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Vincent Bouchet, Jean-Charles Pavard, Maria Holzmann, Mary McGann, Eric Armynot Du Châtelet, et al.. The invasive Asian benthic foraminifera *Trochammina hadai* Uchio, 1962: identification of a new local in Normandy (France) and a discussion on its putative introduction pathways. *Aquatic Invasions*, 2023, 18 (1), pp.23-38. 10.3391/ai.2023.18.1.103512 . hal-04248088

HAL Id: hal-04248088

<https://normandie-univ.hal.science/hal-04248088v1>

Submitted on 9 Feb 2024

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Research Article

The invasive Asian benthic foraminifera *Trochammina hadai* Uchio, 1962: identification of a new local in Normandy (France) and a discussion on its putative introduction pathways

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Academic editor: Tammy Robinson

Received: 12 December 2022

Accepted: 15 February 2023

Published: 18 April 2023

Citation: Bouchet VMP, Pavard J-C, Holzmann M, McGann M, Armynot du Châtelet E, Courleux A, Pezy J-P, Dauvin J-C, Seuront L (2023) The invasive Asian benthic foraminifera *Trochammina hadai* Uchio, 1962: identification of a new local in Normandy (France) and a discussion on its putative introduction pathways. *Aquatic Invasions* 18(1): 23–38, <https://doi.org/10.3391/ai.2023.18.1.103512>

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Abstract

The invasive benthic foraminifera *Trochammina hadai* has been found for the first time in Europe along the coast of Normandy. Its native range of distribution is in Asia (Japan and Korea), and it has also been introduced along the coasts of western North America, Brazil and Australia. Morphological and molecular assessments confirm that specimens found in Le Havre and Caen-Ouistreham harbors belong to the Asiatic type. Like in Asia, *T. hadai* was found in transitional waters with muddy sediments. It exhibited high relative abundances (up to about 40%) confirming that *T. hadai* is a highly competitive species. In the present study, it was nearly absent from natural transitional waters and very abundant in heavily modified habitats like harbors, suggesting that ballast waters may likely be the vector of introduction. It was not recorded farther north along the coast of the Hauts-de-France. It is further hypothesized that the finding of a few specimens outside the harbor may facilitate the expansion of *T. hadai* in the English Channel by means of propagules dispersion.

Key words: English Channel, harbor, non-indigenous species, ballast waters, benthic unicellular eukaryote, competitor

Introduction

Ocean shipping accounts for about 80% of international trade by volume (United Nations Conference on Trade and Development 2021, Review of maritime transport). It leads to intense exchanges between countries and continents worldwide. This makes ports one of the main gateways for the introduction of non-indigenous species (NIS) worldwide (Gouletquer et al. 2002; Occhipinti-Ambrogi et al. 2011; Zenetos et al. 2017; Mosbahi et al. 2021). Noticeably, about 44% of NIS are thought to have

been introduced in Europe by shipping (Nunes et al. 2014). When not carrying cargo or not enough cargo, ships typically fill their ballast tanks with seawater from the port of origin to ensure stability and maneuverability during a voyage. Eventually, ballast water will be discharged in the port of destination when ships pick up cargo. Ballast water and ballast sediment often contain organisms from the port of origin that will end up in the port of destination and eventually settle there (Drake et al. 2001; Gollasch et al. 2002; Gollasch 2006). This process is of tremendous proportion as the International Maritime Organization (2019, Ballast water management – the control of harmful invasive species) estimates that about 7,000 aquatic species are transported in ballast water every single day. Hull biofouling, *i.e.* organisms attached to ship surfaces, is another means of species transport between ports (Gollasch 2002; Drake and Lodge 2007; Georgiades et al. 2021). Quite a number of organisms may hence be introduced outside their natural range of distribution, where a few may survive and eventually flourish in the port of discharge, and ultimately colonize the surrounding habitats, where they may become invasive (Stiger-Pouvreau and Thouzeau 2015).

Along the coast of Normandy in the eastern English Channel, a total of 152 NIS have been recorded up to 2018 (see review in Pezy et al. 2021). In particular, Le Havre harbor has often been the first site where these species were observed, suggesting that it may be the main NIS entry pathway in Normandy (Breton 2014; Pezy et al. 2021; Dauvin et al. 2022). Ballast waters have been identified as the vector of introduction for most of the NIS found in Le Havre harbor (Pezy et al. 2021). Noticeably, it is connected to about 650 harbors worldwide through numerous shipping routes in all continents (Fig. 1, Haropa Port 2022, Rapport d'activité 2021), making it the 1st French harbor for international trade and the 4th in northern Europe by volume (Haropa Port 2022, Rapport d'activité 2021).

In the context of a survey of seven transitional waters in the eastern English Channel in Normandy (Bay of Veys, Orne estuary, Caen-Ouistreham and Le Havre

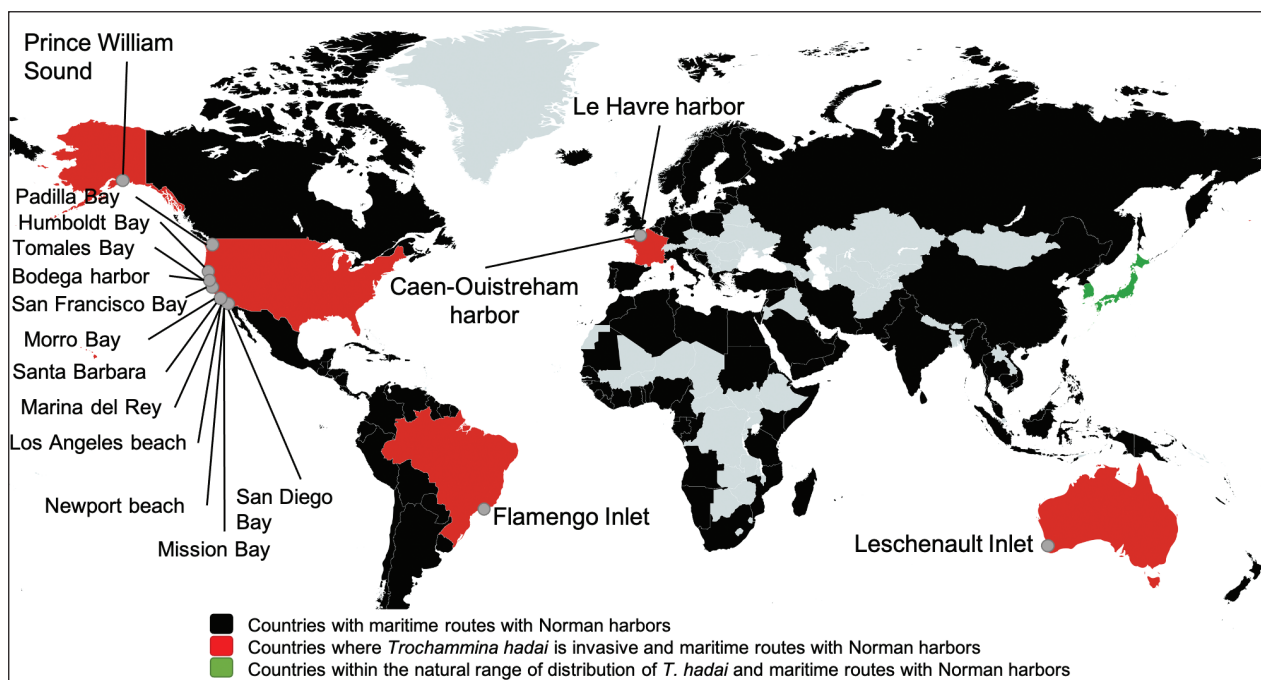


Figure 1. Map showing countries having commercial maritime routes with the Norman harbors of Le Havre harbor and Caen-Ouistreham in France (in black, red and green, sources: Le Havre harbor website: <https://www.haropaport.com/fr/havre/offre-maritime>, grey countries does not have maritime trade with Normandy). Red and green countries are known for the presence of *Trochammina hadai*, invasive or natural range of distribution, respectively. Places where *T. hadai* was introduced are also indicated (filled grey circles).

harbors) and in the Hauts-de-France (Authie estuary, Calais and Dunkirk harbors), living foraminiferal specimens resembling *Trochammina hadai* Uchio, 1962, were found for the first time in Europe in Normandy in Le Havre and in the Caen-Ouistreham harbors. To the best of our knowledge, the only *Trochammina* species observed in harbors and transitional waters of the eastern English Channel is the indigenous *T. inflata* (Montagu 1808) see Armynot du Châtelet et al. (2018a) for a review. In its native distribution range in Japan (Uchio 1962; Matsushita and Kitazato 1990) and South-Korea (Fig. 1, Lee et al. 2012; Lee et al. 2016), *T. hadai* flourishes in transitional environments like brackish waters-lakes, estuaries, harbors and sheltered bays. The species was first reported in 1995 as an invasive (*sensu* Blackburn et al. 2011) species along the American coast of the Pacific Ocean in San Francisco Bay (McGann 1995); subsequent work documented its first appearance in the bay in 1983 (McGann 2014). Later *T. hadai* was found to be present in estuaries and harbors along the western coast of the United States from the Mexico-USA border up to Prince William Sound in Alaska (Fig. 1, McGann et al. 2000; McGann et al. 2012). It has since been reported as an invasive species in Brazil (Fig. 1, Eichler et al. 2018) and in western Australia (Fig. 1, Tremblin et al. 2022). In all cases, ballast water and sediment are suspected as the main vector of introduction of *T. hadai* outside its natural Asiatic range of distribution (McGann et al. 2000; Eichler et al. 2018; Tremblin et al. 2022). The presence of a population of introduced *T. hadai* has also been recorded in the Gulf of Mexico (Moss et al. 2016).

In this context, the aim of the present study is to determine whether the living specimens of *Trochammina* found in Le Havre and the Caen-Ouistreham harbors belong to a non-indigenous species. This was achieved through the combination of thorough morphological and molecular taxonomical diagnoses. The possible invasive status of the species is discussed based on high relative abundances, as well as shipping as their putative introductory pathway in Europe.

Material and methods

Sampling procedure

Sampling stations were situated in eight transitional waters of the eastern English Channel along the French coasts (Fig. 2): four in Normandy in the Bay of Veys (BV: intertidal), the Orne estuary (O1: subtidal, O2: intertidal), the Caen-Ouistreham harbor (CO: subtidal) and Le Havre harbor (LHP: intertidal, recreational area, H1 to H5: subtidal, international shipping area) and three in the Hauts-de-France in the Authie estuary, Calais and Dunkirk harbors. In September 2019 in Normandy and September 2020 in the Hauts-de-France, one surface sediment sample was collected for grain size analysis and three replicates for total organic carbon and nitrogen. A Van Veen grab was used for the subtidal stations and a hand corer was used for the intertidal stations. For foraminiferal morphological analysis, three replicate cores were sampled at each station, with a Reineck corer for subtidal stations and a hand corer for intertidal ones (56 cm² in surface). *Trochammina hadai*-like specimens were only found in Normandy in Le Havre and Caen-Ouistreham harbors. Additional sediment samples were taken with a small Van Veen grab in May 2022 in Le Havre harbor for molecular investigations of *Trochammina hadai*-like specimens.

Sediment analysis

To assess sediment granulometry, laser diffraction particle-size analysis was carried out. Sediment grain size distribution has been subdivided in three fractions: clay



Figure 2. Sampling stations along the coasts in the eastern English Channel with a focus on Normandy where *Trochammina hadai* was found (black filled circle and unfilled triangle: presence and absence of *T. hadai*, respectively).

(<2 μm), silt (2 to 63 μm) and sand (63 to 2000 μm) for physical characterization (*i.e.*, energy of the environment). The three replicates of sediment samples for TOC and TN analysis were first frozen and then freeze-dried. They were preserved at $-20\text{ }^{\circ}\text{C}$ at the laboratory. Total organic carbon and nitrogen content was determined with an elemental analyzer (ThermoFisher Flash 2000, Laboratory of Oceanology and Geosciences in Wimereux-France) and expressed as the % of Corg and Norg per total weight of dry sediment. The C/N ratio was calculated at each station to determine the terrestrial or marine origin of the organic matter. The amount of inorganic carbon and nitrogen (measured in samples heated at $550\text{ }^{\circ}\text{C}$ for 5 hours) was subtracted.

Foraminiferal analysis

Samples for morphological identification were preserved in ethanol and Rose Bengal solution (2 g L^{-1}). In the laboratory, samples were sieved through a $63\text{ }\mu\text{m}$ -mesh and the fraction $>63\text{ }\mu\text{m}$ was dried at $50\text{ }^{\circ}\text{C}$ in an incubator. Foraminifera were then concentrated by flotation using trichloroethylene (density = 1.46). At least 300 living (stained) benthic foraminifera individuals were collected and identified for each sample. Behavioral observations of this species confirmed that living

specimens are present at sampling sites (unpublished data). Relative abundances of living *Trochammina hadai*-like specimens were then calculated. Sediment samples for molecular analysis were preserved in seawater at *in-situ* temperature (15 °C) and sieved at the laboratory on a 125 µm-mesh the day after sampling. Living specimens of *T. hadai* were placed on a microslide, dried at ambient temperature and sent to the University of Geneva, Switzerland. Specimen images were taken with a stereomicroscope using reflected light.

Morphological diagnosis of *Trochammina* spp.

Living *Trochammina* specimens were morphologically identified based on the original type descriptions (Table 1).

Table 1. Morphological characteristics of *Trochammina hadai* Uchio, 1962 and *T. inflata* (Montagu 1808).

<i>Trochammina hadai</i> Uchio, 1962
Description: Chambers inflated, somewhat subglobular, trochospiral with chambers usually gradually sometimes rapidly increasing in size as added. Dorsal side convex, umbilical area rather flat but deeply umbilicate in well preserved specimens, usually covered by fine particles. Consisting of 3 to 4 whorls, all visible from the dorsal side, only the last one from the ventral side. Sutures slightly curved dorsally, more depressed and nearly radial ventrally. Usually five occasionally four chambers in the last whorl. Finely arenaceous, wall of sand grains and a variable amount of cement, outer surfaces fairly even, color reddish brown to yellowish brown. Aperture on umbilical side, at the base of the apertural face of the last chamber forming an arched slit.
<i>Trochammina inflata</i> (Montagu, 1808)
Description: Inflated test, trochospiral with chambers increasing in size as added. Spiral side, all chambers visible, sutures depressed and radial to slightly curved. 5–6 chambers in the outer whorl, with a deep umbilicus. Agglutinated wall. Aperture on umbilical side, at the base of the final chamber forming a narrow lip.

DNA extraction, PCR amplification and sequencing

Five *Trochammina* specimens were extracted individually using guanidine lysis buffer (Pawlowski 2000). Semi-nested PCR amplification was carried out for the 18S barcoding fragment of foraminifera (Pawlowski and Holzmann 2014) using primers s14F3 (acgcamgtgtgaaacttg)-sB (tgatccttctgcaggttcacctac) for the first and primers s14F1 (aagggcaccacaagaacgc)-sB for the second amplification. Thirty-five and 25 cycles were performed for the first and the second PCR, with an annealing temperature of 50 °C and 52 °C, respectively. The amplified PCR products were purified using the High Pure PCR Cleanup Micro Kit (Roche Diagnostics). Sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and analyzed on a 3130XL Genetic Analyzer (Applied Biosystems). The resulting sequences were deposited in the NCBI/GenBank database. Isolate and Accession numbers are specified in Table 2.

Phylogenetic analysis

The obtained sequences were added to 44 sequences belonging to textulariids and Reophacidae that are part of the publicly available 18S database of foraminifera (NCBI/Nucleotide; <https://www.ncbi.nlm.nih.gov/nucleotide/>). All sequences were aligned using the default parameters of the Muscle automatic alignment option, as implemented in SeaView vs. 4.3.3. (Gouy et al. 2010). The alignment contains 49 sequences with 1192 sites used for analysis.

The phylogenetic tree was constructed using maximum likelihood phylogeny (PhyML 3.0) as implemented in ATGC: PhyML (Guindon et al. 2010). An automatic model selection by SMS (Lefort et al. 2017) based on Akaike Information

Table 2. Isolate, accession numbers and sampling localities of analysed foraminiferal species.

Species	Isolate	Accession number	Sampling locality
<i>Arenoparrella mexicana</i>	229	AJ307741	USA, Sapelo Island
<i>Balticammina pseudomacrescens</i>	32	MZ479306	Russia, White Sea, Chupa Inlet
<i>Balticammina pseudomacrescens</i>	35	MZ479307	Russia, White Sea, Chupa Inlet
<i>Bigenerina</i> sp.	31	AJ504688	Puerto Rico
<i>Cyrea</i> sp.	n.a.	X86095	France, Mediterranean Sea, St.Cyr
<i>Cyrea szymborska</i>	17247	LN886773	France, Mediterranean Sea, St. Claire
<i>Eggerelloides scaber</i>	ce1	MZ475350	Denmark, Faroe Islands
<i>Eggerelloides scaber</i>	12302	FR839728	Denmark, Aarhus
<i>Entzia macrescens</i>	418	HG425225	GBR, Dovey Estuary
<i>Entzia macrescens</i>	420	AJ307742	GBR, Dovey Estuary
<i>Entzia</i> sp.	505	MK121743	France, Camargue
<i>Haplophragmoides wilberti</i>	417	AJ312436	GBR, Dovey Estuary
<i>Liebusella goesi</i>	R3	FR754403	Norway, Oslo Fjord
<i>Liebusella goesi</i>	R6	FR754401	Norway, Oslo Fjord
<i>Reophax curtus</i>	9713	MK121734	Russia, White Sea, Chupa Inlet
<i>Reophax pilulifera</i>	8206	MF770994	Antarctica
<i>Reophax scorpiurus</i>	E17	AJ514850	Norway, Svalbard
<i>Reophax spiculifer</i>	3895	MF770993	Antarctica
<i>Siphoniferoides</i> sp.	655	AJ504690	Japan
<i>Spiroplectammina</i> sp.	cs1	MZ475343	Chile, Patagonia
<i>Spiroplectammina</i> sp.	2646	AJ504689	Norway, Svalbard
<i>Srinivasania sundarbanensis</i>	EC4	MN364400	India, Sundarbans
<i>Srinivasania sundarbanensis</i>	EC5	MN364401	India, Sundarbans
<i>Srinivasania sundarbanensis</i>	EC7	MN364402	India, Sundarbans
<i>Textularia agglutinans</i>	17015	LN879399	Israel, Eilat
<i>Textularia agglutinans</i>	17016	LN879402	Israel, Eilat
<i>Textularia gramen</i>	13633	LN848740	Denmark, Faroe Islands
<i>Textularia gramen</i>	13634	MF771001	Denmark, Faroe Islands
<i>Trochammina hadai</i>	95	AJ317979	Japan, Hamana Lake
<i>Trochammina hadai</i>	96	MF771005	Japan, Hamana Lake
<i>Trochammina hadai</i>	97	MF771008	Japan, Hamana Lake
<i>Trochammina hadai</i>	Troch1B3	MZ475344	USA, San Francisco
<i>Trochammina hadai</i>	Troch1B4	MZ475345	USA, San Francisco
<i>Trochammina hadai</i>	Troch1B9	MZ475346	USA, San Francisco
<i>Trochammina hadai</i>	21189	MZ707232	West Australia, Leschenault Inlet
<i>Trochammina hadai</i>	21190	MZ707233	West Australia, Leschenault Inlet
<i>Trochammina hadai</i>	21522	OP288014	France, Le Havre, harbour
<i>Trochammina hadai</i>	21523	OP288015	France, Le Havre, harbour
<i>Trochammina hadai</i>	21524	OP288016	France, Le Havre, harbour
<i>Trochammina hadai</i>	21525	OP288017	France, Le Havre, harbour
<i>Trochammina hadai</i>	21527	OP288018	France, Le Havre, harbour
<i>Trochammina inflata</i>	13847	MZ475341	Germany, Bottsand Lagune
<i>Trochammina inflata</i>	16337	MZ707242	Germany, Bottsand Lagune
<i>Trochammina inflata</i>	16343	MZ707245	Germany, Bottsand Lagune
<i>Trochammina pacifica</i>	Troch1B1	MF771002	USA, San Francisco
<i>Trochammina pacifica</i>	Troch1B2	MF771003	USA, San Francisco

Species	Isolate	Accession number	Sampling locality
<i>Trochammina pacifica</i>	Troch3B7	MF771004	USA, San Francisco
<i>Trochammina</i> sp.	1	MZ479320	Russia, White Sea, Chupa Inlet
<i>Trochammina</i> sp.	3	MZ479321	Russia, White Sea, Chupa Inlet

Criterion (AIC) was used, resulting in a GTR+R substitution model being selected for the analysis. The initial tree is based on BioNJ. Bootstrap values (BV's) are based on 100 replicates.

Results

Environmental parameters

Most stations were characterized by the dominance of silt, with the exception of stations BV3, BV4 and O1 which were composed of a balanced mix of sand and silt had more than 40% of sand (Table 3). Total organic carbon content was similar between stations, typically ranging between 0.8 and 1.64% (Table 3). Total nitrogen content did not change between stations and was relatively low, around 0.02–0.04% (Table 3).

Table 3. Environmental parameters of sampling stations (September 2019) along the coast of Normandy.

Site	Stations	Tidal	Clay (%)	Silt (%)	Sand (%)	TOC (%)	TN (%)
Bay of Veys	BV1	Intertidal	0.02	69.48	30.50	0.93	0.06
	BV2	Intertidal	0.01	77.93	22.06	1.84	0.14
	BV3	Intertidal	0.02	54.45	45.53	0.81	0.07
	BV4	Intertidal	0.02	48.80	51.18	1.43	0.10
Caen Ouistreham harbor	CO1	Subtidal	0.01	88.47	11.51	3.03	0.27
	CO2	Subtidal	0.02	80.45	19.53	1.95	0.16
	CO3	Subtidal	0.02	78.69	21.29	1.87	0.14
	CO4	Subtidal	0.00	71.80	28.20	3.83	0.37
Orne estuary	O1	Subtidal	0.02	59.78	40.20	1.57	0.13
	O2	Intertidal	0.02	67.06	32.92	1.36	0.11
Le Havre harbor	LHP	Intertidal	0.02	92.51	7.46	3.18	0.23
	H1	Subtidal	0.02	90.71	9.27	2.10	0.20
	H3	Subtidal	0.03	93.81	6.16	2.60	0.25
	H5	Subtidal	0.08	93.31	6.61	2.96	0.25

Morphological identification of *Trochammina inflata* and *T. hadai*

The indigenous *Trochammina inflata* exhibits inflated chambers, gradually increasing in size (see Table 1 for details). According to the type description (Montagu 1808), it has depressed sutures that are radial to slightly curved, a rounded periphery and a smoothly agglutinated wall surface.

The *Trochammina* specimens found in the Caen-Ouistreham and Le Havre harbors were distinct and characterized by a less lobulate periphery and shell is composed of big grains clearly visible under binocular (Fig. 3). In addition, chambers were subglobular, increasing in size during growth, and sutures were slightly curved dorsally, more depressed and nearly radial ventrally (Fig. 3). The morphological features strongly suggested that they belong to *T. hadai* (Table 1) and the assignment to the latter species was confirmed by molecular analysis.

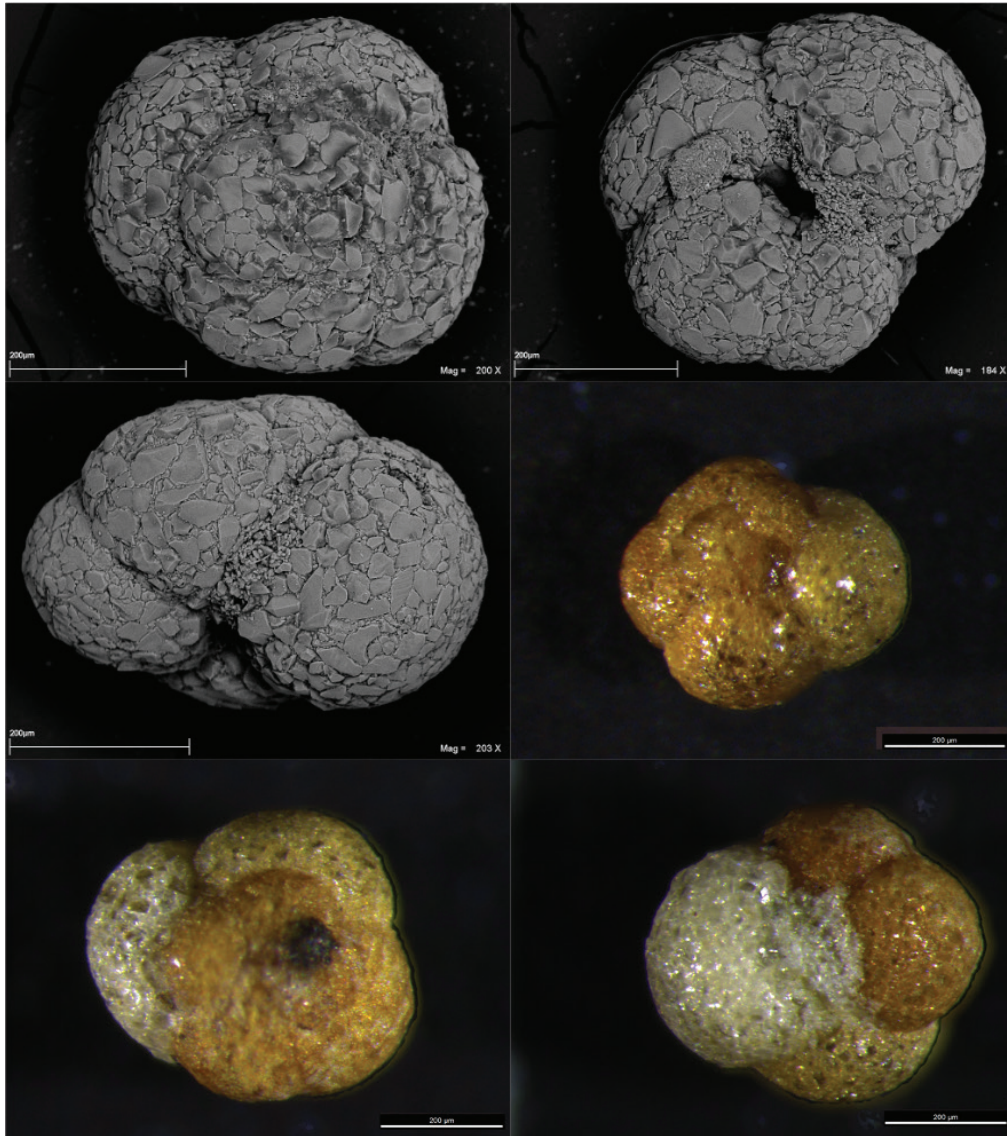


Figure 3. Pictures of living *Trochammina hadai* specimens sampled in Le Havre harbor in May 2022. Photographs by Jean-Charles Pavard and Maria Holzmann.

Phylogeny

The phylogenetic tree (Fig. 4) contains 49 sequences of agglutinated foraminifera and is rooted in Reophacidae (*R. scoriurus*, *R. spiculifer*, *R. curtus*, *R. pilulifera*). The obtained sequences cluster with *T. hadai*, supported by a bootstrap value (BV) of 100%. *Trochammina hadai* is part of a clade that contains *Srinivasania sundarbanensis*, *Eggerelloides scaber* and *Trochammina pacifica*. The clade is not supported by bootstrap value. Three other clades are present in the tree. The second clade, also without BV support, consists of *Textularia agglutinans*, *Siphoniferoides* sp., *Textularia gramen*, *Bigenerina* sp. and *Cyrea* spp. A third clade without BV support contains *Trochammina* sp. and *Spiroplectammina* sp. A fourth clade contains *Entzia macrescens*, *Entzia* sp., *Balticammina pseudomacrescens*, *Haplophragmoides wilberti* and *Arenoparrella mexicana* and is highly supported by BV (93%). *Liebusella goesi* and *Trochammina inflata* are branching separately. Species represented by more than one sequence are well supported by BV (78–100%).

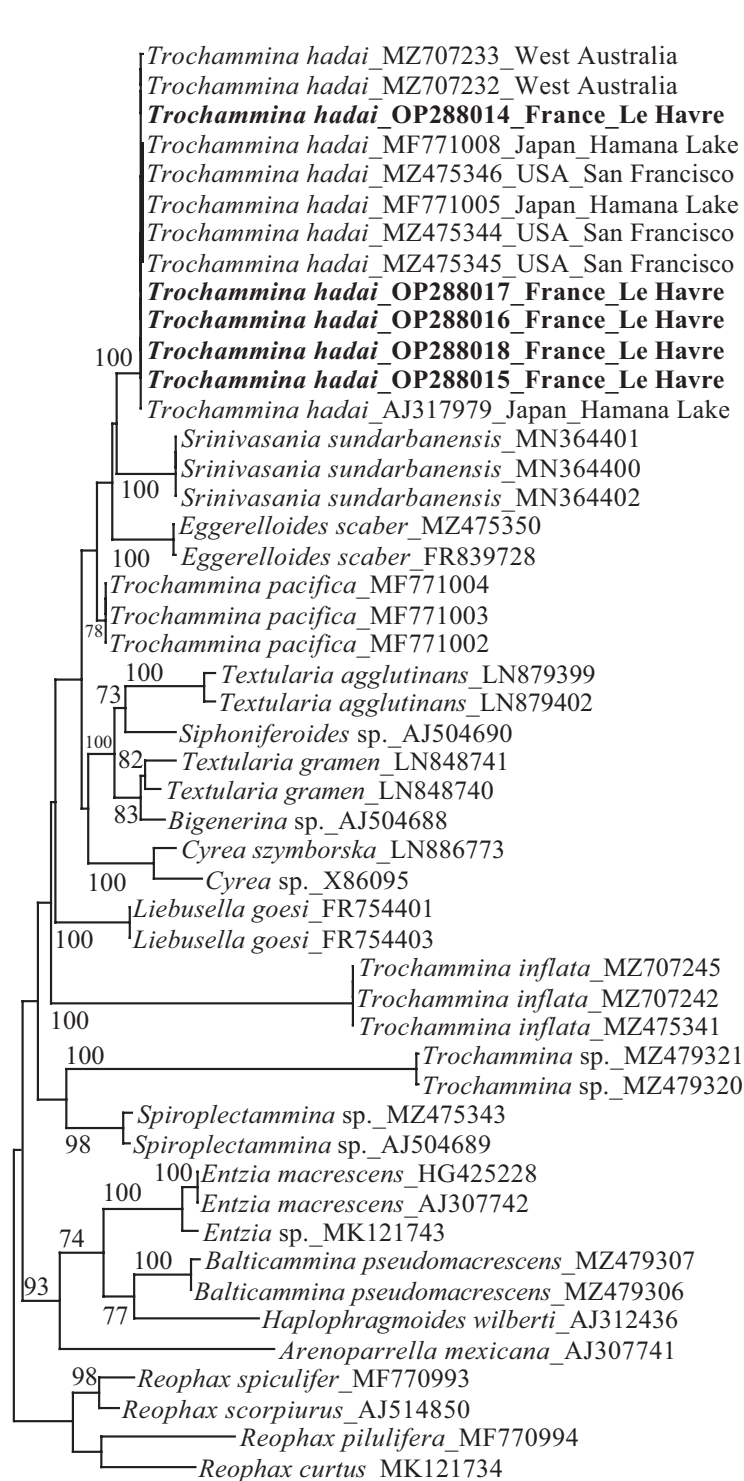


Figure 4. PhyML phylogenetic tree based on the 3' end fragment of the SSU rRNA gene, showing the evolutionary relationships of 49 agglutinated foraminiferal taxa. Specimens marked in bold indicate those for which sequences were acquired for the present study. The tree is rooted in Reophacidae (*R. scorpiurus*, *R. spiculifer*, *R. curtus*, *R. pilulifera*). Sequenced specimens are identified by their accession numbers. Numbers at nodes indicate bootstrap values (BV). Only BV larger than 70% are shown.

Relative abundances of living *Trochammina hadai* and *T. inflata* in Normandy

There were large differences in the relative abundances of *Trochammina hadai* along the coast of Normandy (Fig. 5). Subtidal stations in Le Havre and in the

Caen Ouistreham (CO3) harbors exhibited the highest relative abundances between 20 and 34% (48 ± 51 – 85 ± 19 ind. 50 cm^{-2} , mean \pm standard deviation). Conversely, *T. hadai* was barely found at the intertidal stations in the Orne estuary and in Le Havre harbor, and was completely absent in the Bay of Veys (Fig. 5). The indigenous *T. inflata* was only observed in the Bay of Veys at very low abundances (0.3–0.7%, 1 ± 1 ind. 50 cm^{-2}).

Trochammina hadai was not found along the coast of the Hauts-de-France (Authie estuary, Boulogne, Calais and Dunkirk harbors) while few *T. inflata* specimens were recorded in the Authie estuary.

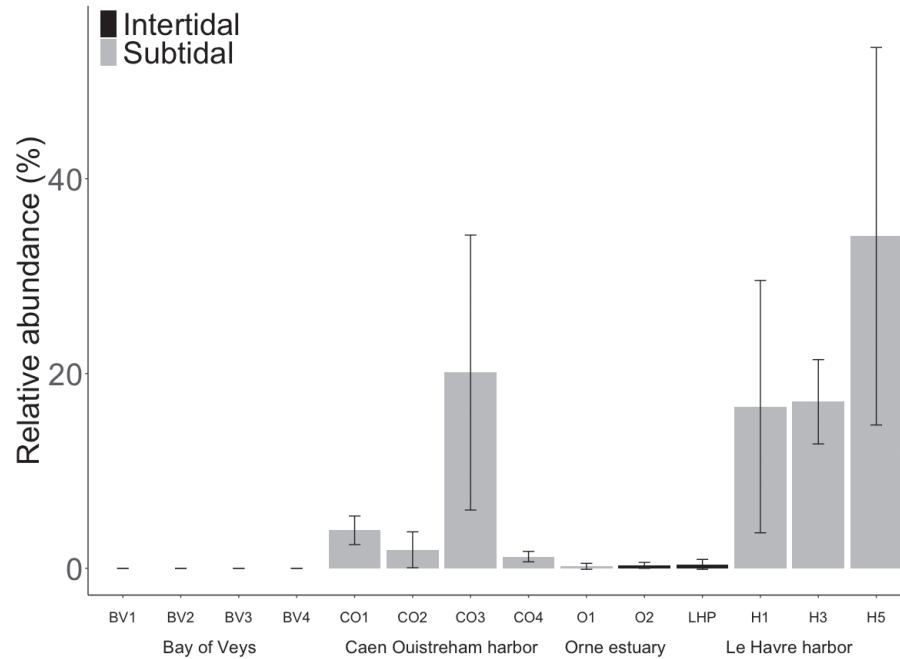


Figure 5. Mean relative abundances (error bars: standard deviation) of living *Trochammina hadai* at sampling stations in Normandy.

Discussion

Trochammina hadai: a new invasive species in Normandy

Until the present study, only three living benthic foraminiferal species from the Trochamminidae family were known to occur in the English Channel *i.e.* *Trochammina inflata*, *Lepidodeuterammmina ochracea* (Williamson 1858) and *L. eddystonensis* (Brönnimann and Whittaker 1990) (see review in Armynot du Châtelet et al. 2018b). Morphological and molecular assessments of the Trochamminidae found along the coast of Normandy in the harbors of Le Havre and Caen-Ouistreham showed that they belong to *T. hadai*. Conversely, this species is not yet present farther north in the Hauts-de-France region. This work therefore represents the first known record of this species in European waters.

The natural range of distribution of *Trochammina hadai* is in Asia, specifically in Japan and Korea (Matsushita and Kitazato 1990; Lee et al. 2012; Lee et al. 2016). It usually flourishes in polluted or naturally stressed environments (Toyoda and Kitazato 1995; Lee et al. 2012), which may be considered as an ecological advantage over native species where it is introduced. This hypothesis is consistent with the polluted water of Le Havre Harbor (Hamdoun et al. 2015), where *T. hadai* exhibited high relative abundances up to 40%. The dominance of this NIS species in Caen-Ouistreham and Le Havre harbors suggests that this species is a strong competitor which most likely led to a significant shift in the foraminiferal community

composition. Hence, *T. hadai* may be considered an invasive species in Normandy. The previous records of this species outside its native range of its distribution, in northwest America (McGann et al. 2000; McGann et al. 2012), Brazil (Eichler et al. 2018) and lately in Australia (Tremblin et al. 2022), consistently reported an invasive behavior. It is nevertheless stressed that *T. hadai* was only found in high abundances in heavily modified habitats in Normandy such as harbors. Only a few specimens were found outside harbors at the mouth of the Orne River. Though this may suggest an early stage of colonization or a limited potential for colonization outside highly polluted habitats, foraminiferal resting stages, *i.e.*, propagules are nevertheless easily transported by currents (Alve and Goldstein 2003, 2010). Although speculative, this mean of dispersion is a way by which the invasive foraminifera *T. hadai* could extend its distribution from Normandy to the whole eastern English Channel, as it did from its point source in San Francisco Bay (McGann and Sloan 1996) before colonizing the whole United States West Coast (McGann et al. 2000). Regular surveys are then suggested as an absolute prerequisite to assess the future possible expansion of *T. hadai* in Normandy and farther in the eastern English Channel, as previously successfully implemented for other invasive species, in particular the crabs *Hemigrapsus sanguineus* and *H. takanoi* (Gothland et al. 2013; Gothland et al. 2014).

Trochammina hadai: introduced via ballast waters?

Apart from species deliberately introduced for aquaculture purposes, vectors of NIS introductions are ballast waters and/or ballast sediments, ship hull fouling, accidental releases associated to shellfish activities and ichthyochory (Carlton 1992; Gollasch 2002, 2006; Guy-Haim et al. 2017). It is noticeably not rare to find benthic foraminifera in ballast waters (McGann et al. 2003), which have subsequently often been mentioned as their major mean of introduction (Bouchet et al. 2007; Calvo-Marcilese and Langer 2010; Deldicq et al. 2019; McGann et al. 2019), especially compared to shellfish activities (McGann et al. 2000; Bouchet et al. 2007).

In the present study, *Trochammina hadai* specimens were essentially found in harbors exhibiting intense international shipping. Noticeably, Le Havre harbor is connected with about 650 harbors in all continents (Haropa Port 2022, Rapport d'activité 2021), and most of the NIS recorded in this harbor were introduced by ballast waters (Pezy et al. 2021). In agreement with previous records of *T. hadai* outside its natural range of distribution (McGann et al. 2000; Eichler et al. 2018; Tremblin et al. 2022), ballast waters are likely to be responsible for its introduction to harbors in Normandy. This hypothesis is consistent with the distribution pattern of *T. hadai* which is only found in Le Havre harbor in the area dedicated to international shipping, in sharp contrast with the part of the harbor dedicated to recreational boats which seems to be free of *T. hadai*. The exact country of origin remains uncertain. As explained above, Le Havre and Caen-Ouistreham harbors have international connections with all continents, noticeably Asia, South America, North America and Australia. As a result, it may be hypothesized that it could be a primary introduction directly from its natural range of distribution in Asia, or it could be a secondary spread from one of the areas where it has already been introduced and now proliferate such as in the United States, Brazil, or Australia. The molecular analyses performed in this study do not allow to choose between these two hypotheses. Noticeably, phylogenetic analyses of the small subunit (SSU) ribosomal DNA (rDNA) of rDNA nucleotide sequences in populations of *T. hadai* (18S) exhibited a low molecular genetic differentiation between the different populations, like previously observed in *Virgulinema fragilis* (Tsuchiya et al. 2009).

In the future, conducting a retrospective study based on fossil foraminifera would be relevant to determine when *Trochammina hadai* appeared in Normandy, like it was done for other NIS foraminifera (McGann et al. 2012; Polovodova Asteman and Schönfeld 2015; Deldicq et al. 2019; Stulpinaite et al. 2020). Further works are also required to understand from where it was introduced. This may be done through an assessment of the presence and nature of the benthic foraminifera present in ballast waters and associated sediments from incoming ships. Finally, because the identification of this Asiatic invasive foraminifera in Le Havre harbor was fortuitous, it emphasizes the need to implement a survey plan in French harbors in order to thoroughly track and document the presence of NIS, particularly in the context of the European marine strategic framework directive. Given the intense maritime traffic occurring between Europe and Asia or North/South America, *T. hadai* may likely be already present or is soon to be in other French/European harbors.

Funding declaration

The Foram-INDIC project received financial support from the “Agence de l’Eau Artois Picardie” and the “Agence de l’Eau Seine Normandie” (Grants No. 58183 and No. 1082222)2019, respectively). The molecular work was supported by a Schmidheiny Foundation grant (MH).

Authors contribution

Research conceptualization: V.M.P. Bouchet. Sampling design and methodology: V.M.P. Bouchet, J.C. Pavard, J.-C. Dauvin and J.P. Pezy. Investigation and data collection: E. Armynot du Châtelet, M. McGann, M. Holzmann, A. Courleux and J.-C. Pavard. Data analyses and interpretation: V.M.P. Bouchet, J.-C. Pavard and M. Holzmann. Funding provision: V.M.P. Bouchet and M. Holzmann. Writing – original draft: V.M.P. Bouchet and M. Holzmann. Writing – review and editing: all co-authors. Figures and tables were produced by V.M.P. Bouchet and M. Holzmann.

Acknowledgments

Thanks to the crew of the Oceanographic Vessel Sepia II and Louis Lanoy for their help during sampling, Camille Hennion for the CTD data, Romain Abraham for grain size analyses and Gwendoline Duong for the organic matter data. The Cellule du Suivi du Littoral Normand and Pierre Balay are thanked for their help in sampling in May 2022. J.-C.P. PhD fellowship received funding supports from the Agence de l’Eau Artois-Picardie and the University of Lille. We are grateful to Dr Orit Hyams-Kazphan for providing suggestions on an earlier version of this manuscript. We are thankful to the Tammy Robinson (associate editor), Pr Martin Langer and one anonymous reviewer for their comments that helped to improve this paper. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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