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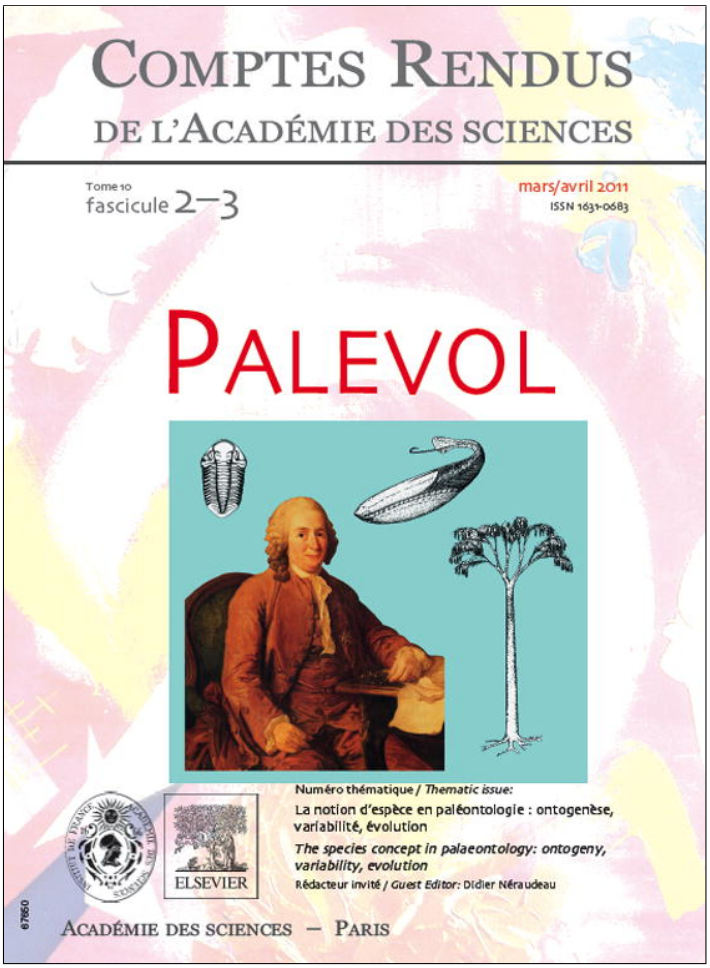

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Contributions of molecular phylogenetics to foraminiferal taxonomy: General overview and example of *Pseudoeponides falsobeccarii* Rouvillois, 1974

Contributions de la phylogénie moléculaire à la taxonomie des foraminifères : aperçu général et cas pratique avec Pseudoeponides falsobeccarii *Rouvillois, 1974*

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ABSTRACT

Molecular phylogenetics gives new insights into the taxonomy of foraminifera, independent of their morphology. After a survey of the present knowledge on how molecular phylogeny can contribute to foraminiferal taxonomy, we present an applied example. The comparison of ribosomal DNA (rDNA) sequences belonging to the SSU (Small Subunit) and LSU (Large Subunit) genes of *Pseudoeponides falsobeccarii* with other similar sequences of rotaliids available in GenBank shows that this species actually belongs to the genus *Ammonia*, because it groups inside the other *Ammonia* sequences instead of forming a distinct clade. Moreover, *Ammonia falsobeccarii* forms a clade well separated from other *Ammonia* phylotypes, meaning that it can be considered as a distinct species, and not as an ecophenotype of one of the other *Ammonia* species.

RÉSUMÉ

Grâce à la phylogénie moléculaire, il est possible d'aborder la taxonomie des foraminifères sous un nouvel angle, indépendant de la morphologie. Après un aperçu général des apports de la phylogénie moléculaire à la taxonomie des foraminifères, nous présentons un cas pratique. Des séquences appartenant aux gènes de la petite (18S) et de la grande sous-unités (28S) de l'ADN ribosomique (ADNr) de *Pseudoeponides falsobeccarii* ont été comparées à des séquences semblables de rotaliides disponibles dans GenBank. Leur analyse phylogénétique démontre que *P. falsobeccarii* appartient au genre *Ammonia*, parce que les séquences d'ADN de cette espèce se retrouvent à l'intérieur du clade formé par toutes les séquences d'*Ammonia*. À l'intérieur de ce clade, *Ammonia falsobeccarii* forme un groupe bien distinct des autres phylotypes décrits pour *Ammonia*, ce qui signifie que ce taxon peut être considéré comme une espèce séparée, plutôt qu'un écophénotype d'une autre espèce d'*Ammonia*.

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1. Introduction

1.1. Particulars of foraminifera from a phylogenetic point of view

The first foraminiferal DNA sequences were published in the middle of the 1990s (Pawlowski, 2000, and references therein). Compared to other Eukaryotes, there was and still is some delay in the development of foraminiferal phylogenetics. This is due to the difficulty in culturing-foraminifera in axenic conditions as well as the peculiarity of their genome.

It is rather easy to maintain foraminifera alive under controlled conditions, but it is much more difficult to actually culture them and make them reproduce (Bowser et al., 2006). The absence of foraminifera from "strain catalogues" means that acquiring foraminiferal DNA is not yet a routine task, and that a better knowledge of how to sample them is still needed.

Another difficulty with foraminifera is that the genes whose sequences are known differ from the ones of other Eukaryotes. For example, the ribosomal genes (Small (SSU) and Large (LSU) Subunits of ribosomal DNA (rDNA)) of foraminifera are much longer than those of other Eukaryotes due to typical insertions (Pawlowski, 2000). A few other foraminiferal genes have been successfully amplified such as actin, RNA polymerase II largest subunit, ubiquitin and tubulin (see Bowser et al., 2006 for references). However, despite many attempts, it was not yet possible to amplify mitochondrial DNA, which is widely used for barcoding in many other organisms (Pawlowski and Lecroq, 2010). The fact that foraminiferal DNA is very divergent compared to other Eukaryotes also means that this group does not appear in DNA environmental surveys where biodiversity is targeted with universal eukaryotic primers (Berney et al., 2004).

1.2. Comparison between phylogenetic studies of planktic and benthic foraminifera

Phylogenetic studies on planktic foraminifera showed that genetic variation was usually much higher than the described morphological variation in this group (e.g. Kucera and Darling, 2002, and references therein). Cryptic (species separated by genetics but undistinguishable morphologically) or pseudocryptic (morphological distinction possible after re-examination) species are regularly described in planktic foraminifera (e.g. Aurahs and Grimm, 2009; de Vargas et al., 1999; Huber et al., 1997).

Among benthic foraminifera, allogromiids (foraminifera with an organic test) also present a high level of genetic diversity compared to a rather low level of morphological diversity (Habura et al., 2008; Pawlowski et al., 2002). In the group of rotaliids (foraminifera with a calcitic hyaline bilamellar perforate test), *Ammonia* Brünnich, 1772 was the first genus studied at a large scale. At a first view, this cosmopolitan genus showed a similar tendency, with genetic diversity being much higher than morphological diversity (Hayward et al., 2004; Holzmann, 2000). However, when looking in more detail, it appears that *Ammonia* morphotypes may have been subject to extreme lumping; only one to three species are routinely used by most foraminiferologists, although over 40 species have been described on the basis of small morphological differences (Hayward et al., 2004). In this context, the discovery of 13 different phylotypes in a worldwide study of *Ammonia* (Pawlowski and Holzmann, 2008) was not really a surprise. Further studies on rotaliids confirmed that there is a fairly good correspondence between phylotypes and morphospecies (e.g. Schweizer et al., 2005, 2009; Tsuchiya et al., 2000, 2008).

In many other taxonomic groups it is possible to fix a threshold in the divergence between sequences beyond which they will be considered as belonging to different species (see Hayward et al., 2004 for references). In foraminifera, the evolutionary rates are so heterogeneous that it is not possible to define any general threshold (Pawlowski and Lecroq, 2010). However, there are other methods to investigate species boundaries. Some variable regions of the SSU rDNA such as 37/f are rather accurate to discriminate the different morphospecies of foraminifera (Pawlowski and Lecroq, 2010). Moreover, a general observation of the phylogenetic tree will usually show phyloclades well separated by branches longer than the branches inside them. In the case of rotaliids, these phyloclades correspond to known morphospecies most of the time.

1.3. Contributions of phylogenetic studies for enhancing the taxonomy of foraminifera

1.3.1. Taxonomy and classification of higher levels

For the time being the classification and taxonomy of foraminifera at suprageneric levels are still exclusively based on the wall structure and the morphology of the test (Sen Gupta, 2002), without taking into account biological aspects such as ecology (except for the group of planktic foraminifera), life cycle or DNA. However, phylogenetic studies were able to shed new light and/or to confirm some aspects of the classification of foraminifera previously based on the characteristics of the test.

Some authors have considered the composition and ultrastructure of the test as subsidiary features compared to the overall form of the test and the chamber arrangement (e.g. Hofker, 1956; Mikhalevich and Debenay, 2001), but for the majority of recent authors, the wall composition remains the most important criterion to separate the different foraminiferal orders (Haynes, 1981; Loeblich and Tappan, 1987; Sen Gupta, 2002). However, phylogenetic studies have shown that the absence of shell was sometimes due to a secondary loss instead of being a primary state (Pawlowski et al., 1999) and that allogromiids and textulariids (foraminifera with an agglutinated test) formed paraphyletic clades (Bowser et al., 2006; Pawlowski, 2000; Pawlowski et al., 2003). A very interesting example, showing that the chamber arrangement could be a more important criterion than wall composition, is *Miliammina fusca* (Brady, 1870). This foraminifer has an agglutinated test, but with a chamber arrangement similar to the one found in most miliolids (foraminifera with a porcellaneous, non perforate test). Traditionally, *M. fusca is* placed in the order Textulariida on the basis of the wall composition, which is agglutinated (Loeblich

and Tappan, 1987). However, DNA analyses clearly show that this species groups with porcelaneous foraminifera and therefore belongs to the order Miliolida (Fahrni et al., 1997; Habura et al., 2006).

Foraminifera with a calcitic hyaline bilamellar perforate test (rotaliids *sensu lato*, Bowser et al., 2006) have been separated in three different orders in some classifications based on the test morphology (e.g. Haynes, 1981; Loeblich and Tappan, 1992; Sen Gupta, 2002). The order Globigerinida includes all planktic foraminifera, whereas benthic rotaliids are separated into Rotaliida and Buliminida on the basis of the coiling of the test (low/high), the toothplate (absence/presence) and the shape of the aperture (arched/loop-shaped). Phylogenetic studies based on the SSU rDNA showed that rotaliids *sensu lato* form three main phyloclades mixing Buliminida and Rotaliida (Schweizer et al., 2008) and that planktic foraminifera are polyphyletic (Darling et al., 1997, 2009; Ujiié et al., 2008), originating from different phyloclades of benthic rotaliids (Bowser et al., 2006).

Surprisingly, the wall structure, which was considered a main criterion to distinguish superfamilies in rotaliids in a former classification (Loeblich and Tappan, 1964), was still used to separate closely related genera such as *Cibicides* de Montfort, 1808 and *Cibicidoides* Thalmann, 1939 in different superfamilies in a later classification (Loeblich and Tappan, 1987), although this criterion had already been dismissed some years before (Deutsch Conger et al., 1977; Towe and Cifelli, 1967). Phylogenetic studies confirmed that the wall structure was not an important criterion because genera such as *Cibicides* and *Cibicidoides* are closely related genetically (Schweizer et al., 2009). Moreover, the effort to separate plano- and biconvex cibicidids into *Cibicides* and *Cibicidoides*, respectively, appears to be incorrect, since the shape of the test varies in function of the form of the substrate to which the foraminifer is fixed (Schweizer et al., 2009). The coiling mode has also been considered as a major morphological criterion to distinguish superfamilies and families (Haynes, 1981; Loeblich and Tappan, 1987). However, phylogenetic analyses (Schweizer et al., 2008, 2009) have shown that some genera with very different coiling modes grouped together in phyloclades (e.g. *Melonis* de Montfort, 1808 (planospiral) with *Cibicides*, *Cibicidoides* and *Hanzawaia* Asano, 1944 (trochospiral); *Ammonia* (trochospiral) with *Elphidium* de Montfort, 1808 and *Haynesina* Banner and Culver, 1978 (planospiral)).

Conversely, other morphological criteria, which were previously considered less important for taxonomy, gained new weight after re-examination of the morphology in the light of phylogenetic results. The coiling direction in planktic foraminifera, which was usually thought to be influenced by ecological factors, could have a genetic origin (Darling et al., 2006), and also the size and number of pores seem, at least in some benthic and planktic foraminifera, to be determined genetically rather than influenced by environmental factors (Holzmann and Pawlowski, 1997; Huber et al., 1997; Morard et al., 2009; Schweizer et al., 2009).

1.3.2. Choice of generic names

Many generic names have been described for rotaliids (about 640 retained by Loeblich and Tappan, 1987). For the time being, intrageneric phylogenetic variability has only been studied in some detail for a handful of rotaliid genera: *Ammonia* (Hayward et al., 2004), *Bulimina* d'Orbigny, 1826 (Tsuchiya et al., 2008), *Cibicides*/*Cibicidoides* (Schweizer et al., 2009), *Glabratella* Dorreen, 1948 and related genera (Tsuchiya et al., 2000), *Nummulites* Lamarck, 1801 and related genera (Holzmann et al., 2003), *Uvigerina* d'Orbigny, 1826 and related genera (Schweizer et al., 2005).

Among the three genera of uvigerinids studied by Schweizer et al. (2005), *Trifarina* Cushman, 1923 is traditionally distinguished from *Uvigerina* by having a triangular cross section instead of a round one, whereas *Rectuvigerina* Mathews, 1945 has a uniserial arrangement of the final chambers compared to a triserial one throughout for *Uvigerina*. However, the species *Trifarina earlandi* (Parr, 1950) and *Rectuvigerina phlegeri* Le Calvez, 1959 form a clade with *Uvigerina peregrina* Cushman, 1923, which is the sister-group (the closest clade) of the clade formed by *Uvigerina mediterranea* Hofker, 1932 and *U. elongatastriata* (Colom, 1952) in partial SSU rDNA analyses (Schweizer et al., 2005). Therefore, *Trifarina* and *Rectuvigerina* should rather be included in the genus *Uvigerina,* demonstrating that generic distinctions based on the section and the seriality of the test are probably not relevant in that case.

For cibicidids, the phylogenetic results were less clear, although six morphospecies have been sequenced for the SSU rDNA gene. In a first study with partial SSU sequences, the cibicidids were forming a clade and it was decided to call them all *Cibicides,* which is the senior generic name, although there were two clearly separated subgroups in the clade (Schweizer, 2006). However, in a later study with more complete SSU sequences (Schweizer et al., 2009), some doubt was shed on this monophyly by the position of *Melonis*, which was separating the two cibicidid subgroups in some analyses. More sequences of *Melonis* and *Cibicides*/*Cibicidoides* are needed to elucidate this problem. For the time being, Schweizer et al. (2009) decided to give different names to the two subgroups of cibicidids (*Cibicides* and *Cibicidoides*) in order to avoid future problems if the clade turns out to be polyphyletic with *Melonis* positioned in the middle.

1.3.3. Endemism and cosmopolitanism in rotaliids

In benthic foraminifera, very similar morphotypes are often determined using different species names in different areas. In such cases, an important question is whether taxonomical differences between such morphologically close specimens from different regions reflect true endemism or are just the consequence of a poorly constrained taxonomy and regional traditions.

Phylogenetic analyses show that in some cases endemism may be substantial. For instance, *Uvigerina akitaensis* Asano, 1950, a species described from Japan, has been considered by Scott et al. (2000) as a synonym of the cosmopolitan species *U. peregrina*. Although further investigations are needed with more variable parts of the DNA, the SSU sequences of *U. akitaensis* (Ertan et al., 2004) seem slightly different from comparable ones of *U. peregrina* (Schweizer, 2006). Moreover, many shallow water cibicidid species, such as *Cibicides refulgens* de Montfort, 1808 or *Cibicidoides dispars* (d'Orbigny, 1839) are often con-

sidered synonymous with *Cibicidoides lobatulus* (Walker and Jacob, 1798) (e.g. Hageman, 1979; Heron-Allen and Earland, 1932). However, these species are well separated genetically from *C. lobatulus*, and even the morphospecies *C. lobatulus* itself could be a mosaic of different species (Schweizer et al., 2009, Schweizer et al., submitted). On the other hand, *Virgulinella fragilis* Grindell and Collen, 1976 and some deep-sea species such as *Cibicidoides wuellerstorfi* (Schwager, 1866) and *Epistominella exigua* (Weltner, 1895) have genetically very homogeneous populations throughout large geographical spaces, making them cosmopolitan species (Pawlowski et al., 2007; Tsuchiya et al., 2009).

1.3.4. Ecophenotypic versus genetic variations in rotaliids

When DNA is extracted from single specimens for which the morphology is known (either by taking SEM pictures prior to the destruction of the test during DNA extraction or by using extraction buffers which can preserve the test (e.g. Morard et al., 2009)), it is possible to directly compare morphological and genetic variations. In the case of ecophenotypy, morphological variation will be higher than genetic variability and influenced by the environment, whereas genetic variation will be higher than morphological for cryptic species.

We have seen already that genetic variation is apparently high in comparison with the rather restrictive commonly used taxonomic concepts in *Ammonia* and *C. lobatulus*. In these cases, only a limited number of morphospecies (*Ammonia*) or just one (*C. lobatulus*) are routinely used, whereas extensive morphological variability is observed within them. For *Ammonia*, a re-examination of the material following genetic analyses allowed us to distinguish a larger morphological variation correlated with genetic variation (Hayward et al., 2004). A morphological re-examination of the material for *C. lobatulus* connected with a phylogenetic study could also help to determine the limits of morphological intraspecific variation, but it has yet to be done. The *Elphidium excavatum* (Terquem, 1876) plexus is another example where the traditionally distinguished subspecies or ecophenotypes (e.g. Feyling-Hanssen, 1972; Goubert, 1997; Miller et al., 1982) correspond to different phylotypes, and are the results of genetic variation instead of environmental influence (Schweizer et al., 2010).

At present, there are very few well-documented examples of ecophenotypy in foraminifera confirmed by DNA analyses. A population of *U. peregrina* from the Oslo Fjord showed some morphological variation although very variable DNA regions (Internal Transcribed Spacer (ITS) rDNA) were similar (Schweizer et al., 2005). *Uv. peregrina* sampled in the Bay of Biscay are morphologically distinct from the Oslo Fjord populations, but have identical SSU sequences (unpublished data). Further studies with more variable regions of DNA (e.g. ITS) are needed to check if these populations are indeed identical genetically. Another example of possible ecophenotypy is provided by *Virgulinella fragilis*, which tests vary in the size and density of pores among genetically similar populations (Tsuchiya et al., 2009).

It is evident that genetic analyses can unearth new evidence in longstanding taxonomic problems. One of these problems, which has intrigued us for a long time, is the status of the genus *Pseudoeponides* Uchio, 1950, and of the species *Pseudoeponides falsobeccarii* Rouvillois, 1974 in particular. In this paper, we will use this species as an example to show how phylogenetic studies can contribute to the solution of complex taxonomic questions.

1.4. The enigmatic case of P. falsobeccarii

The genus *Pseudoeponides* was established by Uchio (1950), with *Pseudoeponides japonica* (Uchio, 1950) as the type species. According to the original description, the genus is a basic trochospiral form resembling *Eponides*, and is characterised by secondary openings on both sides. The original description mentions elongate slits parallel to the sutures on the dorsal side and loop-shaped secondary openings, positioned along each suture radiating from the umbilicus on the ventral side.

This generic description has been emended by Hofker (1958) who demonstrated that Uchio wrongly described and figured the secondary openings on the dorsal side. In fact, these openings are not areal (parallel to the sutures), as figured by Uchio (figure reproduced on Pl. 667, Fig. 12 in Loeblich and Tappan, 1987), but are entirely sutural, positioned at the junctions of spiral and chamber sutures (Fig. 1). In the earlier part of the test, the openings are rather circular; they become more or less triserial toward later ontogenetic stages. Hofker (1958) also mentioned that the dorsal supplementary openings are surrounded by a poreless area. He presented very precise drawings of the ventral secondary openings, which are positioned at the external side of triangular chamber extensions pointing into the umbilical area. Hofker remarked that these umbilical features were similar to those found in the genus *Streblus* (now *Ammonia*). He showed that the only difference between *Pseudoeponides* and *Ammonia* was the position of the toothplate, which extends to the chamber wall, and is connected to the dorsal secondary openings in *Pseudoeponides*, but does not reach the chamber wall in *Ammonia*. In all other aspects, *Pseudoeponides* is similar to *Ammonia*. Therefore, Hofker concluded that *Pseudoeponides* is a *Streblus* (now *Ammonia*) in which the chambers are slightly turned towards the ventral side, so that the toothplate reaches the dorsal wall, the poreless area becomes very prominent and a secondary opening is formed (in fact, this turning of the chambers towards the ventral side causes the spiral to be higher in *Pseudoeponides* than in *Ammonia*). For Hofker (1958), there is not enough difference to justify a separate genus, and the species *Pseudoeponides japonica* should be included in the genus *Streblus*. Unfortunately, the very judicious emendation of Hofker (1958) has been largely overlooked, and the description and figures of the genus *Pseudoeponides* in Loeblich and Tappan (1964, 1987) faithfully reproduce the error of Uchio.

P. falsobeccarii is a common species in continental shelf environments in the Mediterranean and north-eastern Atlantic. It was first described by Rouvillois (1974), who indicated that this species had previously often been determined erroneously as *Ammonia beccarii* (Linnaeus, 1758), which is morphologically similar, but lacks the dorsal sec-

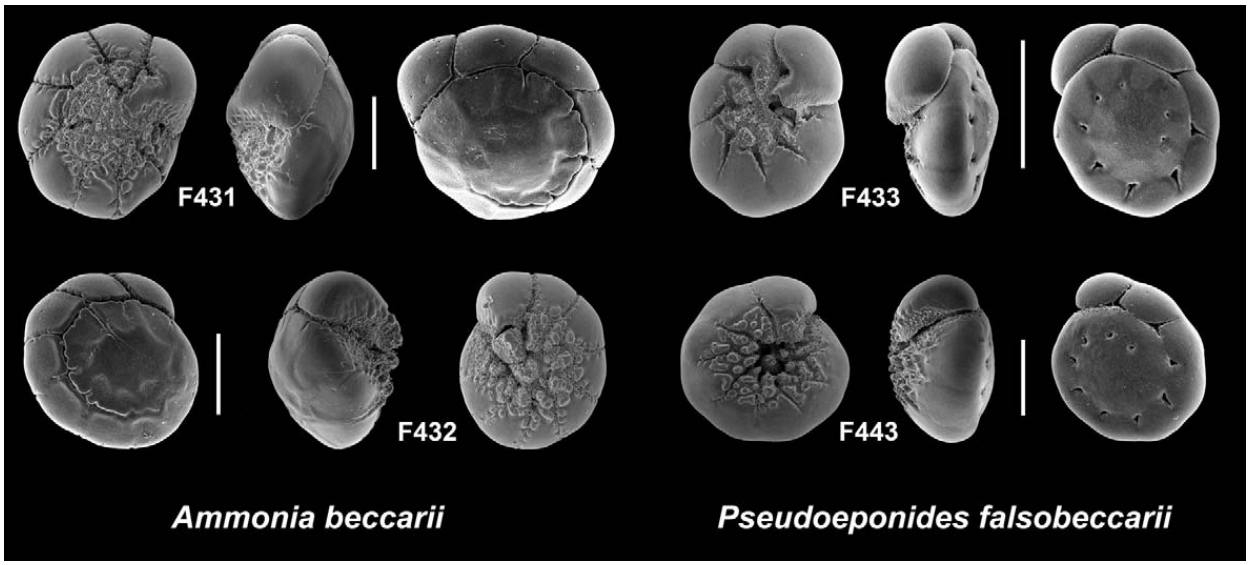

Fig. 1. SEM pictures of specimens identified as *Ammonia beccarii* (F431, F432) and *Pseudoeponides falsobeccarii* (F433, F443). F431, F432, F433 come from the Rhône prodelta and F443 from the Bay of Biscay. Scale bar: 200 μm.
Fig. 1. Photos au MEB de spécimens identifiés comme *Ammonia beccarii* (F431, F432) et *Pseudoeponides falsobeccarii* (F433, F443). F431, F432, F433 proviennent du prodelta du Rhône et F443 du Golfe de Gascogne. Échelle : 200 μm.

ondary openings (Fig. 1). Unfortunately, confusion between both taxa has continued until today. For example, *P. falsobeccarii* has been incorrectly determined as *A. beccarii* or *A. batava* (Hofker, 1951) by Haake (1977) (Pl. 1, Figs 1-2), Vénec-Peyré, 1983 (ecophenotype 4: Pl. 1, Fig. 6; Pl. 2, Fig. 5; Pl. 5, Fig. 4) and Hayward et al. (2004, Pl. 4, T3S). This list is far from exhaustive, many other examples exist.

P. falsobeccarii has been described in recent sediments (thanatocoenoses) from the Adriatic Sea (Jorissen, 1988), where it accounts for up to 10% of the faunas, at water depths between 20 and 60 m. It is mainly found in the centre of the coast-parallel mud belt, which slightly deepens from 10 to 30 m depth in front of the Po delta to 40 to 60 m depth off Pescara. In live faunas from a sediment core from 35 m depth off Ancona, Pucci et al. (2009) found about 20 specimens per 50 cm^2, corresponding to about 8% of the total fauna. Mojtahid et al. (2009) found abundant live populations of *P. falsobeccarii* between 60 and 90 m depth in the Rhône prodelta. In the Bay of Biscay, Rouvillois (1974) described *P. falsobeccarii* at water depths between 60 and 130 m. This larger water depth in the Bay of Biscay has recently been confirmed by Fontanier et al. (2002), Duchemin et al. (2005) and Langezaal et al. (2006), who described numerous live (Rose Bengal stained) specimens in the Bay of Biscay, on silty clays between 100 and 140 m depth. In the five previously cited studies on live specimens, *P. falsobeccarii* shows a clear infaunal tendency; an abundance maximum is systematically observed at a depth of several centimeters, in anoxic sediments. Pucci et al. (2009) incubated several multicores sampled at a 35 m deep site in the Adriatic Sea in oxic as well as strongly dysoxic conditions, for various periods of time, to a maximum of 69 days. In both experimental setups, *P. falsobeccarii* specimens migrated to the uppermost sediment level. In comparison with the other dominant taxa, standing stocks of *P. falsobeccarii* decreased rapidly over time, suggesting that this taxon resisted badly to the experimental conditions (without added food), in oxic as well as strongly dysoxic conditions. Summarising, it appears that *P. falsobeccarii* has a clear preference for organically enriched fine-grained sediments, such as those present in front of major deltaic systems. It appears well adapted to support the phenomena of oxygen deficiency associated with such systems, as shown by its presence in deeper, anoxic, sediment layers.

Jorissen (1988) suggested that *P. falsobeccarii* could be an ecophenotype of the *Ammonia parkinsonia* (d'Orbigny, 1839) group (which includes also *A. tepida* Cushman, 1926, but not *A. beccarii*, which is characterised by an abundant ornamentation, and deeply incised sutures on the dorsal side). He hypothesised that this morphospecies could have adapted to the stressed conditions of the enriched clay belt environments by a more intensive use of the canal system, leading to the formation of supplementary apertures (outlets of the channel system) on the dorsal side.

The question we want to address in this article is twofold:

- is the genus *Pseudoeponides* sufficiently different from *Ammonia* from a genetic point of view to justify a separated genus?
- is *P. falsobeccarii* an ecophenotype of one of the *Ammonia* species, or is it different enough genetically to be considered as a separate species?

2. Material and methods

Four live specimens of *P. falsobeccarii* have been sampled, three in the Rhône prodelta at the station 15 (Goineau et al., 2011) and one in the Bay of Biscay at the station

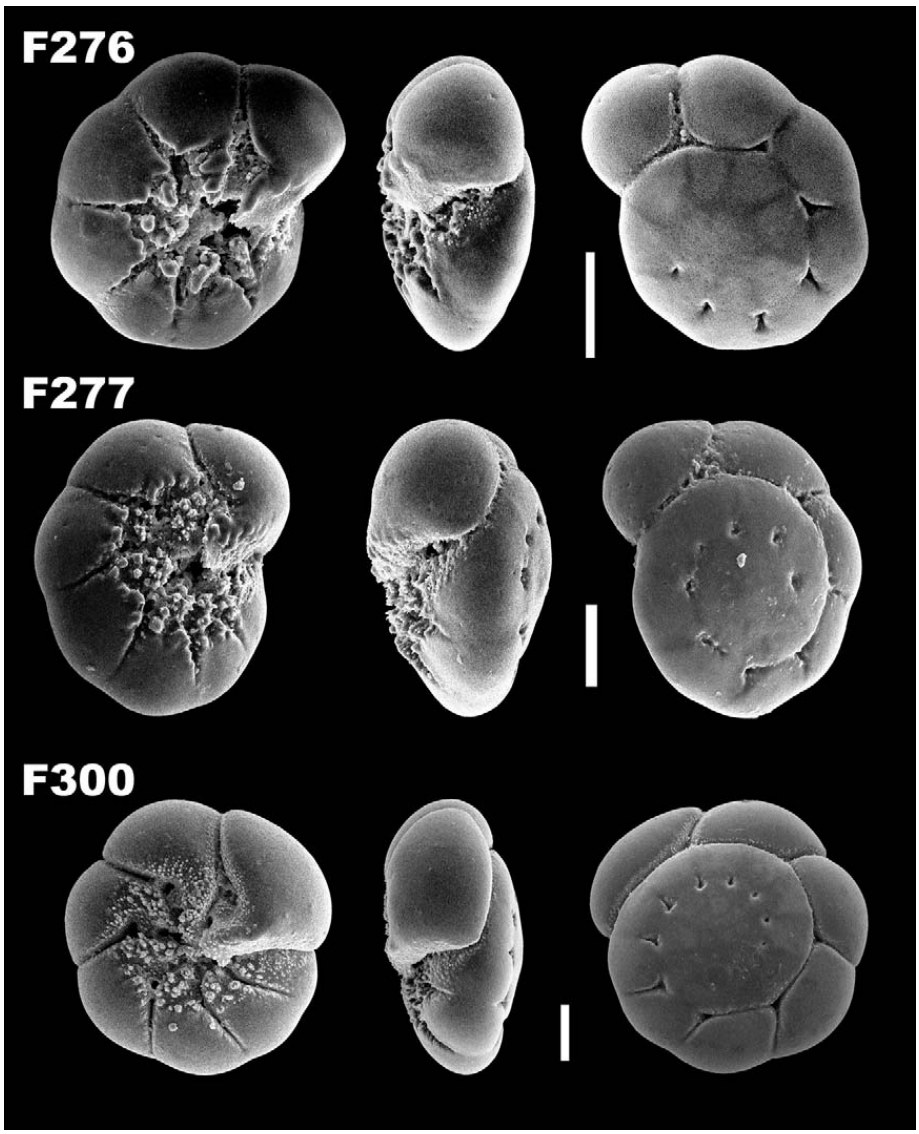

Fig. 2. SEM pictures of the specimens sequenced and DNA identification numbers. Scale bar: 100 μm. N.B. No picture was taken for specimen F187 and DNA amplification was negative for specimen F276.
Fig. 2. Photos au MEB des spécimens séquencés, avec leur numéro d'identification ADN. Échelle : 100 μm. N.B. Il n'y a pas de photo pour le spécimen F187 et l'amplification d'ADN n'a rien donné pour le spécimen F276.

D (Fontanier et al., 2002) (Fig. 2, Table 1). The specimens were picked, photographed and sequenced according to the protocol described in Schweizer et al. (2010).

The new sequences (one partial SSU and three partial LSU ones) have been deposited in the EMBL/GenBank database (accession numbers given in Table 1). These sequences were aligned manually with related sequences available from GenBank in two separate datasets (SSU and LSU) using Seaview (Gouy et al., 2010). The genus *Elphidium* was chosen as the out-group taxon because it is the closest known relative of *Ammonia* (Schweizer et al., 2008).

The regions, which were impossible to align properly were removed and 847 and 348 sites were respectively used for SSU and LSU analyses. Maximum likelihood (ML) analyses were performed with 100 bootstrap (BS) replicates by PhyML 2.4.4 (Guindon and Gascuel, 2003) under the HKY (Hasegawa, Kishino, Yano) model (Hasegawa

Table 1
List of new partial rDNa SSU and LSU sequences with the location of DNA samples, the water depth and the GenBank accession numbers for both genes. The samples of the Rhône prodelta were taken from station 15 (43°17'055 N and 04°45'148 E, Goineau et al., 2011) and the one from the Bay of Biscay from station D (43°42' N and 01°34' W, Fontanier et al., 2002).
Tableau 1
Liste des nouvelles séquences partielles d'ADNr 18S et 28S, avec la localisation des échantillons, la profondeur d'eau et les numéros d'accès GenBank pour les deux gènes. Les échantillons du prodelta du Rhône proviennent de la station 15 (43°17'055 N et 04°45'148 E, Goineau et al., 2011) et celui du Golfe de Gascogne de la station D (43°42' N et 01°34' W, Fontanier et al., 2002).

DNA number	Location	Water depth (m)	SSU sequence	LSU sequence
F187	Rhône delta	60	Negative	HM448840
F276	Rhône delta	60	Negative	Negative
F277	Rhône delta	60	HM448841	HM448838
F300	Bay of Biscay	140	Negative	HM448839

et al., 1985) for the SSU and the LSU alignments. In order to correct for among-site rate variations, the proportion of invariable sites (I) and the alpha parameter of gamma distribution (Γ), with six rate categories, were estimated by PhyML (HKY+I+Γ). In addition, BIONJ phylogenetic trees (Gascuel, 1997) were inferred with Seaview under the K2P (Kimura's two parameter) evolution model (Kimura, 1980) with non-parametric bootstrapping (1000 replicates).

3. Results and discussion

Two regions of the rDNA gene cluster are investigated here: the 3' end fragment of the SSU which is widely used in

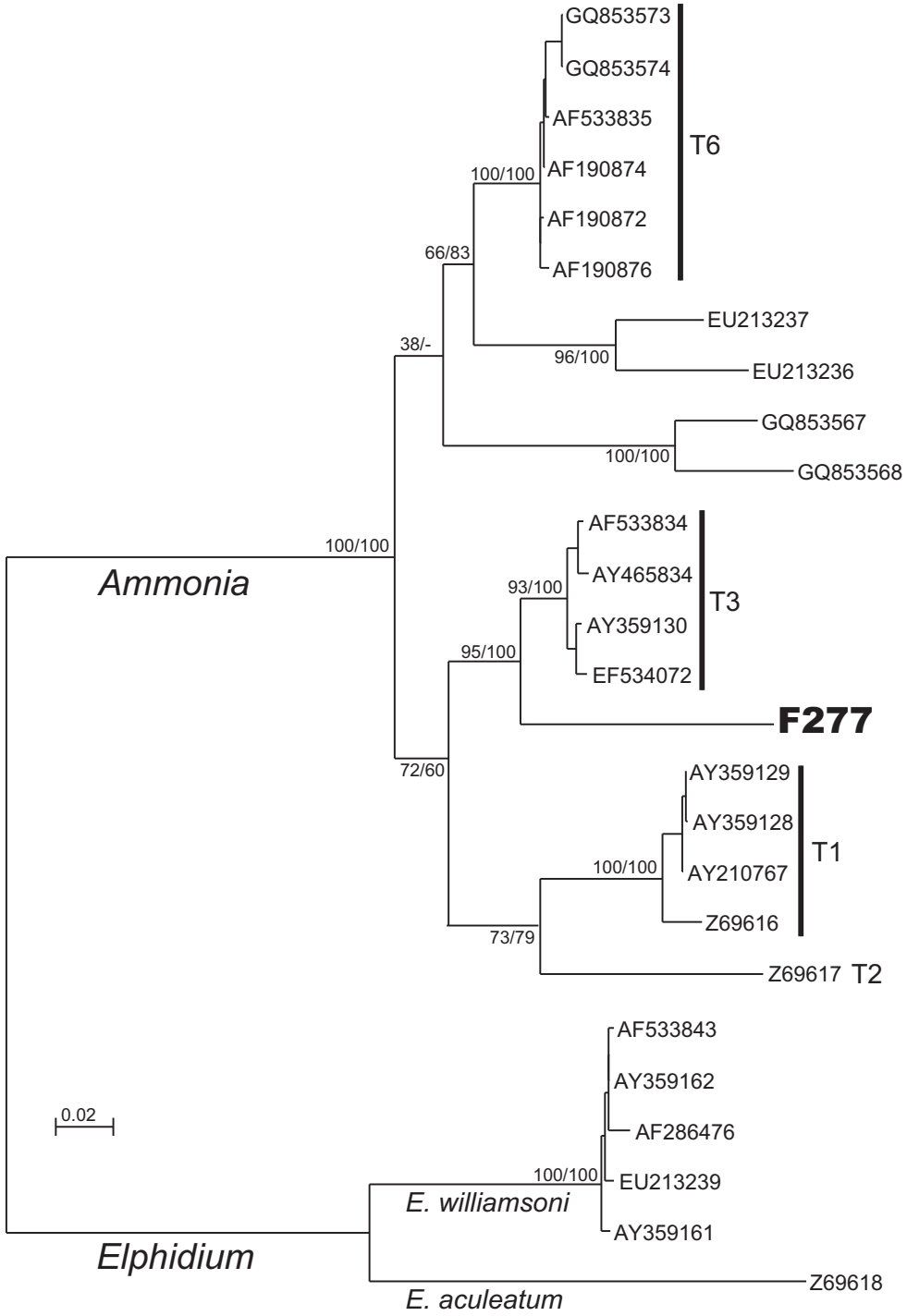

Fig. 3. Molecular phylogeny of selected *Ammonia* species based on partial SSU rDNA sequences inferred using the ML method (model HKY+I). Tree is rooted on *Elphidium williamsoni* and *E. aculeatum* and bootstrap values for ML and BIONJ analyses are indicated at the nodes.
Fig. 3. Phylogénie moléculaire de représentants du genre *Ammonia*, basée sur des séquences partielles d'ADNr 18S et calculée d'après la méthode du maximum de vraisemblance (ML) (modèle HKY+I). L'arbre est enraciné sur *Elphidium williamsoni* et *E. aculeatum* et les valeurs de *bootstrap* pour les analyses ML et BIONJ sont indiquées aux nœuds.

foraminiferal phylogenetic studies (Pawlowski, 2000) and the 5' end of the LSU which was used to define 13 different phylotypes of *Ammonia* by Hayward et al. (2004). In the SSU analysis (Fig. 3), only four phylotypes of *Ammonia* are represented: T1, T2, T3 and T6. The *P. falsobeccarii* sequence is the sister group of the phylotype T3. The same topology, also well supported, is observed in the LSU analysis with three sequences of *P. falsobeccarii* and the 13 phylotypes of *Ammonia* previously defined (Fig. 4).

3.1. *Generic assignation and taxonomic status of* Pseudoeponides falsobeccarii

Both SSU and LSU phylogenetic analyses show that *Pseudoeponides falsobeccarii* sequences have a central position in the *Ammonia* sequences (Figs. 3 and 4). Therefore, it is not justified to give this species a separate generic name, *Pseudoeponides.* These phylogenetic results confirm the earlier conclusion of Hofker

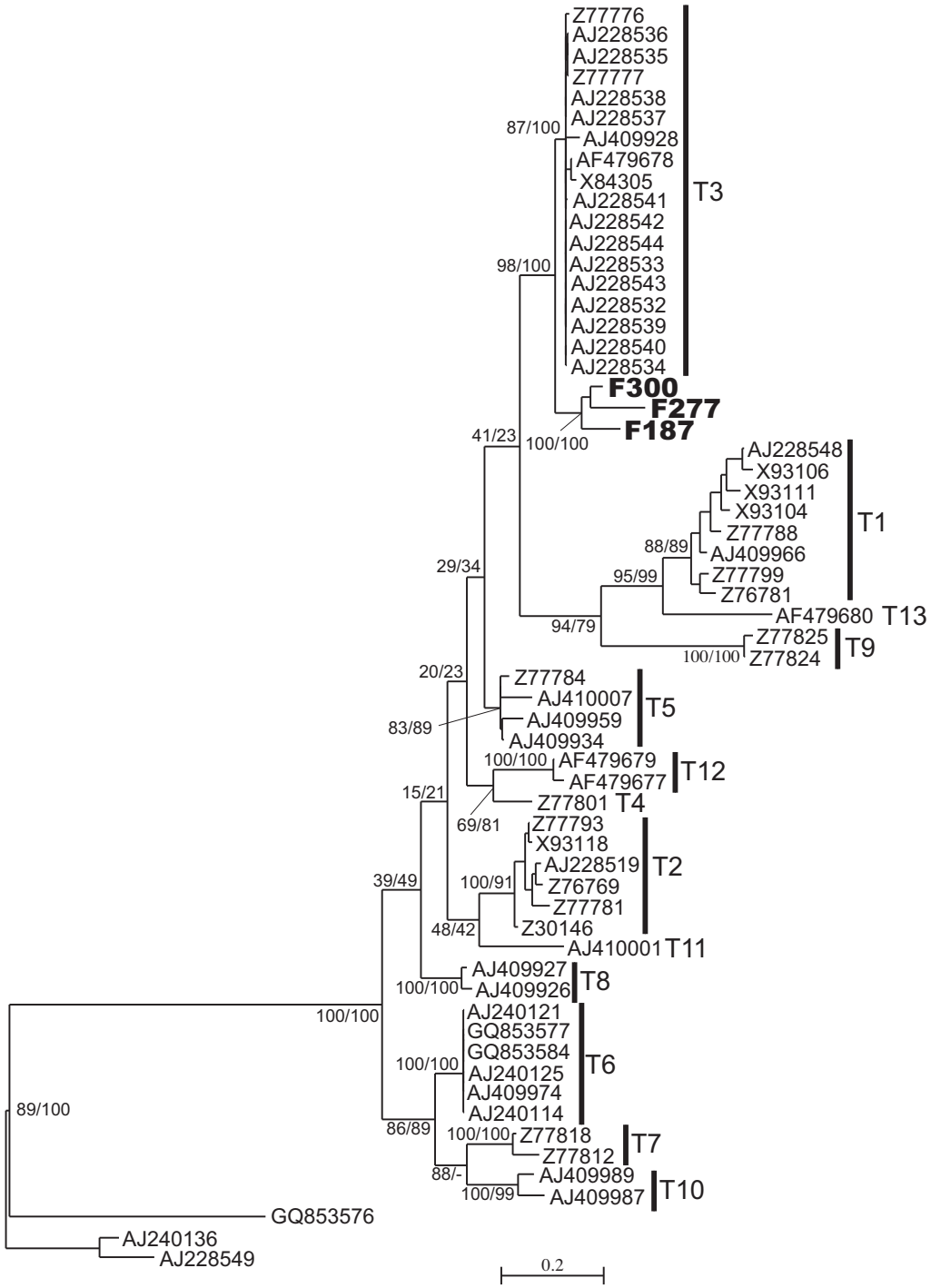

Fig. 4. Molecular phylogeny of selected *Ammonia* species based on partial LSU rDNA sequences inferred using the ML method (model HKY+I+Γ). Tree is rooted on *N. calcar*, *P. nipponica* and *H. germanica* and bootstrap values for ML and BIONJ analyses are indicated at the nodes.

Fig. 4. Phylogénie moléculaire de représentants du genre *Ammonia*, basée sur des séquences partielles d'ADNr 28S et calculée d'après la méthode ML (modèle HKY+I+Γ). L'arbre est enraciné sur *N. calcar, P. nipponica* et *H. germanica* et les valeurs de *bootstrap* pour les analyses ML (100 réplicats) et BIONJ sont indiquées aux nœuds.

Fig. 5. Map of Europe with sampling sites for specimens T3 analysed by Hayward et al. (2004) (grey diamonds) and *A. falsobeccarii* from the present study (white stars).
Fig. 5. Carte d'Europe avec indication des sites d'échantillonnage pour les spécimens T3 analysés par Hayward et al. (2004) (losanges gris) et les *A. falsobeccarii* de la présente étude (étoiles blanches).

(1958) based on a morphological analysis of both genera.

DNA sequences of *Ammonia falsobeccarii* form a well-supported clade, closely related but clearly separated from the phylotype T3 in both SSU and LSU analyses (Figs. 3 and 4).

3.2. *Relationships between* Ammonia falsobeccarii *and the phylotype T3*

The phylotype T3 has been sampled in Sweden, the Channel, the Bay of Biscay and the Gulf of Lions (Hayward et al., 2004) (Fig. 5). Although this heavily ornamented phylotype was not identified as *A. beccarii* by Hayward et al. (2004), because it is morphologically slightly different from the topotypes of that species, similar specimens from Vendée (Bay of Biscay) have previously been commonly attributed to *A. beccarii* (e.g. Debenay et al., 1998). The northern specimens (e.g. Sweden, Channel) are usually identified as *Ammonia batava* (Hayward et al., 2004). Although specimens genetically identified as T3 have been presented with different species names, they all clearly share a common morphology (Hayward et al., 2004).

Ammonia falsobeccarii and the phylotype T3 can be found in the same locations. However, it is usually relatively easy to morphologically discriminate them. *Ammonia falsobeccarii* has more lobulated chambers and unfilled secondary openings at the junctions of the radial sutures with the spiral one on the spiral side (Figs. 1 and 2), whereas the T3 phylotype has deeply incised fissured sutures on the spiral side and a more ornamented umbilical side (Fig. 1).

The fact that *A. falsobeccarii* is well separated morphologically and genetically from T3 strongly suggests that it can be considered as a new phyloclade.

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