Presence of Artemia franciscana (Branchiopoda, Anostraca) in France: morphological, genetic, and biometric evidence

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Research Article

Presence of *Artemia franciscana* (Branchiopoda, Anostraca) in France: morphological, genetic, and biometric evidence

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Abstract

New parthenogenetic and gonochoristic populations of *Artemia* were found along the French Atlantic and Mediterranean coasts. The taxonomic identity of these new populations was determined based upon: i) an analysis of the variation in the *caudal* gene, ii) morphology of the penis and frontal knob of male specimens using scanning electronic microscopy (SEM) and iii) a principal coordinate analysis of selected biometric traits. This analysis showed that all French gonochoristic populations of *Artemia* were comprised of the New World species *A. franciscana* (Kellogg, 1906) and not the Mediterranean native species, *A. salina.* As well, the parthenogenetic populations of *Artemia* in France are being rapidly replaced populations by the North America *A. franciscana*. This is a concern for all the European Atlantic and Mediterranean regions and is another example of a New World invasive species potentially decreasing European biodiversity.

Key words: brine shrimp; invasion; Atlantic coast; Mediterranean coast; SEM; *caudal* gene; biometry; replacement

Introduction

The genus *Artemia* (Leach, 1819) (Branchiopoda, Anostraca) is a cosmopolitan taxon having gonochoristic and parthenogenetic strains and is typically restricted to saline habitats such as saltmarshes, saltlands or lakes (Daday 1910; Persoone and Sorgeloos 1980; Vanhaecke et al. 1987; Triantaphyllidis et al. 1998). Because *Artemia* (brine shrimp) is one of the most important live feeds in aquaculture, the systematic of this genus has been relatively well studied in recent years (e.g., Mura 1990; Perez et al. 1994; Abatzopoulos et al. 2002; Gajardo et al. 2004; Mura and Brecciaroli 2004; Mura et al. 2005; Munoz et al. 2008, 2010), except for the taxonomic status of all the parthenogenetic populations. Since the first observations of *Artemia* near Marseille and in the Hérault department during the 1800s (Audouin 1836; Joly 1840), the all populations in France were considered to be parthenogenetic *Artemia* (Bowen and Sterling, 1978) (Simon 1886; Labbé 1925; Artom 1931; Mathias 1932, 1937) based on the absence of male specimens. In the last two centuries, parthenogenetic populations have been reported in three regions of France: i) along the Mediterranean coast; ii) along the Atlantic coast; and iii) in the inland saltmarshes of the Eastern region of Lorraine (Appendix 1; Godron 1863; Briquel 1881; Simon 1886; Daday 1910; Vanhaecke et al. 1987). Preliminary results seems to indicate that the parthenogenetic trait appeared several times in different *Artemia* lineages and that the taxonomic identity of the previous *A. parthenogenetica* species does not exist, although a larger study should be conducted, in the future, including more parthenogenetic populations of the Old World (Baxevanis et al. 2006). Recently, some populations from the Atlantic (Mesquer; Guérande) and Mediterranean French coasts (Sète; Villeneuve; Joly 1840; Labbé 1925; Artom 1931; Mathias 1932) have been re-classified as gonochoristic due to frequent observations of males (Maillard and Baudet 1980; Thiéry et al. 1990, 1992). The authors suggested that the gonochoristic and French Mediterranean populations were *Artemia salina* (Linnaeus, 1758) or *A. tunisiana* (Bowen and Sterling, 1978), two

synonymous names for the native gonochoristic species from the Mediterranean Basin (Triantaphyllidis et al. 1997; Munoz et al. 2008). The possible introduction of *A. franciscana* (Kellogg, 1906), a North American gonochoristic species exported commercially around the world, into France was considered but not verified by several authors (Maillard and Baudet 1980; Defaye et al. 1998).

Some recent studies confirm the presence of *A. franciscana* along the Western Mediterranean coast (Amat et al. 2005, 2007). One of these coastal sites (Aigues Mortes) currently has a mixed population of the invasive *A. franciscana* and the native diploid parthenogenetic strains, while reports less than fifteen years earlier described this population as obligately parthenogenetic (Vanhaecke et al. 1987; Thiéry et al. 1990). Recent studies indicate that the New World *A. franciscana* species is a threat to *Artemia* biodiversity worldwide (e.g., Iran, Iraq, Morocco, Spain, Italy, Portugal; Amat et al. 2005; Mura et al. 2006; Abatzopoulos et al. 2006; Mohammed et al. 2010) because it is replacing the native *Artemia* species (Amat et al. 2007). This new information about the spread of *A. franciscana* makes the assignment of the gonochoristic French populations to *A. salina* questionable. To address the issue of the species identity of gonochoristic populations of *Artemia* in France, this study used a combined analysis of variation in the *caudal* gene (Copf et al. 2003), several morphological traits (form of frontal knob, spine on the penis); and selected metrics (e.g., total length of male specimen, width of head, distance between eyes, width of first antenna).

Material and methods

Sampling

Samples were collected from twelve locations in Eastern, Western and Southern to determine the *Artemia* species present. Samples of *Artemia* were collected from seven localities of saltlands along the Atlantic coast (Mesquer; Guérande; Noirmoutier; St Hilaire de Riez; Ile d´Olonne; Talmont St Hilaire; Ile d´Oléron), four locations along the Mediterranean coast (Gruissan; La Palme; Aigues-Mortes; Giraud), and one historical location in Eastern France (Luneville) between summer 1997 and autumn 2001 (Table 1, Figure 1, Appendix 1 and 2). Adult male *Artemia* specimens to be used for the morphological study were fixed in 4% formalin, while those used for genetic analysis were preserved in absolute ethanol. For each locality, the proportion of males was determined from samples consisting of several hundred adults (Table 1). Archived samples in the Muséum National d´Histoire Naturel (MNHN responsible D. Defaye) were examined to determine the species identity in samples collected in Luneville and Guérande sites during the 1800s.

Molecular systematics and phylogenetical analyses

To identify the different *Artemia* strains or species present in the French populations, a genetic study using the *caudal* gene was performed for each sample available (Appendix 2). Genomic DNA was extracted from either, at least five, adult specimens or on several hundred of dehydrated eggs, depending to the population. The samples preserved in alcohol or dehydrated eggs were rehydrated, washed, and placed in an extraction buffer (TE: Tris 0.1M, pH 9, EDTA 0.1M, SDS 1%) with 1/10 of potassium acetate (8M) and refrigerated for 30 min after mixing. The solution was then centrifuged for 90 minutes at 13 000 rpm/min. The pellet was cleaned with 70% ethanol, dried and re-suspended in 400 ml of TE + RNase (Tris 10 mM, EDTA 1 mM, RNase 50 mg/ml). We then added SDS (sodium dodecyl sulfate) and proteinase K to a final concentration of 0.5% and 100 mg/ml, respectively. After digestion for 3 hours at 50°C, a phenol/phenol-chloroform/chloroform extraction was performed. The DNA was again precipitated, washed with ethanol and taken up to a final volume of 20 ml.

Polymerase Chain Reactions (PCR) were carried out on the DNA extracts using the following primers *Artemia*-AS (5'-TAACTCT AGAAGAAAACATAAACAGTTTATT-3') and *Artemia*-S (5'-TAACGGATCCAAGAAACGAG AAGAAGTGACA-3') (Copf et al. 2003). PCRs were performed in a volume of 20 ml with 50-100 ng of DNA, 200 mM deoxynucleotides and 0.5 mM of each primer. The PCR programs were as follows: 5 minutes at 94°C, 40 cycles (1 minute at 94°C, 1 minute at 55°C, 1 minute at 72°C), and 10 minutes at 72°C as final elongation. The products of PCRs were purified and cloned in a vector « T-overhang » and ten clones for each population were sequenced by using the «Thermosequenase sequencing kit (Amersham)» for a length of fragments of the 3'

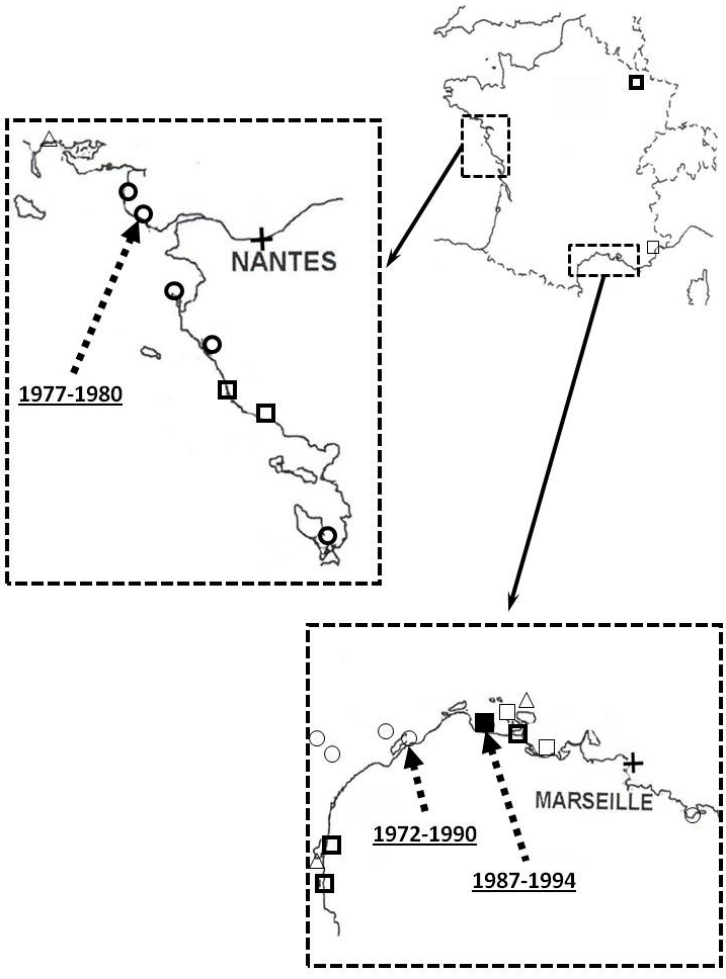

Figure 1. Distribution of *Artemia* populations in France. The symbols without bold correspond to the localities of the *Artemia* populations present in the literature (Appendix 1) while the bold symbols correspond to the localities of our study listed in Table 1. The empty triangles indicate *Artemia* observation (unknown species). The empty squares indicate the parthenogenetic populations while the empty circles indicate the populations of *A. franciscana*. The full squares indicate the presence of one mixed population of *A. franciscana* and parthenogenetic *Artemia* strains. The dates indicate the period of introduction of the French localities by the commercial *Artemia franciscana*.

region of *caudal* gene varying from 511 to 516 pb (Appendix 3 and 4). The sequences were aligned manually and a phylogenetical analysis was carried out by the Neighbour-joining (NJ) with correction of Kimura 2-parameters and by the Maximum of Parsimony (MP) method using the software MEGA version 2.1 (Kumar et al. 2001). In addition to the nine French *Artemia* populations (all the studied populations, except Luneville in the Eastern, Talmont St Hilaire in the Western and Gruissan in the Southern parts of France), three reference samples of *Artemia* species were used in this study. Two Mediterranean populations (from Tunisia and Egypt) were used as reference for the native gonochoristic *A. salina* species (Persoone and Sorgeloos 1980; Vanhaecke et al. 1987). These populations were provided by Gilbert Van Stappen from the "Artemia Reference Center" (ARC) of the Ghent University (Belgium) and correspond to the collection numbers n° 1290, 1492 and 1493. Adult specimens from the population of Ile d´Olonne were considered as reference for the native parthenogenetic *Artemia* strain because no males were found in our sample of 762 individuals. Finally, adult specimens of the commercial stock ("GSL Artemia") from Great Salt Lake, Utah, USA, were used as the reference for *A. franciscana* (Persoone and Sorgeloos 1980; Vanhaecke et al. 1987). A total of thirteen accessions (for a total of 74 adult individuals and several hundreds of eggs) were investigated in the genetic study (Appendix 2).

Table 1. Results of the morphological, biometrical and genetic studies for studied *Artemia* populations. Determination of species of each population was based on the combination of morphological, biometric, and genetic analyses together. New localities found during this study are represented in bold, while reference populations used for the morphological, biometric, and genetic studies are in italics. Bp= collection number of MNHN; Nind.= number of individuals studied.

Localities	Countries	Collectors or collections	GPS coordinates Lat/Long	Date of sample	% sex-ratio ($N_{ind.}$)	Determination of *Artemia* species
Luneville	France	MNHN: Bp 89 / 91 / 93-101	48°34´34"N / 06°30´04"E	1879	0.0% (246)	parthenogenetic strain
Mesquer (St Molf)	France	Rabet N.	47°24´30"N / 02°24´05"W	16.07.1997	48.5% (33)	*A. franciscana*
		Rabet N.	47°24´30"N / 02°24´05"W	02.08.2001	48.8% (215)	*A. franciscana*
Guérande	France	MNHN: Bp 108 / 110	47°18´03"N / 02°26´43"W	bef. 1886	0% (7)	parthenogenetic strain
	France	MNHN: Bp 621	47°18´03"N / 02°26´43"W	1911	0% (7)	parthenogenetic strain
	France	Rabet N.	47°18´03"N / 02°26´43"W	16.07.1997	47.5% (1266)	*A. franciscana*
	France	Rabet N.	47°18´03"N / 02°26´43"W	10.08.2000	38.1% (42)	*A. franciscana*
	France	Rabet N.	47°18´03"N / 02°26´43"W	03.08.2001	63.4% (235)	*A. franciscana*
Noirmoutier	France	Rabet N.	46°59´17"N / 02°16´44"W	12.08.2000	50.7% (75)	*A. franciscana*
St Hilaire de Riez	France	Oger A.	46°43´12"N / 01°56´15"W	20.09.2001	56.7% (97)	*A. franciscana*
Ile d´Olonne	France	Talon C. and Eveno Y.	46°33´36"N / 01°47´24"W	04.09.2001	0.0% (762)	parthenogenetic strain
Talmont St Hilaire	France	Levet P.	46°26´40"N / 01°38´12"W	10.09.2001	0.0% (1004)	parthenogenetic strain
Ile d´Oléron	France	Degryse P.	45°51´46"N / 01°13´44"W	23.09.2001	65.3% (75)	*A. franciscana*
Gruissan	France	Rabet N.	43°05´54"N / 03°05´22"E	05.08.2001	0.0% (26)	parthenogenetic strain
La Palme	France	Cart J.F.	42°58´36"N / 03°01´17"E	01.04.1998	0.0% (19)	parthenogenetic strain
	France	Cart J.F.	42°58´36"N / 03°01´17"E	01.06.2000	0.0% (215)	parthenogenetic strain
Aigues Mortes	France	Rabet N.	43°32´51"N / 04°09´40"E	01.04.1994	33.1% (1029)	*A. franciscana* & parthenogenetic strains
	France	Compagnie du Midi	43°32´51"N / 04°09´40"E	02.10.2000	8.7% (1065)	*A. franciscana* & parthenogenetic strains
	France	Compagnie du Midi	43°32´51"N / 04°09´40"E	01.10.2001	58.0% (1115)	*A. franciscana* & parthenogenetic strain
Giraud	France	Compagnie du Midi	43°22´48"N / 04°43´32"E	01.10.2001	30.9% (730)	*A. franciscana* & parthenogenetic strains
Wadi Natron	Egypt	ARC: 1290	30°26´14"N / 30°15´01"W	1994	reference	*A. salina*
Sfax	Tunisia	ARC: 1492	34°42´33"N / 10°44´37"W	1997	reference	*A. salina*
	Tunisia	ARC: 1493	34°42´33"N / 10°44´37"W	2000	reference	*A. salina*
Sebkha Ez-Zemoul	Algeria	Amarouayache M.	35°52´58"N / 06°33´41"W	2008	reference	*A. salina*
Utah	USA	Commercial stock	41°08´22"N / 112°49´33"W	2001	reference	*A. franciscana*
Lake Abert	USA	Rogers C.	42°38´40"N / 120°10´55"W	15.05.1990	reference	*A. franciscana*
Cabo Frio	Brasil	Rabet N.	22°56´59"S / 42°02´42"W	26.06.1993	reference	*A. franciscana*

Morphological systematics and biometry

To confirm the results of the molecular investigation, morphological and biometrical observations were conducted on adult male specimens with binocular and scanning electronic microscopes. Morphological observations were made on two male specimens per gonochoristic population using a Scanning Electron Microscope (SEM; model Jeol Scanning Microscope 6100). Two characters were retained for the morphological analysis: the shape of the frontal knob (Mura et al. 1989a, 1989b; Mura 1990; Mura and Brecciaroli 2004) and the presence or absence of spine at the base of penis (Mura and Brecciaroli 2004). Thirty-two adult males coming from sixteen accessions were investigated in the morphological study (Appendix 2).

Four biometric traits measured on twelve adult male specimens of each gonochoristic French population except Noirmoutier where only eight specimens were available. The biometric measures were: total body length (TL) measured as the distance from a point between the base of the eyes of the individual and the end of the tail; head width (WH) measured as width at the base of the first antennas; distance between eyes (SE) measured as distance between the two eyes; and width of antenna (WA), which corresponds to the maximal width found along the first antenna. A Principal Coordinate Analysis (PCA) based on the four biometric traits was performed using the statistical package SPSS version 14.0 (SPSS Inc., Chicago, Illinois, USA) to discriminate *A. franciscana* from the other European *Artemia* strains (parthenogenetic strains and *A. salina*), as previously done by Amat et al. (2005).

Morphological and biometric traits were measured on adult male specimens from the same population sampling, four French Atlantic and two Mediterranean populations at the exception of the population of Mesquer, which was not included in the PCA analysis of the biometrical study. Similar reference populations were used for both studies: one Algerian population (Sebkha Ez-Zemoul) representing the gonochoristic autochthonous *A. salina* species (Amarouayache et al. 2012) and three New World populations (Great Salt Lake and Albert Lake in USA and Cabo Frio in Brazil; Persoone and Sorgeloos 1980; Vanhaecke et al. 1987) corresponding to the gonochoristic allochthonous *A. franciscana* species. In total, eighty-nine adult males coming from ten populations were examined in the biometrical study (Appendix 2).

Results and discussion

Evolution of the sex-ratio along the French coasts

Within our twenty different collections from French *Artemia* shore sites, six localities had previously been determined to be inhabited by a parthenogenetic strain (Luneville; Mesquer; Guérande; Aigues-Mortes; La Palme; Giraud; Triantaphyllidis et al. 1998) while six had never been examined: five on the Atlantic coast (Noirmoutier; St Hilaire de Riez; Ile d'Olonne; Talmont St Hilaire; Ile d'Oléron) and one on the Mediterranean coast (Gruissan) (Table 1, Appendix 1 and 2). Within the new French *Artemia* sites, males were present in three populations (Noirmoutier; St Hilaire de Riez; Ile d'Oléron) and absent from the remaining three (Ile d'Olonne; Talmont St Hilaire; Gruissan; Table 1). The archived samples from the Muséum National d'Histoire Naturelle coming from Luneville (year: 1879) and Guérande (year: before 1886 and 1911) also lacked males (Table 1). Males were observed at four sites (Mesquer; Guérande; Aigues-Mortes; Giraud) that previously (two decades ago) only had populations of parthenogenetic strains (Maillard and Baudet 1980; Simon 1886; Vanhaecke et al. 1987; Table 1). The sex-ratio determination of the twenty French collections is based on the count of 8263 adult *Artemia* specimens, in total.

The caudal gene as molecular marker in Artemia systematic

Within our samples from nine French *Artemia* sites and four *Artemia* reference populations, nine different genetic sequences were discovered and grouped in three clusters corresponding to the three reference populations of *Artemia* (Table 1, Appendix 2, 3, 4 and 5). In contrast to earlier reports (Maillard and Baudet 1980; Thiéry et al. 1990, 1992), we did not detect any gonochoristic Mediterranean species *A. salina* in France, i.e. no "As" allele specific to this species were found in our French samples. Instead, five "Ap" alleles (1Ap to 5Ap) specific to the parthenogenetic strains were found in four different French sites (Ile d´Olonne; La Palme; Aigues Mortes; Giraud)

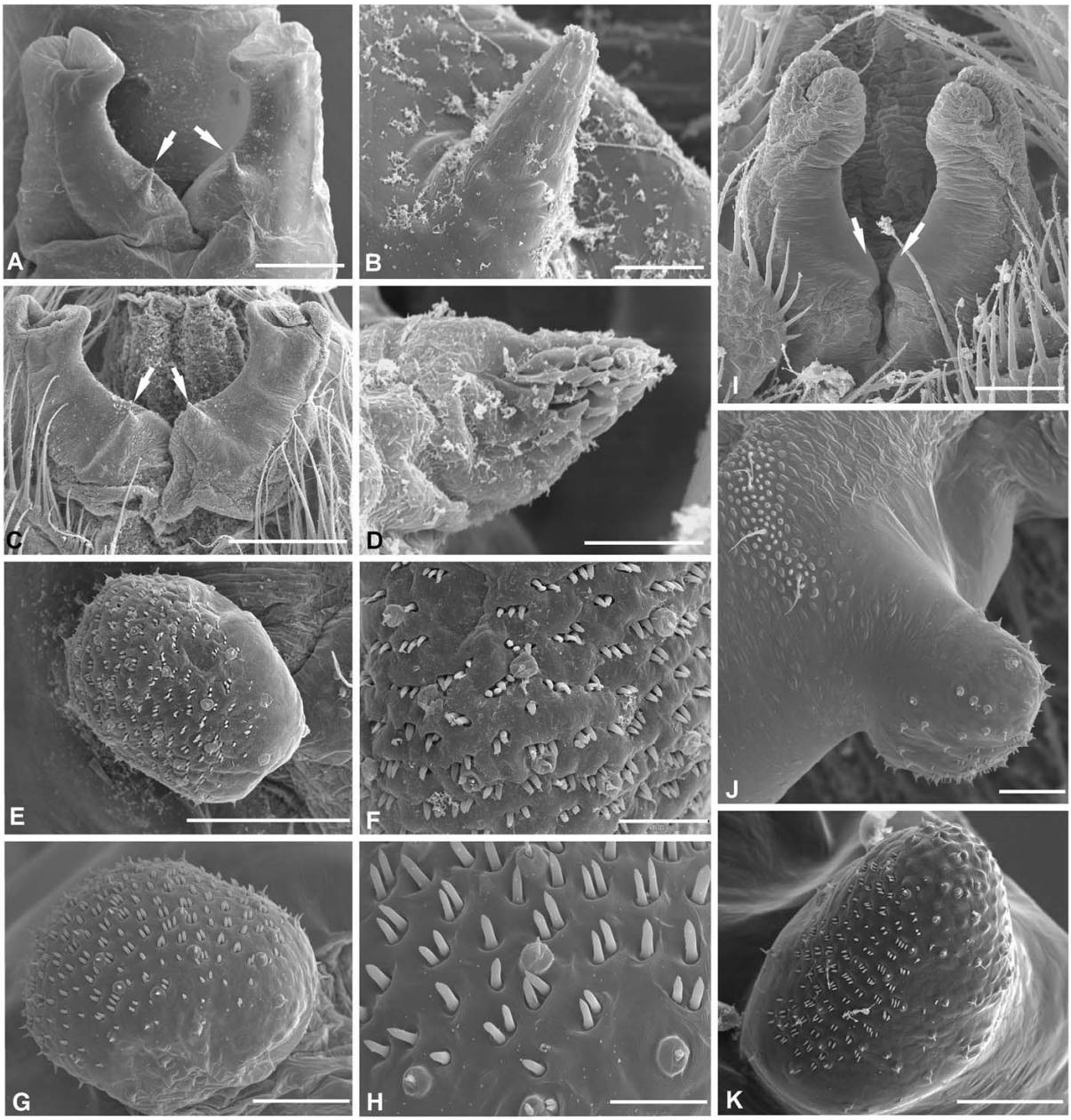

Figure 2. SEM micrographs of penis and frontal knobs: The morphological study focuses on the penis morphology (Figure 2A, C, I) and on the shape of the frontal knob (Figure 2E, F, G, H, J, K). SEM micrographs present penis (Figure 2A, C), spine on penis (Figure 2B, D) and the frontal knobs (Figure 2E, G) with numerous setae on its surface (Figure 2F, H) from French *Artemia franciscana* male specimens. These specimens in the panels came from: Aigues Mortes (A, B); Ile d´Oléron (C, D); Salins de Giraud (E, F); Guérande (G, H). Scare bar of 200 µm for A and C; 100 µm for E and F; 50 µm for G and H; 20 µm for B; 10 µm for D. SEM micrographs present penis (Figure 2I) and frontal knobs (Figure 2J) with numerous setae on its surface (Figure 2K) from Algerian *Artemia salina* male specimens. Scare bar of 200 µm for I; 100 µm for J; 50 µm for K. Photographs by Scalone and Rabet.

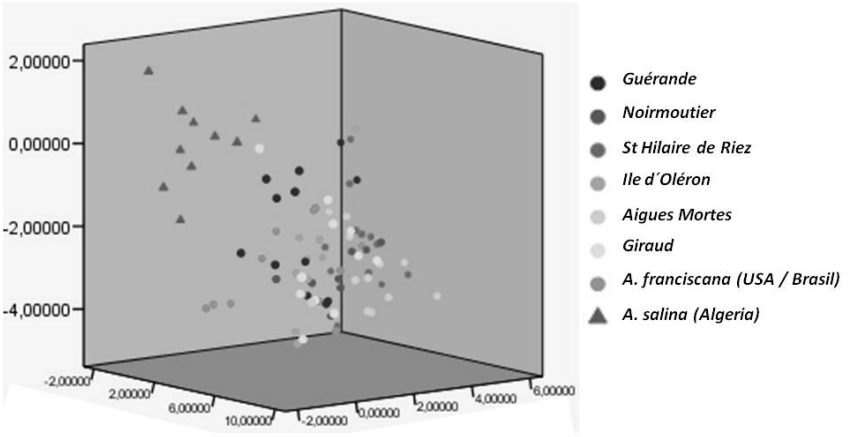

Figure 3. Principle Coordinate Analysis (PCA) representation of male specimens from gonochoristic French populations together with the reference populations of *A. salina* and *A. franciscana*. The triangles represent the male specimens identified belonging to *A. salina* and the circles to *A. franciscana*.

and two "Af" alleles (2Af; 3Af) specific to the New World species *A. franciscana* were present in seven French sites (Mesquer; Guérande; Noirmoutier; St Hilaire de Riez; Ile d´Oléron; Aigues Mortes; Giraud) (Table 1, Appendix 2, 3, 4 and 5). It is important to mention that two sites present a large diversity of different "Ap" alleles (n = 4) (Aigues Mortes; Giraud) while two other are monoallelic (2Ap in Ile d´Olonne; 5Ap in La Palme), although the DNA extraction of each site was done with at least five adults specimens. One "Af" allele was only observed in the commercial stock (1Af) while one (3Af) was present only in the French introduced populations (Mesquer; Aigues Mortes; Giraud) and not in the New World samples. The more frequent allele (2Af) in our sampling was the one specific of the New World species, and it was present in four different introduced populations all along the Atlantic French coast and in the commercial stock from its native area. However, more populations from the native area should be investigated genetically to determine the origin of these "Af" alleles. Moreover, this genetic analysis shows that the sequences of *caudal* gene are sufficiently divergent to differentiate three *Artemia* species but probably, because of their close localization near the coding region, not enough variability for a large multi-population study as previously done with mitochondrial cytochrome C oxidase subunit 1 or cytochrome b (cox1 and cytB; Perez et al. 1994; Hou et al. 2006; Munoz et al. 2008, 2010), nuclear ITS1 region (Baxevanis et al. 2006; Hou et al. 2006) or with DNA-fingerprinting of type micro-satellite (Munoz et al. 2009), RFLP (Gajardo et al. 2004; Mura et al. 2006) or RAPD (Abatzopoulos et al. 2002; Camargo et al. 2002).

Morphology and biometry of the Artemia males in France

All males from French populations observed by SEM had two spines of variable aspect (Figure 2B and 2D) at the base of penis (Figure 2A and 2C). Recent studies showed that all species of *Artemia* have these spines on the penis except for *A. salina*. The absence of spines in *A. salina* was confirmed in the reference specimens from the Algerian population (Figure 2I; Mura and Brecciaroli 2004).

The general shape of the frontal knob for all males from French populations was sub-spherical (Figure 2E and 2G) with numerous clusters of setae at the surface (Figure 2F and 2H). Earlier studies state that a sub-conical form of the frontal knob is specific to *A. salina* (Figure 2J and 2K) while a sub-spherical form is specific to *A. franciscana* (Mura et al. 1989a, 1989b; Mura 1990).

Our PCA showed a clear segregation between the Algerian *A. salina* specimens and the other *Artemia* specimens coming from France, USA or Brazil (Figure 3, Appendix 6). This clear separation when added to the morphological results excludes the presence of *A. salina* species from the French sites where male specimens were collected and confirms the presence of *A. franciscana* within seven sites along the French Atlantic and Mediterranean coasts.

The presence of New World Artemia in Atlantic and Mediterranean French coasts

The genetic, morphological, and biometrical results were consistent and supported the use of a multidisciplinary approach in investigations of the taxonomic status of *Artemia* populations as recommended by Mura et al. in 2005. Our results confirmed those reported by Amat et al. (2005) about the presence of *A. franciscana* and the parthenogenetic strains in Aigues Mortes saltworks. The date of our earlier collection in Aigues Mortes (1994) moves back the year of introduction of the New World species. Vanhaecke et al. (1987) did not detect male specimens in this locality in 1987 and Amat et al. revealed the presence of this exotic invasive species since 2002 (Amat et al. 2005); however, our results suggest that *A. franciscana* was introduced to this site sometime between 1987 and 1994. The potential year of introduction is even shorter (between 1977 and 1980) for the localities of Guérande and Mesquer on the Atlantic French coast (Fontaine 1977; Maillard and Baudet 1980; Appendix 1). The determination of these short and precise periods when the introductions probably occurred can facilitate future investigations on the origins of the introduction of *A. franciscana* and can help us to understand the rapid success of the invasion of this species around the world. Finally, the actual absence of parthenogenetic strains within the Guérande saltworks confirms the loss of parthenogenetic *Artemia* diversity in France coupled with the invasion of the New World species (Amat et al. 2007). These parthenogenetic strains already disappeared or are on the way to extinct for the other French infested sites before the investigations of their relationships within France and within the Old World.

The results of Amat et al. (2007) are consistent with our results obtained for La Palme during 1998 and 2000 but partially inconsistent with our results for the Giraud site. Amat et al. (2007) did not find any specimens of *A. franciscana* in Giraud during 2007 in contrast with our results showing that male specimens of the invasive species were present since 2001. This apparent contradiction may be due to: i) different areas within the large saltwork site of Giraud being sampled in the two studies; ii) the samples were collected at different times in the *Artemia* seasonal cycle (May/June for Amat et al. 2007 and October for our study); and iii) the entire area had yet to be fully colonized by the New World species in 2007. New studies focusing on the possibility of temporal coexistence of the French parthenogenetic strains with the New World species within one single site are in process for the locality of Aigues Mortes. These new studies will give us more details about the process of the extinction of the French strains by competition and permit elaboration strategies for conservation of the French parthenogenetic strains in the future.

Conclusions

To conclude, the recent reviews of French *Artemia* populations observed during the 1800s and located along the Atlantic and Mediterranean coasts showed that: i) two out the four populations (Guérande; Mesquer) that were suspected to be the native Mediterranean, gonochoristic *A. salina* species were in fact the New World species, *A. franciscana* and ii) a majority of French sites are now inhabited by this non-native species. In Aigues Mortes, one mixed population is present and could be an intermediary phase leading to the extinction of the native parthenogenetic strains. Indeed, the extinction of these genetically diverse parthenogenetic strains when confronted by the New World species has been demonstrated experimentally in laboratory (Browne 1988; Browne and Halanych 1989). Different resistances to cestode parasitism have been also revealed between them (Georgiev et al. 2007) and could explain partially the extinction of the parthenogenetic strains. The invasion of *A. franciscana* is a serious danger for the French *Artemia* diversity in the Mediterranean as well as in the Atlantic hypersaline environments and need rapid and strong measures to protect the native and parthenogenetic *Artemia* populations.

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Supplementary material

The following supplementary material is available for this article.

Appendix 1. List of French *Artemia* localities.

Appendix 2. Intermediary results of the morphological, biometrical and genetic studies.

Appendix 3. Alignment of *Artemia* sequences from the 3' region of the *caudal* gene.

Appendix 4. List of the accession numbers for the Artemia 3' regions of c*audal* gene.

Appendix 5. Branch constructed from the *Artemia* 3' regions of c*audal* gene.

Appendix 6. Biometric measures of male specimens used for the Principal Coordinate Analysis.

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