

## Marine Record

**Cite this article:** Gouillieux B, Sorbe J-C, Bachelet G, Daffe G (2024). *Incisocalliope aestuarius* (Crustacea: Amphipoda: Pleustidae), an old introduced species in the Gironde Estuary (SW France). *Journal of the Marine Biological Association of the United Kingdom* **104**, e116, 1–6. <https://doi.org/10.1017/S0025315424000997>

Received: 2 October 2023  
Revised: 11 September 2024  
Accepted: 11 October 2024

### Keywords:

Atlantic coasts; non-indigenous species; *Parapleustes*; *Parapleustinae*; record

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Marine Biological Association of the United  
Kingdom



# *Incisocalliope aestuarius* (Crustacea: Amphipoda: Pleustidae), an old introduced species in the Gironde Estuary (SW France)

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## Abstract

The amphipod *Incisocalliope aestuarius* was recently collected since 2015 in the mesohaline part of the Gironde Estuary (SW France) with a van Veen grab and in the lower intertidal part in oyster bed by hand-picking. A look back at past studies showed that this species was present in this estuary since 1976, whereas the original description of this amphipod comes from Watling and Maurer in 1973, from the East coast of America. The validity of the *Incisocalliope* genus is herein questioned, and it is proposed to transfer species of the genus *Incisocalliope* to *Parapleustes* genus.

## Introduction

The family Pleustidae has been widely studied and the subject of various major publications creating new sub-families, genera and species (Ishimaru, 1984; Bousfield and Hendrycks, 1994, 1995; Hendrycks and Bousfield, 2004). In European waters 20 species are reported out of the 145 valid species in the world. These European species are distributed in nine genera (De Broyer *et al.*, 2007; Horton *et al.*, 2022): *Austropleustes* K.H. Barnard, 1931, *Mesopleustes* Stebbing, 1899, *Neopleustes* Stebbing, 1906, *Incisocalliope* J.L. Barnard, 1959, *Parapleustes* Buchholz, 1874, *Pleustes* Bate, 1858, *Pleustomesus* Guryanova, 1972, *Pleusymtes* J.L. Barnard, 1969 and *Stenopleustes* Sars, 1895. These genera are mainly composed of open sea or deep sea species (Stephensen, 1938; Ledoyer, 1986). *Incisocalliope* is the single brackish water genus belonging to the Pleustidae currently known in European waters (Faasse and van Moorsel, 2003), where it is represented by a unique species, *I. aestuarius* (Watling and Maurer, 1973). This species, native to the East coast of the United States, recorded on hydroids and oysters reefs from Maryland to Georgia, is considered as an alien species in European waters (Marchini & Cardeccia, 2017) where it has been recorded since the 1990s in The Netherlands and Belgium, 2016 in Germany, and since 1976 in France (Watling and Maurer, 1973; Fox and Bynum, 1975; Ysebaert *et al.*, 2000; Faasse and van Moorsel, 2003; Bachelet *et al.*, 2020; Leitingner *et al.*, 2021; present study). In the present paper, we report the occurrence of *I. aestuarius* in the Gironde Estuary and confirm the identification by molecular analyses, and we propose to move the genus *Incisocalliope* into the closely genus *Parapleustes*.

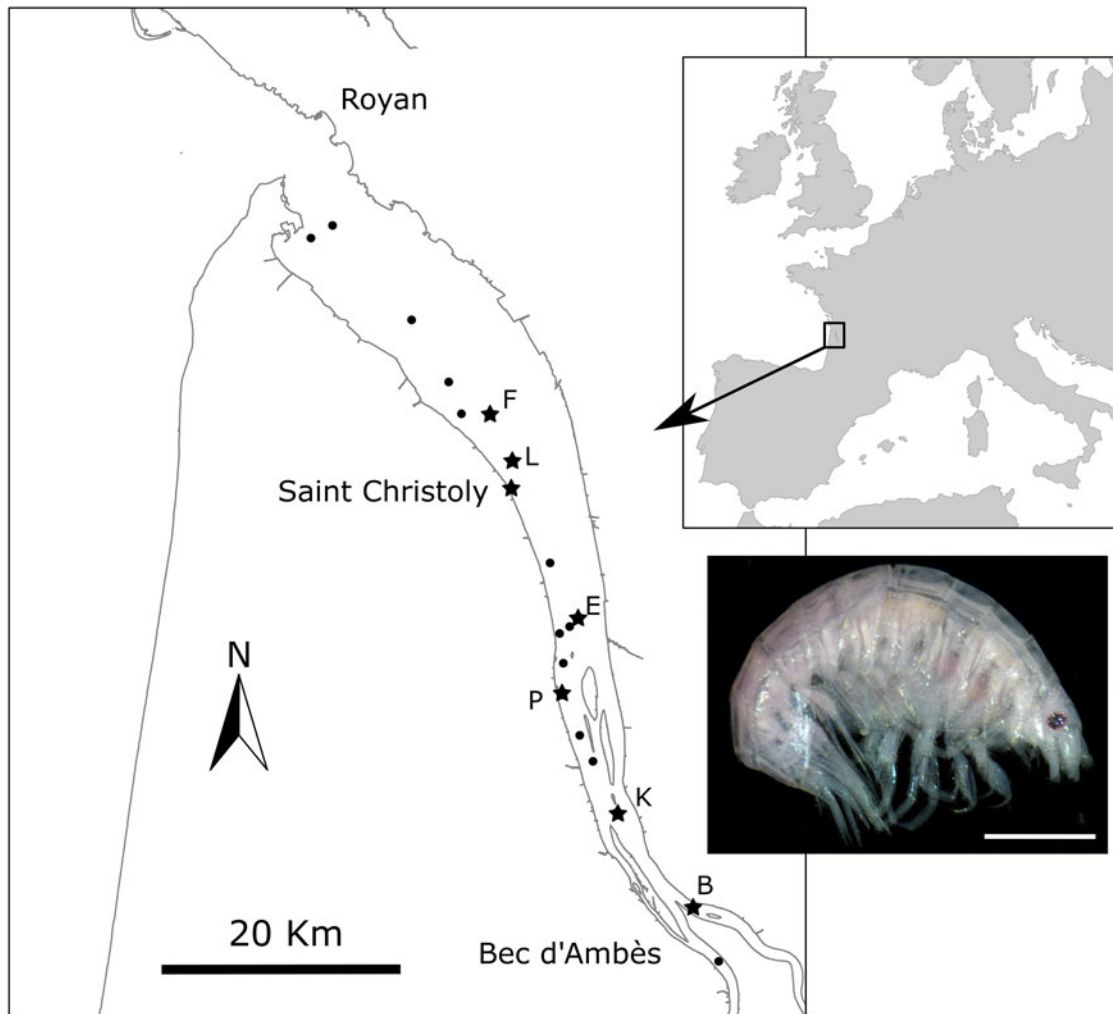
## Materials and methods

### Study area

The Gironde Estuary is the largest SW European estuary, with a total haline area of 625 km<sup>2</sup>; intertidal mudflats occupy only approximately 8% of this area. Water depth in the navigation channel ranges between 6 m near Bec d'Ambès and 30 m at the mouth of the estuary. The sampling stations were located on the western bank in the middle estuary (Figure 1). Water salinity and temperature fluctuates annually within the range 0.3–14 PSU and 5–25°C, respectively, near the Bec d'Ambès, and 21–29 PSU and 9–21°C, respectively, near the mouth of the estuary (<http://www.somlit.fr>; see also Lheureux *et al.*, 2021). Although oyster farming has been prohibited since 1996 due to cadmium pollution, wild oysters occur on muddy bottom and hard intertidal substrates along the haline part of the estuary. The Gironde Estuary is the seventh largest French maritime port and receives international maritime traffic, with 8.5 million tons of freight circulating annually, of which approximately 300 000 tons with North America (<https://www.bordeaux-port.fr/fr/le-port-de-bordeaux/chiffres-cles>).

### Sampling method

We can separate two main periods during which monitoring was carried out where the species was found: (1) as part of the ecological study of the Gironde Estuary from October 1975 to December 1979: 663 samples were collected (283 sub-surface and 380 bottoms) between Bec d'Ambès and Royan. From October 1975 to March 1976, a mesh WP2 net of 1.4 mm



**Figure 1.** Sampling stations in the Gironde Estuary, France. Black stars: stations where *Incisocalloipe aestuarius* (Watling and Maurer, 1973) was sampled at least once. Black circles: stations without *I. aestuarius*. Photograph of a specimen from the Gironde Estuary, July 2015, station F. Scale bar: 1 mm.

was used, and from April 1978 to December 1979, a net with a rectangular opening of 770 × 545 mm with a 0.5 mm mesh. (2) Since 2005, ecological monitoring of the Blayais nuclear power plant in the Gironde Estuary consisting of sampling three subtidal stations E, F and K with a van Veen grab, five samples of 0.1 m<sup>2</sup> per station, once a month during 9 months per year (February, April to November). The samples were sieved on a 0.5 mm mesh.

An additional specific sampling was carried out in November 2016 at Saint-Christoly: oysters *Magallana gigas* (Thunberg, 1793) still submerged were sampled by hand at low tide in the lowest part of the foreshore, and washed over a 0.5 mm mesh sieve to collect epifauna. To genetically compare specimens of *Incisocalloipe aestuarius* sampled in the Gironde Estuary with those of the type locality, a single specimen was successfully sampled in the Delaware Bay (USA).

#### Molecular data and analyses

Extraction of DNA was done with QIAamp DNA Micro Kit (QIAGEN) following protocol supplied by the manufacturers. Approximately 380 bp of the 18S gene were amplified, using primers: Aoro18SF (GATCCTGCCAGTAGTCATATGC) with Aoro18SR (GCCTGCTGCCGTCCTTGACG) design for this study. Polymerase chain reaction (PCR) occurred with GoTaq® G2 Flexi DNA Polymerase Kit (PROMEGA, France) in 25 µl mixtures containing: 5 µl of 5X Green GoTaq® Reaction Buffer (final

concentration of 1X), 1.5 µl of MgCl<sub>2</sub> (25Mm) solution, 0.5 µl of PCR nucleotide mix (final concentration of 0.2 mM each dNTP), 0.25 µl of each primer (final concentration of 1 µM), 0.1 µl of Taq DNA polymerase (5 U/µl), 0.5 µl template DNA and 16.9 µL of nuclease-free water. The temperature profile was as follows: 94°C/600s–(94°C/60s–59°C/30s–72°C/90s)×40 cycles–72°C/600s–16°C. PCR success was verified by electrophoresis in a 1% p/v agarose gel stained with ethidium bromide. Amplified products were sent to Eurofins Genomics Company to complete double strain sequencing, using the same set of primers as used for PCR. Overlapping sequence (forward and reverse) fragments were merged into consensus sequences and aligned using Geneious Prime 2019.0.4. All sequences obtained in this study were deposited in GenBank. Pair-wise Kimura 2-parameter (K2P) genetic distance was performed using MEGA version 7.0.26.

#### Results

Number of specimens collected with ecological data are in the Supplementary Table S1

#### Systematics

Order Amphipoda Latreille, 1816  
Family Pleustidae Buchholz, 1874

Subfamily Parapleustinae Bousfield and Hendrycks, 1994  
 Genus *Incisocalliope* J.L. Barnard in J.L. Barnard and Reish, 1959  
*Incisocalliope aestuarius* (Watling and Maurer, 1973)

**Material examined**

France, Gironde Estuary, Point F, subtidal station: 10 specimens, 2.70–5.99 mm (body length, BL), 09 July 2015, MNHN-IU-2016-3385; 1 dissected female specimen, 5.45 mm (BL), 09 July 2015, MNHN-IU-2016-3384; 2 specimens, 3.67 and 4.06 mm (BL), 06 September 2016, MNHN-IU-2016-3383; 3 specimens, 2.91–3.21 mm (BL), 04 August 2018; 5 specimens, 2.30–4.42 mm (BL), 26 July 2019.

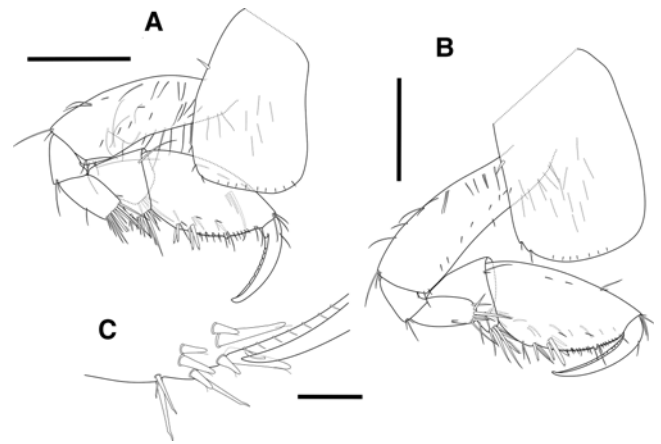
France, Gironde Estuary, Saint-Christoly, intertidal station: 16 specimens, 1.81–6.32 mm (BL), 15 November 2016, on oysters *M. gigas* (Thunberg, 1793), MNHN-IU-2016-3382.

USA, New Jersey, Delaware Bay, subtidal station: 1 brooding female, 4.75 mm (BL), on oyster *Crassostrea virginica* (Gmelin, 1791) bed, 22 May 2017, MNHN-IU-2016-3386.

Due to the loss of collected specimens between 1976 and 1978, the identification of the species for this period was made on the description and figures that were made and conserved at that time.

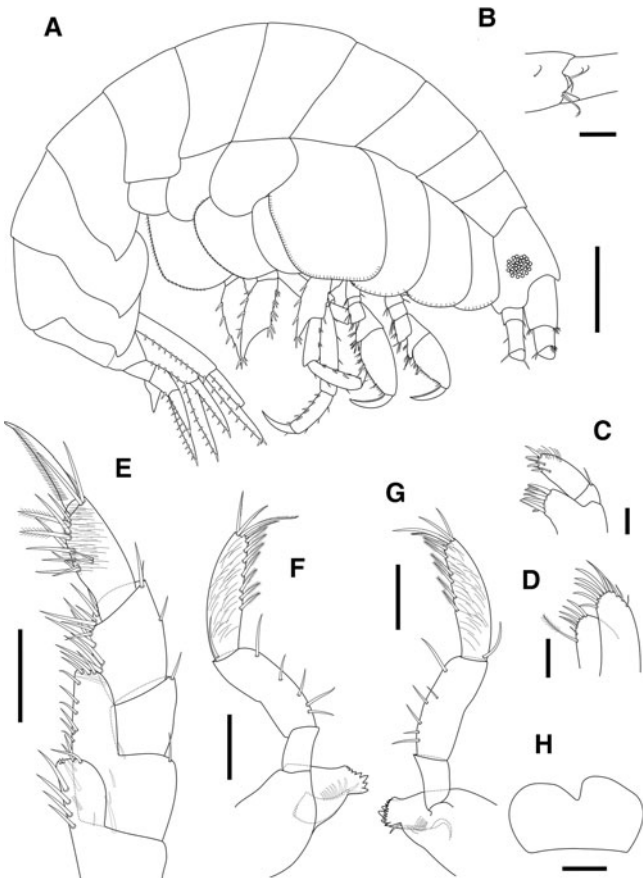
**Diagnosis (Figures 2–4)**

Body without dorsal processes; eyes circular; lateral cephalic lobe rounded; antenna 1 at least as long as first 5 pereonites, accessory flagellum very small, scale-like, looks absent (sometimes covered by A1 peduncle article 3); antenna 2 peduncle longer than antenna 1 peduncle, peduncle article 4 and 5 subequal; upper lip deeply incised, lobes asymmetrical; non-triturative mandibular

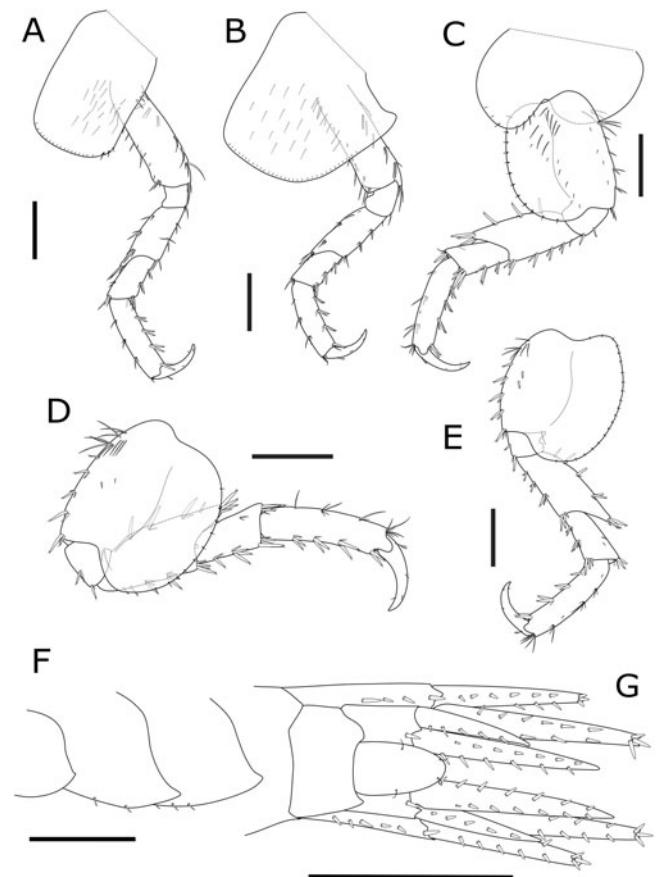


**Figure 3.** *Incisocalliope aestuarius* (Watling and Maurer, 1973). Specimen from the Gironde Estuary: (A) gnathopod 1; (B) gnathopod 2; (C) gnathopod 2, ventrolateral view of palmar corner. Scale bars: A–B: 1 mm; C: 0.5 mm.

molar; mandibular palp article 1 distal tooth absent; gnathopods subequal, carpus with posterior lobe poorly developed, two or three clusters of spines delimit the palms, palmar margin with a small triangular tooth; coxae 1–3 posterior margin with one to three robust setae and with two to four posteroventral notches; coxa 1–4 with setae on the mesial face; gnathopod 1 basis anterior margin densely setosed; pereopod 3 carpus distinctly shorter than merus; pereopod 7 basis posterodistal lobe variously expanded (extending or not beyond the ischium), dactylus about half length



**Figure 2.** *Incisocalliope aestuarius* (Watling and Maurer, 1973). Specimen from the Gironde Estuary: (A) lateral view; (B) antenna 1 peduncle article 3, accessory flagellum and flagellum article 1; (C) maxilla 1; (D) maxilla 2; (E) maxilliped; (F) right mandible; (G) left mandible; (H) upper lip. Scale bars: A, D–F: 0.1 mm; B, C, G: 0.05 mm.



**Figure 4.** *Incisocalliope aestuarius* (Watling and Maurer, 1973). Specimen from the Gironde Estuary: (A–E) pereopods 3–7; (F) epimeral plates 1–3; (G) uropode 2–3 and telson, dorsal view. Scale bars: A–E: 1 mm; F: 0.25 mm; G: 0.5 mm.

of propodus; epimeral plate 3 posterodistal corner acuminate; uropod 2 outer ramus slightly shorter than the inner ramus.

### DNA analyses

The amplification product of 380 bp for 18S gene was published at NCBI GenBank for two specimens. Similarity between *I. aestuarius* specimen from the Gironde Estuary (France) (accession number OQ561302) and specimen from the type locality (Delaware Bay – USA, specimen MNHN-IU-2016-3386) (accession number PQ302303) was 100% ( $e$ -value = 0). Another sequence was registered in GenBank database (accession number KT808759) (Verheye *et al.*, 2016) corresponding to a specimen from Hoedekenskerke (The Netherlands). This last sequence contains a certain number of indeterminations, however its percentage of similarity remains high (K2P distance between French specimen and Netherlands specimen = 0%)

### Discussion

#### *Incisocalliope aestuarius* in European waters

*Incisocalliope aestuarius* was recorded in The Netherlands, Germany, Belgium and France (Faasse and van Moorsel, 2003; Bachelet *et al.*, 2020; Leitinger *et al.*, 2021). Concerning the French coasts, the species is currently known only in the Gironde Estuary (Bachelet *et al.*, 2020). Leitinger *et al.* (2021) report the species on the French coasts from the Aquanis database. After verification on the Aquanis website, the species is not referenced in the English Channel and the French North Sea, but only in the Gironde Estuary. Thereby, Curd (2019a, 2019b) references in Leitinger *et al.* (2021) should not be taken into account. In the Gironde Estuary, between 1975 and 1979, only 28 specimens were sampled by JC Sorbe. When the first specimens were sampled, the species was considered new to science and a description was started. Later, it was learned that the species had been recently described from the USA, the description was stopped, and its presence was not mentioned in France either. A first hypothesis at the time to explain the presence of the species only during September and October was that the species migrated for reproduction in the water column, while the rest of the year the species had a benthic way of life. Since the beginning of ecological monitoring of the Blayais nuclear power plant, only 23 specimens were collected in more than 2000 samples, and only in the station 'F' around the summer period (June to September). The species has been found abundant when associated with oysters, hydroids or fouling (Cory, 1967; Watling and Maurer, 1973; Faasse and van Moorsel, 2003; present study). In the Gironde Estuary, the species was only sampled out of these habitats during the period when estuary temperatures are highest. Opportunistic sampling with a net or a van Veen grab on habitats devoid of hydroids and oysters would seem to confirm the hypothesis of a migration in the water column during the period of highest temperatures.

In the original description, *I. aestuarius* was collected from the base of hydroids attached to oysters between 3 and 5 m depth in the Delaware Bay, where salinity and temperature ranged between 10 to 33 PSU and  $-1.8$  to  $28.9^{\circ}\text{C}$ , respectively (Watling and Maurer, 1973). In the Gironde Estuary, the species was collected with typical species of the mesohaline environment (temperature, salinity and associated species – see Table S1) and many specimens were collected while sampling a subtidal oyster bed. Unfortunately, no data are available on the associated fauna for the period 1975–1979.

The species was described by Watling and Maurer in 1973 with specimens from the Delaware Bay region, but the first observation of the species in the USA was 1963 in Maryland

(‘Amphipod species A’ in Cory, 1967, see Watling and Maurer, 1973 comments). In European waters, the first record of the species was in the Gironde Estuary in 1976 (present paper). The Gironde Estuary has an important commercial port through which transit thousands of boats and tons of goods from Europe, but also from North America (data from Bordeaux port: Archives/GPMB/1975). The presence of the species is therefore most likely due to an introduction by maritime traffic with hull fouling on ships or ballast water, as was also suggested by Faasse and van Moorsel (2003) and Leitinger *et al.* (2021).

Identification of *I. aestuarius* in European waters was mainly based on Watling and Maurer (1973) and Bousfield and Hendrycks (1995). Specimens recorded in the Gironde Estuary are in accordance with the original description apart from (1) the presence of setae on the mesial face on coxa 1–4; (2) coxa 1–3 with less posterior robust setae (1–3 vs 2–5) and (3) posterior lobe of pereopod 7 basis is variously expanded, exceeding or not ischium. Faasse and van Moorsel (2003) showed some differences between their specimens from The Netherlands and the original description of *I. aestuarius* (anterodistal lobe of the basis of pereopods 5–7 and uropod 2 outer ramus vs inner ramus size) but concluded that these differences were not enough to erect their specimens to a new species. Leitinger *et al.* (2021) specimens from Germany are in accordance with the original description, but showed some morphological differences with Bousfield and Hendrycks (1995) description (length of uropod 2 rami and length of pereopod 7 dactylus in relation to the propodus). Genetic analyses having shown that no differences exist between specimens from the type locality, the Gironde Estuary and The Netherlands, these slight morphological differences are therefore due to an intraspecific variability.

#### Taxonomical comments on *Incisocalliope* and *Parapleustes*

The genus *Parapleustes* was created by Buchholz in 1874 for *P. gracilis* from East Greenland, and characterized by the following distinguishing features: small rostrum; antenna 1 longer than antenna 2; upper lip short and wide, deeply emarginated; mandibular incisor with small teeth associated with a dentate lacinia mobilis, molar process poorly developed.

*Parapleustes aestuarius* was described by Watling and Maurer (1973) from the Delaware Bay region. This estuarine species is in good accordance with the original diagnosis of the genus *Parapleustes*. Barnard and Reish (1959) created the genus *Incisocalliope* for the new species *I. newportensis* from Newport Bay (California), with the following features: accessory flagellum minute, scale-like; upper lip deeply incised; lower lip with broad inner lobes; palp of maxilla 1 large; gnathopods moderately large, propodus much longer than carpus, both articles not slender or elongated; rami of uropod 3 unequal in size and length. This new genus was probably created in relation with the deeply incised morphology of its upper lip. Nevertheless, it was first ascribed to Calliopiidae, a family generally characterized by a rounded distal margin of upper lip, except for some species (*Stenopleura atlantica* Stebbing, 1888; *Tylosapis dentatus* [Stebbing, 1888]; *Bouvierella carcinophila* [Chevreux, 1889]; *Pontogeneoides dubia* [Ruffo, 1949]; and *Stenopleuroides macrops* Birstein and M. Vinogradov, 1964). Subsequently, Barnard and Karaman (1991) properly transferred *Incisocalliope* to the family Pleustidae, and synonymized it to the former genus *Parapleustes*.

Later on, Bousfield and Hendrycks (1994, 1995) wisely subdivided the family Pleustidae into 11 subfamilies, proposed a new identification key to Parapleustinae and took the opportunity to create many new genera in this subfamily. In this way, they resurrected the genus *Incisocalliope*, creating the new species

*I. nipponensis* and transferring many *Parapleustes* species in this new genus: *I. dilatatus* (Ishimaru, 1984), *I. bairdi* (Boeck, 1871), *I. derzhavini* (Gurjanova, 1938), *I. makiki* (J. L. Barnard, 1970), *I. aestuarius* (Watling and Maurer, 1973) and *I. filiaris* (Hirayama, 1988). In their new identification key, *Incisocalliope* was distinguished from *Parapleustes* by the following characters (couplet 3): antennae normally elongate (*vs* short), antenna 1 flagellum distinctly longer than peduncle (*vs* little longer); antenna 2 flagellum distinctly longer than peduncle (*vs* shorter); gnathopods, anterior margin of carpus >0.25x propodus length (*vs* <0.25x), hind lobe about half as wide as its anterior margin (*vs* small, narrow); epimeron 3 with acuminate hind corner (*vs* small hook).

If we follow this couplet 3 to distinguish *Incisocalliope* to *Parapleustes*, we notice that due to (1) gnathopod 1 carpus more than ¼ length of propodus (ratio between 0.4 and 0.8) for *Parapleustes assimilis* (Sars, 1882), *P. bicuspides* Nagata, 1965, *P. gracilis* (Buchholz, 1874), *P. major* (Bulyčeva, 1952), *P. monocuspis* (Sars, 1893), *P. trianguloculatus* (Bulyčeva, 1952), *P. tricuspis* Ishimaru, 1984, (2) gnathopod 2 carpus more than ¼ length of propodus (ratio about 0.3–0.4) for *P. ischimarui* Bousfield and Hendrycks, 1995 and *P. sinuipalmus* Dunbar, 1954, or (3) antenna 1 distinctly longer than peduncle (ratio about 3.8 whereas ratio for *Incisocalliope* species between 3.1 and 4.3) for *P. bicuspidis* (Krøyer, 1838), all these species should be placed into the genus *Incisocalliope*. Concerning *P. mielcki* (Sokolowsky, 1925), the original description is too insufficient to conclude. Thereby, characters used in the key are only based on *P. americanus* Bousfield and Hendrycks, 1995 and do not apply to all *Parapleustes* species.

According to the new classifications of Lowry and Myers (2013, 2017) and to Bousfield and Hendrycks (1995), *Incisocalliope* is part of the family Pleustidae (suborder Amphilochidea) and not of the family Calliopiidae (suborder Senticaudata). But as characters used in the original diagnoses of *Incisocalliope* and *Parapleustes* do not make it possible to distinguish the species between the two genera, and due to inconsistencies in Bousfield and Hendrycks (1995) in the morphological criteria used in the key and in *Parapleustes* diagnosis (body smooth while dorsally toothed for *P. bicuspidis*, *P. bicuspides*, *P. monocuspis* and *P. tricuspis*; epimeral plate 3 hind corner weakly acuminate while subquadrate for *P. assimilis*, *P. gracilis*, *P. ischimarui* and *P. trianguloculatus*), we propose to group the species *Incisocalliope* and *Parapleustes* into the genus *Parapleustes*, as Barnard and Karaman (1991) did.

## Conclusion

*Incisocalliope aestuarius* can be a rare or abundant species, depending on the water salinity or the substratum (Cory, 1967; Feeley and Wass, 1971; Watling and Maurer, 1973; Fasse and van Moorsel, 2003, present paper). In the Gironde Estuary, *I. aestuarius* was collected in the same type of habitat as in the type locality (abundant on oyster bed) as well as in the water column (rare). The Gironde Estuary being an important port area, the introduction of the species could be due to maritime traffic between North America and France. Due to its low abundance and likely reproductive cycle, the lack of a strong sampling strategy, or targeted sampling, may explain why the species was not found between 1978 and 2015. A revision of Pleustidae is necessary with the grouping of the genera *Incisocalliope* and *Parapleustes* as it has already been done because even if Bousfield and Hendrycks (1995) did a great work, many errors exist that can lead to misidentification.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S0025315424000997>

**Acknowledgments.** Authors have a thought for Jean-Claude Sorbe who sadly passed away in 2019. This contribution is dedicated to his memory. We thank the SOMLIT network for providing temperature and salinity data, and EDF (Electricité De France) for macrofauna data from ecological monitoring of the Blayais nuclear power plant. Authors also want to thank Jenny Paterno Shinn (Rutgers University, USA) for kindly providing the specimen used in molecular analyses.

**Author contributions.** Benoit Gouillieux and Jean-Claude Sorbe made the identification and the main text; Guy Bachelet participated in the writing and corrections of the manuscript; Guillemine Daffe provided genetic analyses.

**Financial support.** There was no financial support.

**Competing interest.** None.

**Ethical statement.** Normal scientific ethical practices have been respected.

**Data availability.** Data of temperature and salinity are provided from the SOMLIT network, data of macrofauna from EDF (Electricité De France) ecological monitoring of the Blayais nuclear power plant.

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