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Unravelling the distribution of three *Ammonia* species (Foraminifera, Rhizaria) in French Atlantic Coast estuaries using morphological and metabarcoding approaches

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ABSTRACT

Assessing the distribution of species in natural environments is essential for their use in environmental surveys. Here, we investigate the distribution of three pseudo-cryptic species formerly lumped in the morphospecies *Ammonia tepida* (Cushman, 1926), commonly found on estuarine mudflats along the European coasts: *Ammonia veneta* Schultze, 1854 (T1), *Ammonia aberdoveyensis* Haynes, 1973 (T2) and *Ammonia confertitesta* Zheng, 1978 (T6). We studied their distribution at 51 sites located in seven estuaries of the French North Atlantic coast (Elorn, Aulne, Odet, Crac'h, Auray, Vilaine, Vie), using both morphological and molecular identification methods. *Ammonia veneta* was detected by both approaches at most of the stations. While *A. aberdoveyensis* was frequently identified by the morphological method but not detected with metabarcoding, the presence of *A. confertitesta* in the eDNA data often contrasted with its absence in the morphological analysis. The absence of *A. aberdoveyensis* in eDNA of sites where it was identified morphologically could be the consequence of its relative scarcity, and eventually a patchy distribution. Concerning *A. confertitesta,* we hypothesise that these contradictory results can be explained by the supposedly invasive character of this species. Despite the widespread presence of *A. confertitesta* genetic material (including adults, juveniles and propagules), a mature population has not yet fully developed everywhere. The seven investigated estuaries seem to represent different stages of replacement of the autochthonous species *A. veneta* and *A. aberdoveyensis* by *A. confertitesta*. Our study demonstrates that the combination of visual observations and molecular approaches is ideal for monitoring the progressive spreading of exotic species.

1. Introduction

Evaluating the spatial arrangement of species in natural settings is indispensable for their incorporation in environmental assessments. Foraminifera (Eukaryota, Rhizaria) are distributed worldwide in all marine environments from estuaries and coastal areas to the deep sea, and their distribution is widely studied both by micropalaeontologists and biologists. The first use them for palaeo-environmental reconstructions to trace past climate and oceanographic changes (Debenay, 1995; Horton and Edwards, 2006). The second investigate their distribution to evaluate the environmental conditions, for instance in the context of biomonitoring of human activities (Dimiza et al., 2016; Jorissen et al., 2018; Bouchet et al., 2021). In this context, assessing the distribution of species is essential for their use in environmental surveys.

Ammonia was one of the first erected foraminiferal genera (Brünnich, 1771). This genus is characterised by a hyaline wall and trochospirally coiled chambers and is found in coastal open marine as well as estuarine habitats (Hayward et al., 2021). For decades, the different morphotypes of *Ammonia* were considered as ecophenotypes, often as variants of a single species (e.g., Schnitker, 1974; Jorissen, 1988; Walton and Sloan,

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1990). Today, the combination of DNA barcoding and detailed morphometric studies has allowed the validation and the redescription of 26 distinct species (Hayward et al., 2004, 2021; Richirt et al., 2019; Bird et al., 2020). Among the formerly described morphospecies, *Ammonia tepida* (Cushman, 1926) was considered as cosmopolitan with high abundances in intertidal environments (Debenay et al., 2000), often being one of the dominant genera in estuarine ecosystems (Cearreta, 1988; Alve and Murray, 1994; Castignetti, 1996). However, individuals found along the European Atlantic coasts (Saad and Wade, 2016; Hayward et al., 2021) and previously identified as *Ammonia tepida* were shown to belong to three different phylotypes, initially named T1, T2 and T6 (Hayward et al., 2004). A few years ago, Richirt et al. (2019) proposed a method to distinguish phylotypes T1, T2 and T6 morphologically. These authors demonstrated that the examination of two morphological characters (pore diameter and flushed or raised sutures on the spiral side) under a Scanning Electron Microscope (SEM) is sufficient to discriminate these three phylotypes morphologically with a success rate of >90%. Recently, the three phylotypes have been erected to the rank of species and renamed *Ammonia veneta* Schultze, 1854 for T1, *Ammonia aberdoveyensis* Haynes, 1973 for T2 and *Ammonia confertitesta* Zheng, 1978 for T6 (Hayward et al., 2021). Today, a more deep-going understanding to disentangle the ecology of these different species is needed, so that they can be used more efficiently in environmental studies.

These three species have been found in various types of environments: intertidal saltmarshes, mudflats along estuaries, shallow marine environments and harbours (Saad and Wade, 2016). Few studies have attempted to disentangle ecological preferences of *A. aberdoveyensis, A. confertitesta* and *A. veneta* in estuarine environments (Saad and Wade, 2016; Richirt et al., 2019; Bird et al., 2020; Hayward et al., 2021; Pavard et al., 2021, 2023b). Bird et al. (2020) suggested that *A. aberdoveyensis* could have higher abundances at higher elevation on the mudflats. However, this observation concerns only a few stations (i.e., three stations on a mudflat in the Dart estuary). Other authors suggested that *A. confertitesta* could have a higher tolerance for brackish water (Schweizer et al., 2011), or for hypoxia/anoxia (Richirt et al., 2022) compared to *A. veneta* and *A. aberdoveyensis.* In addition, even though these three species are found along the European coast, *A. confertitesta* shows two disjunct distributional areas, the eastern coasts of Asia (i.e., Japan, Toyofuku et al., 2005; China, Hayward et al., 2004), and the European coasts (Schweizer et al., 2011; Richirt et al., 2020). This disjunct distribution, and its present occurrence in areas where no representatives of *Ammonia* had been observed in the historical past (Schweizer et al., 2011) has led to the hypothesis that *A. confertitesta* could be an introduced species in Europe, originating from eastern Asia (Pawlowski and Holzmann, 2007; Pavard et al., 2023b). Some authors suggested that this species could have been introduced through ballast waters (Pawlowski and Holzmann, 2007), but there is still no consensus concerning the vector and period of introduction.

Here, we will study the distribution of these three *Ammonia* species in seven estuaries along the French Atlantic coast. We will apply two different methods: 1) morphological determination and 2) molecular identification using DNA metabarcoding (eDNA). The first objective of this study is to investigate whether the three species have the same distributions and densities in each of the seven estuaries, to obtain more information about their ecological preferences. The second objective is to investigate whether morphological and eDNA datasets lead to similar conclusions. If not, then the combined use of both methods should give a more complete vision of the presence of the three *Ammonia* species at the studied sites.

2. Material and methods

2.1. Study area

This study focuses on seven estuaries located along the north French Atlantic coast; from north to south the Elorn, Aulne, Odet, Crac'h, Auray, Vilaine and Vie estuaries (Fig. 1). The location of the sampling stations is detailed on Figs. 2 and 3. The Elorn and Aulne estuaries are both rias (drowned river valleys) located in the inner part of the roadstead of Brest, an enclosed marine bay. The Odet, Crac'h and Auray estuaries are also rias; the former two are directly connected to the Atlantic Ocean, whereas the latter flows into the Morbihan Gulf. The Vilaine estuary is a typical lowland estuary, open to the Atlantic Ocean, whereas the Vie estuary can be characterised as a lowland bar-built estuary. Its mouth is deflected northwards by a sandy spit. All studied estuaries are subjected to a meso- to low macrotidal regime with a tidal range of 4 to 5.5 m at the entrance, except for the Vilaine estuary, where the tidal range is higher (about 7.5 m at the entrance). (See Table 1.)

2.2. Sampling

All samples were collected during low tide. Environmental parameters, such as the altitude of sampling stations, the distance of the sampling point to the sea divided by the length of the salt intrusion, the percentage of organic matter and the sediment fine fraction (percentage of sediment <63 μm) were measured according to the protocol detailed in Fouet et al. (2022). For foraminiferal morphological analyses, at each station, three tubes with an internal diameter of 9.6 cm were randomly placed at one or two meters from each other, and pushed into the sediment. The top 1 cm of the sediment cores was sliced and preserved in 96% ethanol and stained with 2 g/l Rose Bengal, following the FOBIMO protocol (Schönfeld et al., 2012). In addition, replicate surface sediment samples were taken for eDNA analyses and stored rapidly at −20 °C prior to DNA extractions (details below). In total, 51 stations were studied for the morphological inventories, but three stations could not be sampled for eDNA analysis, so the eDNA data set concerns 48 stations.

2.3. Morphological analysis

Samples were sieved on a 125 μm mesh, all Foraminifera were picked wet using a Leica MZ16 stereomicroscope and stored on micropalaeontological slides. For each station, about 40 *Ammonia* specimens were selected randomly. In practice, a randomly generated number was assigned to each *Ammonia* specimen on the slide using the Excel function RAND(). After sorting in ascending order, the 40 specimens having the lowest values were selected. When the total number of *Ammonia* specimens was below 40, we used all available specimens.

Overview images of the spiral side for all 1739 selected individuals were acquired with a Scanning Electron Microscope (SEM, Hitashi TM4000). Based on these images, specimens were determined using the criteria of Richirt et al. (2019). In some cases, species assignation was not possible, for instance for specimens with a damaged test or small individuals with not fully developed distinctive criteria (some specimens are shown in Fig. A.1).

As *Ammonia* can be very numerous in the foraminiferal community, only a part of the total *Ammonia* assemblages was analysed, specifically up to 40 randomly chosen individuals per station. Then, the proportions of the three *Ammonia* species in the total foraminiferal assemblage were estimated by multiplying the relative proportions of the group *Ammonia* spp. in the total foraminiferal assemblage with the relative frequencies of each species (i.e., *A. veneta*, *A. aberdoveyensis* and *A. confertitesta*) in the subset of 40 specimens analysed morphologically, considering only specimens that could be assigned to one of the three species.

2.4. DNA extraction, amplification, sequencing

Environmental DNA (eDNA) was extracted from the sediment using the DNeasy PowerMax Soil (one replicate of 10 g) and the DNeasy Nucleospin Soil (two replicates of 250 mg, Macherey Nagel) (Vie, Vilaine) and the FastDNA Spin Kit for Soil (two replicates of 5 g, MP

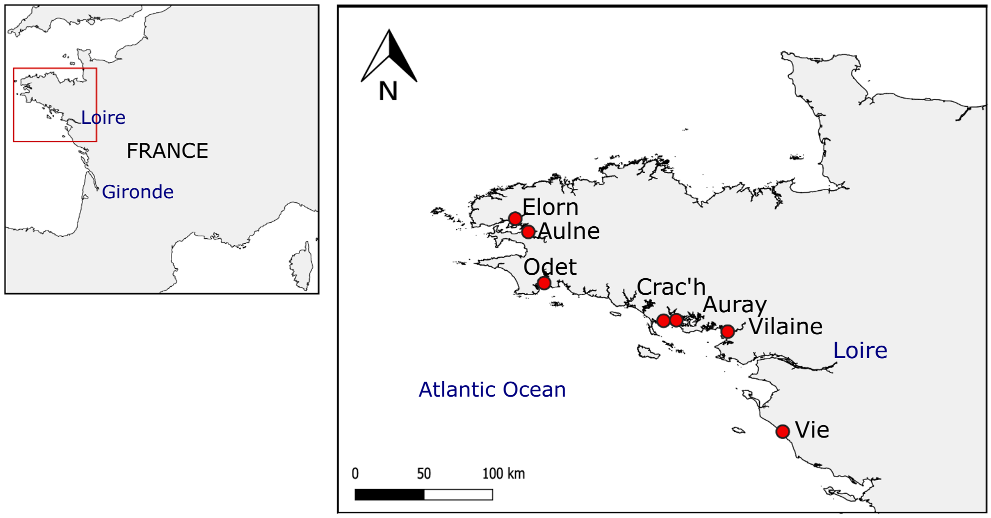

Fig. 1. Location of the studied estuaries along the French Atlantic coast. Studied estuaries are indicated with a red dot.

Biomedicals) (Elorn, Aulne, Odet, Crac'h, Auray) according to the manufacturers' instructions. These DNA extraction kits were shown to be efficient to extract foraminiferal DNA (Brinkmann et al., 2023; Singer et al., 2023). AccuPrime™ Taq DNA Polymerase High Fidelity (Thermo Fisher Scientific) was used to carry out the PCR. The specific foraminiferal primers s14F1 (Pawlowski, 2000) and s15r (Lejzerowicz et al., 2014) were used to amplify the 37f hypervariable region (Pawlowski et al., 2014) (amplicon size: 135–190). Three PCR replicates were done for each DNA extractions. The PCR conditions consisted of an initial denaturation of 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 50 °C for 45 s and extension at 68 °C for 90 s plus a final extension at 68 °C for 10 min. PCR product replicates were pooled and then quantified using the QuBit HS dsDNA (Invitrogen). Each sample was then pooled with the same amount of DNA and purified using Sera-Mag™ Magnetic carboxylate modified particles (GE Healthcare). Library preparation and MiSeq (paired-end, 2x250bp) sequencing were performed at the ANAN platform (SFR 4207 QUASAV, INRAE, University of Angers, Institut Agro, Beaucouzé, France) for the Vie and Vilaine and at ID-Gene Ecodiagnostics (Geneva, Switzerland) for the Elorn, Aulne, Odet, Crac'h and Auray samples. The methodology used for DNA extraction is further detailed and discussed in Singer et al. (2023).

2.5. Bioinformatics and taxonomic assignment and statistical analysis

Tags and primers were removed from the sequences using cutadapt v. 3.4 (Martin, 2011). Clustering of the reads was done using R (version 4.0.4, R Core Team, 2014) and the R package DADA2 (v. 1.16; Callahan et al., 2016). Raw reads were quality controlled by truncating the reads (forward and reverse length of 120 bp) and filtering to a maximum number of two 'expected errors'. Amplicon sequence variants (ASVs) were dereplicated if identical, clustered and pair-end reads merged using a minimum overlap of 12 bp and maximum mismatch of 0 bp. Chimeras were removed using the 'pooled' method. The ASVs were first taxonomically assigned using VSEARCH v. 2.18.0 (Rognes et al., 2016) using our custom foraminifera reference database based on NCBI reference database. Then, all ASVs affiliated to the genus *Ammonia* were verified by comparison with the GenBank database using BLAST and quick neighbour joining tree analyses were performed to attribute phylogenetically ambiguous ASVs to *A. aberdoveyensis*, *A. confertitesta* and *A. veneta*. The total number of reads of the ASVs were finally merged for each species.

All statistical analyses were performed on R (version 4.0.4, R Core Team, 2014). In order to use a semi-quantitative approach of the eDNA results, numbers of reads were log-transformed as applied by Pochon et al. (2015).

For all correlation tests, the normality of the data set was tested using the Shapiro–Wilk normality test, and the homogeneity of variance (homoscedasticity) with the Bartlett test. Because the data were not normally distributed, Spearman correlation tests were applied ($\alpha < 0.05$) using R software (version 4.3.2) (R Core Team, 2014).

3. Results

3.1. Morphological identification

Among the 1739 analysed individuals, 206 (11.8%) specimens were determined as *A. aberdoveyensis*, 444 (25.5%) as *A. confertitesta* and 952 (54.7%) as *A. veneta*, whereas the remaining 137 specimens (7.9%) could not be identified with sufficient reliability.

Detailed counting results for the three *Ammonia* species are presented in Table A.1. Of the 51 investigated stations, four did not contain any of the three studied species (Elorn-1; Crac'h-4, Vie-1, Vie-2). At the 47 remaining stations, *A. aberdoveyensis, A. confertitesta* and *A. veneta* together accounted for 0.3% to 96.7% of the total foraminiferal community. *Ammonia aberdoveyensis* was observed at 33 stations, *A. confertitesta* at 29 stations, and *A. veneta* occurred at 42 stations. At 19 stations, all three *Ammonia* species were observed, whereas only two species were found at 19 other stations (14 stations with *A. aberdoveyensis* and *A. veneta* and five stations with *A. confertitesta* and *A. veneta*). Finally, at five stations in the Vilaine estuary (1B, 1C, 2 A, 2B, 3) only *A. confertitesta* was observed and at four stations (Aulne-3, Auray-2 A, 2B and 8 A) only some specimens of *A. veneta* were observed (Table A.1).

The relative frequency of the three *Ammonia* species is presented in Fig. 2. Stations with <20 individuals are indicated with a red asterisk. These samples will not be discussed individually, considering that with a small number of specimens it is not possible to obtain a reliable estimate of the relative frequencies of the three species. Considering the average proportion of each species in the total assemblage, *A. veneta* accounted for 12.1% $_{(\pm 9.3\%)}$, with a maximum frequency of 29% (Vie-10B). *Ammonia aberdoveyensis* was less frequent, with an average percentage of 2.5% $_{(\pm 2.8\%)}$, and a maximum of about 10% (Elorn-2, Vie-10B). Finally, *A. confertitesta* was generally rare (0–2%), but was observed in large numbers in the Vie and Vilaine estuaries and at a single station in the Crac'h estuary. At these sites, its part of the total foraminiferal community increased substantially, attaining maximum values of 97% (Vilaine 2 A), 23% (Vie-10 A) and 7% (Crac'h-1), respectively.

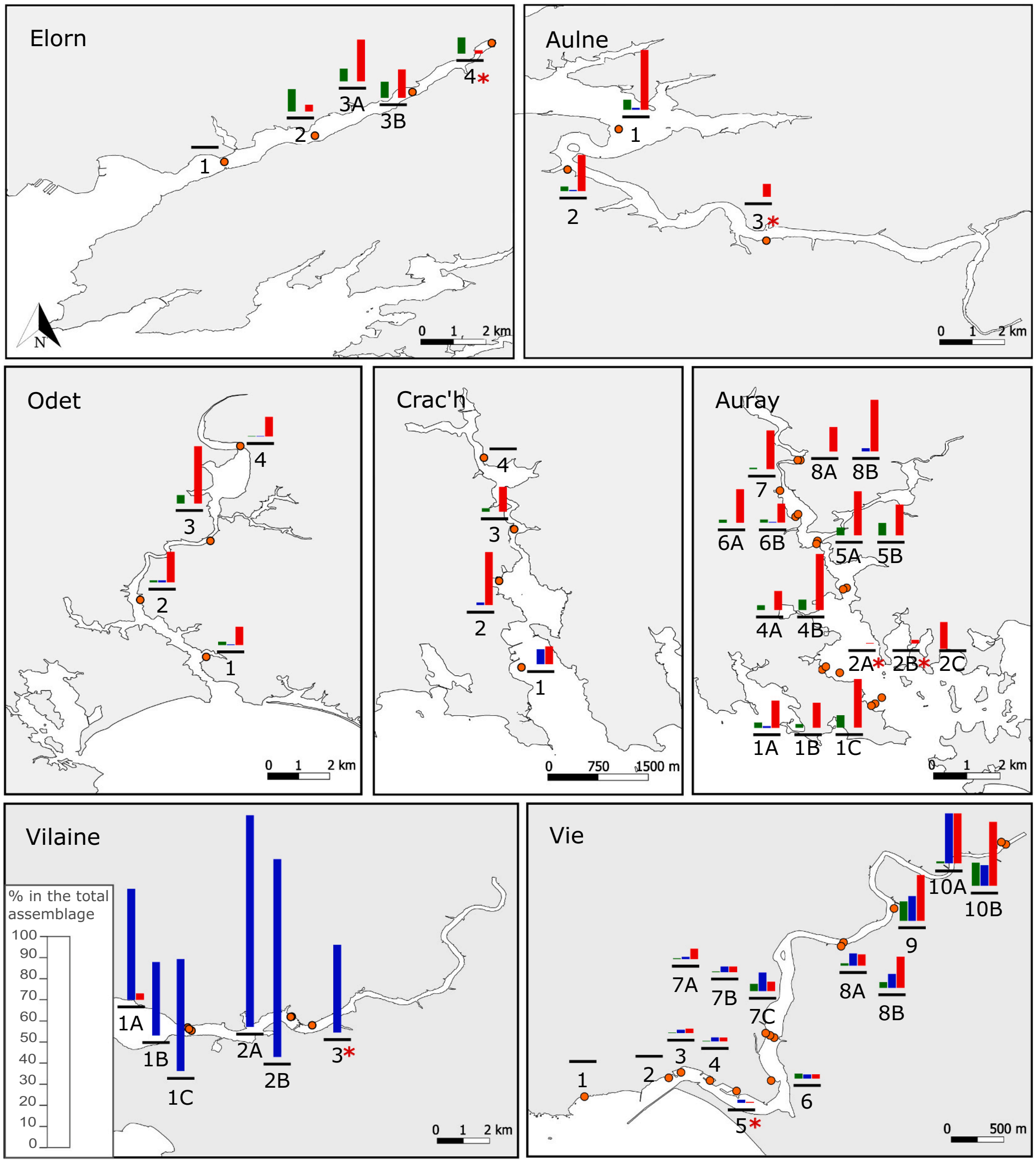

Fig. 2. Distribution of the three Ammonia species (*A. aberdoveyensis* in green, *A. confertitesta* in blue, *A. veneta* in red) at all stations in the seven estuaries. Stations with less than twenty individuals are marked with a red asterisk. The length of the barplot varies in function of the relative abundance of the taxon in the total foraminiferal assemblage, as shown on the scale on the bottom-left. The localisation of the different estuaries is presented in Fig. 1.

When considering for each estuary all stations together, all species were present in all estuaries, except in the Vilaine estuary, where *A. aberdoveyensis* was not observed, and the Elorn estuary where no *A. confertitesta* was found (Fig. 2). In view of our data, the seven estuaries can be divided into three groups:

1 The Aulne, Elorn, Odet, Crac'h and Auray estuaries showed a majority of *A. veneta* (Fig. 2), and very low numbers of *A. confertitesta*.
2 The Vie estuary showed comparable frequencies of *A. veneta* and *A. confertitesta*.
3 Finally, the Vilaine estuary stood out by the dominance of *A. confertitesta*, whereas *A. veneta* was only observed in very low numbers at a single station (Vilaine-1 A) and *A. aberdoveyensis* was not found.

Regarding the upstream-downstream estuarine gradient, no clear trends were visible, except in the Vie estuary, where all three *Ammonia* species were more abundant in the inner part of the estuary. There was no correlation between the distance to the sea and abundances of the three *Ammonia* species (Fig. A.2). In terms of absolute elevation on the mudflats, in the Vie and Auray estuaries, *A. aberdoveyensis* (and *A. veneta* to a lesser degree) showed a higher relative abundance at stations lower on the mudflat. In fact, the relative densities of *A. aberdoveyensis* showed a significant negative correlation with the absolute elevation (Spearman correlation test, R: −0.33, *p*-value: 0.017). This was not the case for *A. confertitesta* (Fig. A.2), for which no preference for a specific part of the mudflats was observed. Next, the relation with the percentage of grain size <63 μm and the percentage of organic matter was tested. Except a correlation between the percentage of grain size <63 μm and *A. confertitesta* (R: 0.58, p-value <0.001), most correlations tests were not significant (Fig. A2).

3.2. eDNA analysis

A table with the number of reads per species and per station is provided as Table A.2. Fig. 3 shows the eDNA distribution of

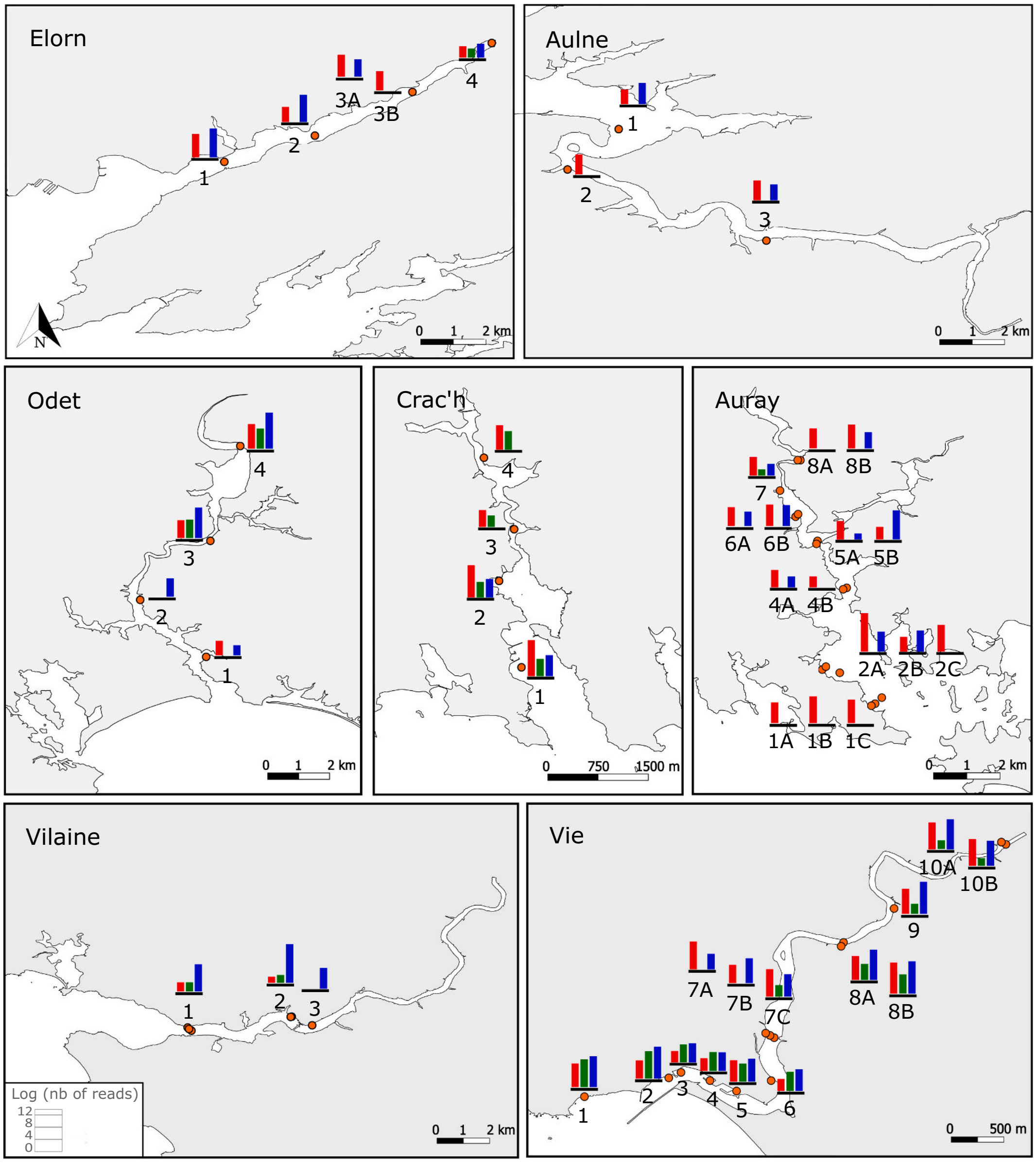

Fig. 3. Distribution of the three Ammonia species (*A. veneta* in red, *A. aberdoveyensis* in green, *A. confertitesta* in blue) based on eDNA sequencing analysis (the data present the log transformed number of reads, as shown on the scale on the bottom-left). The localisation of the different estuaries is presented in Fig. 1.

Table 1
Overall characteristics of the seven studied estuaries. Values marked with [1] come from Office français de la Biodiversité and marked with [2] from Banque Hydro.

Estuary	Elorn	Aulne	Odet	Crac'h	Auray	Vilaine	Vie
Number of sampling stations	5	3	4	4	15	6 (only 3 for eDNA)	14
Sampling campaign	October 2020	October 2020	October 2020	October 2020	September 2020	May 2019	October 2018
Estuary type	Ria	Ria	Ria	Ria	Ria	Lowland estuary	Lowland estuary partly closed by a bar
[1] Salt water penetration	15 km	28.8 km	20 km	13 km	19.8 km	12 km	8.25 km
[1] Catchment area	385 km^2	1797 km^2	715 km^2	64 km^2	324 km^2	10,536 km^2	751 km^2
[1] Width at the mouth	570 m	1460 m	1000 m	1016 m	950 m	4400 m	200 m
[2] Flood discharge	54 m^3/s	330 m^3/s	76 m^3/s	–	31 m^3/s	810 m^3/s	20–25 m^3/s
[2] Low flow discharge	1.1 m^3/s	1.5 m^3/s	0.79 m^3/s	–	0.18 m^3/s	5.50 m^3/s	0.01 m^3/s
[2] Mean annual discharge volume	5.59 m^3/s	25.00 m^3/s	7.45 m^3/s	–	2.72 m^3/s	74.00 m^3/s	1.18 m^3/s

A. aberdoveyensis, A. confertitesta, and *A. veneta* in the seven estuaries, indicating the log-transformed number of reads for each species. For the 48 stations investigated, reads of *A. aberdoveyensis*, *A. confertitesta* and *A. veneta* were detected at 22, 38 and 46 stations, respectively.

The co-existence of several *Ammonia* species is common in our samples. At 20 of the 48 stations, all three *Ammonia* species were detected, whereas at 18 stations only two species were found. When two species were observed together, in most cases (16 stations) these were *A. veneta* and *A. confertitesta*, whereas at two stations *A. veneta* and *A. aberdoveyensis* were present. At the remaining 10 stations, a single species was observed, which was *A. veneta* at eight stations and *A. confertitesta* at the remaining two stations. *A. aberdoveyensis* was never observed alone (Fig. 3).

In view of the eDNA results, the seven estuaries can be divided into three groups:

1 In the Elorn, Aulne and Auray estuaries, only a few reads were assigned to *A. aberdoveyensis*. In the Aulne estuary, this species was not observed at all, whereas it was only found in the innermost part of the other two estuaries. *A. confertitesta* and *A. veneta* were observed at most stations, with a few exceptions in the downstream part of Auray estuary, where only *A. veneta* was observed.
2 In the Crac'h, Odet and Vie estuaries, reads corresponding to *A. aberdoveyensis* were detected at most of the stations, whereas the other two species were generally well represented (except Crac'h 3 and 4 where *A. confertitesta* was not present).
3 Finally, in the Vilaine estuary, the results showed a large number of reads for *A. confertitesta* at all stations, whereas only a few reads were assigned to the other two species.

All three species were detected in all estuaries, except in the Aulne estuary where no reads were assigned to *A. aberdoveyensis*.

Concerning the upstream-downstream gradient in the seven estuaries, the three *Ammonia* species did not show a clear and systematic preference for specific parts of the estuary. However, in the Elorn, Auray and Odet estuaries, the data showed reads of *A. aberdoveyensis* only in stations located in the innermost parts.

4. Discussion

4.1. Morphometric discrimination of the three Ammonia species

Of the 1739 analysed specimens, 137 (i.e., 7.9%) could not be assigned. The proportion of unassigned specimens was around 9–12% in most of our estuaries, it was lower in Vilaine and Vie estuaries (2.5 and 0.5%), and higher in Crac'h estuary (25%). The inability to identify these specimens, which encompassed specimens from all estuaries, arose from *i)* their small size (7 specimens concerned), *ii)* the presence of deformation and/or dissolution (12 specimens), or, *iii)* indecisive morphological characteristics (118 specimens) between *A. veneta* and *A. confertitesta*. In Auray estuary, numerous specimens showed intense traces of dissolution, as described by (Daviray et al., 2023) and therefore were excluded from our dataset.

Such assignation difficulties are mentioned in previous studies (e.g., for damaged individuals; Richirt et al., 2019; Pavard et al., 2021). In fact, for their respective datasets, Richirt et al. (2019, 2021) estimated an accuracy of ≥ 90% and 95%, respectively, for their morphological determination method. However, the specific challenging discrimination between *A. veneta* and *A. confertitesta* was not mentioned in these earlier studies.

Richirt et al. (2021) present a dichotomous determination procedure. The small average pore diameter is the primary criterion to distinguish *A. aberdoveyensis* from the two other species, with a threshold value of 1.4 μm. Next, the main criterion to distinguish *A. veneta* and *A. confertitesta* is the elevation of the sutures on the central part of the dorsal side, flush in *A. confertitesta* versus raised in *A. veneta*. In our material, this difference was evident in typical representatives of both species, but there were also numerous specimens with an intermediate morphology, showing slightly raised sutures.

Richirt et al. (2019, 2021) proposed the average pore diameter as a secondary criterion to distinguish *A. veneta* from *A. confertitesta*, with a threshold value of 2.4 μm. All specimens with an average pore diameter larger than 2.4 μm should be *A. confertitesta*, whereas specimens with a smaller pore diameter could belong to either of the two species. In our study, the range of the mean pore diameter for *A. aberdoveyensis* was 0.59–1.40 μm (n = 78), similar to the literature data (Hayward et al., 2004; Richirt et al., 2019, 2021; Pavard et al., 2021). The range of the mean pore diameter for *A. confertitesta* was 1.42–3.14 μm (n = 222), similar to those found in Pavard et al., 2021, but these values almost perfectly overlap the ones measured for *A. veneta* (1.40–3.15 μm, n = 278). Surprisingly, in our dataset, many typical *A. veneta* specimens (with clearly raised sutures on the dorsal side) had a pore diameter well above the threshold value of 2.4 μm indicated by Richirt et al. (2021, 2019). In fact, the range of the mean pore diameter for *A. veneta* was not statistically different from that of *A. confertitesta* (t-test, p-value: 0.72) in our dataset. Consequently, contrary to the observations of Richirt et al. (2019), in our study, the criterion "average pore diameter" was efficient to discriminate *A. aberdoveyensis* from *A. veneta* and *A. confertitesta*, but not suitable to distinguish *A. veneta* from *A. confertitesta*.

The third criterion proposed by Richirt et al. (2021) to distinguish *A. veneta* and *A. confertitesta* is the number of incised sutures (between successive chambers) in the last whorl of the spiral side. *Ammonia confertitesta* shows two or less incised sutures, whereas specimens with more than two incised chamber sutures only occur in *A. veneta*. Therefore, for these two species, our morphological determination was based exclusively on two criteria, flush or raised sutures in the centre and the number of incised sutures in the last whorl. However, because of the presence of numerous specimens with intermediate dorsal suture characteristics (partly or slightly raised), the distinction between *A. veneta* and *A. confertitesta* was very challenging and some adult specimens could not be assigned to a species with sufficient reliability. Some image examples are presented in Fig. A.1.

4.2. Distribution of the three Ammonia species according to the morphological inventory

At an intra-estuary scale, along the upstream-downstream gradient, none of the three species showed a clear preference for a specific part of the estuary. No correlation between species relative abundances and the percentage of organic matter was found. While the percentage of sediment <63 μm and the relative abundance of *A. aberdoveyensis* and *A. veneta* showed no correlation, a positive correlation was found for *A. confertitesta*, suggesting that this species has a preference for stations with a finer sediment. Next, the distribution according to absolute elevation was examined. The absolute elevation determines the emersion time at low tide, when the organisms are exposed to potentially harsh conditions, such as elevated temperature, low or high salinity and predation. Consequently, several authors have suggested that elevation should be a primary control of the distribution of foraminifera in estuarine environments (e.g., Horton and Murray, 2007; Francescangeli, 2017; Armynot du Châtelet et al., 2018; Jorissen et al., 2022). In our study, *A. aberdoveyensis* showed a slight preference for stations located lower on the mudflats. This observation is in disagreement with Bird et al. (2020), who found more specimens of *A. aberdoveyensis* higher on the shore in a shoreline transect in Dartmouth estuary. However, the relative abundances of the three species showed no clear relation with the tested parameters and results showed at best correlations with low R^2 value (0.11 and 0.34). These results do not support major differences in the ecological preferences of the three species. When comparing the different species distributions, some recurrent distributional patterns can be observed. First, *A. veneta* was most common and was often the dominant *Ammonia* species (densities of up to 300 specimens per 50cm^3

in the Odet and Auray estuaries; on average 10% of the total foraminiferal community). *Ammonia aberdoveyensis* was present as a subsidiary species, with a maximum density of 79 specimens per 50cm^3 (in the Vie estuary). This species represented 2% of the total foraminiferal community on average. Finally, the density of *A. confertitesta* was much more variable compared to the other two species. All northern estuaries (Elorn, Aulne, Odet, Crac'h and Auray) showed very low densities of *A. confertitesta* (from 0 to 10 ind/50 cm^3, <1%, except station Crac'h-1 and Auray-8B with 34 and 39 ind/50 cm^3 respectively). Conversely, much higher densities were encountered in the two southern estuaries, with up to 202 ind/50 cm^3 (23%) in the Vie estuary, and up to 1497 ind/50 cm^3 (33 to 97%) in the Vilaine estuary. These observations suggest that the three *Ammonia* species have various degrees of opportunistic behaviour, with *A. confertitesta* being the most and *A. aberdoveyensis* the least opportunistic taxon.

All three *Ammonia* species were present in all estuaries, except *A. confertitesta,* that was not observed in the Elorn estuary. Additionally, in the Aulne estuary, the presence of *A. confertitesta* (based on two atypical specimens) is questionable. The fact that the three *Ammonia* species occur together at many different sampling stations in most of our estuaries contrasts with previous studies. Both Saad and Wade (2016) and Bird et al. (2020) observed that *A. confertitesta* only rarely co-exists with the other two species. Similarly, Richirt et al. (2021) suggested that the co-existence of different *Ammonia* species at the same station is rare. However, most of these observations are based on small numbers of sequenced specimens (10 or less), which could be statistically insufficient to detect the co-existence of the three *Ammonia* species at single sampling stations. On the contrary, our results show a co-occurrence of the three *Ammonia* species in half of the stations, whereas at only few stations, a single species was found (mostly *A. confertitesta*). The combination of *A. aberdoveyensis* and *A. confertitesta* (without *A. veneta*) was not observed in our study, but was described in the Gironde estuary (Pavard et al., 2021).

4.3. Comparison of morphological observation and eDNA data

In Table 2, we compare the presence-absence data for our morphological observations and eDNA sequencing. The results show large differences. In fact, both methods give the same results only for 14 of the 48 stations (i.e. 29.1%). In this study, a potential bias in the number of reads of abundances of the different phylotype could be due to the different amounts of sediment used for DNA extraction (10 g, 5 g or 250 mg). It was shown that DNA extracted from a small amount of sediment (0.5-1 g) represented more foraminiferal propagules, whereas DNA extracted from 10 g of sediment was more representative of the foraminiferal adult population (Brinkmann et al., 2023). Nevertheless, to reduce this potential bias we have used presence/absence dataset in our analyses.

When comparing the two methods, several observations can be made:

1) At the four stations where no *Ammonia* species were observed in the morphological study (Elorn 1, Crac'h 4 and Vie 1 and 2), eDNA revealed the presence of at least two different species (*A. veneta* and *A. confertitesta* in Elorn 1, *A. veneta* and *A. aberdovenyensis* in Crac'h 4, and the three species in Vie 1 and 2).
2) Morphological and eDNA data showed a good correspondence for *A. veneta*, which was detected by both methods at 41 of the 48 stations.
3) In the Elorn, Aulne and Auray estuaries, *A. aberdoveyensis* was present at most stations in the morphological inventory, but was rarely detected with the eDNA approach. In general, *A. aberdoveyensis* was better represented in the morphological dataset (31 stations) than in the eDNA dataset (22 stations).
4) A major difference between the two data-sets concerns *A. confertitesta*, which was more frequently detected within the eDNA dataset (38 stations) than the morphological one (25 stations). This difference concerns especially the northern estuaries (Elorn, Aulne and Auray), where this taxon was very rare in the morphological survey, while detected with the eDNA approach.

These important discrepancies can be explained by the different characteristics of both methods, which can lead to an entirely different picture of the diversity. Morphological analyses are based on adult specimens, larger than >125 µm. The choice of the >125 µm mesh size is motivated by the difficulty, and even the impossibility, to discriminate smaller individuals of the three *Ammonia* species. Conversely, environmental DNA sequencing analyses are based on the total sediment, without size selection. Metabarcoding data therefore include adult specimens of large species (> 125 µm), but also juveniles (Pawlowski et al., 2014), propagules (Brinkmann et al., 2023) and juveniles/adult individuals of small species. Even the presence of eDNA preserved in dead specimens can be envisaged. Both approaches give therefore different, but complementary results (Lejzerowicz et al., 2013; Pitsch et al., 2019; Brinkmann et al., 2023). Here, observations based on morphological analyses indicate the presence of a population of active adult specimens, whereas the eDNA analysis reveals the presence of genetic material of the investigated species. However, eDNA analysis does not allow the distinction between an active population interacting with the environment, a stock of propagules awaiting the appropriate conditions to develop, or exceptionally preserved DNA of dead specimens.

By considering both approaches, three distinct scenarios/cases can be identified.

1) *Ammonia veneta* was detected by both approaches at most of the stations. It appears therefore that the population of this species is active at most stations where genetical material is present. This suggests that the environmental conditions were generally favourable for the development of this taxon.
2) *Ammonia aberdoveyensis* was frequently detected with the morphological method but not with the eDNA method. This difference can not be explained by a difference between active and inactive populations. This discrepancy could be related to the sampling procedure. The morphological analysis showed that *A. aberdoveyensis* was widely present at the sampled stations but always in low numbers. In fact, the volume of sediment analysed is an order of magnitude higher for morphological analysis (three replicates of ~80 g each) than for eDNA analysis (two or three replicates with a total of 10-12 g). The absence of *A. aberdoveyensis* in many eDNA samples may be the consequence of its relative scarcity, eventually in combination with a patchy distribution. In other words, we hypothesise that the volume of sediment analysed for eDNA was too small to systematically detect this scarce species. This issue concerning the quantity of sampled material was earlier mentioned by Pawlowski et al. (2014) as a potential bias.
3) The presence of *A. confertitesta* in the eDNA data often contrasted with an absence in the morphological analysis. In this case, it appears that the eDNA results could reveal the presence of propagules and possibly juveniles <125 µm, whereas an active adult population has not (yet) developed, most probably because the environmental conditions were not appropriate.

An explanation could be found in the distributional history of the three species. Several studies have suggested that *A. confertitesta* is an exotic species (Pawlowski and Holzmann, 2007; Schweizer et al., 2011) that would progressively replace the autochthonous species *A. aberdoveyensis* and *A. veneta*. If this hypothesis is correct, the difference between morphological and eDNA data for this taxon could be explained by the fact that today, genetic material of *A. confertitesta* is present in all estuaries, but this species has not yet established active adult populations in all these estuaries.

Table 2
Presence-absence matrix for three Ammonia species with both approaches: morphological observations and eDNA analysis.

		Ammonia aberdoveyensis		Ammonia confertitesta		Ammonia veneta	
		Morphology	eDNA	Morphology	eDNA	Morphology	eDNA
Elorn	Elorn-1				*		*
	Elorn-2	*			*	*	*
	Elorn-3 A	*			*	*	*
	Elorn-3B	*				*	*
	Elorn-4	*	*		*	*	*
Aulne	Aulne-1	*		*	*	*	*
	Aulne-2	*		*		*	*
	Aulne-3				*	*	*
Odet	Odet-1	*		*	*	*	*
	Odet-2	*		*	*	*	
	Odet-3	*	*		*	*	*
	Odet-4	*	*	*	*	*	*
Crac'h	Crac'h − 1		*	*	*	*	*
	Crac'h − 2		*	*	*	*	*
	Crac'h − 3	*	*			*	*
	Crac'h − 4		*				*
Auray	Auray-1 A	*		*		*	*
	Auray-1B	*				*	*
	Auray-1C	*				*	*
	Auray-2 A				*	*	*
	Auray-2B				*	*	*
	Auray-2C	*		*		*	*
	Auray-4 A	*			*	*	*
	Auray-4B	*				*	*
	Auray-5 A	*			*	*	*
	Auray-5B	*			*	*	*
	Auray-6 A	*			*	*	*
	Auray-6B	*		*	*	*	*
	Auray-7	*	*		*	*	*
	Auray-8 A					*	*
	Auray-8B			*	*	*	*

		Ammonia aberdoveyensis		Ammonia confertitesta		Ammonia veneta	
		Morphology	eDNA	Morphology	eDNA	Morphology	eDNA
Vilaine	Vilaine-1 A		*	*	*	*	*
	Vilaine-2 A		*	*	*		*
	Vilaine-3			*	*		
Vie	Vie-1		*		*		*
	Vie-2		*		*		*
	Vie-3	*	*	*	*	*	*
	Vie-4	*	*	*	*	*	*
	Vie-5		*	*	*	*	*
	Vie-6	*	*	*	*	*	*
	Vie-7 A	*		*	*	*	*
	Vie-7B	*		*	*	*	*
	Vie-7C	*	*	*	*	*	*
	Vie-8 A	*	*	*	*	*	*
	Vie-8B	*	*	*	*	*	*
	Vie-9	*	*	*	*	*	*
	Vie-10 A	*	*	*	*	*	*
	Vie-10B	*	*	*	*	*	*

4.4. Presumed invasive behaviour of Ammonia confertitesta

Several authors have suggested that *A. confertitesta* is an introduced species in Europe, originating from eastern Asia (e.g., Pawlowski and Holzmann, 2007; Schweizer et al., 2011; Richirt et al., 2021). The two main lines of evidence supporting this hypothesis are 1) the disjoint geographical distribution: Eastern Asia (Toyofuku et al., 2005) and European coasts (Schweizer et al., 2011) and 2) the recent appearance of *A. confertitesta* at sites in Europe where it has never been identified in the past (e.g., the Baltic Sea (Flensburg Fjord in Polovodova et al. (2009); Kiel Fjord in Schweizer et al. (2011)) or the North Sea (Grevelingenmeer in Petersen et al. (2016), 2016; Elbe estuary in Francescangeli et al. (2021)). This species could have been transported from Asia through ballast waters (Pawlowski and Holzmann, 2007) and is now widely present along the European coasts, from the Baltic Sea to France (Bird et al., 2020).

Recently, Richirt et al. (2021) studied the distribution of the three *Ammonia* species along the English Channel and Great Britain coasts. They hypothesised that marine currents could be the main vector of transport of foraminiferal propagules away from their source population (important harbours). When arrived, *A. confertitesta* would replace the autochthonous species *A. aberdoveyensis* and *A. veneta,* except for some refuge zones, mainly in high marshes, where the latter two species could persist.

In our study, the morphological observations of the distribution of the three *Ammonia* species showed a clear difference between the Vilaine estuary, with a very strong dominance of *A. confertitesta*, the Vie estuary, where *A. confertitesta* co-occurred with the other two species (with comparable frequencies of *A. confertitesta* and *A. veneta*), and the other five estuaries (Elorn, Aulne, Odet, Crac'h and Auray) where this species was rare or absent.

Together with the apparently greater opportunistic potential of *A. confertitesta*, deduced from the fact that it attains a much higher absolute and relative densities than the other two species at several stations (i.e., for *A. aberdoveyensis* until 10% of the total assemblage, for *A. veneta* until 29% and for *A. confertitesta* until 97% of the total assemblage), these observations seem to corroborate the "invasive species hypothesis". Our data suggest that *A. confertitesta* has fully colonised the Vilaine estuary, that the colonisation of the Vie estuary is in progress, whereas the colonisation of the other five estuaries is still at an early stage.

If true, this pattern could be explained by two complementary features: 1) the proximity of the source area(s), and 2) the ease of access to the various estuaries.

Richirt et al. (2021) suggested that major harbours (e.g., Cardiff, Le Havre, Rotterdam) should be source areas, in view of the intense international maritime traffic and the introduction of exotic ballast waters. This hypothesis seems to be confirmed by the dominance of *A. confertitesta* in major harbours along the French coast, such as Le Havre harbour located in the Seine estuary (Pavard et al., 2023a), and Bordeaux located in the upper part of the Gironde estuary (Pavard et al., 2021, 2023b).

In our study area, the major commercial harbour nearby is Nantes-St. Nazaire, on the Loire estuary (Fig. 1). The *Ammonia* assemblages of this estuary is indeed largely dominated by *A. confertitesta* (Fouet, 2022; Thibault de Chanvalon et al., 2022). The complete colonisation of the Vilaine estuary, immediately northward of the Loire, and the ongoing colonisation of the Vie estuary, immediately south of the Loire, would be logical if the Loire estuary is indeed the source area of *A. confertitesta* in this region. As would be the fact that the colonisation of all northern estuaries of this study area, much farther away from the Loire estuary, is still at an early stage. The presence of *A. confertitesta* in further north areas of Europe (e.g., British Isles (Bird et al., 2020; Richirt et al., 2021), North Sea (Schweizer et al., 2011; Richirt et al., 2020), Skagerrak (Brinkmann et al., 2023)) shows that differences of climatic conditions do not explain the differences of distribution between northern and southern estuaries in our studied area.

The second parameter, the easiness of access of the various estuaries, is related to their morphology. The two southern estuaries, Vilaine and Vie, are both lowland estuaries. The Vilaine estuary shows a wide mouth, the Vie estuary is partly closed with a sand spit. Conversely, all five northern estuaries are rias, flooded river valleys with a steep relief, and often with sills at the entrance. It appears therefore that the estuaries already inhabited by large populations of *A. confertitesta* could have an easier access than those in which this taxon is still at an early stage of colonisation. The importance of the morphology of the estuaries as a factor facilitating or hampering the introduction of *A. confertitesta* seems to be confirmed in scientific literature. A closer inspection of the sites studied by Saad and Wade, 2016, Bird et al., 2020, and Richirt et al., 2021 shows that *Ammonia* assemblages dominated by *A. confertitesta* are mainly found in open estuaries, whereas ria-type estuaries are dominated by *A. veneta* or *A. aberdoveyensis* (Table A.3).

However, our eDNA data detected the presence of *A. confertitesta* in these northern estuaries. This observation suggests that the colonisation of this taxon does not only depend on spreading mechanisms. Once potentially present, the species needs appropriate conditions to develop and replace autochthonous taxa *A. veneta* and *A. aberdoveyensis*. The ecological requirements of the three species appear to be comparable, but *A. confertitesta* stands out by its potentially higher degree of opportunism. The replacement of both autochthonous species by *A. confertitesta* could result from events that would force the foraminiferal community to recolonise the estuarine mudflat. In estuaries, such conditions may happen after major river floods, which can annihilate the foraminiferal community, creating empty environments suitable for the settlement of more opportunistic species. The importance of major river floods as a factor causing the re-colonisation by highly opportunistic species was earlier shown by Goineau et al. (2012). At a station located in front of the Rhone prodelta (Mediterranean Sea), the foraminiferal assemblage sampled two days after a major river flood, contained a very dense, almost monospecific population of *Leptohalysis scottii* (Chaster, 1892). The authors concluded that *L. scottii* is a pioneer species that could colonise the newly formed empty habitat first due to its greater reproduction and/or dispersal rates. Similarly, after each major river flood, Foraminifera have to recolonise the intertidal mudflats. If *A. confertitesta* is indeed a more opportunistic species (e.g., with a higher reproduction rate or a more efficient feeding strategy), it would ultimately replace the other two species, as proposed by Pavard et al. (2023b) in the case of the Gironde estuary. Although our distributional data do not show a significant ecological preference difference between the three species, a higher tolerance to low salinity could be an additional factor favouring *A. confertitesta* (Polovodova et al., 2009; Schweizer et al., 2011), even though results of this study do not corroborate this hypothesis.

5. Conclusions

This study investigates the distribution of *A. aberdoveyensis*, *A. confertitesta* and *A. veneta* on intertidal mudflats in seven estuaries along the north French Atlantic coast. None of the species showed a clear preference for a specific part of the estuaries, although *A. aberdoveyensis* and *A. veneta* were slightly more frequent on the lower parts of the mudflats. This suggests that the three species have comparable ecological requirements. However, whereas *A. confertitesta* and *A. veneta* can be dominant species in the foraminiferal community, *A. aberdoveyensis* was always a minor species. This difference could be indicative of a more opportunistic life strategy for the former two species. The morphology and eDNA based methods give different information. The presence of the investigated *Ammonia* species in the eDNA dataset can be due to the presence of adults, juveniles, propagules and preserved DNA from dead individuals. Their detection in the visual inventory (Rose Bengal stained) of the >125 μm fraction certifies the presence of an active and mature population. In this study, there were some important differences between the eDNA and morphology-based data. *Ammonia aberdoveyensis*, for which adult specimens were observed at many stations, in all estuaries, was often absent in the eDNA inventories. This could be explained by the relative scarcity of this taxon. *Ammonia confertitesta* was well represented in the eDNA data set, at most sites in all estuaries, whereas this taxon was rare or even absent in the morphological inventory of the five northern estuaries (Elorn, Aulne, Odet, Crac'h and Auray). Conversely, it was common in the Vie estuary, and attained very high densities at all stations in the Vilaine estuary. These observations corroborate the hypothesis of the invasive nature of *A. confertitesta*. The consistent detection of this taxon in eDNA, despite its absence, or scarcity, in the mophological inventories of northern estuaries, suggests that the species has not replaced the autochthonous species *A. veneta* and *A. aberdoveyensis* everywhere yet, even though its genetic material is present. The fact that genetic material of *A. confertitesta* is present in each estuary, while this species is absent or scarce in the visual inventories of northern estuaries indicates that it has not yet replaced the native species *A. veneta* and *A. aberdoveyensis* everywhere. If true, the seven estuaries could present different replacement stages: completed in the Vilaine, ongoing in the Vie, and still in an early stage in the other five estuaries. These different stages of colonisation by *A. confertitesta* could be explained by: 1) the relative distance to the potential source area, 2) the facility of access in each of the estuaries, and 3) the presence of favourable conditions for the development of *A. confertitesta*. We hypothesise that such favourable conditions could be brought by major flooding events which create empty ecological niches. *Ammonia confertitesta* could be more successful in recolonising such empty habitats than the other two taxa, because of its more opportunistic lifestyle, and maybe, because of its higher tolerance to low salinity conditions. Finally, this study underlines the strength of the combination of morphological and eDNA metabarcoding approaches to assess the distribution of foraminiferal species. This combination allows a better understanding of complex distributional patterns, by distinguishing between potential and observed assemblages, and finally bring important clues about species ecological preferences.

Credit authorship contribution statement

Marie P.A. Fouet: Conceptualisation, Field work, Methodology, Data analysis, Visualization, Writing - original draft. **Magali Schweizer**: Field work, Methodology, Data analysis, Writing. **David Singer**: Field work, Methodology, Data analysis, Writing. **Julien Richirt**: Methodology, Data analysis, Writing. **Sophie Quinchard**: Field work, Methodology. **Frans. J. Jorissen**: Conceptualisation, Field work, Data analysis, Writing.

CRediT authorship contribution statement

Marie P.A. Fouet: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Magali Schweizer:** Writing – review & editing, Validation, Supervision, Methodology, Investigation. **David Singer:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation. **Julien Richirt:** Writing – original draft, Methodology, Data curation. **Sophie Quinchard:** Methodology, Investigation. **Frans J. Jorissen:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are available in appendices.

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Appendix A. Appendices

Table A.1
Estuary, sampling station, sampling month/year, total abundance of foraminifera, relative frequency of Ammonia species in the total foraminiferal assemblage, total number of individuals analysed, numbers assigned to each of the three Ammonia species and the number of non assigned specimens.

Estuary	Station	Sampling period (month/year)	Abundance of foraminifera (ind/50 cm3)	% of the three *Ammonia* spp. within the total assemblage	Number of individuals	*Ammonia aberdoveyensis*	*Ammonia confertitesta*	*Ammonia veneta*	Undetermined	Estuary	Station	Sampling period (month/year)	Abundance of foraminifera (ind/50 cm3)	% of the three *Ammonia* spp. within the total assemblage	Number of individuals	*Ammonia aberdoveyensis*	*Ammonia confertitesta*	*Ammonia veneta*	Undetermined
	Elorn-1		319	0.0	0	0	0	0	0		Vilaine-1 A		2954	53.6	39	0	35	2	2
	Elorn-2		446	13.2	40	27	0	8	5		Vilaine-1B		1257	33.5	40	0	39	0	1
Elorn	Elorn-3 A	10/20	709	24.9	40	8	0	26	6	Vilaine	Vilaine-1C	5/19	285	51.1	40	0	39	0	1
	Elorn-3B		71	20.4	22	8	0	14	0		Vilaine-2 A		231	96.7	40	0	39	0	1
	Elorn-4		62	8.8	8	5	0	1	2		Vilaine-2B		652	90.3	40	0	40	0	0
	Aulne-1		649	32.8	40	5	1	30	4		Vilaine-3		7	40	2	0	2	0	0
Aulne	Aulne-2	10/20	361	19.1	40	4	1	32	3		Vie-1		50	0.0	0	0	0	0	0
	Aulne-3		70	5.9	6	0	0	5	1		Vie-2		220	0.0	0	0	0	0	0
	Odet-1		504	10.1	40	5	1	29	5		Vie-3		597	3.8	63	4	25	34	0
Odet	Odet-2	10/20	773	15.7	40	2	2	32	4		Vie-4		623	3.8	33	2	16	15	0
	Odet-3		1334	29.8	40	4	0	28	8		Vie-5		169	1.8	9	0	7	2	0
	Odet-4		866	9.5	40	1	1	35	3		Vie-6		627	6.1	57	21	17	18	1
	Crac'h-1		499	14.9	40	0	15	18	7		Vie-7 A		1742	6.2	48	3	8	36	1
Crac'h	Crac'h-2	10/20	209	25.3	40	0	1	22	17	Vie	Vie-7B	10/18	1282	5.3	59	3	28	28	0
	Crac'h-3		861	12.8	40	4	0	29	7		Vie-7C		905	16.0	68	14	36	18	0
	Crac'h-4		0	0	0	0	0	0	0		Vie-8 A		1798	11.7	55	5	26	24	0
	Auray-1 A		1178	15.8	40	6	2	30	2		Vie-8B		1078	23.2	44	5	12	27	0
	Auray-1B		665	13.1	40	4	0	28	8		Vie-9		888	41.1	52	11	14	26	1
	Auray-1C		259	27.9	40	8	0	31	1		Vie-10 A		882	46.7	51	1	25	25	0
Auray	Auray-2 A	9/20	242	0.3	1	0	0	1	0		Vie-10B		202	49.0	42	9	8	25	0
	Auray-2B		89	1.6	1	0	0	1	0										
	Auray-2C		524	13.7	40	5	1	25	9										
	Auray-4 A		37	10.9	6	1	0	4	1										
	Auray-4B		1363	30.4	40	5	0	27	8										

(*continued on next page*)

Table A.1 (*continued*)

Estuary	Station	Sampling period (month/ year)	Abundance of foraminifera (ind/50 cm3)	% of the three *Ammonia* spp. within the total assemblage	Number of individuals	*Ammonia aberdoveyensis*	*Ammonia confertitesta*	*Ammonia veneta*	Undetermined	Estuary	Station	Sampling period (month/ year)	Abundance of foraminifera (ind/50 cm3)	% of the three *Ammonia* spp. within the total assemblage	Number of individuals	*Ammonia aberdoveyensis*	*Ammonia confertitesta*	*Ammonia veneta*	Undetermined
	Auray-5 A		842	23.6	40	6	0	34	0										
	Auray-5B		166	19.9	40	11	0	27	2										
	Auray-6 A		204	16.6	40	3	0	35	2										
	Auray-6B		136	10.2	40	5	1	31	3										
	Auray-7		501	18.3	40	1	0	29	10										
	Auray-8 A		184	11.2	33	0	0	29	4										
	Auray-8B		2560	25.1	40	0	2	31	7										

Table A.2
Number of ASV reads corresponding to Ammonia aberdoveyensis, A. confertitesta and *A. veneta* per sample obtained by eDNA extractions. For more details, see Section 2.4.

Estuary	Station	*Ammonia aberdoveyensis*	*Ammonia confertitesta*	*Ammonia veneta*	Estuary	Station	*Ammonia aberdoveyensis*	*Ammonia confertitesta*	*Ammonia veneta*
Elorn	1	0	396	117	Vilaine	1 A	4	264	4
	2	0	265	16		2 A	3	3747	2
	3 A	0	28	79		3	0	59	0
	3B	0	0	43	Vie	1	305	604	117
	4	4	12	7		2	288	795	34
Aulne	1	0	63	14		3	32	41	7
	2	0	0	50		4	38	36	9
	3	0	19	48		5	31	102	68
Odet	1	0	5	14		6	42	74	8
	2	0	33	0		7 A	0	18	314
	3	34	562	31		7B	0	148	32
	4	53	2090	147		7C	7	87	279
Crac'h	1	27	64	2122		8 A	20	539	123
	2	18	35	837		8B	43	867	664
	3	7	0	25		9	5	819	161
	4	38	0	151		10 A	4	529	260
Auray	1 A	0	0	59		10B	3	165	243
	1B	0	0	247					
	1C	0	0	111					
	2 A	0	50	3509					
	2B	0	64	15					
	2C	0	0	241					
	4 A	0	6	27					
	4B	0	0	6					
	5 A	0	2	36					
	5B	0	405	9					
	6 A	0	14	40					
	6B	0	62	72					
	7	2	7	35					
	8 A	0	0	49					
	8B	0	21	121					

Table A.3
Geographic distribution of the three Ammonia spp. in the literature (T1: *A. veneta*, T2: A. aberdoveyensis, T6: A. confertitesta). The locations are classified in five types: saltmarsh, open marsh, lowland estuary (e.g., Vilaine), lowland estuary semi-enclosed (e.g., Vie), ria/fjord (e.g. Elorn).

	Site	Latitude	Longiture	Type	*Ammnonia* spp.
Richirt et al., 2021	Authie	50°22′23.80″N	1°35′44.00″E	Lowland estuary	T6 (*n* = 4)
	Biezelingse Ham	51°26′53.40″N	3°55′49.79″E	Lowland estuary	T6 (*n* = 51)
	Ouistreham	49°16′16.40″N	0°14′12.20″W	Lowland estuary semi-enclosed	T1 (*n* = 1) T2 (*n* = 1) T6 (n = 5)
	Rade de Brest	48°24′13.10″N	4°21′16.00″W	Ria/fjord	T2 (n = 2)
	Seine estuary	49°26′31.30″N	0°16′25.20″E	Lowland estuary	T6 (*n* = 32)
	St. Vaast-La-Hougue	49°34′38.60″N	1°16′38.80″W	Open marsh	T1 (n = 1) T2 (n = 3)
	Veerse Meer	51°33′12.24″N	3°52′25.34″E	Lowland estuary	T2 (n = 5) T6 (n = 4)
Bird et al., 2020	Cromarty	57°40′45.59″N	04°02′28.12″W	Ria/fjord	T2 (*n* = 1)
	Torry Bay	56°03′28.3″N	03°35′02.5"W	Lowland estuary	T6 (*n* = 8)
	Cramond	55°58′54.2″N	03°17′56.5″W	Lowland estuary	T6 (*n* = 52)
	Loch na Cille	55°57′36.00″N	05°41′24.00″W	Ria/fjord	T2 (*n* = 13)
	Whiterock	54°29′05.42″N	05°39′12.58″W	Ria/fjord	T2 (*n* = 18)
	Den Oever	52°56′24.8″N	05°01′30.6″E	Lowland estuary	T6 (n = 1)
	Norfolk	52°49′02.41″N	00°21′46.16″E	Lowland estuary	T6 (*n* = 30) T2 (n = 1)
	Laugharne Castle	51°46′12.00″N	04°27′00.00″W	Lowland estuary	T6 (n = 2)
	Cork	51°38′29.40″N	08°45′44.50″W	Lowland estuary	T1 (n = 2) T2 (*n* = 28)
	Cardiff	51°29′25.40″N	03°07′19.50″W	Lowland estuary	T6 (*n* = 20)
	Dartmouth	50°21′04.84″N	03°34′11.33″W	Lowland estuary	Upper shore T2 (*n* = 6) Mid shore T1 (n = 2) T2 (*n* = 12) Lower shore T1 (n = 2) T2 (*n* = 49)
Saad and Wade, 2016 – modified by Richirt et al., 2021	Baie de l'Aiguillon	46°15′17.00″N	01°08′27.00″W	Lowland estuary	T6 (n = 2)
	Bangor	53°14′02.41″N	04°07′04.26″W	Open marsh	T1 (n = 5)
	Barmouth	52°43′17.26″N	04°02′27.43″W	Lowland estuary	T1 (*n* = 9) T6 (n = 1)
	Barrow-in-Furness	54°05′24.16″N	03°14′29.61″W	Open marsh	T6 (n = 9)
	Barton-upon-Humber	53°41′50.86″N	00°26′40.08″W	Lowland estuary	T6 (n = 9)
	Brancaster Staithe	52°58′11.78″N	00°40′ 05.05″E	Saltmarsh	T2 (n = 7)
	Braunton	51°05′55.09″N	04°09′52.15″W	Lowland estuary	T6 (*n* = 10)
	Burnham Overy Staithe	52°58′06.76″N	00°40′05.08″E	Saltmarsh	T6 (n = 10)
	Galmpton	50°23′31.53″N	03°34′31.15″W	Lowland estuary	T2 (n = 4)
	Hambleton	53°52′40.15″N	02°57′52.46″W	Lowland estuary	T6 (n = 2)
	Lymington	50°45′16.36″N	01°31′39.34″W	Lowland estuary semi-enclosed	T2 (n = 8)
	Pembroke Dock	51°41′59.66″N	04°55′14.72″W	Lowland estuary	T6 (n = 8)
	Pen Clawdd	51°38′36.28″N	04°06′20.18″W	Lowland estuary	T6 (n = 10)
	Queenborough	51°25′01.47″N	00°44′21.15″W	Lowland estuary	T6 (*n* = 11
	Severn Beach	51°33′17.99″N	02°40′11.37″W	Lowland estuary	T6 (n = 6)
	Shoreham-By-Sea	50°49′49.04″N	00°16′30.79″W	Lowland estuary semi-enclosed	T1 (n = 1) T2 (n = 7) T6 (n = 2)
	South Queensferry	55°59′34.28″N	03°24′38.18″W	Lowland estuary	T6 (n = 6)
	St Osyth	51°47′54.83″N	01°03′50.32″W	Lowland estuary	T6 (n = 9)
	Thornham	50°57′59.35″N	00°34′20.09″E	Open marsh	T6 (n = 6)

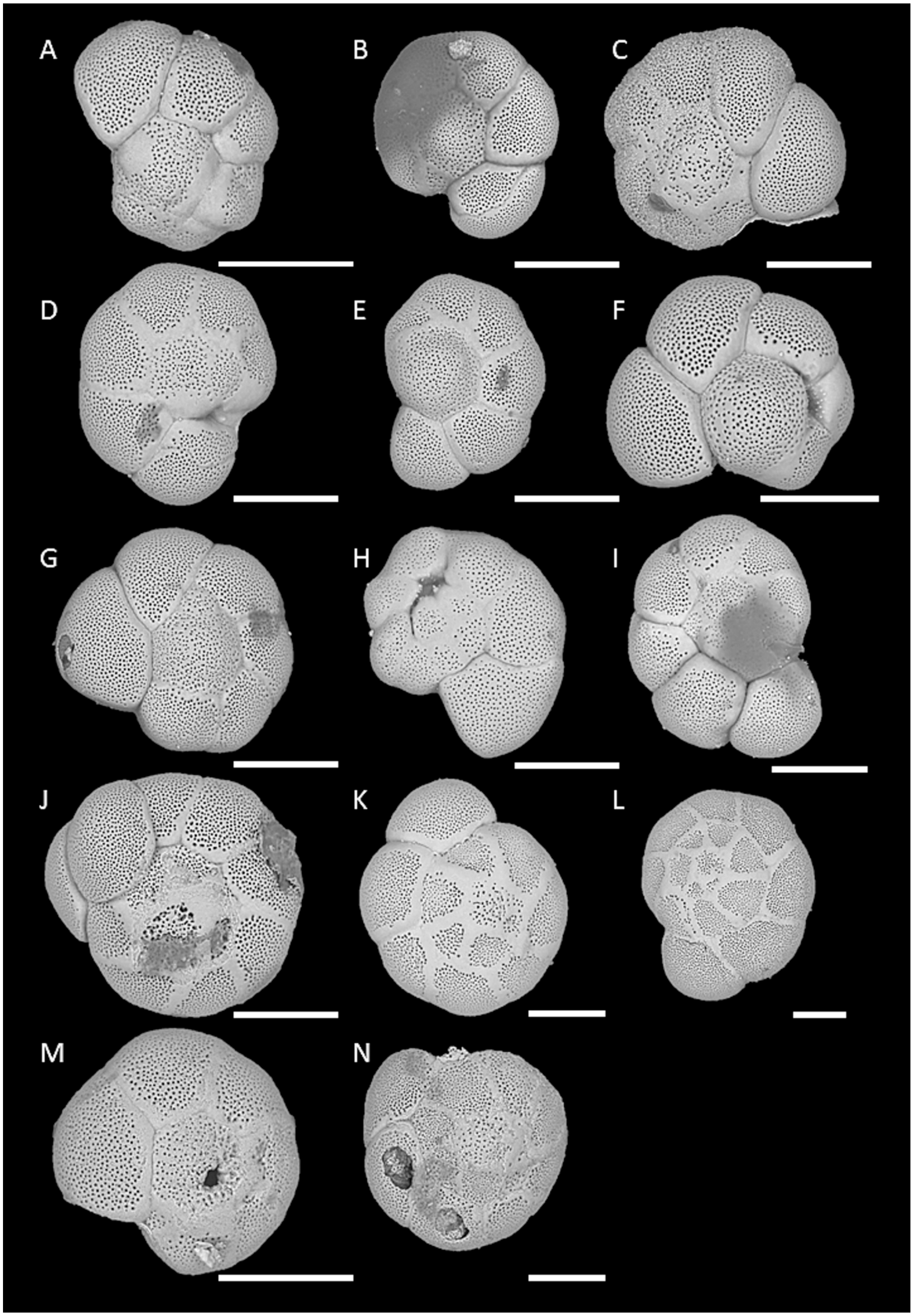

Fig. A.1. Examples of individuals classified as undetermined. A: Auray_2C (28); B: Auray_7(20); C: Auray_1A(40); D: Auray_1B(32); E: Auray_6B(23); F: Auray_6B (25); G: Auray_7(30); H: Elorn_4(01); I: Vie_7A(87); J: Vie_9(09); K: Vilaine_1A(04); L: Vilaine_1 A(12); M: Aulne_1(37); N: Aulne_1(02). Scale bar: 100 μm.

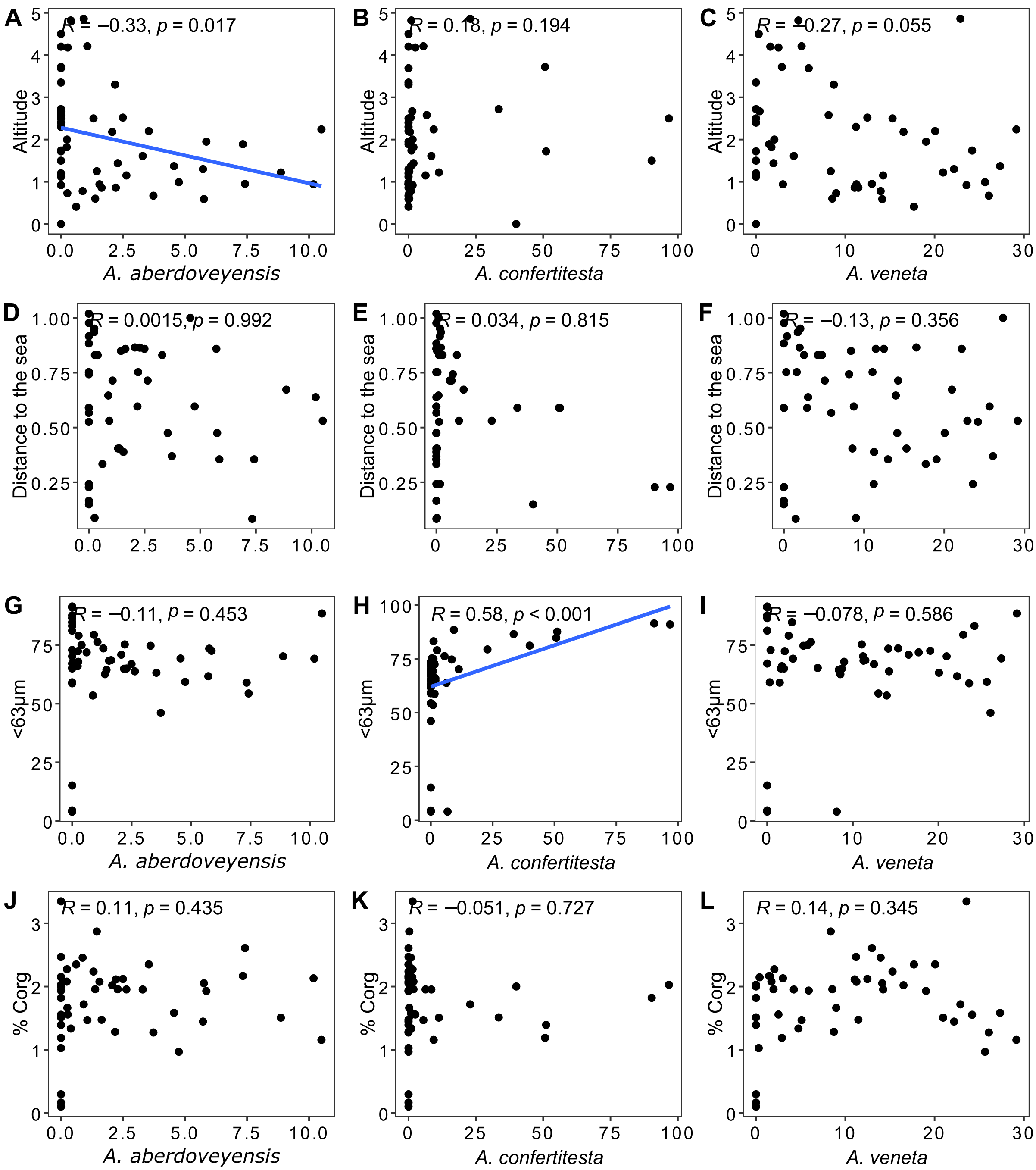

Fig. A.2. Relation between A,B,C: absolute elevation from the lowest astronomical tide, D,E,F: distance of the sampling point to the sea, G,H,I: sediment fine fraction (percentage of sediment < 63 μm), J,K,L: percentage of organic matter and the percentage of Ammonia spp. in the total assemblage.

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