′59.00″ N/8°50′14.00″ W, 6 April 2020 on glass eel fishing bycatch; deposited as NatMIP-CMDe-0041.

Diagnosis: Rostrum slightly upcurved with seven to eight dorsal teeth and three to five ventral teeth, two of the dorsal teeth behind the orbit; lower half of the rostrum without red-pigmented spots; pereopod 2 carpus equal to or slightly longer than merus [2,22].

Global distribution: Northeast Atlantic from Germany to Morocco and Mediterranean Sea [22,25].

Distribution in Portugal: Species recorded on the estuaries of the rivers Mira [90], Sado [91] and Tejo [92].

Ecological notes: On estuarine and brackish waters [22].

Superfamily Processoidea Ortmann, 1896

Family Processidae Ortmann, 1896

Genus *Processa* Leach, 1815

Processa modica Williamson, 1979 (Figure 3D).

Processa modica Williamson & Rochanaburanon, 1979: 12–25, Figs. 1, 2, 3F–I, 4, 6A,D,G, and 9D [93]; Smaldon, 1993: 96, Fig. 36 [2].

Type material.

Paratype: Ten specimens on larval stage, collected at Dogger Bank, UK on 8 September 1976, deposited at Natural History Museum of London (NHMUK) catalogue number 1977.150 [33].

Type locality: The Hague, Netherlands [93].

Material examined: One adult specimen, size 12 mm length, collected at the international Minho River, Caminha 41°52′44.80″ N, 8°50′26.25″ W, 14 Mar. 2021 on glass eel fishing bycatch; deposited as NatMIP-CMDe-0047.

Diagnosis: Carapace with an antennal spine, second pair of pereopods equal in length, pereopod 2 merus with 11 segments (Figure 3D) [2,93].

Global distribution: Northeast Atlantic from the Netherlands to the Iberian Peninsula and Mediterranean [2].

Distribution in Portugal: Species recorded along the Portuguese west coast [94].

Ecological notes: Marine; depth range from 0.5 to 100 m; protandrous hermaphrodite [2].

Superorder Peracarida Calman, 1904

Order Cumacea Krøyer, 1846

Family Bodotriidae Scott, 1901

Genus *Iphinoe* Bate, 1856

Iphinoe tenella Sars, 1878 (Figure 4A,B).

Iphinoe tenella Sars, 1878: 505–506, Pls. 15–16 [95]; Stebbing, 1913: 47 [96]; Ledoyer, 1965: 259–260, Pl. II Figs. 2A,B, and 3A,B, Pl. IV Fig. 1A,B, Pl. VI, Figs. 2–3, Pl. VII, Fig. 1, Pl. X, Figs. 2–3, Pl. XII, Fig. 1, Pl. XIV, Figs. 2,3, Pl. XVI, Fig. 1 [27]; Jones, 1976: 22, Fig. 6K–N [23]; Corbera & Garcia-Rubies, 1998: Fig. 4A [97]; Mazziotti & Lezzi, 2020: 347–348 [28].

Type material: Unknown.

Type locality: Siracusa, Italy [95].

Material examined: Six adult specimens, four female (size 11 mm) and two males, size range 9.4 to 10.1 mm, collected at the international Minho River, Caminha 41°52′59.00″ N/8°50′14.00″ W, 6 April 2020 on glass eel fishing bycatch; deposited as NatMIP-CMCu-0049.

Diagnosis: Male and female with teeth on dorsal midline of carapace; antennula with a single aesthasc; antenna as long as the body size on male; telson with six perianal setae [27].

Global distribution: Atlantic Ocean from British isles to the Iberian Peninsula and Mediterranean [22,23].

Distribution in Portugal: Species recorded on Troia [98], Alvor estuary, Lagoa de Albufeira [99] and Ria Formosa [100,101].

Ecological notes: From intertidal to shallow sublittoral, on muddy sand [22].

Iphinoe trispinosa (Goodsir, 1843) (Figure 4C,D).

Iphinoe trispinosa Ledoyer, 1965: 259, Pl. III Fig. 1A,B, Pl. VII Fig. 1, Pl. XI Fig. 1, Pl. XV Fig. 1 [27]; Jones, 1976: 22, Fig. 6A–E [23]; Hayward & Ryland, 2017: 315, Fig. 8.13 [22]; Mazziotti & Lezzi, 2020: 348–351 [28].

Synonyms: *Cuma trispinosa* Goodsir, 1843: 126–127, Pl. II Fig. 19, Pl. III Fig. 1, Pl. IV Fig. 16 [102].

Halia trispinosa Bate, 1856: 459–460, Pl. XIV, Fig. V [103].

Venilia gracilis Bate 1856: 460, Pl. XV, Fig. VII [103].

Type material.

Holotype: Collected at Firth of Forth, Scotland; deposited at Natural History Museum of London (NHMUK), catalogue number 1966.12.16.1 [33].

Type locality: Firth of Forth, Scotland [33].

Material examined: Five adult specimens, four female (size 9 mm) and one male (size 11.2 mm), collected at the international Minho River, Caminha 41°52′59.00″ N/8°50′14.00″ W, 6 April 2020 on glass eel fishing bycatch; deposited as NatMIP-CMCu-0048 and NatMIP-CMCu-0050.

Diagnosis: Female with two to six teeth on dorsal midline of carapace, smooth in male; antennula with a single aesthasc; antenna as long as the body size on male; telson with two perianal setae [22,27].

Global distribution: Northeast Atlantic Ocean from Norway to the Iberian Peninsula and Mediterranean Sea [22].

Distribution in Portugal: Species recorded along the west coast [94,99,104].

Ecological notes: From coastal intertidal up to depths of 150 m, on fine sand [22].

Family Diastylidae Bate, 1856
Genus *Diastylis* Say, 1818

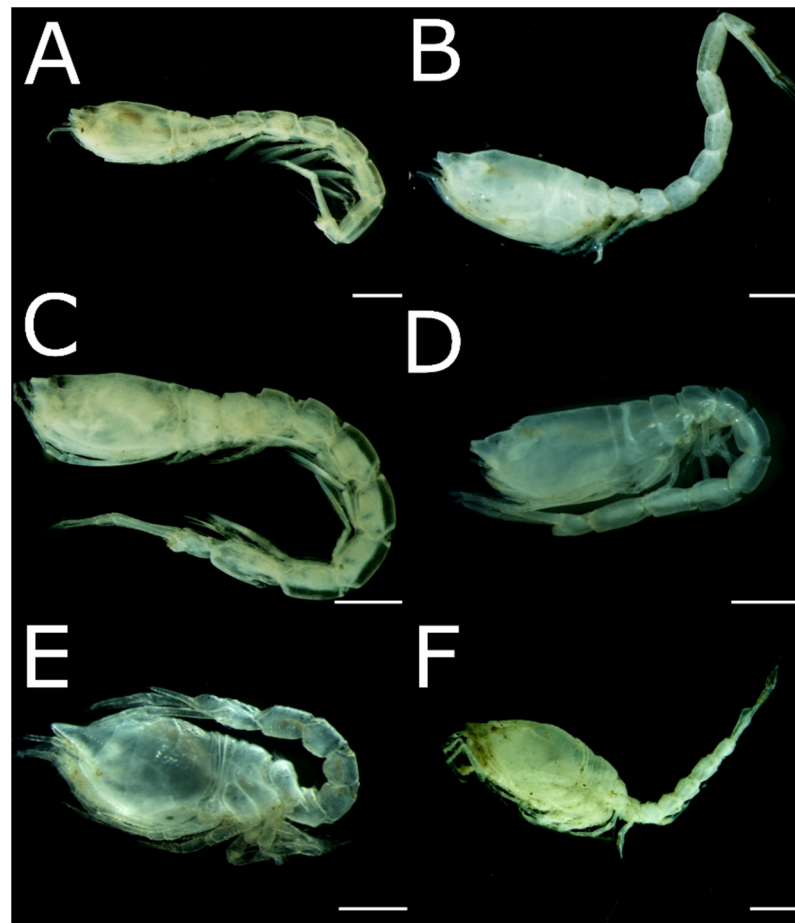


Figure 4. *Iphinoe tenella* Sars, 1878: male side view (A), female side view (B); *Iphinoe trispinosa* (Goodsir, 1843): male side view (C), female side view (D); *Diastylis bradyi* Norman, 1879 (E); and *Diastylis cornuta* (Boeck, 1864) (F). Scale bars: (A–F) = 1 mm.

***Diastylis bradyi* Norman, 1879 (Figure 4E).**

Diastylis bradyi Norman, 1879: 59–60 [105]; Jones, 1976: 52, Fig. 17F–J [23].

Type material: Unknown.

Type locality: Lough Swilty, Donegal, Ireland [105].

Material examined: Six specimens, two male adults, size range 12.8 to 13.5 mm and four female adults, size range 9 to 10.1 mm, collected at the international Minho River, Caminha 41°52′59.00″ N/8°50′14.00″ W, 6 April 2020 on glass eel fishing bycatch; deposited as NatMIP-CMCu-0051.

Diagnosis: Female carapace with denticles; postero-lateral corners of pereonite 5 with acute prolongations; propodus less than twice as long as dactylus [23].

Global distribution: Northeast Atlantic from Skagerak to Bay of Biscay [23].

Distribution in Portugal: Species recorded along the Portuguese southwest coast [99].

Ecological notes: On coarse sediments; depth range 0–30 m depth [23].

***Diastylis cornuta* (Boeck, 1863) (Figure 4F).**

Diastylis cornuta Norman, 1879: 55 [105]; Sars, 1900: 45–47, Pls. XXXVI–XXXVII [106]; Jones, 1976:56, Fig. 19A–C [23].

Synonyms: *Cuma cornuta* Boeck, 1863: 190 [107].

Diastylis bispinosa Sars, 1864: 164–166 [108].

Diastylis cornutus Stebbing, 1913: 96–97, Figs. 55–57 [96].

Type material: Unknown.

Type locality: Unknown.

Material examined: Two male adult specimens, size range 8 to 13.2 mm, collected at the international Minho River, Caminha 41°52'59.00" N/8°50'14.00" W, 6 April 2020 on glass eel fishing bycatch; deposited as NatMIP-CMCu-0052.

Diagnosis: Female carapace with numerous spines with two pairs specially large, reduced to tubercles on males; pleonites with various small spines; telson with 8 to 10 pairs of spines on each side [106].

Global distribution: Northeast Atlantic from Norway to the Iberian Peninsula and Mediterranean [33].

Distribution in Portugal: Species recorded at Portinho da Arrábida [99].

Ecological notes: Marine on soft bottom [104].

Order Mysida Boas, 1883

Family Mysidae Haworth, 1825

Genus *Gastrosaccus* Norman, 1868

Gastrosaccus spinifer (Goës, 1864) (Figure 5A).

Gastrosaccus spinifer Norman, 1892: 154 [109]; Zimmer, 1909: 61, Figs. 104–107 [31]; Makings, 1977: 588, Fig. 2K [30]; Hayward & Ryland, 2017: 323, Fig. 8.18B [22].

Synonyms: *Mysis spinifera* Goës, 1864: 174–175 [110].

Acanthocaris livingstoneana Sim, 1872: 185–186, Pl. IV Fig. B [111].

Gastrosaccus spiniferus Stebbing, 1880: 114–118, Pl. III [112].

Type material: Unknown.

Type locality: Gullmaren, Sweden [110].

Material examined: Six adult specimens, size range 13 to 17 mm, collected at the international Minho River, Caminha 41°52'59.00" N/8°50'14.00" W, 6 April 2020 on glass eel fishing bycatch; deposited as NatMIP-CMMY-0043.

Diagnosis: Dorsal fifth segment with a dorsal ridge forming a spine visible on lateral view [22,30,31].

Global distribution: Northeast Atlantic from Baltic Sea to Northwest Africa and Mediterranean Sea [22].

Distribution in Portugal: Common species along the Portuguese coast and estuaries [12,94,113].

Ecological notes: Benthic on coastal shallow to deep waters [30].

Genus *Neomysis* Czerniavsky, 1882

Neomysis integer (Leach, 1814) (Figure 5B).

Neomysis integer Tattersall & Tattersall, 1951: 399–409, Figs. 109–110 [114]; Makings, 1977: 592, Fig. 2V [30]; Hayward & Ryland, 2017: 330, Fig. 8.19F [22].

Synonyms: *Praunus integer* Leach, 1814: 401–402 [115].

Mysis integer Leach, 1815: Figs. 7–8 [61].

Mysis scoticus Thompson, 1829: 30–31 [116].

Mysis vulgaris Thompson, 1829: 30, Pl. 1 [116].

Neomysis vulgaris Czerniavsky, 1882: Pl. XVIII Figs. 18–22, Pl. XXX Figs. 12–14 [117]; Zimmer, 1909: 166–168, Figs. 368–373 [31].

Type material: Unknown.

Type locality: Lochranza, Scotland [115].

Material examined: Three adult specimens, size range 18.4 to 19.7 mm, collected at the international Minho River, Caminha 41°52'59.00" N/8°50'14.00" W, 6 April 2020 on glass eel fishing bycatch; deposited as NatMIP-CMMY-0042.

Diagnosis: Antennal scale tapering to a point, with setae all round; telson lateral sides converging gradually to a narrow almost pointed apex; uropods less than twice as long; endouropod with a comb of spines on the inner ventral side [22,30].

Global distribution: Northeast Atlantic [22].

Distribution in Portugal: Species recorded at Ria de Aveiro [118], Cávado [67] and Tejo [119] rivers.

Ecological notes: Common in estuaries, brackish pools, on hypersaline pools up to freshwater and all intermediate salinity concentrations; occasionally on the open sea [22,30].

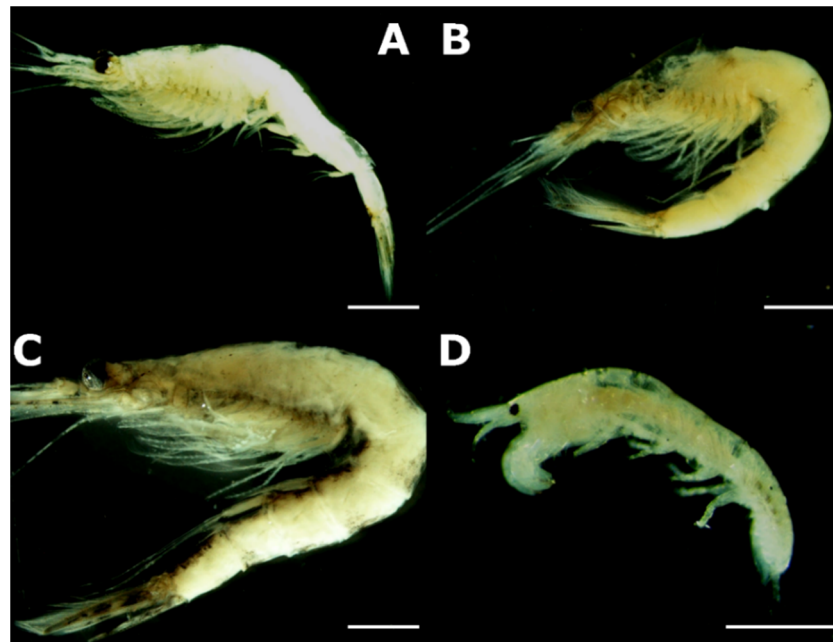


Figure 5. *Gastrosaccus spinifer* (Goës, 1864): side view (A); *Neomysis integer* (Leach, 1814): side view (B); *Praunus neglectus* (Sars, 1869): side view (C); and *Heterotanais oerstedii* (Krøyer, 1842): female side view (D). Scale bars: (A–C) = 2 mm; (D) = 0.5 mm.

Genus *Praunus* Leach, 1814

Praunus neglectus (Sars, 1869) (Figure 5C).

Praunus neglectus Makings, 1977: 591, Fig. 2A–B [30]; Hayward & Ryland, 2017: 326, Fig. 8.17A [22].

Synonyms: *Mysis neglecta* Sars, 1869: 341 [120].

Macromysis neglecta Norman, 1892: 252–253 [109].

Synmysis neglecta Czerniavsky, 1882: 26 [117].

Macromysis nigra Keeble & Gamble, 1904 [121].

Type material: Unknown.

Type locality: Christiania, Denmark [120].

Material examined: Four adult specimens, size range 20 to 29 mm, collected at the International Minho River, Caminha 41°52'59.00" N/8°50'14.00" W, 6 April 2020 on glass eel fishing bycatch; deposited as NatMIP-CMMY-0044.

Diagnosis: Antennal scale five to six times as long as broad; anterior lobe slightly longer than subterminal spine; telson with 18–24 lateral spines [22,30].

Global distribution: Northeast Atlantic [22].

Distribution in Portugal: Species recorded in Ria de Aveiro [122].

Ecological notes: Associated with weeds on shallow waters [30].

Order Tanaidacea Dana, 1849

Suborder Tanaidomorpha Sieg, 1980

Family Leptocheliidae Lang, 1973

Genus *Heterotanais* Sars, 1882

Heterotanais oerstedii (Krøyer, 1842) (Figure 5D).

Heterotanais oerstedii Norman, 1906: 169, Pl. VI Figs. 2–3 [123]; Holdich & Jones, 1983: Figs. 4A,B, and 6A [124]; Barnes, 1994: 169, Fig. 75 [24]; Hayward & Ryland, 2017: 321, Fig. 8.15 [22].

Synonyms: *Tanais oerstedii* Krøyer, 1842: 183–184 [125].

Tanais curculio Krøyer, 1842: 184–185 [125].

Tanais balticus Müller, 1852: 89–90 [126].

Tanais rhynchites Müller, 1852: 88–89 [126].

Heterotanais gurneyi Norman, 1906: 168–169, Pl. V, Pl. VI Fig. 1 [123].

Type material.

Syntypes: Collected in Norfolk, England in 1907; deposited at Natural History Museum of London (NHMUK), catalogue numbers 1956.10.10.104–108 and 1911.11.8.7243–7262 [33].

Type locality: Öresund, Baltic Sea [125].

Material examined: Three female specimens size 1.9 mm, collected at the saltmarsh of the international Minho River, Caminha 41°52'30.42" N/8°49'52.97" W, March 2006 with Van Veen grab sampler; deposited as NatMIP-CMTa-0053.

Diagnosis: Male with anterior part of the carapace abruptly narrower than posterior part; eyes prominent on both sexes; antenna 1 with three articles on female and five on male; uropod exopodite with two articles and endopodite with four [22].

Global distribution: Northeast Atlantic from Norway to northern France [22].

Distribution in Portugal: Species recorded at the Minho River estuary [15], Ria de Aveiro [113] and Tejo estuary [99].

Ecological notes: From coastal to brackish habitats, constructing tubes on algae and hydroids, or on muddy substrates [22,24].

Subclass Phyllocarida Packard, 1879

Order Leptostraca Claus, 1880

Suborder Nebaliacea Calman, 1904

Family Nebaliidae Samouelle, 1819

Genus *Nebalia* Leach, 1814

Nebalia strausi Risso, 1826 (Figure 6A).

Nebalia strausi Risso, 1826: 84–85 [127]; Dahl, 1985: 155–157, Figs. 63–78 [128]; Moreira et al., 2004: 84–92, Figs. 1–6 [129]; Moreira et al., 2009a: 271, Fig. 1E [130]; Moreira et al., 2009b: 101–102, Pl. 1, Figs. 1–3 [29]; McCormack et al., 2016: Fig. 3D [131].

Type material.

Lectotype: One female from Bay of Naples, Italy deposited at Natural History Museum of London (NHMUK) [129].

Type locality: Unknown.

Material examined: Three adult specimens, size range 7.1 to 8.7 mm, collected at the international Minho River, Caminha 41°52'59.00" N/8°50'14.00" W, 6 April 2020 on glass eel fishing bycatch; deposited as NatMIP-CMLe-0046.

Diagnosis: Pleonites 6–7 with distally acute denticles; antennular flagellum with more than 10 articles; antennular scale more than twice as long as wide [29].

Global distribution: Northeast Atlantic from northwest France and British isles to the Iberian Peninsula and Mediterranean Sea [29,131].

Distribution in Portugal: New record (this study).

Ecological notes: On coarse sediments and rhodoliths-environments, at depths of 4–23 m [2,131].

Genus *Sarsinebalia* Dahl, 1985

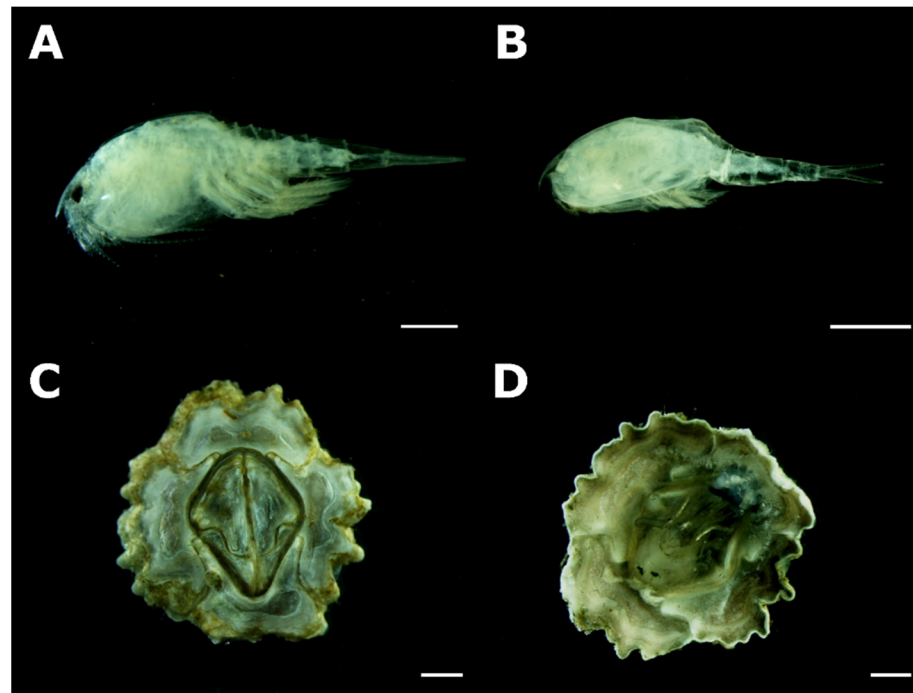


Figure 6. *Nebalia strausi* Risso, 1826: side view (A); *Sarsinebalia cristoboi* Moreira, Gestoso & Troncoso, 2003: side view (B); *Austrominius modestus* (Darwin, 1854): dorsal view (C), and ventral view (D). Scale bars: (A,C,D) = 1 mm; (B) = 2 mm.

Sarsinebalia cristoboi Moreira, Gestoso & Troncoso, 2003 (Figure 6B).

Sarsinebalia cristoboi Moreira et al., 2003: 191–200, Figs. 1–7, 15A [132]; Moreira et al., 2009a: 282, Fig. 1C [29].

Type material.

Holotype: 1 female collected at Ria de Vigo, Spain 42°13'54" N 8°46'30" W on October 2000, 14 m depth; deposited at Museo Nacional de Ciencias Naturales, Madrid, catalogue number MNCN 20.04/5326a [132].

Paratypes: Three females collected at Ria de Vigo, Spain 42°13'54" N 8°46'30" W on October 2000, 14 m depth; deposited at Museo Nacional de Ciencias Naturales, Madrid, catalogue number MNCN 20.04/5326c [132].

Type locality: Ria de Vigo, Spain [132].

Material examined: One adult specimen, size 8.5 mm, collected at the International Minho River, Caminha 41°52'59.00" N/8°50'14.00" W, 6 April 2020 on glass eel fishing bycatch; deposited as NatMIP-CMLe-0045.

Diagnosis: Antennal flagellum with thin long setae; supraorbital scale extending beyond the distal end of the eye; pigmented eye; pleonite 4 with acute denticles [29].

Global distribution: Only known from the west Iberian coast [94,132].

Distribution in Portugal: Species recorded along the Portuguese west coast with a southern limit at Aljezur [94].

Ecological notes: Collected at depths between 9 and 33 m, coarse sand bottoms with a high presence of red non-geniculate calcareous algae (Rhodoliths) [132].

Class Thecostraca Gruvel, 1905

Subclass Cirripedia Burmeister, 1834

Superorder Thoracicalcarea Gale, 2015

Order Balanomorpha Pilsbry, 1916

Superfamily Elminioidea Chan, Dreyer, Gale, Glenner, Ewers-Saucedo, Pérez-Losada, Kolbasov, Crandall & Høeg, 2021

Family Elminiidae Foster, 1982

Genus *Austrominius* Buckeridge, 1983

Austrominius modestus (Darwin, 1854) (Figure 6C,D).

Austrominius modestus Hayward & Ryland, 2017: 302, Fig. 8.8 [22].

Synonyms: *Elminius modestus* Darwin, 1854: 350–351, Pl. 12 Figs. 1A–E [133]; Foster, 1978: 95, Pl. 12C Fig. 57 [134]; Barnes, 1994: 154, Fig. 67 [24].

Elminius sinuatus Hutton, 1879: 328 [135].

Type material.

Lectotype: Collected in New Zealand, deposited at NHMUK catalogue number 1981.274 [33].

Type locality: New Zealand [133].

Material examined: One specimen, diameter 6 mm, collected at the international Minho River, Caminha 41°52'00" N/8°51'15.90" W, March 2021, on rock, deposited as NatMIP-CTBa-0055.

Diagnosis: Shell with four wall plates, low conical shape, orifice diamond-shaped [22,133].

Global distribution: Native to New Zealand and introduced to northwest Europe, present from Britain to south Portugal [22].

Distribution in Portugal: Species recorded at Setúbal, Faro [136] and Madeira island [137].

Ecological notes: Estuarine and costal, intertidal and shallow subtidal, on rocks, shells, algae, other crustaceans and artificial structures [22].

Update to Isopods from the Minho River [14]:

Order Isopoda Latreille, 1817

Suborder Asellota Latreille, 1802

Superfamily Janiroidea Sars, 1897

Family Janiridae Sars, 1897

Genus *Jaera* Leach, 1814

Jaera (Jaera) albifrons Leach, 1814 (Figure 7A,B).

Jaera (Jaera) albifrons Kussakin, 1988: 138–142, Figs. 115–117 [138]; Hayward & Ryland, 2017: 347–348, Fig. 8.25 [22].

Synonyms: *Jaera albifrons* Leach, 1814: 434 [115]; Hansen, 1916:12–13 [139]; Naylor, 1972: 54, Figs. 15A,B, 16A, and 17A [6].

Oniscus marinus Fabricius, 1780: 252 [140].

Jaera nivalis Kröyer, 1838: 303–306, Table IV, Fig. 21 [141].

Jaera marina Sars, 1899: 104–105, Pl. XLIII [142]; Richardson, 1905: 450–451, Figs. 503–504 [143].

Jaera albifrons albifrons Forsman, 1949: 455–456, Figs. 3–4 [144].

Type material: Unknown.

Type locality: Greenland [140].

Material examined: One adult male, size 2.33 mm length, collected the international Minho River, Caminha 41°52'04.8" N 8°51'18.8" W, June 2021 on beam trawl, deposited as NatMIP-CMIs-0054.

Diagnosis: Body oval in females, males smaller than females with eyes relatively large; lateral margins of both sexes fairly sparsely fringed with spines; pereopods 6 and 7 of male with the distal region of carpus extended as a lobe fringed with spines (Figure 7B) [6].

Global distribution: Northeast Atlantic from Russia to France and northwest Atlantic from Greenland to Maine (USA) [6].

Distribution in Portugal: Species recorded along the Portuguese west coast [113,145].

Ecological notes: Common on sheltered shores, occurring often in estuaries usually beneath stones [6].

Suborder Cymothoidea Wägele, 1989

Superfamily Cymothoidea Leach, 1814

Family Cymothoidae Leach, 1818

Genus *Ceratothoa* Dana, 1852

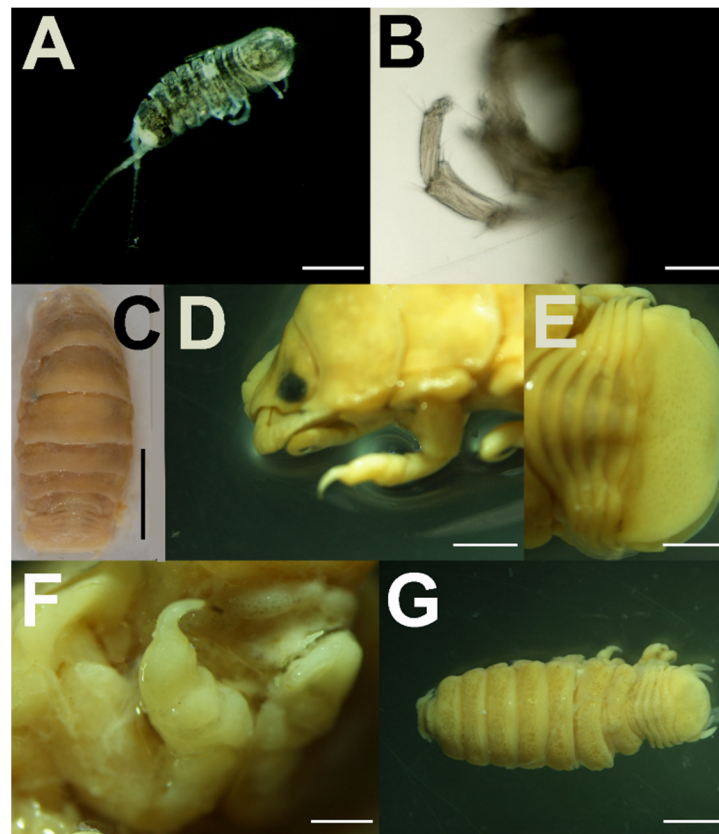


Figure 7. *Jaera (Jaera) albifrons* Leach, 1814: dorsal view (A), pereopod 6 (B); *Ceratothoa* aff. *oestroides* (Risso, 1826): dorsal view (C), cephalon lateral view (D), pleotelson (E), pereopod 1 (F), and juvenile (G). Scale bars: (A) = 0.5 mm; (B) = 0.05 mm; (C) = 1 cm; (D) = 1 mm; (E–G) = 2 mm.

Ceratothoa* aff. *oestroides (Risso, 1826) (Figure 7C–F).

Ceratothoa oestroides Meinert & Schiødte, 1879: 350–356, Table XV (Cym. XXII), Figs. 5–11 [146]; Carus, 1885: 442 [147]; Barrois, 1888: 63 [148]; Sociedad Española de historia natural, 1890: 118 [149]; Gourret, 1891: 14–15, Pl. IV, Figs. 10–11 [150]; Koelbel, 1892: 107, 115 [151]; Graeffe, 1902: 26 [152]; Brian, 1902: 15 [153]; Nobre, 1903a: 61 [154]; Nobre, 1903b: 156 [155]; Trilles, 1991: 122–124 [156]; Horton, 2000: 1047–1048, Fig. 7A–B [9]; Hadfield et al., 2016: 65–69, Fig. 9 [157].

Synonyms: *Canolira oestroides* Risso, 1826: 123 [127].

Cymothoa oestroides Milne Edwards, 1840: 272 [158]; Lucas, 1849: 78, Pl. 8, Fig. 3 [159].

Ceratothoa sargorum Gourret, 1891: 16, Pl. I, Fig. 17, Pl. V, Figs. 1–4 [150].

Meinertia oestroides Nierstrasz, 1915: 89 [160]; Trilles, 1972: 1201–1208, Figs. 90–136, Pl. I, Figs. 6–9, Pl. III, Fig. 20 [161]; Trilles & Raibaut, 1973: 274 [162].

Type material

Holotype: Specimen collected at Nice, France [156].

Syntypes: Two females collected in Nice, France deposited at Musée d’Histoire Naturelle, Paris, catalogue number MNHN-Is431 [9].

Lectotype: One female collected at the Mediterranean Sea, deposited at National Museum of Natural History, Paris, catalogue number MNHN-IU-2014-17478 [157].

Paralectotype: Female specimen deposited at National Museum of Natural History, Paris, catalogue number MNHN-IU-2007-4240 [157].

Type locality: Nice, France [156].

Material examined: One adult female with 30 mm deposited as NatMIP-CMIs-0059 with COI-5P sequence (pereopod 1 missing); one adult female and one juvenile with 30 mm and 9.8 mm deposited as NatMIP-CMIs-0060; one adult male with 26 mm deposited as NatMIP-CMIs-0061; one ovigerous female with 29 mm deposited as NatMIP-CMIs-0062.

All specimens were collected on the mouth of *Alosa alosa* (Linnaeus, 1758) in Vila Nova de Cerveira, International Minho River (41°55'57.67" N/8°45'33.85" W) in May 2013.

Diagnosis: Rostrum acute; pereonite 1 anterolateral angle extending to the middle of the eye (Figure 7D); expansions on merus of pereopods 1–3 (Figure 7F); uropod slightly longer than pleotelson (Figure 7E) [9,157].

Global distribution: Mediterranean Sea and the east Atlantic Ocean from north Africa to France [9,157]

Distribution in Portugal: Species recorded in Póvoa de Varzim, Matosinhos [154], Faro and on Azores archipelago [148].

Ecological notes: Parasitic on fishes: *Boops boops* (Linnaeus, 1758); *Diplodus sargus* (Linnaeus, 1758); *Diplodus annularis* (Linnaeus, 1758); *Diplodus vulgaris* (GeoffroySaint-Hilaire, 1817); *Diplodus bellottii* (Steindachner, 1882); *Spicara maena* (Linnaeus, 1758); *Spicara smaris* (Linnaeus, 1758); *Mullus barbatus barbatus* Linnaeus, 1758; *Trachurus trachurus* (Linnaeus, 1758); *Trachurus mediterraneus* (Steindachner, 1868); *Phycis* (Linnaeus, 1766); *Pagellus acarne* (Risso, 1827); *Pagellus erythrinus* (Linnaeus, 1758); *Sardina pilchardus* (Walbaum, 1792); *Scorpaena notata* Rafinesque, 1810; *Scorpaena porcus* Linnaeus, 1758; *Scorpaena scrofa* Linnaeus, 1758; *Spondylisoma cantharus* (Linnaeus, 1758); *Uranoscopus scaber* Linnaeus, 1758; *Abudefduf saxatilis* (Linnaeus, 1758); *Argyrosomus regius* (Asso, 1801); *Pagellus erythrinus* (Linnaeus, 1758); *Dicentrarchus labrax* (Linnaeus, 1758); *Sparus aurata* Linnaeus, 1758; *Lithognathus mormyrus* (Linnaeus, 1758); *Pomatomus saltatrix* (Linnaeus, 1766); *Rostroraja alba* (Lacepède, 1803); *Zeus faber* Linnaeus, 1758 [163].

Family Gnathiidae Leach, 1814

Genus *Gnathia* Leach, 1814

Gnathia vorax (Lucas, 1849) (Figure 8A,B).

Gnathia vorax Naylor, 1957: 3, Fig. 5A,B [164]; Naylor, 1972: 19, Fig. 4G [6].

Synonyms: *Anceus vorax* Lucas, 1849: 85–86 [159].

Type material: Unknown.

Type locality: Algeria [159].

Material examined: One juvenile (praniza) size 4.73 mm length, collected the international Minho River, Caminha 41°52'59" N 8°50'14" W, May 2020 on glass eel fishing bycatch, deposited as NatMIP-CMIs-0058.

Diagnosis: Male cephalon with central concavity and an acute median projection [165].

Global distribution: North Atlantic from Greenland to the British Isles extending southwards to the Mediterranean [6].

Distribution in Portugal: Species recorded on the Mondego estuary [99] and in Sines [166].

Ecological notes: Pranizas ectoparasitic on fish [167].

Suborder Valvifera Sars, 1883

Family Idoteidae Samouelle, 1819

Genus *Idotea* Fabricius, 1798

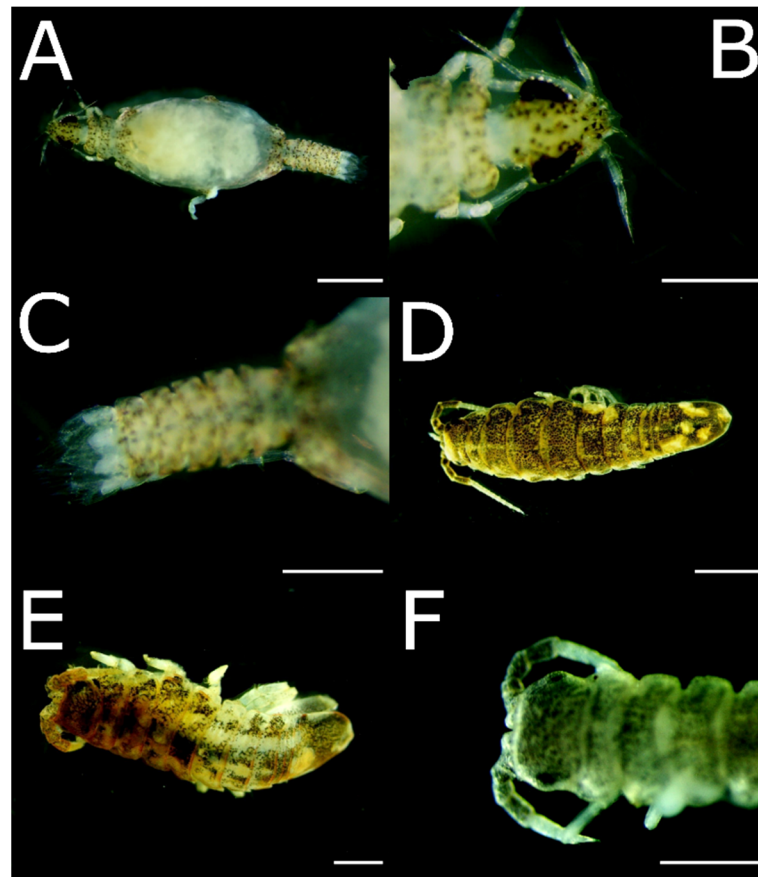


Figure 8. *Gnathia vorax* (Lucas, 1849): dorsal view (A), cephalon (B), pleotelson (C); *Idotea neglecta* Sars, 1897: dorsal view (D); *Idotea pelagica* Leach, 1816: dorsal view (E), and cephalon (F). Scale bars: (A,E) = 1 mm; (B,C,F) = 0.5 mm; (D) = 2 mm.

Idotea neglecta Sars, 1897 (Figure 8C,D).

Idotea neglecta Sars, 1899: 84–85, Pl. XXXV Fig. 1 [142]; Tattersall, 1911: 225–226, Figs. 106–110 [168]; Naylor, 1955: 491–492, Figs. 1, 2, 7, 8, and 11 [165]; Naylor, 1972: 44, Fig. 12A [6].

Type material: Unknown.

Type locality: Norway [142].

Material examined: One specimen, size 8.79 mm length, collected the at international Minho River, Caminha 41°52'59" N 8°50'14" W, May 2020 on glass eel fishing bycatch, deposited as NatMIP-CMIs-0057.

Diagnosis: Body length slightly more than three times the width; antenna 2 flagellum longer than peduncle; antenna 1 reaching the distal end of article 3 of antenna 2 peduncle; pleotelson posterior border laterally rounded with a median obtuse tooth [6,165].

Global distribution: Northeast Atlantic from Norway to France [6].

Distribution in Portugal: Species recorded on Tagus estuary [169] and on Azores archipelago [170].

Ecological notes: Sublittoral on detached algae or fish waste, occasionally on intertidal but also on attached drift algae [6].

Idotea pelagica Leach, 1815 (Figure 8E,F).

Idotea pelagica Leach, 1815a: 365 [171]; Dollfus, 1895: Fig. 23 [172]; Naylor, 1955: 487–489, Figs. 1, 2, 7, 8, and 11 [165]; Naylor, 1972: 44–46, Fig. 13C,F [6]; Hayward & Ryland, 2017: 345, Fig. 8.24 [22].

Synonyms: *Idotea slabberi* Bos, 1874: 35 [173].

Idothea pelagica Sars, 1899: 81–82, Pl. XXXIII [142]; Hansen, 1916: 190 [139].

Type material: Unknown.

Type locality: Scotland [171].

Material examined: Two specimens, size range 2.78 mm to 6.19 mm length, collected the International Minho River, Caminha 41°52'04.8" N 8°51'18.8" W, June 2021 on beam trawl, deposited as NatMIP-CMIs-0056.

Diagnosis: Antenna shorter than other *Idotea* species with flagellum shorter than peduncle; telson rounded or with an obtuse median point on adult specimens [6,165].

Global distribution: Northeast Atlantic from Norway to the Iberian Peninsula [6,174].

Distribution in Portugal: Species recorded along the Portuguese west coast [174].

Ecological notes: Common on exposed shores among barnacles and fucoid algae [6].

Suborder Sphaeromatidea Wägele, 1989

Superfamily Sphaeromatoidea Latreille, 1825

Family Sphaeromatidae Latreille, 1825

Genus *Cymodoce* Leach, 1814

Cymodoce sp. (Figures 9–11).

Material examined: Nine juvenile specimens, size range 2.5 mm to 3.87 mm, one adult female size 6.88 mm length collected the international Minho River, Caminha 41°52'59" N 8°50'14" W, April 2020 on glass eel fishing bycatch, deposited as NatMIP-CMIs-0063 (juvenile and pereopod 1, pleopod 1, antennule and antenna mounted in blades), NatMIP-CMIs-0064 (female and pereopod 1, pleopod 1, antennule and antenna mounted in blades), NatMIP-CMIs-0065 and NatMIP-CMIs-0066; with COI-5P sequence.

Description: Body: Juvenile specimens with uniform color varying bright red to pale greenish yellow (Figure 9A); adult female with orange and yellow bands and molted white spots on pleotelson and most pereonites (Figure 9A,B); head pereon and pleon with a smooth dorsal surface (Figure 9A,B) and pereonite 1 with anterolateral angle extending to the anterior part of the eye on both juvenile and adult female (Figure 9C).

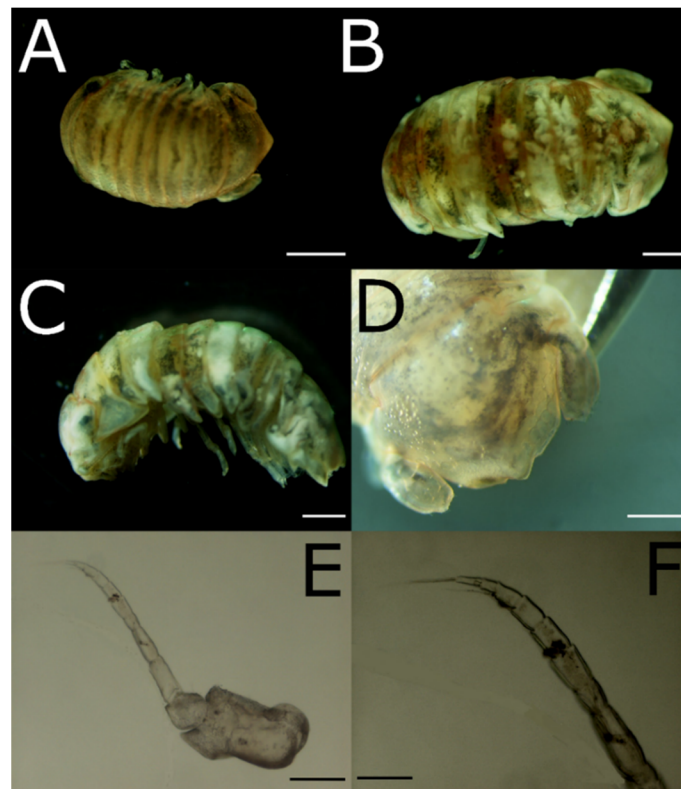


Figure 9. *Cymodoce* sp.: juvenile dorsal view (A), female adult dorsal view (B), female adult lateral view (C), juvenile pleotelson (D), juvenile antennule (E), and juvenile antennule flagellum (F). Scale bars: (A–C) = 1 mm, (D) = 0.5 mm, (E) = 0.2 mm, (F) = 0.05 mm.

Pleotelson: Telson rounded with slightly acute median point (Figure 9D); pleotelson convex on side view (Figure 9C).

Antennule: Juvenile pedunculum with four segments, first segment with an acute corner on inner margin (Figure 9E); flagellum with 7 (Figure 9E) and a large setae on the apex of last segment (Figure 9F); adult female flagellum with 13 segments (Figure 10A,B).

Antenna: Juvenile pedunculum with four segments and flagellum with 10 (Figure 10C); apex of flagellum with three large setae on the apex, posterior end of each segment with a tuft of setae (Figure 10D); adult female pedunculum with four segments and flagellum with 14, posterior end of each segment with a tuft of setae (Figure 10E).

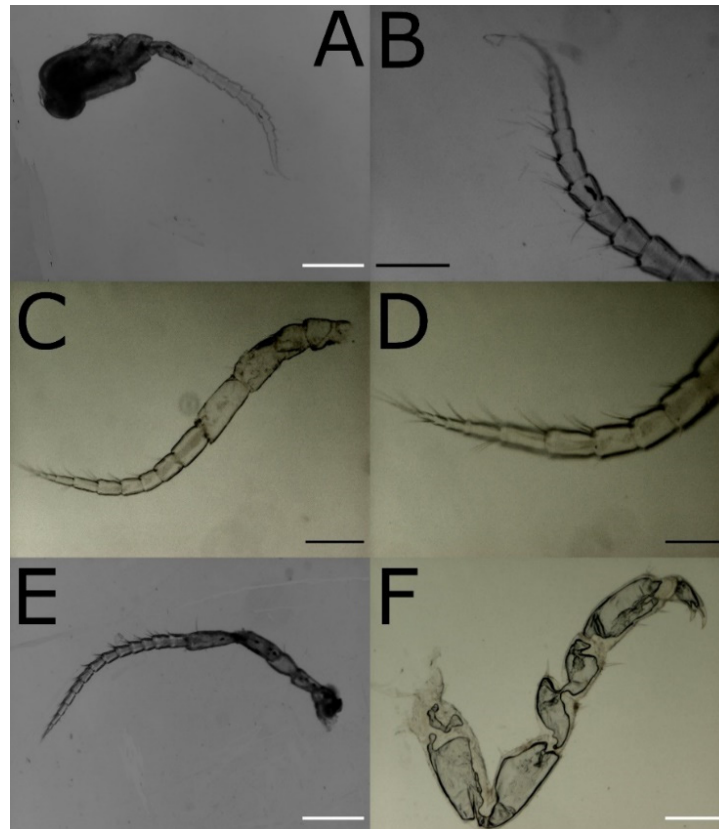


Figure 10. *Cymodoce* sp.: female antennule (A), female antennule flagellum (B), juvenile antenna (C), juvenile antenna flagellum (D) female antenna (E), and juvenile pereopod 1 (F) Scale bars: (A,E) = 0.5 mm; (B) = 0.25 mm; (C,F) = 0.2 mm; (D) = 0.05 mm.

Pereopods: Juveniles with a spine on the middle inner margin of ischium, one spine on each side of the posterior region of merus, one spine on the posterior external margin of carpus, propodus with four spines, one in the middle exterior margin, one on posterior exterior margin and two on posterior inner margin (Figure 10F). Female with four spines on outer margin of carpus (Figure 11A).

Pleopod 1: Juvenile exopod with 19 marginal setae, endopod with 13 on posterior end; anterior margins smooth (Figure 11B), female with smooth basis margins (Figure 11C).

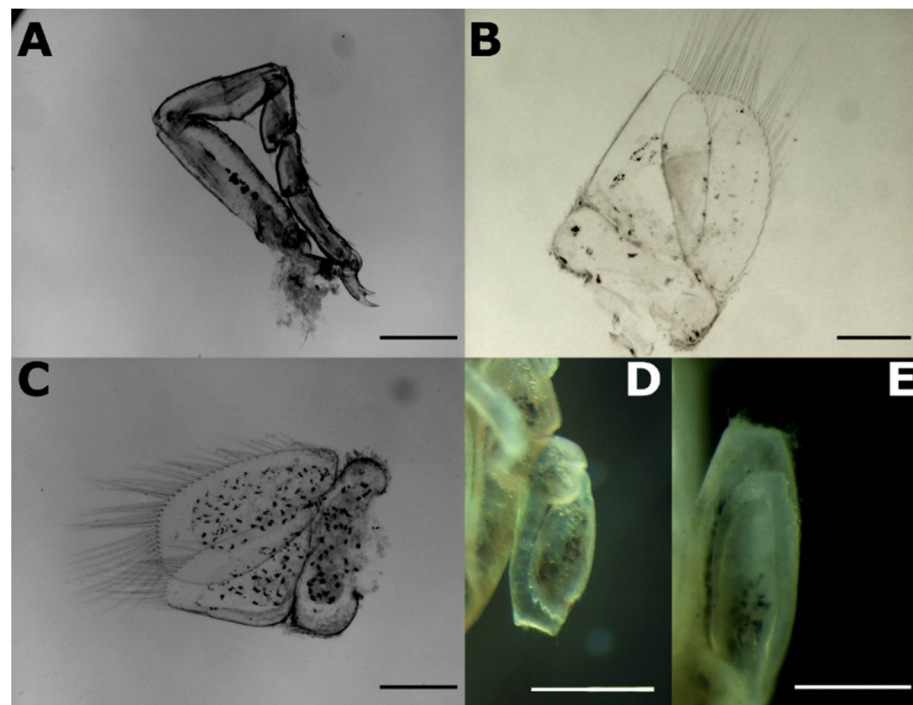


Figure 11. *Cymodoce* sp.: female pereopod 1 (A), juvenile pleopod 1 (B), female pleopod 1 (C), juvenile uropods (D), and female uropods (E). Scale bars: (A,C–E) = 0.5 mm; (B) = 0.2 mm.

Uropod: Juveniles uropods slightly smaller than telson; endopod with marked serrations on inner margin; exopod wither than endopod with subquadrate posterior margin and a slightly rounded inner tip (Figure 11D). Female uropods slightly smaller than telson, endopod with crenulated posterior margin, exopod as juveniles with small crenulations on posterior margin (Figure 10E).

Remarks: The genus *Cymodoce* has six described species for European waters (Atlantic and Mediterranean) [175]. However, antennule, pleopod 1 and uropod shape on both juveniles and female do not seem to match any of the descriptions provided by Dumay [176–179]. The strong acute corner on the inner margin of antennule and pleopod 1 lack of setae and spines on the basis do not match any of the descriptions for females, also juvenile uropod shape has no match with Dumay’s descriptions. As most detailed descriptions are for adult males and none male was examined the identification of these specimens is lacking on this aspect, nonetheless the possibility of this record being for a new species cannot be ruled out.

Genetic Analysis

Both specimens, *Ceratothoa* aff. *oestroides* and *Cymodoce* sp., have not nested on any clade with other publicly available sequences for their respective genera (Figures 12 and 13), nor matched any sequence available on BOLD or GenBank. *Ceratothoa* aff. *oestroides* grouped close to the north Pacific species *Ceratothoa arimae* (Nunomura, 2001), with a sequence divergence of 9.4%, however this branch bootstrap support was 56. In contrast our sequence did not form a group with other *Ceratothoa oestroides* sequences that nested within a clade with *Ceratothoa italica* Schiödte & Meinert, 1883 with an internal sequence divergence of 0.54% (Figure 12). *Cymodoce* sp. grouped closely with a clade formed by sequences of *Cymodoce truncata* Leach, 1814 and *Cymodoce emarginata* Leach, 1818 (sequence divergence of 17%). However, other sequences of *Cymodoce truncata* were placed on other branches (Figure 13).

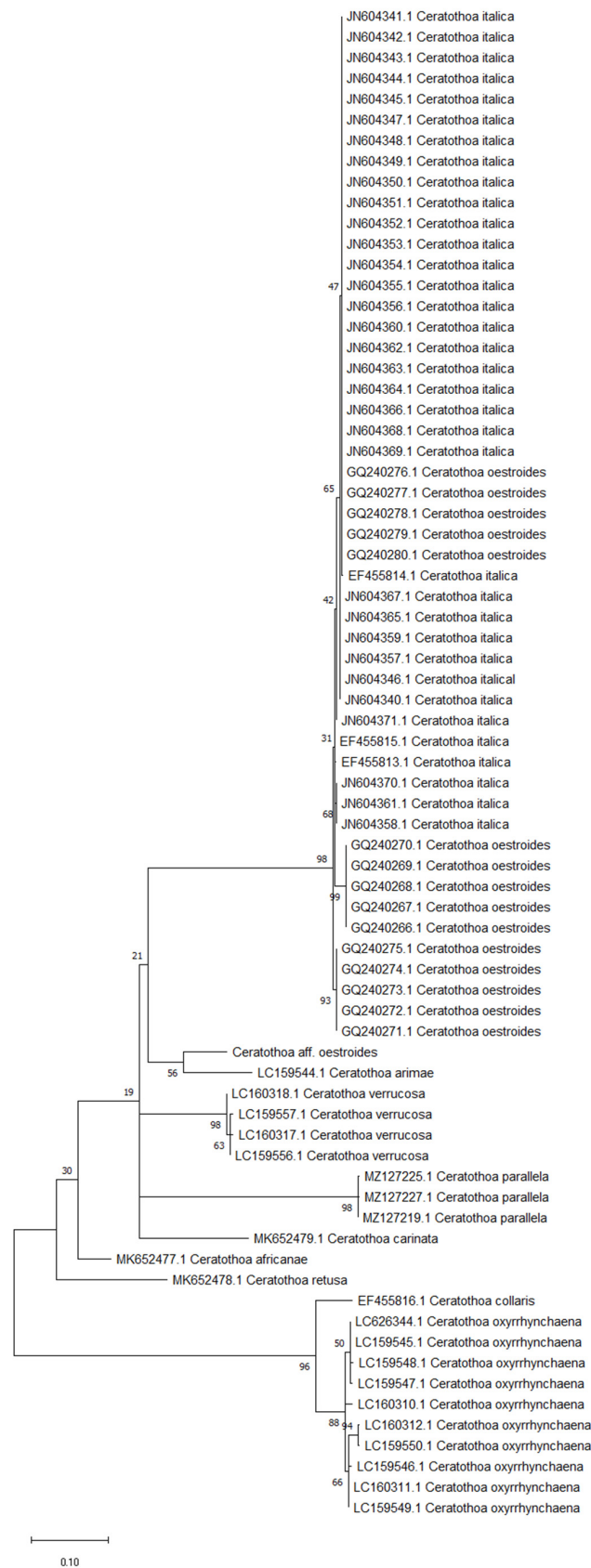


Figure 12. Maximum-likelihood tree obtained from COI-5P sequences of the genus *Ceratothoa*. Value at nodes corresponds to bootstrap support.

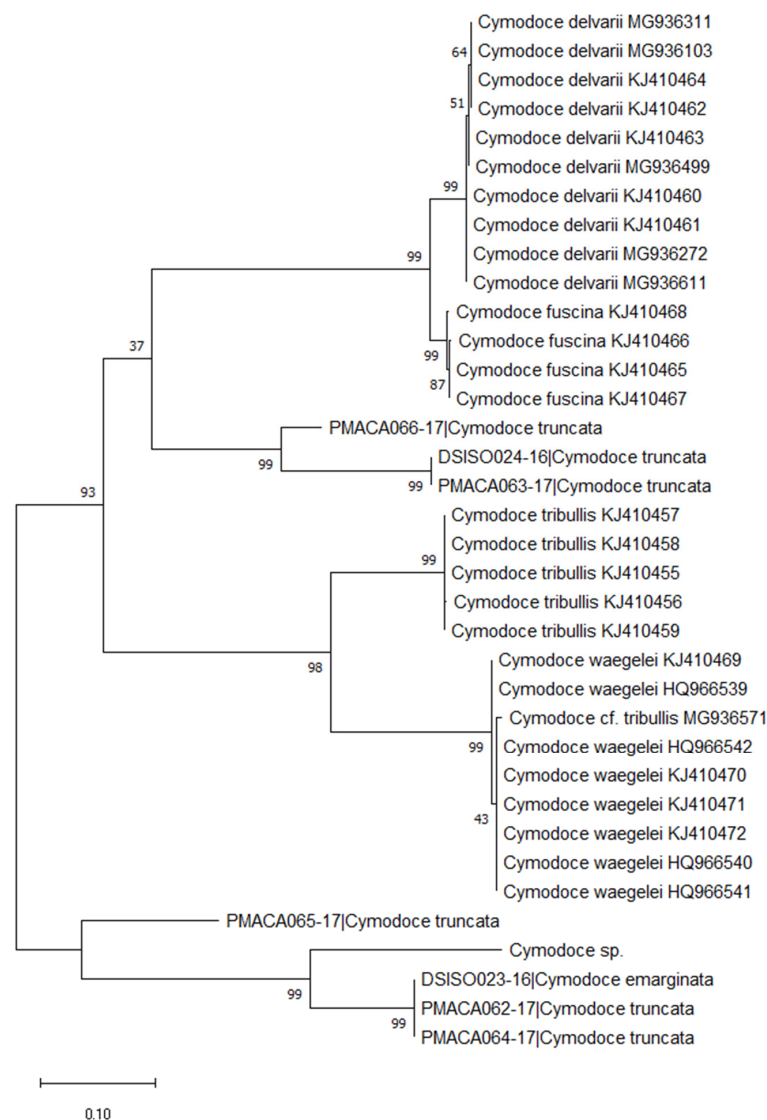


Figure 13. Maximum-likelihood tree obtained from COI-5P sequences of the genus *Cymodoce*. Value at nodes corresponds to bootstrap support.

4. Discussion

From the 23 species collected, six were previously recorded at the Minho River estuary: *Procambarus clarkii* (Girard, 1852), *Carcinus maenas* (Linnaeus, 1758), *Atyaephyra desmarestii* (Millet, 1831), *Crangon crangon* (Linnaeus, 1758), *Gastrosaccus spinifer* (Goës, 1864) and *Heterotanaeis oerstedii* (Krøyer, 1842) [12,13,15,49], with the remaining 17 species being new records (Table S2). Two of those species are exotic to the Minho River *Procambarus clarkia*, introduced in the 90s [49], and *Austrominius modestus*. Decapoda is the most representative group among crustaceans, with freshwater and marine species, while the remaining groups present only marine species on the saltmarsh and near the mouth of the river. Species from the orders Decapoda and Mysida are well documented on the estuary of the Minho River, with four of the six decapod species being already recorded in this area [12,13,49]. Species composition of the order Mysida is similar to assemblages sampled in Ria de Aveiro [113]. Groups like Cumacea, Tanaidacea and Leptostraca are still poorly known in this area.

All species are contained within their documented geographical distribution. *Nebalia strausi* Risso, 1826 was recorded for the first time on Portuguese waters and *Austrominius modestus* (Darwin, 1854) on the northern Portuguese coast expanding the known distribution of this species in Portugal. The first record of parasitism from the isopod *Ceratothoa oestroides* (Risso, 1826) on *Alosa alosa* (Linnaeus, 1758) is also presented, although confirma-

tion on *C. oestroides* identification is still required as COI-5P sequence did not match any of the publicly available sequences for this same species. Although a possible misidentification on the sequences of *C. oestroides* provided by Mladineo et al., 2009 [180] cannot be ruled out as they nest within a clade formed by sequences of *Ceratothoa italica* Schiödte & Meinert, 1883 (internal divergence of 0.54%), provided by different groups of authors (Figure 12). As for *Cymodoce* sp. identification was not possible due to the lack of descriptions and keys for juveniles, being morphologically different from female and male adults, and produced an unmatched COI-5P sequence, requiring a greater effort of adult sampling, identification and posterior DNA barcode sequencing in order to solve problems with juvenile identification or other problematic species in this genus.

Larval stages from *Procambarus clarkii* found in December are in concordance with the findings on Sousa et al., 2013 [49] which placed the presence of juveniles on the Minho River from June to December. The presence of ovigerous females and megalopes of *Palaemon longirostris* also coincides with its known breeding periods (April to August [2]), as well as for the presence of larvae and juveniles of *Carcinus maenas* (starting on March [25]).

5. Conclusions

This investigation represents the first taxonomic study of some crustacean groups from the international Minho River, especially some of the less known groups like Cumacea, Tanaidacea and Leptostraca contributing to the knowledge of the Portuguese and Iberian fauna. The species *Ceratothoa* aff. *oestroides* was found for the first time parasitizing *Alosa alosa*. *Nebalia strausi* had its first record on Portuguese waters. For the genus *Cymodoce* morphological and molecular approaches are recommended in order to solve its problematic species status and assign juveniles and females to the respective male descriptions as those are still lacking. Despite the number of sampling methodologies employed glass eel fishing bycatch seems to be the most efficient method for sampling marine adventitious crustacean fauna, with the possibility of the appearance of other marine species if the sampling efforts were greater and regular over time. To fully understand the composition and distribution of crustaceans on the estuary of the international Minho River, different sampling methods are required concerning different habitats and assemblages.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/hydrobiology1010005/s1>, Table S1: Genbank accession numbers and BOLD sequence ID for sequences used in genetic analysis; Table S2: Examined species list (* new records for the Minho river, NW Iberian Peninsula).

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