Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
www.hcmr.gr
DOI: https://doi.org/10.12681/mms.41784

The spread of *Aoroides longimerus* Ren & Zheng, 1996 across the Mediterranean and the Atlantic: genetic diversity, anthropogenic transport, and ecological implications

José Manuel GUERRA-GARCÍA[1], Andrea DESIDERATO[2], Serena MUCCIOLO[2], Carlos NAVARRO-BARRANCO[1], Macarena ROS[1], Jasmine FERRARIO[3], Paula MORETTI[3,4], Agnese MARCHINI[3], Nawfel MOSBAHI[5], Sofía RUIZ-VELASCO[1], Ignacio GESTOSO[6,7,8], Patrício RAMALHOSA[7,9], João CANNING-CLODE[7,8,9], Celia OLABARRIA[10], Eva CACABELOS[7,11], Jesús S. TRONCOSO[10], Jean-Philippe PEZY[12], Aurore RAOUX[12], Jean-Claude DAUVIN[12], Mathilde CHARBONNELLE[12], Ferdinand SCHLICKLIN[12], Hiroyuki ARIYAMA[13], Emanuele MANCINI[14,15,36], Andrea BONIFAZI[16], Vincent LE GARREC[17], Thomas BUREL[18], Benoit GOUILLIEUX[19], Cristina ESPÍRITO SANTO[20], Paula CHAINHO[21], Romeu S. RIBEIRO[21,22,23], Inês AFONSO[21], Yanrong WANG[24], Jean-Charles LECLERC[25], Marine MOAL[25], Céline HOUBIN[26], Frédérique VIARD[27], Eric THIÉBAUT[25, 26], Victoria FERNANDEZ-GONZALEZ[28], Sandra NAVARRO-MAYORAL[29], Sonia DÍAZ-VERGARA[29], Arjan GITTENBERGER[30], Lauren Elizabeth HUGHES[31], Maria LAMPA[32,33], Giorgos CHATZIGEORGIOU[34], Wanda PLAITIS[34], Francesco TIRALONGO[35,36], Gemma MARTÍNEZ-LAIZ[1], M. Pilar CABEZAS[37,38], Sofia DUARTE[37,38], João P. R. PINHEIRO[37,38], Giovanna O. REIS[2,39], Triana REVANALES[40], Juan SEMPERE-VALVERDE[1,41,42], Eva AYLAGAS[41], Angelo POLISENO[41], Sahar CHEBAANE[7,41,42], Susana CARVALHO[41], Vasilis GEROVASILEIOU[34,43], Inmaculada FRUTOS[2,44] and Pablo SAENZ-ARIAS[1]

[1]Laboratorio de Biología Marina, Department of Zoology, Faculty of Biology, University of Seville, Spain
[2]Department of Invertebrate Zoology and Hydrobiology, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland
[3]Department of Earth and Environmental Sciences, University of Pavia, 27100 Pavia, Italy
[4]Departamento de Biologia Animal (Laboratório de Interações entre Comunidades Marinhas), Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brasil
[5]Laboratoire de Biodiversité Marine et Environment, Faculté des Sciences de Sfax, Université de Sfax, BP 1171, 3038, Sfax, Tunisia
[6]Marine Research Institute (INMAR), Department of Biology, Faculty of Marine and Environmental Sciences, University of Cádiz, Puerto Real, Cádiz, Spain
[7]MARE – Marine and Environmental Sciences Centre / ARNET - Aquatic Research Network, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação, Funchal, Madeira, Portugal
[8]Smithsonian Environmental Research Centre, Edgewater, MD, USA
[9]Faculty of Life Sciences, University of Madeira, Funchal, Madeira Island, Portugal
[10]CIM - Centro de Investigación Mariña and Departamento de Ecoloxía e Bioloxía Animal, Facultade de Ciencias do Mar, Universidade de Vigo, Spain
[11]Centro Oceanográfico de Vigo, Instituto Español de Oceanografía, CSIC, Subida a Radio Faro, 50, 36390 Vigo, Spain
[12]UNICAEN, Laboratoire Morphodynamique Continentale et Côtière, CNRS UMR 6143 M2C, Normandie Univer., 24 rue des Tilleuls, 14000 Caen, France
[13]Osaka Museum of Natural History, Nagai Park, Higashi-Sumiyoshi, Osaka 546-0034 Japan
[14]Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, DiSTeBA, University of Salento, 73100, Lecce, Italy
[15]National Biodiversity Future Center, 90100 Palermo, Italy
[16]Dipartimento Stato dell' Ambiente, ARPA Lazio, 00173 Rome, Italy
[17]Univ Brest, CNRS, IRD, Observatoire, IUEM, F-29280 Plouzané, France
[18]Univ Brest, CNRS, IRD, Ifremer, LEMAR, IUEM, F-29280 Plouzané, France
[19]Université de Bordeaux, CNRS, Bordeaux INP, EPOC, UMR 5805, 33600 Pessac, France
[20]MARE - Marine and Environmental Sciences Centre, Aquatic Research Network, Laboratório de Ciências do Mar, Escola de Ciências e Tecnologia, Universidade de Évora, 7521-903 Sines, Portugal
[21]MARE - Marine and Environmental Sciences Centre, Aquatic Research Network, Faculdade de Ciências, Universidade de Lisboa Campo Grande, 1749-016 Lisboa, Portugal
[22]Escola Superior de Tecnologia de Setúbal, Instituto Politécnico de Setúbal, Estefanilha, 2910-761 Setúbal, Portugal
[23]MARE - Marine and Environmental Sciences Center, Aquatic Research Network, Polytechnic Institute of Setúbal. IPS Campus - Estefanilha. 2910-761 Setúbal, Portugal
[24]Laboratory of Marine Organism Taxonomy and Phylogeny, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[25]Sorbonne Université, CNRS, Station Biologique de Roscoff, UMR7144, Place Georges Teissier, 29680 Roscoff, France
[26]Sorbonne Université, CNRS, OSU STAMAR, UAR 2017, Station Biologique de Roscoff, Place Georges Teissier, 29680 Roscoff, France

[27]ISEM, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France
[28]Department of Marine Science and Applied Biology, University of Alicante, Carretera San Vicente del Raspeig s/n, 03690, Alicante, Spain
[29]Universidad de Las Palmas de Gran Canaria, IU-ECOAQUA, Biodiversidad y Conservación, Parque Científico Tecnológico Marino, 35200 Telde, Las Palmas, Spain
[30]GiMaRIS, Rijksstraatweg 75, 2171 AK Sassenheim, The Netherlands
[31]Life Science Department, Natural History Museum, London, Cromwell Road, South Kensington, United Kingdom
[32]Hellenic Center for Marine Research, Institute of Oceanography, Thalassocosmos, Gournes, Heraklion, Crete, Greece
[33]Marine Ecology Laboratory, Department of Biology, University of Crete, 70013 Heraklion, Greece
[34]Hellenic Center for Marine Research, Institute of Marine Biology Biotechnology and Aquaculture, Thalassocosmos, Gournes, Heraklion, Crete, Greece
[35]Department of Biological, Geological, and Environmental Sciences, University of Catania, Catania, Italy
[36]Ente Fauna Marina Mediterranea, Avola, Italy
[37]Centre of Molecular and Environmental Biology and Aquatic Research Network, Department of Biology, University of Minho, Campus de Gualtar, Braga, Portugal
[38]Institute of Science and Innovation for Bio-Sustainability, University of Minho, Campus de Gualtar, Braga, Portugal
[39]Programa de Pós-Graduação em Biologia Animal, Laboratório de Carcinologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brasil
[40]Departamento de Biología, Instituto Universitario de Investigación Marina, Universidad de Cádiz, Campus Universitario de Puerto Real, 11510, Puerto Real, Cádiz, Spain
[41]Biological and Environmental Sciences and Engineering Division, King Abdullah University of Science and Technology, Thuwal 23955-6900, Saudi Arabia
[42]Notre Grand Bleu, Résidence Kairouan Cap marina Monastir, Monastir 5000, Tunisia
[43]Department of Environment, Faculty of Environment, Ionian University, Zakynthos, Greece
[44]Institut de Systématique, Évolution, Biodiversité ISYEB - UMR 7205 - Muséum national d'Histoire naturelle CNRS, MNHN, UPMC, EPHE, Sorbonne Université, 57 rue Cuvier, CP 26, 75005 Paris, France

Corresponding author: José Manuel GUERRA-GARCÍA; jmguerra@us.es

Contributing Editor: Argyro ZENETOS

Received: 12 June 2025; Accepted: 18 September 2025; Published online: 17 October 2025

Abstract

Accurate knowledge of the global distribution of non-indigenous species (NIS) is essential for understanding their invasion dynamics and for implementing timely management measures. This study reports the first records of the marine amphipod *Aoroides longimerus*, putatively native to the East Asian coast, in Italy (2018), Spain (2019) and Tunisia (2022) and provides the earliest documented record of the species in its introduced European range (Portugal, 2011). Furthermore, it expands the species' known distribution in mainland Portugal (including the northernmost record), Macaronesia (with first records in the Canary Islands and Madeira), and confirms its presence along the Atlantic coasts of France and the Netherlands. Aquaculture facilities, particularly those associated with oyster farming, are probably the primary vector of introduction, while recreational boating may have contributed significantly to secondary dispersal. In comparison to other exotic amphipods, *A. longimerus* remains absent from many marinas, ports and/or aquaculture facilities in the Mediterranean Sea and adjacent regions, such as the Red Sea. This suggests that the species may still be in the early stages of expansion, or that its dispersal ability across marinas and ports is more limited than that of other amphipods. The present study, however, highlights several key ecological traits of *A. longimerus*: (i) it can survive year-round despite seasonal fluctuations, and reach high local densities, (ii) it exhibits strong colonisation capacity, as shown by its rapid establishment on settlement plates, and (iii) it displays opportunistic feeding behaviour, primarily consuming detritus. These characteristics underscore the importance of continuous surveillance and effective communication with stakeholders to prevent further expansion of this species.

Keywords: early detection; non-indigenous; Amphipoda; taxonomic expertise; scientific collaboration; aquaculture; recreational boating.

Introduction

To properly understand how non-indigenous species (NIS) affect marine ecosystems, comprehensive knowledge of their global distribution is essential (Marchini & Cardeccia, 2017; Sun *et al*., 2017). Unfortunately, the lack of biogeographical information and historical records, funding constraints, long detection and reporting time lags (Zenetos *et al*., 2019), limited long-term monitoring programmes, and scarcity of taxonomy experts prevent adequate NIS management (Guerra-García *et al*., 2023 and references therein). Although most efforts have been focused on conspicuous or medium and large-sized organisms with evident economic and/or ecological impacts (Cuthbert *et al*., 2021), interest in updating the distribution of smaller vagile invertebrates, such as amphipods, has increased in recent years.

Among vagile fauna, peracarid crustaceans represent an ecologically highly relevant group due to their widespread occurrence and sensitivity to environmental changes. For instance, they have been proposed as a target group for assessing levels of biocontamination (Saenz-Arias *et al*., 2022a). Within peracarids, amphipods stand out as one of the most interesting taxa due to their dominance in marine habitats, their ability to inhabit fouling communities, and their potential to be transported on vessel hulls (Bell, 1991; Arfianti & Costello, 2020; Martínez-Laiz *et al*., 2022; Saenz-Arias *et al*., 2022b). Recent collaborative efforts have been undertaken to update the distribution of these small, often overlooked amphipods which have been quickly spreading throughout their introduced range (e.g., Beermann *et al*., 2020; Martínez-Laiz *et al*., 2020; Guerra-García *et al*., 2023).

Aoroides longimerus was originally described by Ren & Zheng (1996) from the coast of China. Later, Ariyama (2004) recorded this species along the Japanese coast. Since all species within the genus *Aoroides* have been described from coastal regions of the Pacific Ocean (WoRMS Editorial Board, 2025), this region can be considered its native distribution range. Furthermore, Ariyama (2004) proposed that *Aoroides* may have originated from the genus *Aora* Krøyer, 1845 in the Southwest Pacific and then dispersed northward before spreading into the East Pacific. To our knowledge, *A. longimerus* has only been recorded in China and Japan within its native range.

Outside this putative native range, Gouillieux *et al.* (2015) found specimens of *A. longimerus* from subtidal slipper limpet *Crepidula fornicata* Linnaeus, 1753 beds and *Zostera marina* Linnaeus, 1753 meadows in Arcachon Bay, as well as from floating pontoons in the Bay of Brest, western France, in 2013 and 2014. They also reported two other *Aoroides* species in European waters, namely *A. semicurvatus* Ariyama, 2004 and *A. curvipes* Ariyama, 2004 in Arcachon Bay, located in the southern Bay of Biscay. This was the first record of the genus *Aoroides* in European marine waters. *Aoroides longimerus* was also detected in the Azores in 2013 (Castro *et al*., 2022) and was first recorded in the Mediterranean in 2015, inhabiting hull fouling in Port Camargue marina, France (Ulman *et al*., 2017). More recent records in the NE Atlantic include mainland Portugal in 2019 (CIEMAR, 2021) and the eastern English Channel, France (Dauvin *et al*., 2020). In 2020 and 2021, several specimens were also found inhabiting fouling communities in Delta marina and in Geersdijk harbour in the southern Netherlands (Faasse & Gittenberger, 2021). These scattered reports of *A. longimerus* in Europe outside the native range, covering extensive parts of Europe, have raised important questions about the true extent of its distribution in the Atlantic Ocean and the Mediterranean Sea, as well as the invasion dynamics that might be occurring in other regions. The exchange of information on the occurrence of *A. longimerus* among researchers has encouraged the re-examination of previously collected samples and motivated new sampling efforts, to better understand whether the species is spreading across the Mediterranean, the East Atlantic and elsewhere globally.

Building upon this collaborative approach, the present study specifically aims to: (i) update the global distribution of *A. longimerus*, including new records, (ii) provide taxonomic and ecological knowledge, including morphological and genetic data, from both native and introduced populations; (iii) characterise its diet (as a proxy for feeding habits); and (iv) discuss its invasion dynamics, potential introduction and spreading vectors.

Material and Methods

Sampling surveys and species identification

Several sampling surveys were carried out by different research teams between 1964 and 2024 (Table 1, Table S1). The geographic coverage of these surveys spanned across the Atlantic Ocean (Netherlands, France, Portugal and Spain, including the Macaronesian Islands), the Mediterranean Sea (Spain, Morocco, France, Tunisia, Italy, Croatia and Greece), the Red Sea (Saudi Arabia), and the Indo-Pacific Ocean (Saudi Arabia, Japan and Australia). Samples were mostly collected from artificial substrates in marinas (mainly pontoons, ropes and buoys), but also in ports, on boat hulls, in aquaculture facilities, and occasionally from natural habitats, such as subtidal or shingle beaches and coral reefs. Different sampling techniques were used, including the collection of biogenic substrates (basibionts), scrapings of fouling communities, deployment of PVC settlement plates, and Rapid Assessment Surveys (RAS) among others. After collection, most samples were fixed with 70-96% ethanol (occasionally with 4% formalin seawater solution), sieved with a mesh size between 0.5 and 1 mm, sorted and examined for the presence of *A. longimerus*. In some cases, DNA metabarcoding approach was used to screen for the presence of *A. longimerus* from environmental samples including biofouling and water samples (see Text S1 for a detailed description of all sampling techniques by country/area).

Morphological identification of *Aoroides* specimens was based on key characters established by Barnard & Karaman (1991): (i) accessory flagellum of antenna 1 absent or vestigial, (ii) mandibular palp article 3 rectilinear (except for *A. palfreyensis* Myers, 2009 and *A. vitiosus* Myers, 1995, which lack a mandibular palp), (iii) gnathopod 1 of males merochelate, (iv) gnathopod 2 not carpochelate, and (v) uropod 3 biramous (see Gouillieux *et al*., 2015). The diagnostic characters for the species *A. longimerus* were based on Ren & Zheng (1996) and Ariyama (2004) (see also taxonomic remarks in the discussion section). It is important to note that the keys for identifying *Aoroides* species are currently proposed for

Table 1. Global distribution records of *Aoroides longimerus* Ren & Zheng, 1996. Records from its putative introduced range are shaded. The earliest record for the country is marked in bold. Asterisk (*) indicates the presence of at least one DNA sequence in this study. Atl: Atlantic coast; Med: Mediterranean coast.

Date	Country	Localities	Habitat/substrates	References
Aug 1979-Aug 1980	**Japan**	Kodomari, Takahama Town, Fukui Prefecture	Alga *Sargassum* sp.	Ariyama, 2022; present study
Jul 1981-Mar 2021	Japan	Hannan City, Osaka Bay, Osaka Prefecture	Algae, pontoon, artificial seaweed	Ariyama, 2022; present study
Apr 1982-Mar 2000	Japan	Misaki Town, Osaka Bay, Osaka Prefecture	Algae, sponges, bryozoans, hydrozoans, experimental blocks, artificial seaweed, sandy-mud bottom	Ariyama, 2022; present study
9 Jul 1988	Japan	Off Tanigawa in Misaki, Osaka Prefecture (34.31667º N, 135.11667º E)	Bryozoans, 7 m depth	Ariyama, 2004
10 May 1989	Japan	Off Tanigawa in Misaki, Osaka Prefecture	Brown alga *Sargassum filicinum*	Ariyama, 2004
28 Apr 1991	Japan	Outside of the mouth of the Onosato River in Han 'nan, Osaka Prefecture	Pebble beach	Ariyama, 2004
1 Jul 1992	**China**	Dayawan, Guangdong province (TYPE LOCALITY)	Fouling communities	Ren & Zheng, 1996; Ren, 2006
18 Mar 1994	Japan	Off Tanigawa in Misaki, Osaka Prefecture	Ascidian *Halocynthia dumosa*, 5 m depth	Ariyama, 2004
28 Aug 1995	Japan	Off Tanigawa in Misaki, Osaka Prefecture	Hydroid *Aglaophenia whiteleggei*, 2 m depth	Ariyama, 2004
May 1996-April 2006	Japan	Sennan City, Osaka Bay, Osaka Prefecture	Sponges, algae, experimental blocks, buoys	Ariyama, 2022; present study
10 Jul 1996	Japan	Tanigawa in Misaki, Osaka Prefecture	Experimental board for fouling organisms, l m depth	Ariyama, 2004
Apr 1997, Aug 2010	Japan	Shirahama Town, Wakayama Prefecture	Algae, experimental blocks	Ariyama, 2022; present study
Dec 1998-Jun 2009	Japan	Izumisano City, Osaka Bay, Osaka Prefecture	Algae	Ariyama, 2022; present study
Aug 2001	Japan	Himagajima Is. and Nezumijima Is., Mikawa Bay, Aichi Prefecture	Algae, sandy-mud bottom, shell bottom	Ariyama, 2022; present study
Aug 2008	Japan	Ena, Yura Town, Wakayama Prefecture	Sponges	Ariyama, 2022; present study
May 2020	Japan	Mihogaseki, Matsue City, Shimane Prefecture	Bryozoan	Ariyama, 2022; present study
9 May 2011	**Portugal**	Cascais marina (38.69094º N, 9.41855º W), Sines marina (37.95053º N, 8.86511º W)	Bryozoan *Bugula neritina*, floating pontoons	Present study
2013	Portugal	São Miguel, Azores	Hull fouling	ICES, 2018, DQEM, 2020, Castro *et al*., 2022
Jul 2013	**France** (Atl)	Courbey, Arcachon Bay (44.66667º N, 1.20000º W)	*Zostera marina* meadow, 2 m depth	Gouillieux *et al*., 2015
Jun-Jul 2014	France (Atl)	Thiers, Arcachon Bay (44.65000º N, 1.16667º W)	Slipper limpet bed, 5 m depth	Gouillieux *et al*., 2015
Aug 2014, 2 Jun 2022, 24 Sep 2024	France (Atl)	Château marina, Bay of Brest (48.36667º N, 4.48333º W; 48.37908º N, 4.48950º W) *	Fouling communities on floating pontoons, 1 m depth, among *B. neritina*	Gouillieux *et al*., 2015; present study
Oct 2014	France (Atl)	Arguin, Arcachon Bay (44.56667º N, 1.23333º W)	Pacific oyster reef, 0.1 m depth	Gouillieux *et al*., 2015

Continued

Table 1 continued

Date	Country	Localities	Habitat/substrates	References
May 2015	Japan	Takasu, Kurashiki City, Seto Inland Sea, Okayama Prefecture	Seagrass *Zostera*	Ariyama, 2022; present study
May 2015	Japan	Izu-ōshima Island,Tokyo Prefecture	Sponges	Ariyama, 2022; present study
16-28 May 2015	France (Med)	Le Grau-du-Roi, Port du Plaisance du Port Camargue marina (43.51500º N, 4.13200º E)	Hull fouling	Ulman *et al*., 2017
Jul 2018-Jul 2022	**Italy**	Santa Teresa marina (44.08167º N, 9.881978º E)	Fouling communities on PVC plates	Present study
30 Jan 2019, 25 Jun 2024	Portugal	Quinta do Lorde marina (32.74169º N, 16.71191º W), Madeira *	Fouling communities on PVC plates and artificial structures, 1-1.5 m depth	Present study
12 Feb 2019, Jun 2021-Oct 2022	France (Atl)	Granville marina, Normandy (48.83339º N, 1.60020º W)	Fouling communities on pontoons, piles and PVC plates	Dauvin *et al*., 2020; present study
25 Feb 2019	France (Atl)	Bassin Vauban marina, Le Havre, Normandy (49.49004º N, 0.12375º E)	Fouling communities on pontoons, piles and PVC plates	Dauvin *et al*., 2020; present study
1 Mar 2019, Jun 2021-Oct 2022	France (Atl)	Cherbourg marina, Normandy (49.64782º N, 1.62171º W) *	Fouling communities on pontoons, piles and PVC plates	Dauvin *et al*., 2020; present study
16 Apr 2019	France (Atl)	Saint-Vaast-La-Hougue (49.57298ºN, 1.27139º W)	Brown alga *Sargassum muticum*	Dauvin *et al*., 2020
Apr-Aug 2019*	**Spain** (Atl)	Ría de Vigo: Cangas marina (42.26089º N, 8.78379º W), Davila marina (42.23362º N, 8.74283º W), Moaña port (42.27610º N, 8.73481º W), Museo del Mar harbour (42.22482º N, 8,76865º W), Naútico port (42.24225º N, 8.72229º W), Toralla harbour (42.20196º N, 8.79850º W), Cíes floating pier (42.22632º N, 8.89959º W)	Fouling communities on PVC plates	Present study
2019, 2021, 2022, 2023	Portugal	Porto de Sines (marina and commercial port terminals) (37.95198º N, 8.88690º W; 37.95040º N, 8.86672º W; 37.93850º N, 8.86415º W; 37.93697º N, 8.84805º W)	Fouling communities on PVC plates	CIEMAR, 2021, 2025; present study
9, 23 Oct 2020, 5 Sep 2024	**Netherlands**	Kortgene, Delta marina, Lake Veere (51.55192º N, 3.81062º E; 51.552183º N, 3.81172º E)	Pontoons	Faasse & Gittenberger, 2021; present study
28 May 2021	Netherlands	Geersdijk jetty of port, Lake Veere (51.55468ºN, 3.76229º E)	Fouling communities	Faasse & Gittenberger, 2021
Jul 2021-Jul 2022	Italy	Le Grazie marina (44.06751º N, 9.83570º E), Fezzano marina (44.08022º N, 9.82732º E), Portovenere (44.050961º N, 9.834921º E)	Fouling communities on PVC plates	Present study
Sep 2021	Portugal	Costa Nova marina, Sailing Club (40.61989º N, 8.74832º W), Aveiro	Fouling communities on pontoons	Present study
Oct 2021	Portugal	Setúbal Fishing Port (38.51969º N, 8.89999º W), Sado estuary	Fouling communities on pontoons	Present study

Continued

Table 1 continued

Date	Country	Localities	Habitat/substrates	References
Dec 2021, Jan 2022	Spain (Atl)	Mussel farm, Ría de Vigo (42.27556º N, 8.72495º W)	Fouling communities on aquaculture facilities	Present study
9 May 2022, 12 Jun 2023, 13 Sep 2023, 18 Oct 2024	France (Atl)	Roscoff marina (48.71596º N, 3.96635º W; 48.71523º N, 3.96705º W) *	Fouling communities on pontoons and ropes	Present study
May-Jun 2022	France (Atl)	Trébeurden marina (48.77032º N, 3.58702º W) *, Saint Quay marina (48.64712º N, 2.82010º W), Saint Malo marina (48.63889º N, 2.02592º W) *, Concarneau marina (47.87008º N, 3.91421º W), Le Havre marina (49.48847º N, 0.09561º W), Piriac marina (47.38256º N, 2.54365º W) *	Fouling communities on pontoons	Present study
Sep 2022	Portugal	Alcantara marina (38.70141º N, 9.17107º W), Tagus estuary	Fouling communities on pontoons	Present study
Oct 2022	France (Atl)	Dieppe marina, Normandy (49.92797º N, 1.08197º W)	Fouling communities on pontoons, piles and PVC plates	Present study
Oct 2022	**Tunisia**	Sfax fishing port (34.72083º N, 10.76264º E)	Alga *Ulva* sp. on muddy sediments 2-6 m depth	Present study
27 Jan 2023	Spain (Atl)	Garachico marina (28.37359º N, 16.75579º W), Tenerife, Canary Islands *	Fouling communities on pontoons, ropes and buoys	Present study
28 Jan 2023, 19 Jun 2023	Spain (Atl)	Las Galletas marina (28.00715º N, 16.66107º W), Tenerife, Canary Islands	Fouling communities on pontoons, ropes and buoys, including the bryozoan *Viridentula dentata*	Present study
Mar-Nov 2023	Tunisia	Skhira fishing port (34.28728º N, 10.09555º E)	Algae *Ulva* spp. on muddy sediments, 3-5 m depth	Present study
28 Jun 2023	Spain (Atl)	Gran Tarajal marina (28.20616º N, 14.02637º W), Fuerteventura, Canary Islands	Bryozoan *Viridentula dentata*	Present study
14 Aug 2023	France (Med)	Barcares, Gulf of Lion (42.81000º N, 3.01900º E)	Fouling communities on pontoons	Present study
28 Sep 2023	France (Med)	Leucate harbour, Gulf of Lion (42.87000º N, 3.04660º E)	Fouling communities on pontoons	Present study
Mar-Apr 2024	Tunisia	Kneiss Islands (34.37438º N, 10.29865º E)	Intertidal meadows of *Zostera noltii* and *Halophila stipulacea*	Present study
25 Jun 2024	Japan	Hakotsukuri, Hannan City, Osaka Prefecture (34.34305º N, 135.20250º E) *	Algae	Present study
Jun-Jul 2024	France (Atl)	Dinard, Brittany (48.63830º N, 2.02660º W)	Fouling communities	Present study
6 Aug 2024	France (Atl)	Arcachon harbour, Arcachon Bay (44.65970º N, 1.15190º W)	Fouling communities on pontoons	Present study

adult males. Females and juveniles of different *Aoroides* species can exhibit significant morphological similarity, making identification difficult in the absence of males.

Voucher specimens of *A. longimerus* are deposited in the Museo Nacional de Ciencias Naturales, Madrid (MNCN) and Muséum national d'Histoire Naturelle Paris (MNHN) (see Table S2 for detailed information on the voucher specimens, including Museum Catalogue numbers, sampling locations, coordinates, collection dates, substrates, and numbers of specimens deposited).

Individual specimens barcoding: DNA isolation, amplification and alignment

Total DNA was extracted from two pleopods per specimen (or the whole pleosome for specimens smaller than 3 mm), adapting the protocol from Casquet *et al*. (2012). Out of 107 specimens planned to be sequenced, DNA was successfully extracted and amplified only from 25 (Table S3). Many of the specimens were indeed old or not properly preserved for subsequent DNA analyses. All specimens are available at the University of Lodz in the invertebrate collection of the Department of Invertebrate Zoology and Hydrobiology. Amplifications of both the mitochondrial DNA cytochrome c oxidase subunit 1 (CO1) and 16S rRNA genes were carried out using the primers sets LoboF1 and LoboR1 (Lobo *et al*., 2013), and 16STf (Macdonald *et al*., 2005) and 16Sbr (Palumbi, 1996), respectively. Amplifications were performed in a 15 µl reaction volume mix containing 7.5 µl DreamTaq Green PCR Master Mix (2X), 1.2 µl forward and reverse primers (5 µM), 1 µl template DNA, and nuclease-free water to a final volume of 15 µl, using the polymerase chain reaction (PCR) program described in Hou *et al.* (2007). PCR products were sequenced bidirectionally using the same primers sets used for amplification by Macrogen Europe (Amsterdam, The Netherlands). Raw sequences were trimmed using Geneious R11.0 (https://www.geneious.com), and consensus sequences were aligned using ClustalW (Larkin *et al.*, 2007) with default parameters. CO1 sequences were then visually inspected for stop codons and/or indels to avoid including pseudogenes in the analyses. All the obtained sequences were deposited in the BOLD project and dataset: ALONG and DS-AORLONG respectively (www.boldsystems.org, Ratnasingham *et al.*, 2024; Table S3).

Phylogenetic analyses

To determine the phylogenetic placement of the collected specimens and ascertain their membership to the target species, CO1 and 16S datasets were analysed independently. The sequences of other *Aoroides* species retrieved from public repositories, were used as outgroups: three CO1 sequences of *Aoroides intermedius* Conlan & Bousfield, 1982 from BOLD (BCAMP255-08, BCAMP102-08, NJCGS736-10), three CO1 of *Aoroides columbiae* Walker, 1898 species complex from GenBank (MG936292.1, MG317304.1, JX545451.1) (Clark *et al*., 2016), and two 16S of *A. columbiae* from GenBank (JX545420.1, JX545421.1) (only sequences available). The final alignments consisted of 660 bp for CO1 and 425 bp for 16S. These alignments were used to calculate intra- and interspecific pairwise genetic distances (Kimura 2 parameters- K2P- with 999 bootstrap replicates) in MEGA 11 (Tamura *et al*., 2021) and run a maximum likelihood phylogenetic analysis through PhyML (1000 bootstrap replicates; Guindon *et al*., 2010). The best substitution models (HKY85 + G for CO1 and HKY85 + I for 16S) were tested with the Smart Model Selection (SMS) routine in PhyML using Bayesian information criteria (BIC) as optimality criteria (Lefort *et al*., 2017). To better investigate the molecular variation among the specimens sequenced, a median-joining haplotype network was built using the PopART v1.7 software. For building the network, the sequences were further trimmed (i.e., 564 and 300 bp for CO1 and 16S, respectively) to exclude missing bases at both ends.

Gut contents analysis

To characterise the diet of *A. longimerus*, a total of 125 specimens collected from marinas of the Iberian Peninsula were examined: Sines (May 2011, n = 3 specimens), Cascais (May 2011, n = 20), Cangas (April 2019, n = 15; August 2019, n = 15), Davila (April 2019, n = 12; August 2019, n = 20), and Moaña (April 2019, n = 20; August 2019, n = 20). Dietary analysis followed the methodology proposed by Bello & Cabrera (1999), previously used for amphipod gut content characterisation (see details in Guerra-García *et al*., 2014).

Results

New records of **Aoroides longimerus** *in its introduced range*

Examination of old specimens in the collections enabled us to provide the earliest known record of the species in its introduction range, namely 2011 in Portugal (Cascais and Sines marinas) (Table 1). The present work also provides the first records of *Aoroides longimerus* in Italy (2018), Spain (2019) (Fig. 1) and Tunisia (2022), and reports an expansion of its known distribution in mainland Portugal (including the northernmost record) and Macaronesia (with first records for the Canary Islands and Madeira). Additionally, it also confirms the presence of the species in the Atlantic coasts of France and the Netherlands. Therefore, to date, the introduced range of *A. longimerus* encompasses five ecoregions (North Sea, Celtic Sea, South European Atlantic Shelf, Azores Canaries Madeira and Western Mediterranean) of three biogeographical provinces (Northern European Seas, Lusitanian and Mediterranean Sea), according to Spalding *et al.* (2007).

A total of 161 specimens were collected from four Italian marinas in the Gulf of La Spezia (Le Grazie: 15, Santa Teresa: 79, Fezzano: 26, Portovenere: 41). The species was already present in Santa Teresa marina since July 2018, which represents the first record for Italy (Table 1). In the other three marinas, the species was found in 2021 and 2022 but was absent from 2018 to 2020.

In Ría de Vigo, Galicia, NW Spain, 1,841 specimens of *A. longimerus* (Fig. 1), were found among 20,872 amphipods examined from fouling PVC plates (Table 1), representing 8.82% of the total amphipod abundance (TA). This is the first record of the species in Spain. *Aoroides longimerus* was present at all locations in both sampling periods (three and nine months after deployment, corresponding to April and August 2019), but its abundance was lower in the early stages of macrofouling community

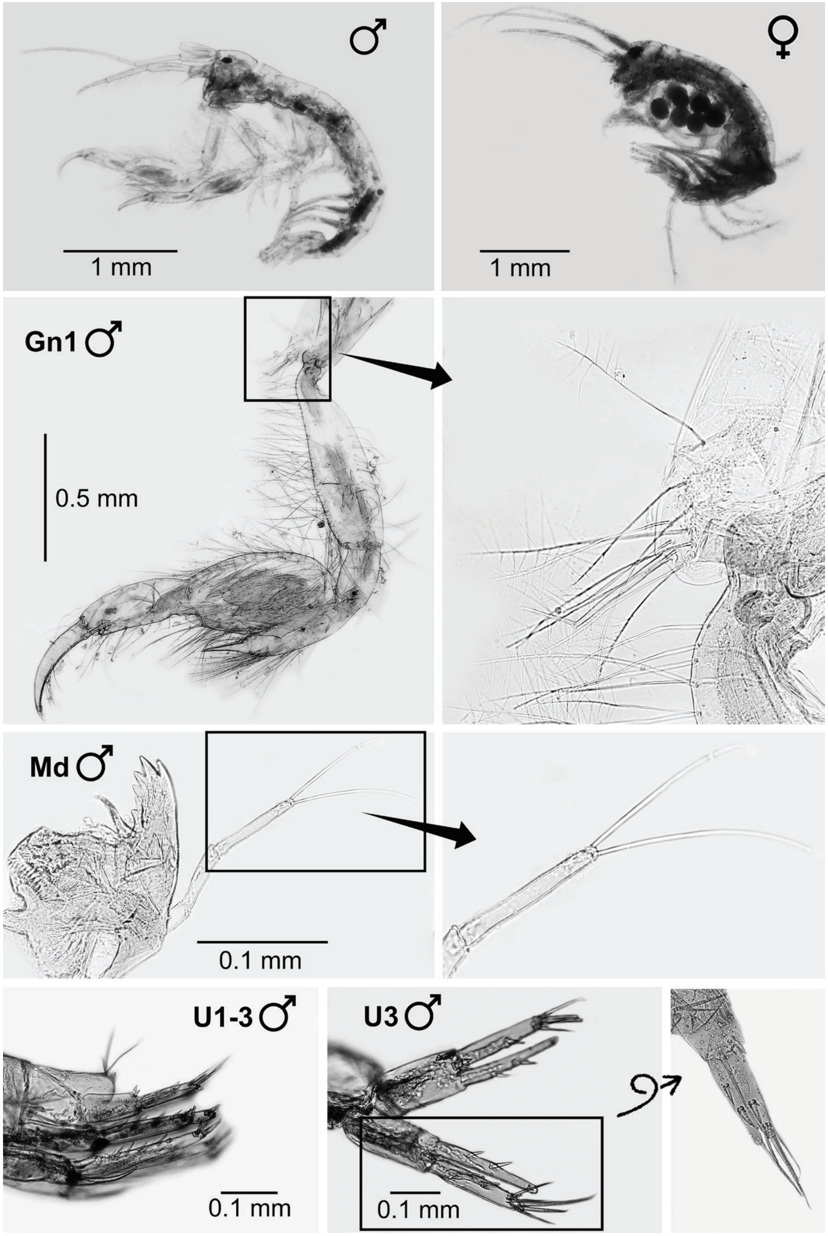

Fig. 1: Lateral view of male (Moaña marina, Galicia, NW Spain, April 2019) and female (Cíes, Galicia, NW Spain, August 2019) specimens of *Aoroides longimerus* Ren & Zheng, 1996. Details of male gnathopod 1 (Gn1) showing the plumose setae on the coxa, mandible (Md) showing the mandibular palp and uropods (U) 1-3, with a detailed view of U3.

development (April 2019: 223 individuals, 2.54% of TA) than in more mature communities (August 2019: 1,618 individuals, 13.39% of TA). These individuals were distributed as follows: 119 individuals in Cíes (23 in April, 96 in August), 197 in Cangas (22 in April, 175 in August), 330 in Davila (10 in April, 320 in August), 862 in Moaña (139 in April, 723 in August), 48 in Museo del Mar (9 in April, 39 in August), 145 in Náutico (13 in April, 132 in August) and 140 in Toralla (7 in April, 133 in August). Additionally, 40 specimens of *A. longimerus* were collected from a mussel farm in the Ría de Vigo between December 2021 and January 2022 (Table 1).

In 2011, *A. longimerus* was found in two (within continental Portugal) of the 42 marinas sampled along the Iberian Peninsula and North Africa, representing the earliest records of the species in its introduced range. Twenty one individuals were found in Cascais, while three were recorded in Sines (Table 1). *Aoroides longimerus* was present in the Port of Sines and its associated marina in subsequent sampling events (2019, 2021, 2022 and 2023). The species was absent from marinas in the southern Iberian Peninsula surveys in 2017 and 2019 (Table S1). However, a sizable population (69 specimens collected) was found inhabiting the Alcantara marina in the Tagus estuary, Portugal (September 2022). The present study also documents a single male specimen in the Sado estuary (Setúbal Fishing Port). The finding in Costa Nova marina, Aveiro (three specimens), represents the northernmost record for mainland Portugal.

Although *A. longimerus* had been previously reported in Macaronesia (Azores, 2013), this study provides the first records for Madeira (2019) and the Canary Islands (2023) (Table 1). In Madeira, the species (ca. 20 specimens) was collected in Quinta do Lorde marina, where it was also present during a sampling programme in June 2024 (>100 specimens identified). Five adult specimens were collected from marinas of Canary Islands (Table 1), confirming the presence of the species on two islands: Tenerife (Garachico and Las Galletas marinas) and Fuerteventura (Gran Tarajal marina).

In Tunisia, 43 specimens were collected from the fishing ports of Sfax and Skhira (Table 1). They were associated with algae (*Ulva* spp.) on muddy sediments at depths of 2-6 metres. The specimens found in Sfax (Gulf of Gabès) in October 2022 represent the first record for Tunisia and the southernmost record in the Mediterranean Sea. Between March and April 2024, 16 specimens were found inhabiting natural intertidal *Zostera noltii* Hornemann, 1832 meadows of the Kneiss Islands (Gulf of Gabès) (Table 1).

A total of 6,393 specimens of *A. longimerus* were collected from four marinas in Normandy, France (Granville, Cherbourg, Le Havre and Dieppe) between June 2021 and October 2022, representing ca. 10% of the total amphipod abundance in fouling communities. *Aoroides longimerus* was previously recorded in the region in 2019 (Table 1; Dauvin *et al*., 2020). More than 100 specimens were also collected from marinas in Brittany, France (May 2022-October 2024) (Table 1). The present study also supports the establishment of the species along the Gulf of Lion in the Mediterranean coast of France (Table 1).

In the Netherlands, the species was first recorded in Lake Veere in 2020. This study confirms its presence four years later in Delta marina, Korgene in September 2024 (Table 1).

Despite extensive sampling efforts conducted to characterise fouling communities in marinas, ports and aquaculture facilities in Morocco, Croatia, Greece, Saudi Arabia, and Australia, the species has not yet been found in these countries (Table S1).

Within the introduced area, the body length of adult specimens of *A. longimerus* ranged from 1.8 to 5.1 mm (males, n = 179) and 1.8 to 5.5 mm (females, n = 170), with the larger sizes corresponding to ovigerous females. Size data from different countries showed similar measurements across global populations. French specimens were, however, slightly larger than those from other populations; Northwestern Spain: 1.8-3.7 mm (males, n = 50) and 2.1-4.4 mm (females, n = 50), mainland Portugal: 2.6-3.6 mm (males, n = 20) and 2.2-3.9 mm (females, n = 20), Madeira: 1.9-3.4 mm (males, n = 20) and 1.9-4.3 mm (females, n = 20). France (Brittany and Normandy): 2.3-5.1 mm (males, n = 62) and 1.8-5.5 mm (females, n = 48), Tunisia: 2.8-4.3 mm (males, n = 27) and 2.9-4.4 mm (females, n = 32).

Phylogenetic analyses

The average genetic distance among *A. longimerus* sequences was 2.1% (SE: 0.4) for CO1, and 1% (SE: 0.2) for 16S. It is noteworthy than one CO1 sequence from a specimen collected in Hakotsukuri, Hannan City, Osaka Prefecture Japan (AORLON86) (Table S3) displayed a greater molecular divergence, with a maximum distance of 6.5% from the most divergent haplotype. For COI, the average distance to the specimens belonging to the congeneric species that were used as outgroups was 18.8% and 19.3% for the species complex *A. columbiae* and *A. intermedius*, respectively (Table S4). For 16S, the average distance to *A. columbiae* was 8.6% (Table S5). Phylogenetic trees clustered all *A. longimerus* sequences together, as compared to the outgroups (Figs. 2 and 3). Haplotype networks showed, for 16S, two haplotypes shared between the native (Japan) and the introduction ranges (Canary Islands and France) and one haplotype shared between two locations within the introduction range (Madeira and France) (Fig. 3). For CO1, no haplotypes were shared between the sequenced specimens, as would be expected given the high polymorphism of the marker. The haplotype network illustrates well the substantial divergence of the Japanese specimen AORLON86, as observed in the phylogenetic tree. Specimens from Madeira were more similar to each other (two to 11 mutational steps) than to specimens from other introduced populations (France and Canary Islands, min. 14 mutational steps).

Habitat use and dietary analysis

Within its introduced range, *A. longimerus* was mainly found on fouling communities of marinas during the present study (Table 1). In Portugal, it was associated to the bryozoan *Bugula neritina* (Linnaeus, 1758) on floating pontoons. In Spain, specimens were found inhabiting fouling communities on PVC plates, aquaculture facilities, and pontoons, ropes and buoys of marinas; in Canary Islands it was associated with the bryozoan *Virididentula dentata* (Lamouroux, 1816) (Table 1). In Normandy, France, populations were found on the bryozoans *B. neritina*, *Tricellaria inopinata* d'Hondt & Occhipinti Ambrogi, 1985 and *Watersipora subatra* (Ortmann, 1890), as well as the ascidians *Botrylloides* spp. and *Botryllus schlosseri* (Pallas, 1766). Based on these Normandy collections, *A. longimerus* appears to inhabit pontoons more readily than piles in marinas, and high abundances were measured on PVC plates, particularly sanded ones compared with smooth ones (Fig. 4, Text S1). In Tunisia, *A. longimerus* was found in natural habitats, associated with algae (*Ulva* spp.) on muddy sediments (2-6 m depth) and seagrass (Table 1).

In the Ría de Vigo, NW Iberian Peninsula, *A. longimerus* coexisted with other amphipods such as *Ampithoe ramondi* Audouin, 1826, *Aora gracilis* (Spence Bate, 1857), *Caprella acanthifera* Leach, 1814, *Caprella equilibra* Say, 1818, *Dexamine spinosa* (Montagu, 1813), *Jassa herdmani* (Walker, 1893), *Monocorophium sextonae* (Crawford, 1937), *Stenothoe monoculoides* (Montagu, 1813), *Stenothoe valida* Dana, 1852, *Phtisica marina* Slabber, 1769, and the NIS *Caprella mutica* Schurin, 1935, *Ericthonius pugnax* (Dana, 1852) and *Jassa slatteryi* Conlan, 1990. In Normandy, the dominant amphi-

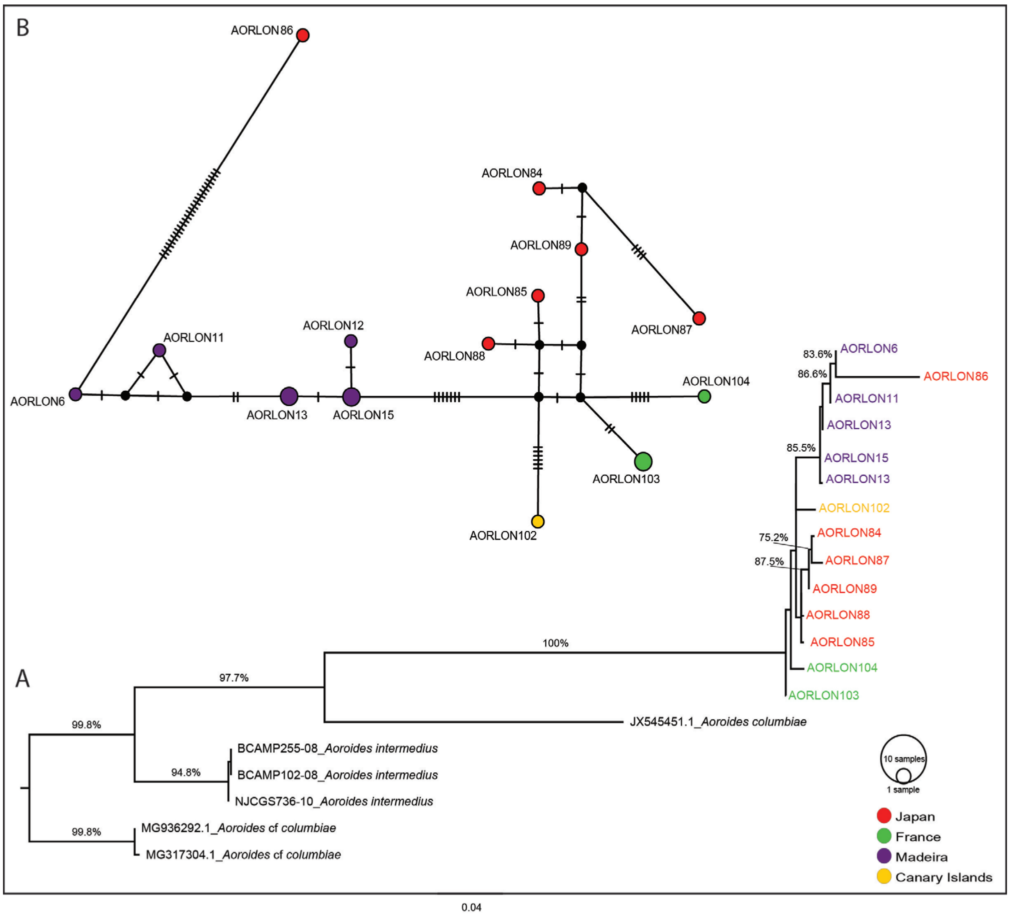

Fig. 2: Maximum likelihood phylogeny of the CO1 haplotypes (A) and relative median joining haplotype network (B). Only bootstrap support values >70% are shown on branches for each subsequent node (1000 bootstrap). The colours refer to the sampling areas and the size of the circles is proportional to the number of specimens with the same haplotype. Black circles refer to missing haplotypes, and small vertical bars on branches represent the mutation steps between two haplotypes.

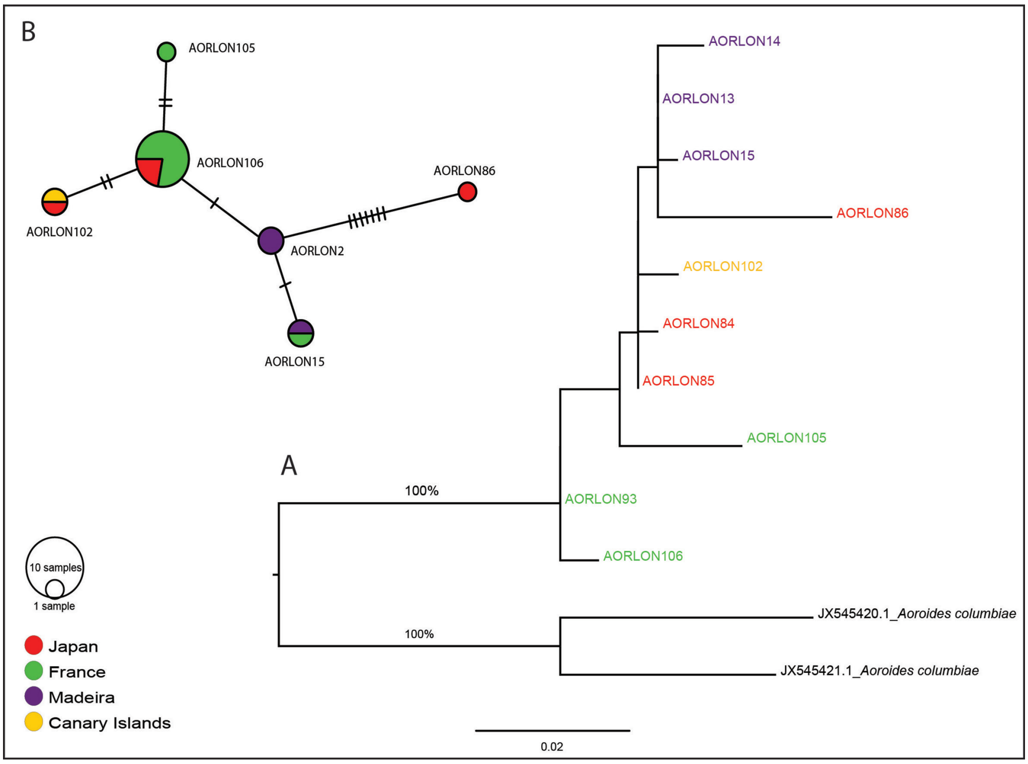

Fig. 3: Maximum likelihood phylogeny of the 16S haplotypes (A) and relative median joining haplotype network (B). For the phylogeny, only bootstrap support values >70% are shown on branches for each subsequent node (1000 bootstrap). The colours refer to the sampling areas and the size of the circles is proportional to the number of specimens with the same haplotype. Black circles refer to missing haplotypes, and small vertical bars on branches represent the mutation steps between two haplotypes.

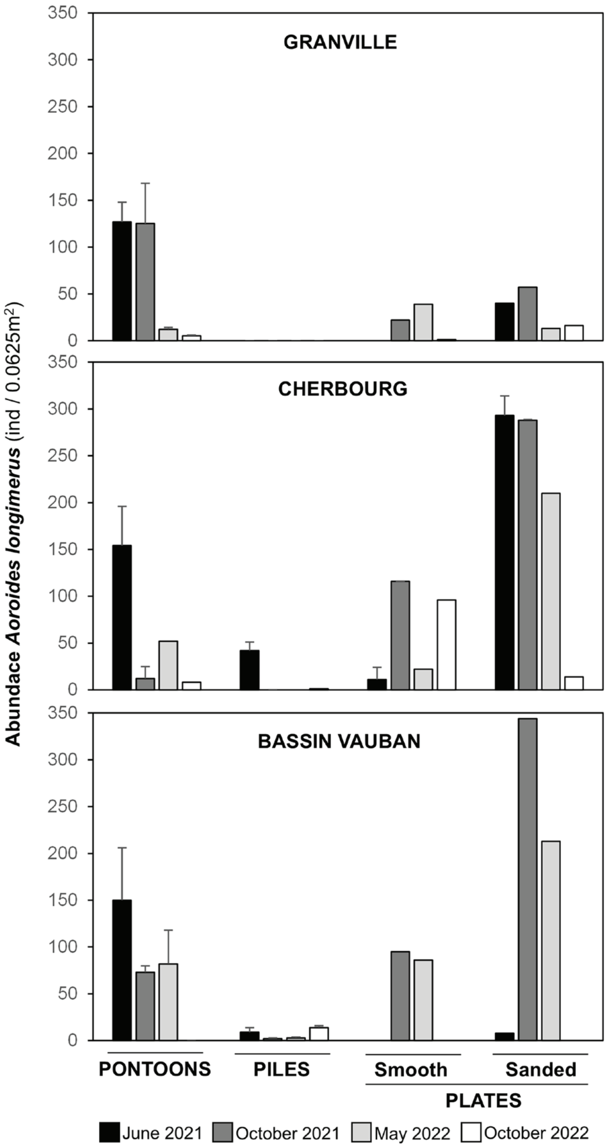

Fig. 4: Abundance of *Aoroides longimerus* Ren & Zheng, 1996 (ind/0.0625m^2) on pontoons, piles and PVC plates (smooth and sanded) of three marinas from Normandy, France (see Text S1 for detailed protocol). Data for pontoons and piles represent mean values with standard error from three replicates; data for plates correspond to a single plate per marina deployed for ca. 6 months prior sampling times (June 2021, October 2021, May 2022 and October 2022). Although the species was also present in Dieppe marina, it was only found in very low abundances in October 2022 and data was not represented in the figure.

pods cohabiting with *A. longimerus* were *Ericthonius punctatus* (Spence Bate, 1857), *Ischyrocerus anguipes* Krøyer, 1838, *Leptocheirus pilosus* Zaddach, 1844, *Leucothoe spinicarpa* (Abildgaard, 1789), *Monocorophium acherusicum* (A. Costa, 1853), *M. sextonae* and the NIS *Caprella mutica*. In the Gulf of La Spezia, Italy, *A. longimerus* was sharing habitat with *Apocorophium acutum* (Chevreux, 1908), *Elasmopus rapax* Costa, 1853, *Ericthonius punctatus* and the NIS *Jassa slatteryi* and *Laticorophium baconi* (Shoemaker, 1834).

Aoroides longimerus clearly showed a detritivorous diet. Digestive contents were found in 102 of the 125 specimens analysed and consisted almost exclusively of detritus in all specimens, regardless of sex, location and sampling time. The total area of the whole digestive tract occupied by detritus ranged from 30% to 100%. Although copepods were also found in the gut contents of six specimens, they were probably ingested accidentally alongside the detritus.

Discussion

Taxonomic remarks on Aoroides

The presence of merochelate gnathopod 1 in *Aoroides* males has also been observed in males of *Aora* Krøyer, 1845, a genus typically found in European waters. However, these two genera can be readily distinguished by the following characteristics, which are even present in females and juveniles: (i) the accessory flagellum in antenna 1 is present in *Aora*, but absent in *Aoroides*; and (ii) the article 3 of the mandibular palp is semifalciform and highly setose (bearing short and pectinate setae) in *Aora*, whereas in *Aoroides* it is rectilinear and poorly setose, with few terminal long setae, except in the two species that lack mandibular palp (Gouillieux *et al*., 2015; Dauvin *et al*., 2020).

Aoroides longimerus was originally described by Ren & Zheng in 1996 based mainly on the setose gnathopod 1 of the male and round-triangle eyes. The genus *Aoroides* currently contains 21 valid species (WoRMS Editorial Board, 2025).

Aoroides columbiae, the type species of the genus, was originally described based on females found in Puget Sound, so information about the male gnathopod 1 was lacking. Although the description and figures were incomplete, the mandibular palp differs from that of *A. longimerus* (three setae on distal article and one seta on second article in *A. columbiae* compared to only two apical setae in *A. longimerus*). Conlan & Bousfield (1982) redescribed the species including male figures and a description. Although the male gnathopod 1 in *A. columbiae* is very setose, as in *A. longimerus*, the dorsal (= anterior) margin of the carpus is bare, except for a distal group of short setae. In contrast, this dorsal margin is densely covered by setae in *A. longimerus*. Furthermore, Conlan & Bousfield (1982) indicated simple setae on the distal end of coxa of gnathopod 1, while they are plumose in *A. longimerus* (Fig. 1).

Aoroides secundus Gurjanova, 1938, was described in the Japan Sea based on a single male specimen. Its eyes are narrow and reniform, differing from those of *A. longimerus*. The species was redescribed by Ariyama (2004), who pointed out that *A. secundus* can be distinguished from *A. longimerus* by the coxae of gnathopod 1 (with plumose setae *in A. longimerus* and lacking plumose setae in *A. secundus*). Indeed, Kim *et al*. (2024) pointed out the presence of only two simple setae in the coxa of *A. secundus*.

Aoroides nahili Barnard, 1970 was described from Oahu, Hawaiian Islands. Despite the incompleteness of its description and illustrations, it can be distinguished from *A. longimerus* by the presence of three setae on the mandibular palp and a non-densely setose male gnathopod 1 (see Barnard, 1970).

Conlan & Bousfield (1982) described four species of *Aoroides* (*Aoroides exilis* Conlan & Bousfield, 1982, *Aoroides inermis* Conlan & Bousfield, 1982, *Aoroides intermedius* Conlan & Bousfield, 1982 and *Aoroides spinosus* Conlan & Bousfield, 1982). They also elaborated a key to distinguish the seven species described so far. While *A. inermis* and *A. intermedius* have a seta on the article 2 of the mandibular palp, this seta is absent in *A. exilis* and *A. spinosus*, as well as in *A. longimerus*. These three species can be further differentiated because the dorsal (= anterior) margin of the carpus is bare or scarcely setose in *A. exilis* and *A. spinosus*, but densely setose in *A. longimerus*.

Myers (1995) described *Aoroides vitiosus* Myers, 1995 from the north coast of Papua New Guinea. The species resembles *A. nahili* from Hawaii in having a scarcely setose gnathopod 1, but it differs by the absence of a mandibular palp. Indeed, the absence of a mandibular palp could be considered sufficient for the establishment of a new genus. However, based on the similarity of this species to *A. nahili*, which has a typical *Aoroides* form mandibular palp, Myers (1995) maintained this species within the genus and suggested that the diagnosis of the genus should be modified to include species lacking a mandibular palp.

Ariyama (2004) described seven species from Osaka Bay, central Japan (*Aoroides columnaris* Ariyama, 2004, *Aoroides curvipes* Ariyama, 2004, *Aoroides ellipticus* Ariyama, 2004, *Aoroides myojinensis* Ariyama, 2004, *Aoroides punctatus* Ariyama, 2004, *Aoroides rubellus* Ariyama, 2004 and *Aoroides semicurvatus* Ariyama, 2004) and redescribed *A. longimerus* and *A. secundus*, providing a key to *Aoroides* species. Males of *A. columnaris*, *A. curvipes* and *A. semicurvatus* have poorly setose gnathopod 1, while the remaining species have densely setose gnathopod 1. However, *A. ellipticus*, *A. punctatus* and *A. rubellus* have a few or no setae on the dorsal (= anterior) margin of the carpus. Ariyama (2004) pointed out that *A. longimerus* can be distinguished from the *A. myojinensis* by the presence of several plumose setae on the anterior margin of coxa 1.

Myers (2009) described two additional species, *Aoroides palfreyensis* Myers, 2009 and *Aoroides parvus* Myers, 2009, from the Great Barrier Reef, Australia. Both species are characterised by a poorly setose male gnathopod 1. Additionally, *A. palfreyensis* lacks a mandibular palp, like *A. vitiosus*.

Another *Aoroides* species, *Aoroides sagamiensis* Ariyama & Kohtsuka, 2022 was described from Japan. Ariyama & Kohtsuka (2022) pointed out the distinctive male gnathopod 1 of this species, which has a poorly setose basis and heavily setose merus, close to *A. rubellus*.

Ariyama & Kawabe (2022) described *Aoroides macrops* Ariyama & Kawabe, 2022, also from Japan. *A. macrops* is probably one of the most morphologically similar species to *A. longimerus*, as it has a heavily setose male gnathopod 1, with the coxa bearing several plumose setae anteriorly, and the uropod 2 with a short inter-ramal process (Ariyama & Kawabe, 2022). However, *A. macrops* can be distinguished from the Japanese specimens of *A. longimerus* by having (i) larger eyes, (ii) shorter peduncular article 4 of the antenna 2 (with a length/body length ratio of 0.11-0.12, compared to ca. 0.14 in *A. longimerus*), (iii) shorter carpus of the male gnathopod 1 (with a length/body length ratio of 0.13-0.16, compared to 0.19-0.21 in A. *longimerus*), (iv) shorter carpus of the pereopods 3 and 4 (with a length/width ratio of ca. 1.7 compared to 1.9-2.3 in *A. longimerus*), and (v) single marginal robust seta each on the uropod 3 rami (usually two or three in *A. longimerus*) (see Ariyama & Kawabe, 2022 for details).

Kim *et al*. (2024) described the species *Aoroides gracilicrus* Kim, Choi, Kim, Im & Kim, 2024 from South Korea. It is also morphologically similar to *A. longimerus* but it can be distinguished by the presence of a single anterior robust and plumose setae in gnathopod 1 coxa (in contrast to the multiple plumose setae in *A. longimerus*), and the absence of setae on both rami of uropod 3 (in contrast to the robust setae present in *A. longimerus*).

Morphological and molecular characteristics ascertaining* Aoroides longimerus *presence in Europe

Among the described *Aoroides* species, three have recently been reported in European waters: *A. longimerus*, *A. semicurvatus* and *A. curvipes* (Gouillieux *et al*., 2015). *Aoroides longimerus* can be easily distinguished from the other two NIS, mainly by the male gnathopod 1 densely setose (Ariyama, 2004; Gouillieux *et al*., 2015).

When the specimens from the present study were compared with the 21 known *Aoroides* species, they were found to fully agree with the detailed description of *A. longimerus* from Japan (see Ariyama, 2004). The main diagnostic characteristics are: (i) fully setose gnathopod 1, with coxa provided with a bunch of plumose setae, (ii) article 2 of the mandible palp without setae and article 3 (distal one) bearing two apical setae, and (iii) both rami of uropod 3 armed with robust setae (see Fig. 1). The description of Japanese specimens (Ariyama, 2004) and those from the introduced range also align with the type specimens from China (Ren & Zheng, 1996), except for larger eyes and a single marginal robust seta on the uropod 3 rami in the Chinese specimens (see Fig. 2-1 and 2-9 respectively) (Ariyama & Kawabe, 2022). Although Figure 2 in Ren & Zheng (1996) shows a cluster of setae on the coxa of gnathopod 1, the description does not indicate whether they are simple or plumose. The type material of *A. longimerus* (four male paratypes 92CA-002, as detailed in Ren & Zheng, 1996) was examined by one of the coauthors (YW). Despite some specimens being poorly preserved and some characters being uncheckable, the examination confirmed the presence of a bunch of plumose setae in the coxa of gnathopod 1. The number of marginal spines in the uropod 3 rami was variable (1 or 2) depending on the paratype. Indeed, Ariyama (2004) pointed out that the number of spines on the outer and inner ramus of uropod 3 ranged from one to three, thus supporting the variability of this character. Consequently, although the uropods of Japanese and European specimens are more armed (Ariyama, 2004; Fig. 1, present study), this character appears to be variable.

Molecular data corroborate morphological evidence. DNA results from the present study indicate that the specimens from Japan and those from the introduced range belong to the same species. However, one female specimen collected in Japan, AORLON86, clearly diverged from all the others, regardless of the method used (Figs. 2 and 3). As identifying females is very difficult, this specimen probably belongs to a different species of *Aoroides*. In fact, *A. secundus* dwells in a similar environment to that of *A. longimerus* in Hannan City (HA, pers. obs.). Alternatively, this finding could also suggest the presence of a species complex. The possible cryptic taxonomic diversity of *A. longimerus* in its native range should be further investigated through morphological studies and deeper molecular analyses (e.g., use of other genes such as 28S) of newly collected specimens. Unfortunately, fresh material of *A. longimerus* from China could not be collected during the present study, and, as a result, Chinese populations could not be incorporated into molecular analyses to confirm that Japanese and Chinese specimens belong to the same species. New collections from the type locality in China are essential to resolve this taxonomic uncertainty. This information is critical to assess the likelihood of multiple taxa being introduced or spreading (secondary introductions) in the future.

Ecological remarks

In its native range, *A. longimerus* lives in association with sessile invertebrates (such as ascidians, bryozoans, hydrozoans and sponges), as well as among algae in shallow subtidal waters, in sediments, on pebble or shell bottoms, under stones in the intertidal, and within artificial substrates (Ren & Zhen, 1996; Ariyama, 2004, 2022; present study) (Table 1). It is interesting to note that the species was first described by Ren & Zheng (1996) based on specimens found in fouling communities. These authors reported 22 species of fouling amphipods from test plates, buoys, mariculture net cages and intertidal zones in Dayawan (Guangdong province, South China Sea), but did not specify whether *A. longimerus* occurred across all these habitats. Most of the current data on its habitat use in the native range come from Japanese populations.

Within its introduced range, *A. longimerus* is primarily associated with fouling communities in aquaculture facilities, marinas and ports (e.g., pontoons, ropes, piles and PVC plates), typically among mussels, ascidians and bryozoans (Gouillieux *et al*., 2015; Dauvin *et al*., 2020; present study). The species has also been recorded on ship hulls (Ulman *et al*., 2017). In addition to artificial habitats, *A. longimerus* has been found in natural environments such as macroalgal beds, slipper limpet aggregations, oyster reefs, and seagrass meadows (Gouilleux *et al*., 2015; present study) (Table 1).

Establishment and introduction pathways

Aoroides longimerus is now recorded across both Mediterranean and Atlantic waters (Table 1). The species was first detected in mainland Portugal in the marinas of Cascais and Sines in 2011. It was subsequently recorded again in Sines (both at the marina and the commercial terminals of the Port of Sines) in 2019, 2022, and 2023, suggesting well-established population in this area. Similarly, *A. longimerus* was found in Madeira in 2019 and again in 2024. It is also well established in France, at least in Normandy (collected in 2019, 2021 and 2022) and in the Bay of Brest (collected in 2014 and 2024), as well as in Italy (collected in the Gulf of La Spezia between 2018 and 2022). In the Netherlands, the species was collected from Kortgene, Delta marina, in 2020 and 2024. In Spain, populations of the Ría de Vigo, Galicia, seem well-established, with collections from April to August 2019, and again during 2021-2022. In Tunisia, the species was collected in several sampling events at Shira fishing port between March and November 2023. Overall, these records indicate the presence of stable populations of *A.*

longimerus in the Netherlands, France, Portugal, Spain, Tunisia, and Italy.

The haplotype network for the 16S marker showed shared haplotypes among Japan, Canary Islands and France (Fig. 3) that may suggest a common origin for the individuals introduced in Europe. However, the notable molecular divergence observed with the CO1 marker between the samples collected in Madeira and those collected in France and the Canary Islands suggests that multiple introductions may have occurred from different source populations within the native range, as often documented in marine species (Rius *et al*., 2015). This hypothesis should be investigated in future genetic studies with a larger sampling across both native and introduced regions.

The original description of the species from China based on specimens collected from fouling communities, supports the idea that this species has been spread to other areas via anthropogenic vectors.

Unlike other small non-indigenous amphipods, such as *Caprella scaura* Templeton, 1836, *Laticorophium baconi* or *Stenothoe georgiana* Bynum & Fox, 1977, which were documented in Australian waters in 1890, 1990 and 2017, respectively (Ros *et al*., 2014; Martínez-Laiz *et al*., 2020; Guerra-García *et al*., 2023), *A. longimerus* has not yet been reported from Australia. Nevertheless, the information provided in this study (Table S1) is mainly based on revised samples collected between 1964 and 2002. Further research is required to ascertain whether the species could have arrived in Australia within the last 20 years. In contrast to other amphipod species such as *L. baconi* or *C. scaura*, which are widely distributed across their introduced ranges (Ros *et al*., 2014; Guerra-García *et al*., 2023), *A. longimerus* is absent from many marinas, ports and aquaculture facilities in the Iberian Peninsula, Italy, Tunisia, the Netherlands, Croatia, Greece and Saudi Arabia (Table S1). This pattern could indicate that, while marinas and port facilities are key for the secondary spread of *A. longimerus*, this species may have a more limited ability to spread within these environments than other non-indigenous amphipods. Alternatively, *A. longimerus* could still be in the early stage of its introduction since this study confirms the capacity to survive during seasonal fluctuations throughout the year and its remarkable colonisation capacity based on its settlement on PVC plates (see Table 1 and Fig. 3). Therefore, special attention must be paid to the potential threat of further spread in the coming years.

Gouillieux *et al.* (2015) suggested that *A. longimerus* could been accidentally introduced in Arcachon Bay and Hossegor Lake through oyster transfers and in the Bay of Brest possibly through both oyster transfers and shipping. Similarly, Dauvin *et al.* (2020) suggested that *A. longimerus* was unintentionally introduced in Normandy through oyster transfers from French production centres of the oyster *Magallana gigas* (Thunberg, 1793). Indeed, Saint-Vaast-la-Hougue (Table 1) is a key location in Normandy for oyster production, with continuous transfers between production centres of the Bay of Biscay and the English Channel (Dauvin *et al*., 2020). Along the Iberian Peninsula, an important population was found in marinas within the Ría de Vigo, Galicia, while the species remains absent from most other sampled marinas across Spain and Portugal (Table S1). The high intensity of aquaculture activities along the Galician coasts (Almón *et al*., 2014), primarily mussel and oyster farms (Ruiz *et al*., 1992), supports the hypothesis that aquaculture may be the main vector for NIS introductions in this region, which is considered a hotspot for invasions (Pascual *et al*., 2010). Indeed, this study confirms the presence of *A. longimerus* in a mussel farm in the Ría de Vigo. In the Gulf of La Spezia, Italy, the species was first observed in 2018 at only one locality (Santa Teresa), which is the only site located in front of a mussel farm. The species was not recorded in other locations until 2021, suggesting initial introduction via aquaculture, followed by secondary spread to adjacent marinas. Additionally, the growing number of oyster hatcheries in France (Iitembu *et al*., 2023) may have played an important role in the secondary spread of *A. longimerus* through the movement of oyster seed stock exported to other European countries. In any case, the current distribution of *A. longimerus* may be explained by multiple vectors and pathways, as often shown for marine NIS (Ojaveer *et al*., 2018; Bailey *et al*., 2020). These authors also underlined the large uncertainties in pathways' assessment for marine NIS, emphasizing the need for caution and continued investigation regarding this topic.

Most records of *A. longimerus* in natural environments are in its native range, while in the introduced range, the species mainly inhabits artificial substrates (Table 1). For example, in the Iberian Peninsula, *A. longimerus* has not yet been found in natural environments despite recent efforts to characterise epifaunal communities inhabiting subtidal algae and other substrates (e.g., Navarro-Barranco *et al*., 2015, 2018, 2019, 2021, 2023). This pattern is common among non-indigenous amphipods in European waters, such as *Caprella scaura* (Ros *et al*., 2014), *Paracaprella pusilla* Mayer, 1890 (Ros & Guerra-García, 2012), *Stenothoe georgiana* (Martínez-Laiz *et al*., 2020) and *Laticorophium baconi* (Guerra-García *et al*., 2023). However, unlike other NIS of the genus *Aoroides* (i.e., *A. curvipes* and *A. semicurvatus*), which are restricted to aquaculture oyster facilities, *A. longimerus* has been observed in diverse subtidal habitats in Arcachon Bay (Gouillieux *et al*., 2015). These authors hypothesised that *A. longimerus* may have dispersed from oyster beds in Arcachon Bay, through natural migration or hull fouling of recreational boats, subsequently establishing successful populations in natural habitats such as seagrass meadows or littoral limpet beds. In fact, the present study also confirms the presence of *A. longimerus* in natural habitats within its introduced range, such as Kneiss Islands in Tunisia. These islands are notable for their mud and sand flats, which are of international importance for their bird diversity. The area was declared a national nature reserve in 1993 and subsequently designated a "Specially Protected Area of Mediterranean Importance" (SPAMI) in 2001, an "Important Bird Area" (IBA) in 2003 and a "RAMSAR site" in 2007 (Moshabi *et al*., 2020). The detection of *A. longimerus* in such ecologically sensitive and protected habitats highlights the urgent need to implement robust monitoring and surveillance programmes aimed at the early detection of further potential introduction and spread of *A. longimerus* into other natural habitats, where it could eventually alter the composition and ecological dynamics of the native communities.

Importance of scientific collaboration

Gouillieux *et al.* (2015) suggested that species of *Aoroides* may have been present in European waters for decades, yet remained unobserved due to the following factors: (i) the genus is not listed in the taxonomic keys generally used to identify amphipods in European waters (e.g., Chevreux & Fage, 1925; Lincoln, 1979; Ruffo, 1982); (ii) specimens are fragile, often loosing gnathopods and antennae during the sieving process, which makes identification difficult and increases the likelihood of confusion with *Aora* genus; (iii) *Aoroides* species occur in particular habitats, such as fouling communities in artificial environments, that are not usually sampled in routine benthic surveys using corers or grabs. In this sense, increased taxonomic and sampling efforts are therefore essential for accurately assessing the status of *Aoroides* in the Mediterranean and adjacent Atlantic waters.

Scientific collaboration, taxonomic expertise, and knowledge dissemination have been crucial to update the distribution of various non-indigenous amphipods in recent years (e.g., Martínez-Laiz *et al.*, 2020; Guerra-García *et al.*, 2023). Indeed, several strategies have been proposed to ensure that NIS information is properly disseminated at all levels (Martínez-Laiz *et al.*, 2020; Carvalho *et al.*, 2023). The following actions are to be taken: (i) facilitation of enhanced opportunities for knowledge exchange between senior and early-career taxonomists; (ii) development of training programmes and online courses to build taxonomic capacity, especially in underrepresented regions and institutions; (iii) establishment of expert networks that bring together taxonomists, molecular scientists and invasion ecologists; (iv) support for participation of researchers in international events such as workshops and conferences focused on taxonomy and bioinvasions; (v) creation of open-access identification keys and field guides for NIS, including both morphological and molecular tools; (vi) development of internationally coordinated long-term monitoring programmes; (vii) increase of opportunities for information exchange between research scientists, environmental management agencies and other stakeholders, including funding agencies; (viii) regular update of open-access NIS databases, ensuring the accuracy and relevance of available information; and (ix) implementation of citizen science initiatives to support early detection and data collection on NIS, encouraging public engagement.

Scientific collaboration has resulted not only in providing an accurate distribution of the species but also backdating previous records (see Galanidi *et al.*, 2025). The present study exemplifies how collaboration and information exchange among researchers can lead to the re-examination of historical material, the collection of new samples, the integration of molecular tools, and the revision of morphological characters. These joint efforts provide a crucial baseline for further monitoring programmes and the development of effective management strategies.

Acknowledgements

The study was conducted within the collaborative initiatives MEDAMP (Mediterranean and Adjacent Seas Amphipod Reference Library) and PoLBOL (Polish Barcode of Life) and was also supported by the Horizon Europe "Biodiversity Genomics Europe" project (Project: 101059492 –BGE– HORIZON-CL6-2021-BIODIV-01). Financial support for this study was provided by the Ministerio de Economía y Competitividad (Project CGL2017-82739-P) co-financed by the ERDF, European Union, and predoctoral grants from the Spanish Government to PS-A (Reference FPU20/04016) and SR-V (Reference FPU21/02406). J-P P was supported by the project CHABIMANOR (Caractérisation des Habitats Biogéniques de substrats durs dans les MArinas NORmandes) financed by "Agence de l'Eau Seine-Normandie". J-C L received funding from the European Union's Horizon 2020 research programme under the Marie Skłodowska-Curie grant agreement no. 899546. IG was supported by EMERGIA contract funded by Junta de Andalucía (DGP_EMEC_2023_00528). PR was financed by Fundação para a Ciência e Tecnologia (FCT) through the strategic project [UIDB/ 04292] granted to MARE UI&I. J C-C was funded by national funds through FCT—Fundação para a Ciência e Tecnologia, IP, under the Scientific Employment Stimulus Institutional Call (CEEC-INST/00098/2018). SC received baseline funding from KAUST. EM and AB thank their colleagues Marco Lezzi, Rocco Auriemma, Giuseppe Catalano and Yann Toso who helped with the sorting of Italian samples. PS-A thanks his colleague Sergio C. García-Gómez for his support and advice during the identification process. We acknowledge the staff of all the marinas, ports, and aquaculture facilities who granted permission for conducting the sampling and observational field studies.

References

Almón, B., Pérez, J., Bañón, R., Trigo, J., 2014. First record of *Caprella mutica* from the Iberian Peninsula: expansion southwards in European waters. *Marine Biodiversity Records*, 7, e30.

Arfianti, T., Costello, M.J., 2020. Global biogeography of marine amphipod crustaceans: latitude, regionalization, and beta diversity. *Marine Ecology Progress Series*, 638, 83-94.

Ariyama, H., 2004. Nine species of the genus *Aoroides* (Crustacea: Amphipoda: Aoridae) from Osaka Bay, Central Japan. *Publications of the Seto Marine Biological Laboratory*, 40 (1-2), 1-66.

Ariyama, H., 2022. Guidebook of Gammaridean Amphipoda. Kaibundo, Tokyo, 160 pp. (in Japanese).

Ariyama, H., Kawabe, K., 2022. Two new species of Aoridae from Chichijima Island, the Ogasawara Islands in Japan (Crustacea: Amphipoda). *Species Diversity*, 27 (1), 113-128.

Ariyama, H., Kohtsuka, H., 2022. Three new species of the family Aoridae collected from Sagami Bay, central Japan (Crustacea: Amphipoda). *Zootaxa*, 5159 (3), 393-413.

Bailey, S.A., Brown, L., Campbell, M.L., Canning-Clode, J., Carlton, J.T. *et al*., 2020. Trends in the detection of aquatic non-indigenous species across global marine, estuarine and freshwater ecosystems: A 50-year perspective. *Diversity and Distributions*, 26 (12), 1780-1797.

Barnard, J.L., 1970. Sublittoral Gammaridea (Amphipoda) of the Hawaiian Islands. *Smithsonian Contributions to Zoology*, 34, 1-286.

Barnard, J.L., Karaman, G.S., 1991. The families and genera of marine gammaridean Amphipoda (except marine gammaroids). *Records of the Australian Museum*, (Suppl. 13) Part 1, 1-417.

Bell, S.S., 1991. Amphipods as insect equivalents? An alternative view. *Ecology*, 72 (1), 350-354.

Bello, C.L., Cabrera, M.I., 1999. Uso de la técnica microhistológica de Cavender y Hansen en la identificación de insectos acuáticos. *Boletín Entomológico de Venezuela*, 14, 77-79.

Beermann, J., Hall-Mullen, A.K., Havermans, C., Coolen, J.W.P., Crooijmans, R.P.M.A. *et al*., 2020. Ancient globetrotters connectivity and putative native ranges of two cosmopolitan biofouling amphipods. *PeerJ*, 8, e9613.

Carvalho, S., Shchepanik, H., Aylagas, E., Berumen, M.L., Costa, F.O. *et al*., 2023. Hurdles and opportunities in implementing marine biosecurity systems in data-poor regions. *BioScience*, 73 (7), 494-512.

Casquet, J., Thebaud, C., Gillespie, R.G., 2012. Chelex without boiling, a rapid and easy technique to obtain stable amplifiable DNA from small amounts of ethanol-stored spiders. *Molecular Ecology Resources*, 12, 136-141.

Castro, N., Carlton, J.T., Costa, A.C., Marques, C.S., Hewitt, C. *et al*., 2022. Diversity and patterns of marine non-native species in the archipelagos of Macaronesia. *Diversity and Distributions*, 28 (4), 667-684.

Chevreux, E., Fage, L., 1925. Amphipodes. Volume 9. Paris: Office Central de Faunistique, Librairie de la Faculté des Sciences.

Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Sayers, E.W., 2016. GenBank. Nucleic Acids Research, 44 (D1), D67-72.

CIEMAR, 2021. Substrato duro subtidal. Monitorização de Ambientes Marinhos do Porto de Sines - MAPSi 2018/2020. Relatório final. Laboratório de Ciências do Mar da Universidade de Évora. 191 pp. (report for the Port of Sines Administration).

CIEMAR, 2025. Substrato duro subtidal. Monitorização de Ambientes Marinhos do Porto de Sines - MAPSi 2021/2023. Relatório final. Laboratório de Ciências do Mar da Universidade de Évora. 275 pp. (report for the Port of Sines Administration).

Cuthbert, R.N., Pattison, Z., Taylor, N.G., Verbrugge, L., Diagne, C. *et al*., 2021. Global economic costs of aquatic invasive alien species. *Science of the Total Environment*, 775, 145238.

Conlan, K.E., Bousfield, E.L., 1982. The superfamily Corophioidea in the North Pacific region. Family Aoridae: systematics and distributional ecology. *National Museum of Natural Sciences (Ottawa), Publications in Biological Oceanography*, 10, 77-101.

Dauvin, J.-C., Pezy, J.-P., Raoux, A., 2020. First records of *Aoroides longimerus* Ren and Zheng, 1996, and *A. semicurvatus* Ariyama, 2004 (Crustacea, Amphipoda), in the English Channel, France. *BioInvasions Records*, 4, 753-762.

DQEM, 2020. Estratégia Marinha. Relatório do 2º ciclo, Parte D - Reavaliação do Estado Ambiental e Definição de Metas, subdivisão dos Açores. Governo dos Açores, Diretiva Quadro Estratégia Marinha, março 2020.

Faasse, M., Gittenberger, A., 2021. De exotische vlokreeften *Aoroides longimerus* en *Erichthonius didymus* bereiken Nederland (Crustacea: Amphipoda). *Nederlandse Faunistische Mededelingen*, 57, 81-88.

Galanidi, M., Gratsia, E., Zenetos, A., 2025. Backdating first records of non-indigenous species in the Mediterranean: are initial findings underestimated? *Mediterranean Marine Science*, 26 (3), 642-656.

Gouillieux, B., Lavesque, N., Leclerc, J.-C., Le Garrec, V., Viard, F. *et al*., 2015. Three non-indigenous species of *Aoroides* (Crustacea: Amphipoda: Aoridae) from the French Atlantic Coast. *Journal of the Marine Biological Association of the United Kingdom*, 96 (8), 1651-1659.

Guerra-García, J.M., Tierno de Figueroa, J.T., Navarro-Barranco, C., Ros, M., Sánchez-Moyano, J.E. *et al*., 2014. Dietary analysis of the marine Amphipoda (Crustacea: Peracarida) from the Iberian Peninsula. *Journal of Sea Research*, 85, 508-517.

Guerra-García, J.M., Revanales, T., Saenz-Arias, P., Navarro-Barranco, C., Ruiz-Velasco, S. *et al*., 2023. Quick spreading of the exotic amphipod *Laticorophium baconi* (Shoemaker, 1934): another small stowaway overlooked? *Mediterranean Marine Science*, 24 (3), 644-665.

Guindon, S., Dufayard, J., Lefort, V., Anisimova, M., Hordijk, W.O.G. *et al.*, 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3 .0. *Systematic Biology*, 59 (3), 307-321.

Gurjanova, E., 1938. Amphipoda, Gammaroidea zalikov Siaukhu i Sudzukhe (Yaponskoe More). [Amphipoda, Gammaroidea of Siaukhu Bay and Sudzukhe Bay (Japan Sea)]. Reports of the Japan Sea Hydrobiological Expedition of the Zoological Institute of the Academy of Sciences USSR in 1934. 1, 241-404.

Hou, Z., Fu, J., Li, S.A., 2007. Molecular phylogeny of the genus *Gammarus* (Crustacea: Amphipoda) based on mitochondrial and nuclear gene sequences. *Molecular Phylogenetics and Evolution*, 45 (2), 596-611.

ICES, 2018. Interim Report of the Working Group on Introductions and Transfers of Marine Organisms (WGITMO). 7-9 March 2018, Madeira, Portugal. ICES CM 2018/HAPISG:11. 179 pp.

Iitembu, J.A., Fitzgerald, D., Altintzoglou, T., Boudry, P., Britz, P. *et al*., 2023. Comparative description and analysis of oyster aquaculture in selected Atlantic regions: production, market dynamics, and consumption patterns. *Fishes*, 8 (12), 584.

Kim, J., Choi, J. H., Kim, Y. J., Im, H.W., Kim, Y. H., 2024. Two new species of the family Aoridae (Crustacea, Malacostraca, Amphipoda) from Korean waters. *ZooKeys*, 1210, 117-132.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A. *et al*., 2007. Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947-2948.

Lefort, V., Longueville, J.-E., Gascuel, O., 2017. SMS: Smart model selection in PhyML. *Molecular Biology and Evolution*, 34 (9): 2422-2424.

Lincoln, R.J., 1979. British marine Amphipoda: Gammaridea. London: British Museum (Natural History).

Lobo, J., Costa, P.M., Teixeira, M.A., Ferreira, M.S.G., Costa, M.H. *et al.*, 2013. Enhanced primers for amplification of DNA barcodes from a broad range of marine metazoans. *BMC Ecology*, 13, 34.

Macdonald III, K.S., Yampolsky, L., Duffy, J.E., 2005. Molecular and morphological evolution of the amphipod radiation of Lake Baikal. *Molecular Phylogenetics and Evolution*, 35, 323-343.

Marchini, A., Cardeccia, A., 2017. Alien amphipods in a sea of troubles: cryptogenic species, unresolved taxonomy and overlooked introductions. *Marine Biology*, 164 (4), 69.

Martínez-Laiz, G., Ros, M., Guerra-García, J.M., Marchini, A., Fernandez-Gonzalez, V., *et al*., 2020. Scientific collaboration for early detection of invaders results in a significant update on estimated range: Lessons from *Stenothoe georgiana* Bynum & Fox 1977. *Mediterranean Marine Science*, 21 (2), 464-481.

Martínez-Laiz, G., MacLeod, C.D., Hesketh, A.V., Konecny, C.A., Ros, M. *et al*., 2022. The journey of hull-fouling mobile invaders: basibionts and boldness mediate dislodgement risk during transit. *Biofouling*, 38 (8), 837-851.

Mosbahi, N., Boudaya, L., Neifar, L., Dauvin, J.C., 2020. Do intertidal *Zostera noltei* meadows represent a favourable habitat for amphipods? The case of the Kneiss Islands (Gulf of Gabès: Central Mediterranean Sea). *Marine Ecology*, 41 (3), 1-16.

Myers, A.A., 1995. The Amphipoda (Crustacea) of Madang Lagoon: Aoridae, Isaeidae, Ischyroceridae and Neomegamphopidae. In Lowry, J.K. (Ed.). The Amphipoda (Crustacea) of Madang Lagoon, Papua New Guinea, Part 1. *Records of the Australian Museum, Supplement*, 22, 25-95.

Myers, A.A., 2009. Aoridae. In: Lowry, J.K., Myers, A.A. (Eds.) Benthic Amphipoda (Crustacea: Peracarida) of the Great Barrier Reef, Australia. *Zootaxa*, 2260, 220-278.

Navarro-Barranco, C., Guerra-García, J.M., Sánchez-Tocino, L., Ros, M., Florido, M. *et al*., 2015. Colonization and successional patterns of the mobile epifaunal community along an environmental gradient in a marine cave. *Marine Ecology Progress Series*, 521, 105-115.

Navarro-Barranco, C., Florido, M., Ros, M., González-Romero, P., Guerra-García, J.M., 2018. Impoverished mobile epifaunal assemblages associated with the invasive macroalga *Asparagopsis taxiformis* in the Mediterranean Sea. *Marine Environmental Research*, 141, 44-52.

Navarro-Barranco, C., Muñoz-Gómez, B., Saiz, D., Ros, M., Guerra-García, J.M. *et al*., 2019. Can invasive habitat-forming species play the same role as native ones? The case of the exotic marine macroalga *Rugulopteryx okamurae* in the Strait of Gibraltar. *Biological Invasions*, 21, 3319-3334.

Navarro-Barranco, C., Moreira, J., Espinosa, F., Ros, M., Rallis, I. *et al*., 2021. Evaluating the vulnerability of coralligenous epifauna to macroalgal invasions. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 31 (9), 2305-2319.

Navarro-Barranco, C., Lanza-Arroyo, P., Gutiérrez-Serrano, J., Moreira, J., 2023. Amphipod assemblages associated with native habitat-forming seaweeds of the Alboran Sea: influence by environmental protection and biogeographical patterns. *Marine and Freshwater Research*, 74, 50-64.

Ojaveer, H., Galil, B.S., Carlton, J.T., Alleway, H., Goulletquer, P. *et al*., 2018. Historical baselines in marine bioinvasions: Implications for policy and management. *PloS ONE*, 13 (8), e0202383.

Palumbi, S.R., 1996. What can molecular genetics contribute to marine biogeography? An urchin's tale. *Journal of Experimental Marine Biology and Ecology*, 203 (1), 75-92.

Pascual, S., Villalba, A., Abollo, E., Garci, M., González, A.F. *et al*., 2010. The mussel *Xenostrobus securis*: a well-established alien invader in the Ria de Vigo (Spain, NE Atlantic). *Biological Invasions*, 12, 2091-2103.

Ratnasingham, S., Wei, C., Chan, D., Agda, J., Agda, J., *et al*. 2024. BOLD v4: A centralized bioinformatics platform for DNA-based biodiversity data. *Methods in Molecular Biology*, 2744, 403-441.

Ren, X., Zheng, C., 1996. Fouling Amphipoda (Crustacea) from Dayawan, Guangdong Province China (South China Sea). *Annual Research Reports*, *Marine Biology Research Station at Dayawan*, *South China Sea Institute of Oceanology*, *the Chinese Academy of Sciences*, 1, 58-78 [in Chinese with English abstract and descriptions of new species].

Rius, M., Turon, X., Bernard, G., Volckaert, F., Viard, F., 2015. Marine invasion genetics: from spatial and temporal patterns to evolutionary outcomes. *Biological Invasions*, 17 (3), 869-885.

Ros, M., Guerra-García, J.M., 2012. On the occurrence of the tropical caprellid *Paracaprella pusilla* Mayer, 1890 (Crustacea: Amphipoda) in Europe. *Mediterranean Marine Science*, 13 (1), 134-139.

Ros, M., Guerra-García, J.M., Navarro-Barranco, C., Cabezas, M.P., Vázquez-Luis, M., 2014. The spreading of the non-native caprellid (Crustacea: Amphipoda) *Caprella scaura* Templeton, 1836 into southern Europe and northern Africa: a complicated taxonomic history. *Mediterranean Marine Science*, 15 (1), 145-155.

Ruffo, S. (Ed.), 1982. The Amphipoda of the Mediterranean. Part 1. Gammaridea (Acanthonotozomatidae to Gammaridae). *Mémoires de l'Institut Océanographique de Monaco*, 13, 1-364.

Ruiz, C., Abad, M., Sedano, F., Garcia-Martin, L.O., Sánchez-López, J.L., 1992. Influence of seasonal environmental changes on the gamete production and biochemical composition of *Crassostrea gigas* (Thunberg) in suspended culture in El Grove, Galicia, Spain. *Journal of Experimental Marine Biology and Ecology*, 155 (2), 249-262.

Saenz-Arias, P., Navarro-Barranco, C., Ros, M., Moreira, J., Guerra-García, J.M., 2022a. Exploring biocontamination in associated macrofaunal assemblages in marinas: Soft bottoms vs artificial hard substrate. Where and what to look for? *Marine Pollution Bulletin*, 185, 114346.

Saenz-Arias, P., Navarro-Barranco, C., Guerra-García, J.M., 2022b. Influence of environmental factors and sessile biota on vagile epibionts: The case of amphipods in marinas across a regional scale. *Mediterranean Marine Science*, 23 (1), 1-13.

Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A. *et al*., 2007. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *BioScience*, 57 (7), 573-583.

Sun, Y., Wong, E., Keppel, E., Williamson, J.E., Kupriyanova, E.K., 2017. A global invader or a complex of regionally distributed species? Clarifying the status of an invasive calcareous tubeworm *Hydroides dianthus* (Verrill, 1873) (Polychaeta: Serpulidae) using DNA barcoding. *Marine Biology*, 164, 1-12.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution*, 38 (7), 3022-3027.

Ulman, A., Ferrario, J., Occhpinti-Ambrogi, A., Arvanitidis, C., Bandi, A. *et al*., 2017. A massive update of non-indigenous species records in Mediterranean marinas. *PeerJ*, 5, e3954.

Walker, A.O., 1898. Crustacea collected by W.A. Herdmann, F.R.S., in Puget Sound, Pacific coast of North America, September 1897. *Transactions of the Liverpool Biological Society*, 12, 268-287.

WoRMS Editorial Board, 2025. World register of marine species. https://www. marinespecies.org. at VLIZ.

Zenetos, A., Gratsia, E., Cardoso, A.C., Tsiamis, K., 2019. Time lags in reporting of biological invasions: the case of Mediterranean Sea. *Mediterranean Marine Science*, 20 (2), 469-475.

Supplementary Data

The following supplementary information is available online for the article:

Text S1. Detailed information on sampling surveys.

Table S1. List of localities sampled by the authors where presence of *Aoroides longimerus* Ren & Zheng, 1996 has not been detected.

Table S2. Detailed information on voucher material of *Aoroides longimerus* Ren & Zheng, 1996 deposited in Museo Nacional de Ciencias Naturales de Madrid (MNCN), Spain, and Muséum national d'Histoire Naturelle Paris (MNHN) France, including Museum Catalogue numbers, sampling locations, coordinates, collection date, substrates and number of specimens.

Supplementary Data

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
www.hcmr.gr
DOI: https://doi.org/10.12681/mms.41784

The spread of *Aoroides longimerus* Ren & Zheng, 1996 across the Mediterranean and the Atlantic: genetic diversity, anthropogenic transport, and ecological implications

José Manuel GUERRA-GARCÍA, Andrea DESIDERATO, Serena MUCCIOLO, Carlos NAVARRO-BARRANCO, Macarena ROS, Jasmine FERRARIO, Paula MORETTI, Agnese MARCHINI, Nawfel MOSBAHI, Sofía RUIZ-VELASCO, Ignacio GESTOSO, Patrício RAMALHOSA, João CANNING-CLODE, Celia OLABARRIA, Eva CACABELOS, Jesús S. TRONCOSO, Jean-Philippe PEZY, Aurore RAOUX, Jean-Claude DAUVIN, Mathilde CHARBONNELLE, Ferdinand SCHLICKLIN, Hiroyuki ARIYAMA, Emanuele MANCINI, Andrea BONIFAZI, Vincent LE GARREC, Thomas BUREL, Benoit GOUILLIEUX, Cristina ESPÍRITO SANTO, Paula CHAINHO, Romeu S. RIBEIRO, Inês AFONSO, Yanrong WANG, Jean-Charles LECLERC, Marine MOAL, Céline HOUBIN, Frédérique VIARD, Eric THIÉBAUT, Victoria FERNANDEZ-GONZALEZ, Sandra NAVARRO-MAYORAL, Sonia DÍAZ-VERGARA, Arjan GITTENBERGER, Lauren Elizabeth HUGHES, Maria LAMPA, Giorgos CHATZIGEORGIOU, Wanda PLAITIS, Francesco TIRALONGO, Gemma MARTÍNEZ-LAIZ, M. Pilar CABEZAS, Sofia DUARTE, João P. R. PINHEIRO, Giovanna O. REIS, Triana REVANALES, Juan SEMPERE-VALVERDE, Eva AYLAGAS, Angelo POLISENO, Sahar CHEBAANE, Susana CARVALHO, Vasilis GEROVASILEIOU, Inmaculada FRUTOS, and Pablo SAENZ-ARIAS

Mediterranean Marine Science, 26 (4) 2025

Text S1. Detailed information on sampling surveys.

Portugal, Spain and Morocco

In May-June 2011, a total of 42 marinas were sampled across the entire Iberian Peninsula and Northern Africa (Ros *et al*., 2015) (Table 1, Table S1). The survey was carried out as part of a general sampling program to characterise the expansion of *Caprella scaura* along the Iberian Peninsula and adjacent areas (see Ros *et al*., 2014). In each marina, three colonies of the erect bryozoan *Bugula neritina* were collected from the submerged portions of floating pontoons and fixed *in situ* in 90% ethanol. In the laboratory, the colonies were washed through a 0.5 mm sieve and their associated communities were checked for the presence of *Aoroides longimerus.* Selected marinas from the Southern Iberian Peninsula were resampled in June-July 2017 and April-May 2019 (Table S1). During the 2017 survey, fouling organisms growing on artificial hard substrates, including pontoons, ropes, wheels, buoys and ship hulls, were sampled. These included red and green algae, hydroids, bryozoans, ascidians and mollusks, along with their associated vagile assemblages. Samples were hand-collected, fixed in 90% ethanol and taken to the laboratory (Martínez-Laiz *et al*., 2018). In 2019, the polychaete *Sabella spallanzanii* (Gmelin, 1791) was sampled from the pontoons and associated fauna was examined for the presence of *A. longimerus*. In addition to marinas, four off-coast aquaculture facilities were also sampled in Spain from 2010 to 2023 (Table S1). These sites (Granada, Murcia, Guardamar del Segura and Tarragona) included sea cages stocked with farmed gilthead sea bream (*Sparus aurata* Linnaeus, 1758) and European sea bass (*Dicentrarchus labrax* (Linnaeus, 1758)). Samples were collected by scraping fouling organisms from shallow ropes of the mooring lines (1-10 m depth). Subsequently, they were sieved through a 0.5 mm mesh with seawater and preserved in 4% formalin seawater solution (see Fernandez-Gonzalez & Sanchez-Jerez, 2017).

In the marina of Quinta do Lorde, Madeira, Portugal (Table 1), several artificial prototype devices consisting mainly of PVC plates (14 cm × 14 cm × 0.3 cm) and CDs (see Diem *et al.*, 2023 for details of the structures) were deployed on 2 July 2018 (3-6 m depth) and collected on 30 January 2019. The associated fauna was sorted and checked for the presence of *A. longimerus*. Additional fouling samples from PVC plates (1-1.5 m depth) (see details in Ramalhosa *et al*., 2019, 2021) were collected at the same marina in June 2024.

In the framework of a study about the potential effects of biofilms on the establishment of sessile macrofouling communities (see Cacabelos *et al*., 2020), associated vagile communities were also examined. For that, a set of PVC settlement plates (14 cm × 14 cm × 0.3 cm) were placed at seven sites in the Ría de Vigo (Galicia, NW Iberian Peninsula) between 2 and 15 km apart, including floating piers (Cíes) and marinas/small harbours (Cangas, Davila, Moaña, Museo del Mar, Nautico, Toralla) (Table 1). Plates were collected in two maturing times, three and nine months after deployment, corresponding to April and August 2019, respectively. In each period, the associated vagile communities were preserved in 96% ethanol. In the laboratory, samples were washed through a 0.5 mm sieve in order to retain macrofaunal specimens. Amphipod assemblages were sorted, identified to species level and counted. Additionally, an aquaculture facility (mussel farm) in the Ría de Vigo was also sampled in December 2021-January 2022 by scraping fouling organisms from shallow ropes of the mooring lines (1-10 m depth) (Table 1).

As a part of a monitoring programme of marine habitats in the Port of Sines, Portugal (2019-2023), PVC plates (10 cm x 10 cm x 0.6 cm) were placed in commercial port terminals and the marina (Table 1). Each replicate consisted of three PVC plates and a polypropylene cable, held in tension in the water column by a weight at the end (CIEMAR, 2021). The plates of each structure were placed at different depths: 1 m below the water surface; in the middle of the water column; and 1 m above the bottom. Structures were collected after six months of immersion.

During different surveys to characterise marine communities of Tagus and Sado estuaries, and Aveiro region (2021-2024), Portugal, fouling communities were also sampled from ports and marinas (Table 1, Table S1).

To determine the potential presence of *A. longimerus* in the Canary Islands, Spain, ports and rhodolith seabeds close to aquaculture facilities were sampled in Gran Canaria. Samples were collected from four sites in this island during September 2023 and October 2024: the Port of Taliarte, the Port of Las Palmas de Gran Canaria (including Muelle de la Luz), and Tufia (Table S1). Samples were collected by scraping ropes colonized by epibionts and hydroids, submerged tires, pontoons, boat hulls, and buoys. When feasible, entire ropes were removed and transported to the laboratory for comprehensive analysis. In the case of rhodolith seabeds close to aquaculture facilities, five random samples were collected using a 20 cm × 20 cm quadrant to ensure representative coverage. Additionally, two more islands were sampled in 2023, Tenerife (marinas of Garachico and Las Galletas) and Fuerteventura (marina of Gran Tarajal). Fouling communities were collected from pontoons, ropes and buoys, and associated fauna of the bryozoan *Virididentula dentata* (Lamouroux, 1816) was also studied (Table 1).

France

As a part of a monitoring programme of sessile and vagile fauna in marinas from Normandy, France (Atlantic coast), PVC plates (0.0625 m^2) (smooth and sanded) were deployed in autumn 2018 in five marinas (Granville, Cherbourg, Ouistreham, Bassin Vauban and Dieppe) (Table 1, Table S1) at 1.5 m depth. PVC plates were collected in June and October 2021, and May and October 2022, and associated fauna was identified. During the four sampling events, scrapings (5 replicates of 0.0625 m^2) were collected from under the pontoons (depth 0.2-0.3 m) and piles (at low tide except for Bassin Vauban and Ouistreham where piles were submerged all the time, 1 m depth). As the surface area of the PVC plates matched that of the scrapings (0.0625 m^2), comparison of *A. longimerus* abundance among pontoons, piles and PVC plates (smooth vs sanded) were possible on a temporal basis from June 2021 to October 2022.

Additionally, floating pontoons and ropes were explored in marinas from the Brittany coast (France) during the period 2014-2022 (Table 1, Table S1) in the framework of several experimental and monitoring programmes conducted by the *Station Biologique de Roscoff* (Leclerc *et al*., 2024).

Floating pontoons in marinas from the Bay of Brest (Brittany, France) were studied since 2014 (Table 1) in the framework of surveys conducted by the *Institut Universitaire Européen de la Mer (IUEM)* (University of Brest).

Fouling communities were sampled in Leucate (Golfe du Lion, Mediterranean coast) and Dinard (Brittany, Atlantic coast) during two surveys organized by the Muséum national d'Histoire Naturelle in Paris in autumn 2023 and summer 2024, respectively (Table 1). In order to characterise the shallow benthic environments, samples were taken by scuba-divers on pontoons and in the harbours. Additionally, in summer 2023 and 2024 pontoons in Arcachon (Arcachon Bay, Atlantic coast) and Barcares (Golfe du Lion, Mediterranean coast) were also sampled ca. 0.5 m depth during a personal collection (Gouillieux, pers. comm) (Table 1).

The Netherlands

In order to streamline and better coordinate various marine NIS monitoring programs and surveys (since 2006), the Marine Alien Species Detection Network of the Netherlands was established in 2021 by the Netherlands Office for Risk Assessment and Research (BuRO) (Gittenberger *et al*., 2023). To cover a wide range of habitats, various sampling methods were employed, including settlement plates (14 cm × 14 cm), scraped samples from jetties and floating docks in ports and marinas, dredges, bottom grabs, and gelatinous zooplankton nets. Sampling sites encompassed not only vector-related sites, like ports and shellfish production areas, but also sites that may serve as stepping stones, such as navigational buoys along the North Sea coast (Table 1, Table S1).

Tunisia

Two off-coast aquaculture facilities, farming gilthead sea bream and sea bass, were sampled in May 2013 (Table S1). Samples were collected by scraping fouling organisms from shallow ropes of the mooring lines (1-10 m depth).

Subsequently, they were sieved through a 0.5 mm mesh with seawater and preserved in 4% formalin seawater solution (see Fernandez-Gonzalez & Sanchez-Jerez, 2017).

As part of Rapid Assessment Surveys (RAS) for NIS in Tunisia (Campbell *et al*., 2007; Chebaane *et al.,* 2019), biofouling samples were collected in August 2021 from six marinas: Bizerte, Sidi Bou Said, Yasmine Hammamet, Kantaoui, Monastir, and Djerba. These samples focused on a range of natural substrates, primarily including seaweeds, hydroids, and bryozoans, collected from the sea surface to a depth of 1 m, to assess the presence of NIS. Samples were preserved in 96% ethanol and sieved through a 0.5 mm mesh to retain both vagile and sessile organisms (Table S1).

Additional sampling was conducted in 2022-2023 as part of a broader effort to monitor benthic biodiversity in the Gulf of Gabès (Tunisia, central Mediterranean Sea) (Table 1). This monitoring encompassed various scientific projects across different habitats, including harbours and intertidal zones. Two sampling methods were employed: the Van Veen grab (0.05 m²) for harbour ecosystems and a corer for intertidal zones (0.0225 m²).

Italy, Croatia and Greece

In April 2011, an aquaculture facility from Follonica, Italy, farming gilthead sea bream and sea bass, was sampled (see Fernandez-Gonzalez & Sanchez-Jerez, 2017) (Table S1). Within a monitoring programme (2018-2022) of marinas in the Gulf of La Spezia, Ligurian Sea, PVC plates (14 cm × 14 cm × 0.3 cm) were placed in four marinas (Le Grazie, Santa Teresa, Fezzano and Portovenere) at 1 m depth (Table 1). Plates were oriented horizontally and facing downwards, kept at 1 m depth with the use of a brick. All the plates were immersed for three months, from the end of April until the end of July of each year. Artificial environments (harbours and marinas) from the Italian regions Lazio, Abruzzo, Sicilia, Puglia, Friuli Venezia Giuli and Emilia Romagna were also investigated for the presence of *A. longimerus* (Table S1). Samples were collected by scraping the fouling organisms from various artificial substrates (wharf wall, floating moorings, ropes, and buoys). A 20 cm × 20 cm hand-operated net (0.2 mm mesh size), provided with a stainless-steel blade on the board, was used. Regarding the mooring lines, an area corresponding to approximately 1 m of rope was scratched. Additional samples of rocky and soft substrates were also taken. Rocky substrates were collected by SCUBA diving (0.2-5 m) using a standard 20 cm x 20 cm sampling square. All material within the square was collected using a metal blade and hammer and immediately placed in a fine mesh bag (0.2 mm) and brought to the surface. The soft substrates were sampled using a 18 litres van Veen grab and sieved using a 1 mm mesh size. All samples were fixed in 80-85% ethanol and preserved in 75% ethanol.

In October 2010, two aquaculture facilities from Croatia, including sea cages stocked with young Atlantic bluefin tuna *Thunnus thynnus* (Linnaeus, 1758), were sampled by scraping fouling organisms from shallow ropes of the mooring lines (1-10 m depth) (see Fernandez-Gonzalez & Sanchez-Jerez, 2017) (Table S1).

Similarly, in September 2010, an aquaculture facility from Greece (farming gilthead sea bream and sea bass) was sampled by scraping fouling organisms from shallow ropes of the mooring lines (1-10 m depth) (see Fernandez-Gonzalez & Sanchez-Jerez, 2017) (Table S1). Additional sampling in several Greek ports, marinas and aquaculture facilities (Table S1) was conducted from 2020 to 2022 as part of different monitoring programmes, including PVC plates, hull fouling scraping, aquaculture facilities and RAS. These surveys targeted biofouling communities on port docks, ropes, buoys, and other floating structures. The RAS conducted in October and November 2022 covered 12 marinas across Greece. This method involved visual assessment and sampling of biofouling communities on a range of substrates, including floating structures and mooring lines. Samples were preserved in 96% ethanol or 4% formalin. To retain small vagile and sessile organisms, the samples were sieved through a 0.5 mm mesh.

Red Sea and Arabian Gulf

Fouling communities were sampled from ports and marinas in Saudi Arabia at three sites per location, covering seven locations in the Red Sea and four in the Arabian Gulf (Table S1), as part of a 2024 project funded by the National Center for Wildlife. At each site, five PVC plates were deployed seasonally, hung facing downward from docks, pontoons, or benthic reef structures at depths between 1 and 2 m, and retrieved after three months. Four retrievals were carried out, during Feb–Mar, May–Jun, Aug–Sep, and Nov–Dec 2024 (Table S1). Additionally, at each retrieval, five samples of mature fouling communities were scraped from vertical surfaces (e.g., pontoons, docks, or other hard substrata), and five seawater samples (~5 litres each) were filtered using 5 µm filters (47 mm diameter) for environmental DNA (eDNA) analysis.

Upon collection, biofouling samples from plates and scrapings were examined under a stereomicroscope, and voucher specimens for each morphospecies were collected. The remaining biofouling material was blended, subsampled, and preserved in 96% ethanol.

Morphological examination of voucher specimens and DNA metabarcoding analysis of three differente DNA sources (DNA from settlement plate biofouling, DNA from mature fouling scrapings, and eDNA from filtered seawater), were followed to screen for the presence of *A. longimerus*. DNA libraries targeting a 313bp fragment of the Cytochrome Oxidase Subunit I (CO1) gene were prepared following a two-step procedure (see detailed protocol in Sempere-Valverde *et al*., 2025) and sequenced on a Illumina MiSeq sequencing platform (v3 chemistry) at KAUST (Bioscience CORE Labs). CO1 sequences obtained from barcoded voucher specimens in this study were subsequently compared against the Amplicon Sequence Variant (ASV) libraries derived from each DNA dataset.

Australia and Japan

Extensive collections as part of different projects focusing on fouling communities from Australian harbours and ports were conducted from 1964 to 2002.

Specimens from Japanese coasts (putative native area) were collected from August 1979 to June 2024 during different sampling surveys (see details in Ariyama, 2004, 2022). Although most records have been already published, some new records for the country are provided by the present study (Table 1).

References

Ariyama, H., 2004. Nine species of the genus *Aoroides* (Crustacea: Amphipoda: Aoridae) from Osaka Bay, Central Japan. *Publications of the Seto Marine Biological Laboratory*, 40 (1-2), 1-66.

Ariyama, H., 2022. Guidebook of Gammaridean Amphipoda. Kaibundo, Tokyo, 160 pp. (in Japanese)

Cacabelos, E., Ramalhosa, P., Canning-Clode, J., Troncoso, J. S., Olabarria, C. *et al*., 2020. The role of biofilms developed under different anthropogenic pressure on recruitment of macro-invertebrates. *International Journal of Molecular Sciences*, 21 (6), 2030.

Campbell, M. L., Gould, B., Hewitt, C.L., 2007. Survey evaluations to assess marine bioinvasions. *Marine Pollution Bulletin*, 55 (7-9), 360-378.

Chebaane, S., Sempere-Valverde, J., Dorai, S., Kacem, A., Sghaier, Y.R., 2019. A Preliminary inventory of alien and cryptogenic species in Monastir Bay, Tunisia: spatial distribution, introduction trends and pathways. *Mediterranean Marine Science*, 20 (3), 616-626.

CIEMAR, 2021. Substrato duro subtidal. Monitorização de Ambientes Marinhos do Porto de Sines - MAPSi 2018/2020. Relatório final. Laboratório de Ciências do Mar da Universidade de Évora. 191 pp. (report for the Port of Sines Administration).

Diem, A., Ramalhosa, P., Cacabelos, E., Ferrario, J., Castro, N. *et al*., 2023. Monitoring Non-Indigenous Species with Passive Sampling Methods in an Oceanic Island. *Journal of Marine Science and Engineering*, 11 (2), 264.

Fernandez-Gonzalez, V., Sanchez-Jerez, P., 2017. Fouling assemblages associated with off-coast aquaculture facilities: an overall assessment of the Mediterranean Sea. *Mediterranean Marine Science*, 18 (1), 87-96.

Gittenberger, A., Rensing, M., Faasse, M.A., van Walraven, L., Smolders, A.A.J. *et al*., 2023. Non-Indigenous Species Dynamics in Time and Space within the Coastal Waters of The Netherlands. *Diversity*, 15, 719.

Leclerc J.-C., Gonzalez, M., Pezy, J.-P., Raoux, A., Houbin, C. *et al*., 2024. Multi-scale patterns in the structure of fouling communities associated with seaweeds in marinas. *Marine Ecology Progress Series*, 742, 1-19,

Martínez-Laiz, G., Ros, M., Guerra-García, J.M., 2018. Marine exotic isopods from the Iberian Peninsula and nearby waters. *PeerJ*, 6, e4408.

Ramalhosa, P., Gestoso, I., Duarte, B., Caçador, I., Canning-Clode, J., 2019. Metal pollution affects both native and non-indigenous biofouling recruitment in a subtropical island system. *Marine Pollution Bulletin*, 141, 373-386.

Ramalhosa, P., Gestoso, I., Rocha, R.M., Lambert, G., Canning-Clode, J., 2021. Ascidian biodiversity in the shallow waters of the Madeira Archipelago: Fouling studies on artificial substrates and new records. *Regional Studies in Marine Science*, 43, 101672.

Ros, M., Navarro-Barranco, C., Cabezas, M.P., Vázquez-Luis, M., 2014. The spreading of the non-native caprellid (Crustacea: Amphipoda) *Caprella scaura* Templeton, 1836 into southern Europe and northern Africa: a complicated taxonomic history. *Mediterranean Marine Science*, 15, 145-155.

Ros, M., Vázquez-Luis, M., Guerra-Garcia, J.M., 2015. Environmental factors modulating the extent of impact in coastal invasions: the case of a widespread invasive caprellid (Crustacea: Amphipoda) in the Iberian Peninsula. *Marine Pollution Bulletin*, 98 (1-2), 247-258.

Sempere-Valverde, J., Aylagas, E., Syomin, V., Teixeira, M.A., Kolbasova, G.D. *et al*., 2025. First assessment of biofouling assemblages in the northern Red Sea, an important region for marine non-indigenous species transfer. *Frontiers in Marine Science*, 12, p.1522723.

Table S1. List of localities sampled by the authors where presence of *Aoroides longimerus* Ren & Zheng, 1996 has not been detected.

Country	Locality	Coordinates	Habitat/Substrates	Date
Australia	Coral Bay, Port Essington, Northern Territory	11.18333º S, 132.05667º E	Fouling communities	1983
Australia	Perkins Wharf, Gove Harbour, Northern Territory	12.19383º S, 136.72000º E	Fouling communities	2001
Australia	Yacht Club, Inverell Bay, Gove Harbour, Northern Territory	12.20000º S, 136.71667º E	Fouling communities	2001
Australia	Cargo Wharf, Gove Harbour, Northern Territory	12.20417º S, 136.68083º E	Fouling communities	2001
Australia	Export Wharf, Gove Harbour, Northern Territory	12.20500º S, 136.67000º E	Fouling communities	2001
Australia	Yacht Club Mooring, Darwin Harbour, Northern Territory	12.43333º S, 130.81667º E	Fouling communities	1998
Australia	Near Stokes Hill Wharf, Darwin Harbour, Northern Territory	12.47000º S, 130.84833º E	Fouling communities	1998
Australia	Fort Hill Wharf, Darwin, Northern Territory	12.47150º S, 130.84667º E	Fouling communities	1999
Australia	Stokes Hill Wharf, Darwin Harbour, Northern Territory	12.47167º S, 130.84917º E	Fouling communities	1999
Australia	Inner Fort Hill Wharf, Darwin Harbour, Northern Territory	12.47200º S, 130.84717º E	Fouling communities	1998
Australia	Quara, Northern Territory, Anchorage, Darwin Harbour, Northern Territory	12.47238º S, 130.85687º E	Fouling communities	1998
Australia	Iron Ore Wharf, Darwin Harbour, Northern Territory	12.47250º S, 130.84283º E	Fouling communities	1998, 1999, 2002
Australia	Stevens Rock, Darwin Harbour, Northern Territory	12.48550º S, 130.78517º E	Fouling communities	2002
Australia	East Arm Port, Darwin Harbour, Northern Territory	12.49178º S, 130.88305º E	Fouling communities	1998
Australia	NW of Middle Point, Darwin Harbour, Northern Territory	12.50000º S, 130.85000º E	Fouling communities	1989
Australia	Channel Island, Middle Arm, Darwin Harbour, Northern Territory	12.55000º S, 130.86667º E	Fouling communities	1985
Australia	Spencer Gulf, near Port Pirie, off mouth of Fifth Creek, South Australia	33.20000º S, 137.90000º E	Fouling communities	1979, 1980
Australia	Port Davis Creek, near Port Pirie, Spencer Gulf, South Australia	33.30000º S, 137.90000º E	Fouling communities	1979, 1980

Continued

Table S1 continued

Country	Locality	Coordinates	Habitat/Substrates	Date
Australia	Port Kembla Jetty, New South Wales	34.48333º S, 150.93194º E	Fouling communities	2000
Australia	Bramble Point, Princess Royal Harbour, Albany, Western Australia	35.05972º S, 117.87177º E	Fouling communities	1988
Australia	Central Port Phillip Bay, Victoria	38.06667º S, 144.93333º E	Fouling communities	1970, 1971
Australia	Port Phillip Bay, Victoria	38.09395º S, 144.83992º E	Fouling communities	1969, 1970, 1971, 1972, 1973, 1990, 1991, 1992, 1994, 1995, 1996
Australia	Western Port, Victoria	38.35631º S, 145.24802º E	Fouling communities	1964, 1965, 1968, 1969, 1970, 1973, 1974, 1976, 1981
Croatia	Ugljan	44.02280° N, 15.21950° E	Fouling communities on fish farm	October 2010
Croatia	Brac	43.29550° N, 16.46000° E	Fouling communities on fish farm	October 2010
France	Fécamp	49.76263° N, 0.36671° E	Fouling communities on pontoons	16 May 2022
France	Cherbourg	49.64623° N, 1.62181° W	Fouling communities on pontoons	25 May 2022
France	Saint-Vaast-la-Hougue	49.58753° N, 1.26427° W	Fouling communities on pontoons	12 May 2022
France	Deauville	49.36338° N, 0.07214° E	Fouling communities on pontoons	16 June 2022
France	Ouistreham marina	49.27406º N, 0.24805º W	Fouling communities on PVC plates, floating pontoons and piles	Jun 2021-Oct 2022
France	Trébeurden	48.77032° N, 3.58702° W	Fouling communities on PVC plates	4 Aug 2014
France	Carantec	48.66656° N, 3.88980° W	Fouling communities of oyster farm	1 Sep 2023
France	Saint-Quay-Portrieux	48.64712° N, 2.82010° W	Fouling communities on ropes	12 Sep 2023
France	Saint-Malo	48.63889° N, 2.02592° W	Fouling communities on ropes	7 Sep 2023
Greece	Evoikos Gulf	38.60106º N, 23.33887º E	Fouling communities on ropes associated with *Mytilus galloprovincialis* and *Pinctada radiata*	Nov 2021
Greece	Nafpaktos marina	38.39247º N, 21.82888º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	14 Nov 2022
Greece	Galaxidi marina	38.37558º N, 22.38711º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	13 Nov 2022
Greece	Patras marina	38.26492º N, 21.73802º E	Fouling communities on artificial substrates	13 Jun 2021
Greece	Patras marina	38.26468º N, 21.73801º E	Fouling communities on artificial substrates	13 Jun 2021

Continued

Table S1 continued

Country	Locality	Coordinates	Habitat/Substrates	Date
Greece	Patras marina	38.26444º N, 20.73811º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	26 Nov 2022
Greece	Elefsina	38.03535º N, 23.51996º E	Fouling communities on ship hull	5 May 2022
Greece	Elefsina	38.03141º N, 23.52833º E	Fouling communities on ship hull	3 Mar 2022
Greece	Piraeus marina	37.93604º N, 23.64970º E	Fouling communities on artificial substrates	26 Sep 2020
Greece	Piraeus marina	37.93588º N, 23.64950º E	Fouling communities on artificial substrates	12 Oct 2021
Greece	Piraeus marina	37.93587º N, 23.64940º E	Soft bottom communities inside	26 Sep 2020
Greece	Kyllini marina	37.93547º N, 21.14767º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	26 Nov 2022
Greece	Piraeus	37.92068º N, 23.55437º E	Fouling communities on ship hull	22 Feb 2021
Greece	Piraeus	37.91656º N, 23.56711º E	Fouling communities on ship hull	22 Feb 2021
Greece	Piraeus	37.91117º N, 23.55621º E	Fouling communities on ship hull	27 Feb 2021
Greece	Agios Nikolaos marina (Zakynthos)	37.90664º N, 20.70742º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	25 Nov 2022
Greece	Piraeus	37.90441º N, 23.56144º E	Fouling communities on ship hull	26 Feb 2021
Greece	Piraeus	37.90332º N, 23.54169º E	Fouling communities on ship hull	8 Aug 2021
Greece	Piraeus	37.90266º N, 23.54730º E	Fouling communities on ship hull	2 Nov 2021
Greece	Piraeus	37.89926º N, 23.54162º E	Fouling communities on ship hull	30 Dec 2021
Greece	Piraeus	37.89628º N, 23.54893º E	Fouling communities on ship hull	8 Jun 2021
Greece	Piraeus	37.89000º N, 23.54642º E	Fouling communities on ship hull	12 Sep 2021
Greece	Alikanas marina (Zakynthos)	37.84419º N, 20.78050º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	10 Oct 2022
Greece	Kavos Psarou marina (Zakynthos)	37.84011º N, 20.81983º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	17 Nov 2022
Greece	Tsilivi marina (Zakynthos)	37.82067º N, 20.86733º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	Nov 2022

Continued

Table S1 continued

Country	Locality	Coordinates	Habitat/Substrates	Date
Greece	Zakynthos Port	37.78278º N, 20.89867º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	1-2 Nov 2022
Greece	Epidaurus, Saronikos	37.76537º N, 23.16102º E	Fouling communities on ropes associated with *Mytilus galloprovincialis* and *Pinctada radiata*	Nov 2021
Greece	Lavrio	37.72903º N, 24.06950º E	Fouling communities on ship hull	20 Oct 2020
Greece	Laganas marina (Zakynthos)	37.71531º N, 20.86400º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	22 Oct 2022
Greece	Keri Lake marina (Zakynthos)	37.68228º N, 20.83742º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	22 Oct 2022
Greece	Nafplio marina	37.56772º N, 22.79525º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	10 Nov 2022
Greece	Rhodes marina	36.43343º N, 28.23931º E	Fouling communities on artificial substrates	22 Sep 2020
Greece	Rhodes marina	36.43325º N, 28.23953º E	Fouling communities on artificial substrates	11 Jun 2021
Greece	Rhodes	36.24414º N, 27.77469º E	Fouling communities on ropes associated with *Pinctada radiata*	May 2021
Greece	Rhodes	36.24414º N, 27.77469º E	Fouling communities on ropes associated with *Pinctada radiata*	Jul 2021
Greece	Crete	35.58410° N, 25.24980° E	Fouling communities on fish farm	September 2010
Greece	Underwater Biotechnological Park of Crete, Gournes	35.35701º N, 25.28275º E	Fouling communities on PVC plates	2021, 2022
Greece	Heraklion marina, Crete	35.34398º N, 25.13720º E	Fouling communities on artificial substrates	3 Sep 2020
Greece	Heraklion marina, Crete	35.34342º N, 25.13687º E	Soft bottom communities	3 Sep 2020
Greece	Heraklion marina, Crete	35.34314º N, 25.13661º E	Fouling communities on PVC plates	12 Oct 2021, 1 Sept 2022
Italy	Trieste harbour, Friuli Venezia Giulia	45.64753º N, 13.74357º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	12 Jul 2024
Italy	Trieste harbour, Friuli Venezia Giulia	45.63720º N, 13.74217º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	12 Jul 2024

Continued

Table S1 continued

Country	Locality	Coordinates	Habitat/Substrates	Date
Italy	Trieste harbour, Friuli Venezia Giulia	45.62240º N, 13.74645º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	13 Jul 2024
Italy	Trieste harbour, Friuli Venezia Giulia	45.61317º N, 13.74550º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	13 Jul 2024
Italy	Ravenna harbour, Emilia Romagna	44.49164º N, 12.29169º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	7 Aug 2024
Italy	Cesenatico, Emilia Romagna	44.20581º N, 12.39722º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	24 Aug 2024
Italy	Follonica	42.91433° N, 10.64233° E	Fouling communities on fish farm	April 2011
Italy	Pescara commercial harbour, Abruzzo	42.46593º N, 14.23388º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-0.5 m depth	8 Aug 2024
Italy	Pescara commercial harbour, Abruzzo	42.46578º N, 14.23296º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-0.5 m depth	8 Aug 2024
Italy	Ortona commercial harbour, Abruzzo	42.34743º N, 14.41489º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-0.5 m depth	12 Jul 2024
Italy	Ortona commercial harbour, Abruzzo	42.34652º N, 14.41537º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-0.5 m depth	12 Jul 2024
Italy	Marina of Fossacesia, Abruzzo	42.23662º N, 14.53781º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-0.5 m depth	14 Jul 2024
Italy	Civitavecchia commercial harbour, Lazio	42.09432º N, 11.78407º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.2-1 m depth	26 July 2024
Italy	Civitavecchia commercial harbour, Lazio	42.09373º N, 11.78668º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.2-1 m depth	25 Jul 2024
Italy	Civitavecchia commercial harbour, Lazio	42.09360º N, 11.78948º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.2-1 m depth	25 July 2024
Italy	Civitavecchia commercial harbour, Lazio	42.08983º N, 11.78861º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.2-1 m depth	26 Jul 2024

Continued

Table S1 continued

Country	Locality	Coordinates	Habitat/Substrates	Date
Italy	Santa Marinella, Marina of Riva di Traiano, Lazio	42.05857º N, 11.81523º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.5 m depth	7 Aug 2024
Italy	Dock of Torre Flavia, Lazio	41.95457º N, 12.05613º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.5 m depth	12 Jul 2024
Italy	Marina of San Nicola, Lazio	41.93181º N, 12.11058º E	Rocky natural substrates, 0.5-4 m depth	22 Aug 2024
Italy	Fiumicino, canale dei pescatori, Lazio	41.71880º N, 12.30325º E	Fouling communities on port docks, ropes, buoys and floating structures, 0-0.5 m depth	24 Sep 2024
Italy	Ostia, Lazio	41.71834º N, 12.30325º E	Rocky natural substrates, 0.5-4 m depth	3 Sep 2024
Italy	Marina of Anzio, Lazio	41.44745º N, 12.63440º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.5 m depth	14 Jul 2024
Italy	Formia harbour, Lazio	41.25644º N, 13.61297º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.5 m depth	9 Sep 2024
Italy	Gaeta Gulf, Lazio	41.24941º N, 13.61009º E	Soft bottoms, 13 m depth	2 Aug 2024
Italy	Marina of Gaeta Gulf, Lazio	41.21641º N, 13.57157º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.5 m depth	2 Oct 2024
Italy	Brindisi commercial harbour, Puglia	40.66073º N, 17.96237º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-0.5 m depth	3 Sep 2024
Italy	Brindisi commercial harbour, Puglia	40.65914º N, 17.96431º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-0.5 m depth	3 Sep 2024
Italy	Taranto, Mar Grande, Puglia	40.43420º N, 17.23842º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-3 m depth	24 Aug 2024
Italy	Taranto, Mar Grande, Puglia	40.43099º N, 17.23938º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-0.5 m depth	24 Aug 2024
Italy	Taranto Mar Grande, Puglia	40.43025º N, 17.24027º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-0.5 m depth	24 Aug 2024
Italy	Otranto harbour, Puglia	40.14873º N, 18.49495º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-0.5 m depth	27 Aug 2024

Continued

Table S1 continued

Country	Locality	Coordinates	Habitat/Substrates	Date
Italy	Gallipoli harbour, Puglia	40.05814º N, 17.98274º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-0.5 m depth	29 Aug 2024
Italy	Palermo, La Cala marina, Sicily	38.11916º N, 13.36834º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	23 Mar 2024
Italy	Trapani harbour, Sicily	38.01583º N, 12.49921º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	5 Jun 2024
Italy	Catania harbour, Sicily	37.49204º N, 15.09882º E	Fouling communities on port docks, ropes, buoys and floating structures, 0-0.5 m depth	15 Jun 2024
Italy	Catania harbour, Sicily	37.49105º N, 15.10208º E	Soft bottoms, 19 m depth	12 May 2024
Italy	Catania harbour, Sicily	37.48249º N, 15.10059º E	Soft bottoms, 18 m depth	12 May 2024
Italy	Porto Empedocle harbour, Sicily	37.28619º N, 13.52721º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	2 Jun 2024
Italy	Augusta harbour, Sicily	37.23196º N, 15.21736º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	9 Mar 2024
Italy	Augusta harbour, Sicily	37.20833º N, 15.22758º E	Soft bottoms, 5 m depth	21 Apr 2024
Italy	Augusta harbour, Sicily	37.20484º N, 15.22927º E	Soft bottoms, 5 m depth	21 Apr 2024
Italy	Siracusa harbour, Sicily	37.06852º N, 15.29127º E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	13 Mar 2024
Italy	Avola harbour, Sicily	36.92015° N, 15.16075° E	Fouling communities on port docks, ropes, buoys and floating structures, 0.5 m depth	13 Sep 2024
Italy	Marzamemi harbour, Sicily	36.74111° N, 15.11873° E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	18 Mar 2024
Italy	Portopalo Capo Passero, Sicily	36.67004° N, 15.12601° E	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	5 Aug 2024
Morocco	Tánger marina	35.78731º N, 5.80594º W	Bryozoan *Bugula neritina* on floating pontoons	31 May 2011
Morocco	Kabila marina	35.71986º N, 5.33550º W	Bryozoan *Bugula neritina* on floating pontoons	30 May 2011
Morocco	M-Diq marina	35.68269º N, 5.31364º W	Bryozoan *Bugula neritina* on floating pontoons	30 May 2011
Netherlands	Den Helder port, Wadden Sea	52.96143º N, 4,78118º E	Fouling communities from jetty	30 Sep 2021, 11 Sep 2022, 11 Sep 2023
Netherlands	Ijmuiden pontplein	52.46292º N, 4,63310º E	Fouling communities from jetty	11 Sep 2023

Continued

Table S1 continued

Country	Locality	Coordinates	Habitat/Substrates	Date
Netherlands	Ijmuiden seaport	52.45865º N, 4.56100º E	Fouling communities from floating docks	28 Oct 2022, 11 Sep 2023
Netherlands	Port of Rotterdam, Petroleumhaven	51.96925º N, 4,06827º E	Fouling communities from floating docks	6 Sep 2021, 6 Sep 2022, 4 Sep 2023
Netherlands	Port of Rotterdam, Beneluxhaven	51.95277º N, 4,12518º E	Fouling communities from floating docks	6 Sep 2021, 6 Sep 2022, 4 Sep 2023
Netherlands	Bommenede, Lake Grevelingen	51.73170º N, 3,97302º E	Fouling communities from floating docks	7 Sep 2021, 6 Sep 2022, 12 Sep 2023
Netherlands	Colijnsplaat, Oosterschelde	51.60278º N, 3,84962º E	Fouling communities from floating docks	27 Sep 2021, 12 Sep 2022, 21 Sep 2023
Netherlands	Kortgene marina, Veerse Meer	51.55202º N, 3.81103º E	Fouling communities from floating docks	27 Sep 2021, 13 Sep 2022, 21 Sep 2023
Netherlands	Sloehaven, Vlissingen, Westerschelde	51.46055º N, 3.67332º E	Fouling communities from floating docks	28 Sep 2021, 30 Sep 2022, 20 Sep 2023
Netherlands	Eemshaven port, Wadden Sea	51.44470º N, 6.82552º E	Fouling communities from floating docks	1 Sep 2021, 21 Sep 2022, 26 Sep 2023
Netherlands	Breskens marina, Westerschelde	51.39575º N, 3.57030º E	Fouling communities from floating docks	28 Sept 2021, 11 Sep 2022, 23 Sep 2023
Portugal	Viana do Castelo marina	41.69421º N, 8.82160º W	Fouling communities on pontoons	Sep 2021
Portugal	Leixões marina	41.18637º N, 8.70494º W	Fouling communities on pontoons	Sep 2021
Portugal	Jardim Oudinot marina	40.64405º N, 8.73185º W	Fouling communities on pontoons	14 Oct 2024
Portugal	Costa Nova marina	40.62012º N, 8.74881º W	Fouling communities on pontoons	14 Oct 2024
Portugal	Nazaré marina	39.58627º N, 9.07180º W	Bryozoan *Bugula neritina* on floating pontoons	9 May 2011
Portugal	Vila Franca de Xira marina	38.95463º N, 8.98513º W	Fouling communities on pontoons	Sep 2016
Portugal	Vila Franca de Xira marina	38.95463º N, 8.98513º W	Fouling communities on pontoons	Sep 2022
Portugal	Parque das Nações marina	38.76560º N, 9.09385º W	Fouling communities on pontoons	Jun 2016
Portugal	Parque das Nações marina	38.76558º N, 9.09385º W	Fouling communities on pontoons	Sep 2022
Portugal	Alcantara marina	38.70204º N, 9.16737º W	Fouling communities on pontoons	Mar 2016
Portugal	Alcantara marina	38.70204º N, 9.16737º W	PVC plates	Sep 2023
Portugal	Cascais	38.69274º N, 9.41794º W	PVC plates	Sep 2022
Portugal	Oeiras marina	38.67601º N, 9.31869º W	Fouling communities on pontoons	Mar 2016
Portugal	Oeiras marina	38.67601º N, 9.31869º W	Fouling communities on pontoons	Sep 2022
Portugal	Albufeira marina	37.08487º N, 8.26617º W	Bryozoan *Bugula neritina* on floating pontoons (2011), polychaete *Sabella spallanzanii* (2019)	10 May 2011, 15 May 2019
Portugal	Vilamoura marina	37.07408º N, 8.12343º W	Fouling communities on pontoons	Sep 2021

Continued

Table S1 continued

Country	Locality	Coordinates	Habitat/Substrates	Date
Portugal	Faro marina	37.01497º N, 7.93669º W	Bryozoan *Bugula neritina* on floating pontoons (2011), Fouling communities (2017), polychaete *Sabella spallanzanii* (2019)	11 May 2011, 26 Jun 2017, 15 May 2019
Saudi Arabia	NEOM, Tabuk Province	Sites from 28.17607º N, 34.64192º E to 28.05413º N, 35.19800º E	PVC plates, fouling communities on artificial substrates, water samples	12-13 Feb 2024, 13-14 May 2024, 12-13 Aug 2024, 4-5 Nov 2024
Saudi Arabia	Jubail, Eastern Province	From 27.12718º N, 49.51521º E to 27.01423º N, 49.67779º E	PVC plates, fouling communities on artificial substrates, water samples	19-20 Mar 2024, 5 Jun 2024, 7-8 Sep 2024, 2-4 Dec 2024
Saudi Arabia	Tarout Island, Eastern Province	From 26.60378º N, 50.08167º E to 26.53970º N, 50.08529º E	PVC plates, fouling communities on artificial substrates, water samples	18-20 Mar 2024, 4 Jun 2024, 5-6 Sep 2024, 5-6 Dec 2024
Saudi Arabia	Dammam, Eastern Province	From 26.50919º N, 50.19781º E to 26.4852º N, 50.20154º E	PVC plates, fouling communities on artificial substrates, water samples	13-17 Mar 2024, 6 Jun 2024, 8-9 Sep 2024, 3-7 Dec 2024
Saudi Arabia	Al Khobar, Eastern Province	From 26.24000º N, 50.22200º E to 26.15660º N, 50.19401º E	PVC plates, fouling communities on artificial substrates, water samples	13-14 Mar 2024, 3 Jun 2024, 4-5 Sep 2024, 3-4 Dec 2024
Saudi Arabia	Al Wajh, Tabuk region	From 26.22584º N, 36.45941º E to 25.02203º N, 37.26420º E	PVC plates, fouling communities on artificial substrates, water samples	26-27 Mar 2024, 25-26 Jun 2024, 24-25 Sep 2024, 16-17 Dec 2024
Saudi Arabia	Yanbu, Al Madinah Province	From 24.16870º N, 37.96528º E to 24.07214º N, 38.05239º E	PVC plates, fouling communities on artificial substrates, water samples	6-7 Mar 2024, 11-27 Jun 2024, 15-30 Sep 2024, 10-11 Dec 2024
Saudi Arabia	Thuwal, Makkah Province	From 22.30460º N, 39.09454º E to 22.27996º N, 39.08821º E	PVC plates, fouling communities on artificial substrates, water samples	26-27 Feb 2024, 20-21 May 2024, 19-20 Aug 2024, 18-19 Nov 2024
Saudi Arabia	Jeddah, Makkah Province	From 21.73788º N, 39.12296º E to 21.45349º N, 39.16747º E	PVC plates, fouling communities on artificial substrates, water samples	28 Feb 2024, 24 Apr 2024, 21-22 May 2024, 20-29 Aug 2024, 19-27 Nov 2024
Saudi Arabia	Al Lith, Tihamah Province	From 20.14038º N, 40.25322º E to 20.12119º N, 40.26432º E	PVC plates, fouling communities on artificial substrates, water samples	3-4 Mar 2024, 25-26 May 2024, 26-27 Aug 2024, 25-26 Nov 2024
Saudi Arabia	Jazan, Jazan Province	From 16.90510º N, 42.53727º E to 1607423º N to 42.68908º E	PVC plates, fouling communities on artificial substrates, water samples	5-6 Mar 2024, 28-29 May 2024, 28-29 Aug 2024
Spain	Gijón marina	43.54411º N, 5.66750º W	Bryozoan *Bugula neritina* on floating pontoons	3 May 2011
Spain	A Graña marina	43.47906º N, 8.25959º W	Bryozoan *Bugula neritina* on floating pontoons	7 May 2011
Spain	Santander marina	43.42838º N, 3.80902º W	Bryozoan *Bugula neritina* on floating pontoons	2 May 2011
Spain	A Coruña marina	43.36830º N, 8.39814º W	Bryozoan *Bugula neritina* on floating pontoons	4 May 2011
Spain	L'Estartit marina	42.05400º N, 3.20603º E	Bryozoan *Bugula neritina* on floating pontoons	25 Jun 2011
Spain	Barcelona marina	41.37725º N, 2.18292º E	Bryozoan *Bugula neritina* on floating pontoons	26 Jun 2011
Spain	Vilanova Geltrú marina	41.21358º N, 1.72906º E	Bryozoan *Bugula neritina* on floating pontoons	27 Jun 2011

Continued

Table S1 continued

Country	Locality	Coordinates	Habitat/Substrates	Date
Spain	Tarragona marina	41.10861º N, 1.25194º E	Bryozoan *Bugula neritina* on floating pontoons	27 Jun 2011
Spain	Tarragona	40.87880º N, 0.58800º E	Fouling communities on fish farm	October 2010
Spain	Benicarló marina	40.41536º N, 0.43367º E	Bryozoan *Bugula neritina* on floating pontoons	27 Jun 2011
Spain	Oropesa del mar marina	40.07533º N, 0.13331º E	Bryozoan *Bugula neritina* on floating pontoons	28 Jun 2011
Spain	Burriana marina	39.86081º N, 0.07275º W	Bryozoan *Bugula neritina* on floating pontoons	28 Jun 2011
Spain	Valencia marina	39.42811º N, 0.33239º W	Bryozoan *Bugula neritina* on floating pontoons	28 Jun 2011
Spain	Dénia marina	38.84551º N, 0.11161º E	Bryozoan *Bugula neritina* on floating pontoons	28 Jun 2011
Spain	Alicante marina	38.33964º N, 0.48631º W	Bryozoan *Bugula neritina* on floating pontoons	29 Jun 2011
Spain	Guardamar del Segura	38.09600º N, 0.60430º W	Fouling communities on fish farm	July 2010, 30 Jun 2022
Spain	Torrevieja marina	37.97472º N, 0.68322º W	Bryozoan *Bugula neritina* on floating pontoons	29 Jun 2011
Spain	Murcia	37.81550° N, 0.69550º W	Fouling communities on fish farm	28 Jul 2022, May 2023
Spain	Granada	37.22983º N, 1.74666º W	Fouling communities on fish farm	May 2010
Spain	El Rompido marina	37.21617º N, 7.12872º W	Bryozoan *Bugula neritina* on floating pontoons (2011), polychaete *Sabella spallanzanii* (2019)	16 May 2011, 16 May 2019
Spain	Isla Cristina marina	37.19600º N, 7.32747º W	Bryozoan *Bugula neritina* on floating pontoons (2011), polychaete *Sabella spallanzanii* (2019)	16 May 2011, 16 May 2019
Spain	Isla Canela marina	37.18650º N, 7.34000º W	Bryozoan *Bugula neritina* on floating pontoons (2011), Fouling communities (2017), polychaete *Sabella spallanzanii* (2019)	16 May 2011, 26 Jun 2017, 16 May 2019
Spain	Carboneras marina	36.99106º N, 1.89633º W	Bryozoan *Bugula neritina* on floating pontoons	30 Jun 2011
Spain	Almería marina	36.83150º N, 2.46167º W	Bryozoan *Bugula neritina* on floating pontoons (2011), polychaete *Sabella spallanzanii* (2019)	1 Jul 2011, 27 Jun 2017, 30 Apr 2019
Spain	Roquetas marina	36.75653º N, 2.60644º W	Bryozoan *Bugula neritina* on floating pontoons	1 Jul 2011
Spain	La Caleta marina	36.74883º N, 4.06731º W	Bryozoan *Bugula neritina* on floating pontoons (2011), Fouling communities (2017)	3 Jul 2011, 28 Jun 2017
Spain	Chipiona marina	36.74486º N, 6.43033º W	Bryozoan *Bugula neritina* on floating pontoons (2011), Fouling communities (2017)	17 May 2011, 1 Jul 2017

Continued

Table S1 continued

Country	Locality	Coordinates	Habitat/Substrates	Date
Spain	Motril marina	36.72417º N, 3.52875º W	Bryozoan *Bugula neritina* on floating pontoons (2011), Fouling communities (2017)	2 Jul 2011, 28 Jun 2017
Spain	Málaga marina	36.70824º N, 4.41282º W	Bryozoan *Bugula neritina* on floating pontoons (2011), polychaete *Sabella spallanzanii* (2019)	3 Jul 2011, 29 Jun 2017, 30 Apr 2019
Spain	Almerimar marina	36.69700º N, 2.79178º W	Bryozoan *Bugula neritina* on floating pontoons	1 Jul 2011
Spain	Rota marina	36.61664º N, 6.35475º W	Bryozoan *Bugula neritina* on floating pontoons (2011), polychaete *Sabella spallanzanii* (2019)	17 May 2011, 17 May 2019
Spain	Benalmádena marina	36.59597º N, 4.51231º W	Bryozoan *Bugula neritina* on floating pontoons	15 May 2011
Spain	Fuengirola marina	36.54161º N, 4.61719º W	Bryozoan *Bugula neritina* on floating pontoons (2011), Fouling communities (2017)	15 May 2011, 29 Jun 2017
Spain	Puerto América marina	36.54144º N, 6.28400º W	Bryozoan *Bugula neritina* on floating pontoons (2011), Fouling communities (2017), polychaete *Sabella spallanzanii* (2019)	17 May 2011, 2 Jul 2017, 18 May 2019
Spain	Sancti Petri marina	36.39619º N, 6.20839º W	Bryozoan *Bugula neritina* on floating pontoons (2011), polychaete *Sabella spallanzanii* (2019)	17 May 2011, 17 May 2019
Spain	Conil marina	36.29494º N, 6.13633º W	Bryozoan *Bugula neritina* on floating pontoons	17 May 2011
Spain	Barbate marina	36.18414º N, 5.93425º W	Bryozoan *Bugula neritina* on floating pontoons (2011), Fouling communities (2017)	17 May 2011, 1 Jul 2017
Spain	La Línea (Puerto Chico) marina	36.15989º N, 5.35756º W	Bryozoan *Bugula neritina* on floating pontoons (2011), Fouling communities (2017)	15 May 2011, 30 Jun 2017
Spain	Ceuta marina	35.88958º N, 5.31619º W	Bryozoan *Bugula neritina* on floating pontoons	29 May 2011
Spain	Muelle de la luz, Gran Canaria Port, Canary Islands	28.14452º N, 15.42061º W	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	18 Oct 2024
Spain	Las Palmas marina, Gran Canaria Port, Canary Islands	28.12835º N, 15.42631º W	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	23 Oct 2024
Spain	Taliarte Port, Gran Canaria, Canary Islands	27.99083º N, 15.36861º W	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	11 Oct 2024

Continued

Table S1 continued

Country	Locality	Coordinates	Habitat/Substrates	Date
Spain	Tufia, Gran Canaria, Canary Islands	27.95871º N, 15.37269º W	Rhodolith seabeds close to aquaculture facilities	11 Sep 2023
Tunisia	Ghar el Melh	37.31660° N, 10.28050° E	Fouling communities on fish farm	May 2013
Tunisia	Bizerte marina	37.27464º N, 9.88108º W	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	16 Aug 2021
Tunisia	Sidi Bou Said marina	36.86692º N, 10.35167º W	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	16 Aug 2021
Tunisia	Yasmine Hammamet marina	36.37333º N, 10.54708º W	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	18 Aug 2021
Tunisia	Kantaoui marina	35.89417º N, 10.59789º W	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	20 Aug 2021
Tunisia	Monastir marina	35.77958º N, 10.83358º W	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	13 Aug 2021
Tunisia	Mahdia	35.45560° N, 11.09460° E	Fouling communities on fish farm	May 2013
Tunisia	Djerba marina	33.88891º N, 10.85622º W	Fouling communities on port docks, ropes, buoys and floating structures, 0.1-1 m depth	19 Aug 2021

Table S2. Detailed information on voucher material of *Aoroides longimerus* Ren & Zheng, 1996 deposited in Museo Nacional de Ciencias Naturales de Madrid (MNCN), Spain, and Muséum national d'Histoire Naturelle Paris (MNHN) France, including Museum Catalogue numbers, sampling locations, coordinates, collection date, substrates and number of specimens.

Catalogue number	Location	Coordinates	Date	Substrates	Number of specimens
MNCN 20.04/41958	Cascais marina, Portugal	38.69094º N, 9.41855º W	9 May 2011	*Bugula neritina* (pontoons)	2 males
MNCN 20.04/41959	Cangas marina, Ría de Vigo, Galicia, Spain	42.26089º N, 8.78379º W	Aug 2019	Fouling communities on PVC plates	1 male, 1 female
MNCN 20.04/41960	Davila marina, Ría de Vigo, Galicia, Spain	42.23362º N, 8.74283º W	Aug 2019	Fouling communities on PVC plates	4 males, 4 females
MNCN 20.04/41961	Moaña marina, Ría de Vigo, Galicia, Spain	42.27610º N, 8.73481º W	Aug 2019	Fouling communities on PVC plates	3 males, 2 females
MNCN 20.04/41962	Quinta do Lorde marina, Madeira, Portugal	32.74169º N, 16.71191º W	25 Jun 2024	Fouling communities on PVC plates	3 males
MNHN-IU-2023-2236	Leucate harbour, Languedoc-Rousillon, Golfe du Lion, France	42.87º N 3.047º E	28 Sep 2023	On pontoon	1 male
MNHN-IU-2020-3924	Leucate harbour, Languedoc-Rousillon, Golfe du Lion, France	42.87º N 3.047º E	28 Sep 2023	On pontoon	1 male
MNHN-IU-2023-2237	Arcachon harbour, Gironde, Arcachon Bay, France	44.6597º N, 1.1519º W	6 Aug 2024	Fouling communities on pontoon	11 males, 12 individuals
MNHN-IU-2020-3914	Arcachon harbour, Gironde, Arcachon Bay, France	44.6597º N, 1.1519º W	6 Aug 2024	Fouling communities on pontoon	1 male
MNHN-IU-2020-3915	Arcachon harbour, Gironde, Arcachon Bay, France	44.6597º N, 1.1519º W	6 Aug 2024	Fouling communities on pontoon	1 male
MNHN-IU-2020-3916	Arcachon harbour, Gironde, Arcachon Bay, France	44.6597º N, 1.1519º W	6 Aug 2024	Fouling communities on pontoon	1 male
MNHN-IU-2020-3917	Arcachon harbour, Gironde, Arcachon Bay, France	44.6597ºN, 1.1519ºW	6 Aug 2024	Fouling communities on pontoon	1 male
MNHN-IU-2020-3918	Arcachon harbour, Gironde, Arcachon Bay, France	44.6597º N, 1.1519º W	6 Aug 2024	Fouling communities on pontoon	1 male
MNHN-IU-2020-3919	Arcachon harbour, Gironde, Arcachon Bay, France	44.6597º N, 1.1519º W	6 Aug 2024	Fouling communities on pontoon	1 male
MNHN-IU-2020-3920	Arcachon harbour, Gironde, Arcachon Bay, France	44.6597º N, 1.1519º W	6 Aug 2024	Fouling communities on pontoon	1 male
MNHN-IU-2020-3921	Arcachon harbour, Gironde, Arcachon Bay, France	44.6597º N, 1.1519º W	6 Aug 2024	Fouling communities on pontoon	1 male
MNHN-IU-2020-3922	Arcachon harbour, Gironde, Arcachon Bay, France	44.6597º N, 1.1519º W	6 Aug 2024	Fouling communities on pontoon	1 male
MNHN-IU-2020-3923	Arcachon harbour, Gironde, Arcachon Bay, France	44.6597º N, 1.1519º W	6 Aug 2024	Fouling communities on pontoon	1 male

Continued

Table S2 continued

Catalogue number	Location	Coordinates	Date	Substrates	Number of specimens
MNHN-IU-2023-2238	Dinard, Ille-et-Vilaine, Brittany, France	48.6383º N, 2.0266º W	28 Jun 2024	Harbour	1 individual
MNHN-IU-2023-2239	Dinard, Ille-et-Vilaine, Brittany, France	48.6486º N, 2.0232º W	2 Jul 2024	Harbour	1 male
MNHN-IU-2020-3913	Barcares harbour, Languedoc-Rousillon, Golfe du Lion	42.81º N 3.019º E	14 Aug 2023	Fouling pontoon	4 males

Table S3. Specimens of *Aoroides longimerus* Ren & Zheng, 1996 processed in the DNA analyses. In grey, specimens with at least one sequence. All the data are available in BOLD in the project and dataset: ALONG and DS-AORLONG respectively.

Process ID	Sample ID	Country	Region	Exact Site	Available sequences
ALONG001-25	AORLON1	Portugal	Madeira	Quinta do Lorde Marina	16S
ALONG002-25	AORLON2	Portugal	Madeira	Quinta do Lorde Marina	16S
ALONG003-25	AORLON3	Portugal	Madeira	Quinta do Lorde Marina	
ALONG004-25	AORLON4	Portugal	Madeira	Quinta do Lorde Marina	
ALONG005-25	AORLON5	Portugal	Madeira	Quinta do Lorde Marina	CO1
ALONG006-25	AORLON6	Portugal	Madeira	Quinta do Lorde Marina	CO1
ALONG007-25	AORLON7	Portugal	Madeira	Quinta do Lorde Marina	
ALONG008-25	AORLON8	Portugal	Madeira	Quinta do Lorde Marina	
ALONG009-25	AORLON9	Portugal	Madeira	Quinta do Lorde Marina	
ALONG010-25	AORLON10	Portugal	Madeira	Quinta do Lorde Marina	
ALONG011-25	AORLON11	Portugal	Madeira	Quinta do Lorde Marina	CO1
ALONG012-25	AORLON12	Portugal	Madeira	Quinta do Lorde Marina	CO1
ALONG013-25	AORLON13	Portugal	Madeira	Quinta do Lorde Marina	CO1, 16S
ALONG014-25	AORLON14	Portugal	Madeira	Quinta do Lorde Marina	CO1, 16S
ALONG015-25	AORLON15	Portugal	Madeira	Quinta do Lorde Marina	CO1, 16S
ALONG016-25	AORLON16	Portugal	Madeira	Quinta do Lorde Marina	16S
ALONG017-25	AORLON17	Portugal	Madeira	Quinta do Lorde Marina	
ALONG018-25	AORLON18	Portugal	Madeira	Quinta do Lorde Marina	
ALONG019-25	AORLON19	Portugal	Madeira	Quinta do Lorde Marina	
ALONG020-25	AORLON20	Portugal	Madeira	Quinta do Lorde Marina	
ALONG021-25	AORLON21	Spain	Galicia	Moaña	
ALONG022-25	AORLON22	Spain	Galicia	Moaña	
ALONG023-25	AORLON23	Spain	Galicia	Moaña	
ALONG024-25	AORLON24	Spain	Galicia	Moaña	
ALONG025-25	AORLON25	Spain	Galicia	Moaña	
ALONG026-25	AORLON26	Spain	Galicia	Moaña	
ALONG027-25	AORLON27	Spain	Galicia	Davila	
ALONG028-25	AORLON28	Spain	Galicia	Davila	
ALONG029-25	AORLON29	Spain	Galicia	Davila	
ALONG030-25	AORLON30	Spain	Galicia	Davila	
ALONG031-25	AORLON31	Spain	Galicia	Davila	
ALONG032-25	AORLON32	Spain	Galicia	Davila	
ALONG033-25	AORLON33	Spain	Galicia	Moaña	
ALONG034-25	AORLON34	Spain	Galicia	Moaña	
ALONG035-25	AORLON35	Spain	Galicia	Moaña	
ALONG036-25	AORLON36	Spain	Galicia	Moaña	
ALONG037-25	AORLON37	Spain	Galicia	Moaña	
ALONG038-25	AORLON38	Spain	Galicia	Moaña	
ALONG039-25	AORLON39	Spain	Galicia	Cangas	
ALONG040-25	AORLON40	Spain	Galicia	Cangas	
ALONG041-25	AORLON41	Spain	Galicia	Cangas	
ALONG042-25	AORLON42	Spain	Galicia	Cangas	
ALONG043-25	AORLON43	Spain	Galicia	Cíes, Portonovo	
ALONG044-25	AORLON44	Spain	Galicia	Cíes, Portonovo	
ALONG045-25	AORLON45	Spain	Galicia	Cíes, Portonovo	

Continued

Table S3 continued

Process ID	Sample ID	Country	Region	Exact Site	Available sequences
ALONG046-25	AORLON46	Spain	Galicia	Cíes, Portonovo	
ALONG047-25	AORLON47	Spain	Galicia	Cíes, Portonovo	
ALONG048-25	AORLON48	Spain	Galicia	Cíes, Portonovo	
ALONG049-25	AORLON49	Spain	Galicia	Cíes, Portonovo	
ALONG050-25	AORLON50	Spain	Galicia	Cíes, Portonovo	
ALONG051-25	AORLON51	Spain	Galicia	Cíes, Portonovo	
ALONG052-25	AORLON52	Spain	Galicia	Cíes, Portonovo	
ALONG053-25	AORLON53	Spain	Galicia	Davila	
ALONG054-25	AORLON54	Spain	Galicia	Davila	
ALONG055-25	AORLON55	Spain	Galicia	Davila	
ALONG056-25	AORLON56	Spain	Galicia	Davila	
ALONG057-25	AORLON57	Spain	Galicia	Davila	
ALONG058-25	AORLON58	Spain	Galicia	Moaña	
ALONG059-25	AORLON59	Spain	Galicia	Moaña	
ALONG060-25	AORLON60	Spain	Galicia	Moaña	
ALONG061-25	AORLON61	Spain	Galicia	Moaña	
ALONG062-25	AORLON62	Spain	Galicia	Moaña	
ALONG063-25	AORLON63	Spain	Galicia	Moaña	
ALONG064-25	AORLON64	Spain	Galicia	Moaña	
ALONG065-25	AORLON65	Spain	Galicia	Moaña	
ALONG066-25	AORLON66	Spain	Galicia	Moaña	
ALONG067-25	AORLON67	Spain	Galicia	Moaña	
ALONG068-25	AORLON68	France	Normandy	Vauban basin, Le Havre	
ALONG069-25	AORLON69	France	Normandy	Vauban basin, Le Havre	
ALONG070-25	AORLON70	France	Normandy	Vauban basin, Le Havre	
ALONG071-25	AORLON71	France	Normandy	Vauban basin, Le Havre	
ALONG072-25	AORLON72	France	Normandy	Vauban basin, Le Havre	
ALONG073-25	AORLON73	France	Normandy	Vauban basin, Le Havre	
ALONG074-25	AORLON74	France	Normandy	Vauban basin, Le Havre	
ALONG075-25	AORLON75	France	Normandy	Vauban basin, Le Havre	
ALONG076-25	AORLON76	France	Normandy	Cherbourg	
ALONG077-25	AORLON77	France	Normandy	Cherbourg	
ALONG078-25	AORLON78	France	Normandy	Cherbourg	
ALONG079-25	AORLON79	France	Normandy	Cherbourg	
ALONG080-25	AORLON80	France	Normandy	Cherbourg	
ALONG081-25	AORLON81	France	Normandy	Cherbourg	
ALONG082-25	AORLON82	France	Normandy	Cherbourg	
ALONG083-25	AORLON83	France	Normandy	Cherbourg	
ALONG084-25	AORLON84	Japan	Osaka prefecture	Hakotsukuri, Hannan City	CO1, 16S
ALONG085-25	AORLON85	Japan	Osaka prefecture	Hakotsukuri, Hannan City	CO1, 16S
ALONG086-25	AORLON86	Japan	Osaka prefecture	Hakotsukuri, Hannan City	CO1, 16S
ALONG087-25	AORLON87	Japan	Osaka prefecture	Hakotsukuri, Hannan City	CO1
ALONG088-25	AORLON88	Japan	Osaka prefecture	Hakotsukuri, Hannan City	CO1
ALONG089-25	AORLON89	Japan	Osaka prefecture	Hakotsukuri, Hannan City	CO1
ALONG090-25	AORLON90	Japan	Osaka prefecture	Hakotsukuri, Hannan City	
ALONG091-25	AORLON91	France	Brittany	Marina du Château, Brest	16S

Continued

Table S3 continued

Process ID	Sample ID	Country	Region	Exact Site	Available sequences
ALONG092-25	AORLON92	France	Brittany	Marina du Château, Brest	16S
ALONG093-25	AORLON93	France	Brittany	Marina du Château, Brest	16S
ALONG094-25	AORLON94	France	Brittany	Marina du Château, Brest	
ALONG095-25	AORLON95	France	Brittany	Marina du Château, Brest	
ALONG096-25	AORLON96	France	Brittany	Marina du Château, Brest	
ALONG097-25	AORLON97	France	Brittany	Marina du Château, Brest	
ALONG098-25	AORLON98	France	Brittany	Marina du Château, Brest	
ALONG099-25	AORLON99	France	Brittany	Marina du Château, Brest	
ALONG100-25	AORLON100	France	Brittany	Marina du Château, Brest	
ALONG101-25	AORLON101	France	Brittany	Marina du Château, Brest	
ALONG102-25	AORLON102	Spain	Canary Islands	Garachico marina	CO1, 16S
ALONG103-25	AORLON103	France	Brittany	Roscoff marina	CO1, 16S
ALONG104-25	AORLON104	France	Brittany	Roscoff marina	CO1, 16S
ALONG105-25	AORLON105	France	Pays de la Loire	Piriac-sur-Mer marina	16S
ALONG106-25	AORLON106	France	Brittany	Trébeurden marina	CO1, 16S
ALONG107-25	AORLON107	France	Brittany	Saint Malo marina	16S

Accession number/Process ID/Sample ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1 JX545451.1_*Aoroides columbiae*		0.019	0.018	0.020	0.018	0.018	0.018	0.018	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.018	0.018	0.017
2 MG317304.1_*Aoroides* cf *columbiae*	0.181		0.002	0.019	0.018	0.018	0.018	0.018	0.019	0.019	0.019	0.019	0.018	0.018	0.018	0.018	0.018	0.019	0.019	0.020	0.016	0.016	0.016
3 MG936292.1_*Aoroides* cf *columbiae*	0.180	0.003		0.020	0.018	0.018	0.018	0.018	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.020	0.016	0.016	0.016
4 AORLON85	0.197	0.194	0.197		0.004	0.003	0.004	0.005	0.005	0.005	0.002	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.007	0.011	0.020	0.020	0.020
5 AORLON103	0.182	0.180	0.183	0.010		0.004	0.000	0.004	0.005	0.005	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.010	0.019	0.019	0.018
6 AORLON89	0.184	0.186	0.189	0.007	0.011		0.004	0.005	0.002	0.004	0.003	0.006	0.006	0.006	0.006	0.006	0.006	0.007	0.007	0.010	0.019	0.019	0.019
7 AORLON106	0.182	0.180	0.183	0.010	0.000	0.011		0.004	0.005	0.005	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.010	0.019	0.019	0.018
8 AORLON104	0.180	0.186	0.189	0.015	0.011	0.015	0.011		0.005	0.006	0.005	0.006	0.006	0.006	0.006	0.006	0.006	0.007	0.007	0.010	0.019	0.019	0.018
9 AORLON84	0.180	0.187	0.192	0.011	0.013	0.003	0.013	0.018		0.003	0.004	0.006	0.006	0.007	0.006	0.007	0.007	0.007	0.007	0.010	0.020	0.020	0.020
10 AORLON87	0.182	0.189	0.194	0.016	0.018	0.008	0.018	0.023	0.008		0.005	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.006	0.010	0.020	0.020	0.020
11 AORLON88	0.182	0.187	0.192	0.004	0.010	0.006	0.010	0.015	0.010	0.015		0.005	0.005	0.006	0.005	0.006	0.006	0.006	0.006	0.010	0.019	0.019	0.019
12 AORLON102	0.188	0.198	0.203	0.014	0.016	0.020	0.016	0.021	0.023	0.028	0.016		0.006	0.007	0.006	0.007	0.007	0.007	0.007	0.010	0.020	0.020	0.020
13 AORLON15	0.181	0.188	0.191	0.017	0.017	0.022	0.017	0.022	0.025	0.030	0.018	0.025		0.002	0.000	0.002	0.002	0.003	0.004	0.009	0.019	0.019	0.019
14 AORLON12	0.182	0.190	0.193	0.018	0.018	0.023	0.018	0.023	0.026	0.031	0.020	0.026	0.002		0.002	0.002	0.002	0.003	0.004	0.009	0.019	0.019	0.019
15 AORLON5	0.181	0.188	0.191	0.017	0.017	0.022	0.017	0.022	0.025	0.030	0.018	0.025	0.000	0.002		0.002	0.002	0.003	0.004	0.009	0.019	0.019	0.019
16 AORLON13	0.183	0.188	0.191	0.018	0.019	0.023	0.019	0.023	0.026	0.031	0.020	0.026	0.002	0.003	0.002		0.000	0.003	0.003	0.009	0.019	0.019	0.019
17 AORLON14	0.183	0.188	0.191	0.018	0.019	0.023	0.019	0.023	0.026	0.031	0.020	0.026	0.002	0.003	0.002	0.000		0.003	0.003	0.009	0.019	0.019	0.019
18 AORLON11	0.187	0.192	0.195	0.023	0.023	0.028	0.023	0.028	0.031	0.028	0.025	0.031	0.006	0.008	0.006	0.005	0.005		0.002	0.009	0.019	0.019	0.019
19 AORLON6	0.184	0.191	0.196	0.025	0.023	0.030	0.023	0.028	0.030	0.025	0.026	0.033	0.008	0.010	0.008	0.006	0.006	0.003		0.009	0.020	0.019	0.020
20 AORLON86	0.186	0.202	0.207	0.059	0.057	0.061	0.057	0.056	0.061	0.056	0.057	0.065	0.050	0.052	0.050	0.052	0.052	0.049	0.045		0.019	0.019	0.019
21 BCAMP255-08_*Aoroides intermedius*	0.165	0.139	0.139	0.197	0.185	0.191	0.185	0.185	0.198	0.200	0.196	0.207	0.187	0.189	0.187	0.189	0.189	0.193	0.197	0.195		0.000	0.002
22 BCAMP102-08_*Aoroides intermedius*	0.164	0.138	0.139	0.196	0.184	0.191	0.184	0.184	0.197	0.199	0.195	0.206	0.186	0.188	0.186	0.188	0.188	0.192	0.197	0.195	0.000		0.002
23 NJCGS736-10_*Aoroides intermedius*	0.165	0.137	0.137	0.200	0.186	0.195	0.186	0.186	0.195	0.197	0.193	0.205	0.190	0.192	0.190	0.192	0.192	0.196	0.197	0.195	0.002	0.002	

Table S4. Kimura 2 parameters pairwise distances of each specimen for CO1 sequences. Below the diagonal the pairwise distances, above the diagonal the standard errors after 999 permutations. In grey the comparisons of *Aoroides longimerus* Ren & Zheng, 1996 with the congeneric species.

Accession number/Sample ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1 JX545420.1_*Aoroides columbiae*		0.011	0.017	0.017	0.017	0.016	0.017	0.017	0.016	0.017	0.017	0.016	0.017	0.016	0.015	0.016	0.016	0.016	0.014	0.019
2 JX545421.1_*Aoroides columbiae*	0.050		0.016	0.016	0.016	0.015	0.016	0.015	0.015	0.015	0.017	0.015	0.016	0.015	0.015	0.015	0.015	0.015	0.014	0.018
3 AORLON86	0.095	0.089		0.008	0.008	0.008	0.008	0.009	0.008	0.009	0.009	0.009	0.009	0.010	0.009	0.010	0.010	0.010	0.008	0.010
4 AORLON14	0.085	0.082	0.023		0.005	0.004	0.007	0.007	0.005	0.006	0.004	0.006	0.005	0.006	0.006	0.007	0.007	0.007	0.004	0.008
5 AORLON15	0.096	0.090	0.025	0.009		0.003	0.008	0.005	0.004	0.004	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.005	0.008
6 AORLON13	0.088	0.082	0.022	0.006	0.003		0.005	0.005	0.003	0.004	0.000	0.003	0.000	0.003	0.003	0.004	0.004	0.004	0.000	0.007
7 AORLON106	0.093	0.087	0.025	0.014	0.021	0.008		0.005	0.004	0.005	0.005	0.003	0.005	0.004	0.004	0.004	0.004	0.004	0.005	0.008
8 AORLON102	0.092	0.083	0.030	0.014	0.011	0.008	0.011		0.004	0.005	0.006	0.005	0.006	0.004	0.004	0.005	0.005	0.005	0.005	0.008
9 AORLON85	0.089	0.080	0.025	0.009	0.005	0.003	0.005	0.005		0.003	0.004	0.000	0.003	0.000	0.000	0.003	0.003	0.003	0.003	0.007
10 AORLON84	0.092	0.083	0.028	0.012	0.008	0.006	0.008	0.008	0.003		0.004	0.000	0.003	0.000	0.003	0.004	0.004	0.004	0.004	0.007
11 AORLON1	0.086	0.083	0.025	0.004	0.014	0.000	0.007	0.011	0.003	0.003		0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.009
12 AORLON107	0.083	0.077	0.026	0.010	0.016	0.003	0.003	0.006	0.000	0.000	0.003		0.004	0.000	0.000	0.000	0.000	0.000	0.003	0.008
13 AORLON2	0.086	0.083	0.024	0.007	0.013	0.000	0.007	0.010	0.003	0.003	0.003	0.006		0.004	0.004	0.004	0.004	0.004	0.003	0.009
14 AORLON91	0.085	0.079	0.029	0.010	0.015	0.003	0.006	0.006	0.000	0.000	0.003	0.000	0.006		0.000	0.000	0.000	0.000	0.003	0.008
15 AORLON92	0.081	0.078	0.028	0.010	0.015	0.003	0.006	0.006	0.000	0.003	0.003	0.000	0.006	0.000		0.000	0.000	0.000	0.003	0.008
16 AORLON93	0.084	0.081	0.031	0.013	0.018	0.006	0.006	0.009	0.003	0.006	0.003	0.000	0.006	0.000	0.000		0.000	0.000	0.004	0.008
17 AORLON103	0.083	0.080	0.031	0.013	0.018	0.006	0.006	0.009	0.003	0.006	0.003	0.000	0.006	0.000	0.000	0.000		0.000	0.004	0.008
18 AORLON104	0.083	0.080	0.030	0.013	0.018	0.006	0.006	0.009	0.003	0.006	0.003	0.000	0.006	0.000	0.000	0.000	0.000		0.004	0.008
19 AORLON16	0.079	0.074	0.022	0.006	0.011	0.000	0.011	0.008	0.003	0.005	0.000	0.003	0.003	0.003	0.003	0.005	0.005	0.005		0.009
20 AORLON105	0.114	0.108	0.036	0.020	0.024	0.019	0.024	0.022	0.016	0.019	0.024	0.019	0.023	0.022	0.024	0.024	0.024	0.023	0.029	

Table S5. Kimura 2 parameters pairwise distances of each specimen for 16S sequences. Below the diagonal the pairwise distances, above the diagonal the standard errors after 999 permutations. In grey the comparisons of *Aoroides longimerus* Ren & Zheng, 1996 with the congeneric species.