

CHAPTER 4

Molecular phylogeny of the foraminiferal genus
Uvigerina based on ribosomal DNA sequences

Abstract

Uvigerina is a common genus of benthic foraminifera, often used as a proxy for paleoclimate and paleoenvironment reconstructions. Better understanding of the phylogeny of *Uvigerina* would improve its proxy value and would allow us to check whether its different morphospecies are real species or ecophenotypes only. Here, we used partial small-subunit ribosomal DNA (SSU rDNA) sequences to examine the phylogenetic relationships within *Uvigerina* and between this genus and other rotaliids. Our analyses show that the family Uvigerinidae forms a well supported clade branching as a sister group to Bolivinidae and Cassidulinidae. Studied individuals of Uvigerinidae include three species described as *Uvigerina* – *U. mediterranea*, *U. elongatastriata* and *U. peregrina* – as well as *Rectuvigerina phlegeri* and *Trifarina earlandi*. As *U. peregrina* is more closely related to *R. phlegeri* and *T. earlandi* than to the other two *Uvigerina*, the taxonomic status of these species needs to be revised. At the intraspecific level, we studied a morphologically highly variable population of *U. peregrina* from the Oslo Fjord. For the sequences obtained from this population of *U. peregrina*, we found almost no divergence inside the internal transcribed spacer (ITS), which is the most variable part of ribosomal DNA. This indicates a high morphological plasticity of *Uvigerina* species, which should be taken into consideration when using this genus as a proxy in paleoecological reconstructions.

Keywords: benthic foraminifera, Rotaliida, *Uvigerina*, ribosomal DNA, molecular phylogeny, morphometry

4.1. Introduction

The benthic foraminiferal genus *Uvigerina* d'Orbigny, 1826 is common in temperate and high latitude regions (Haynes, 1981). Members of this cosmopolitan taxon mainly live in muddy sediment at shallow in-sediment depths, have a vagile mode of life, and prefer relatively cold marine waters of shelf to bathyal zones (Murray, 1991).

Uvigerina is frequently used in reconstructions of Cenozoic marine environments. Initially *Uvigerina* and related morphotypes were, and in the absence of other biostratigraphic markers still are, used as stratigraphic tools for Upper Cretaceous to Neogene sediments (e.g. Lamb, 1964; Hornibrook, 1968; Papp & Schmid, 1971; Douglas, 1973; Boersma, 1984). Since the ecological information carried by benthic foraminifera in general has been recognized, various species of *Uvigerina* have been extensively used as indicator taxa in studies pertaining to marine paleoenvironment and paleoclimate (e.g. Wright, 1980; Woodruff & Douglas, 1981; Boersma, 1986; Casford et al., 2003). In fossil applications, proxy relationships of benthic taxa with environmental factors are often derived from the ecological behaviour observed in Recent representatives of these taxa (Murray, 1991; 2001). This relationship is based on covariance of species abundances and/or benthic assemblage characteristics with environmental parameters (e.g. Bernhard, 1986; Fariduddin & Loubere, 1997; Fontanier et al., 2002; Licari et al., 2003).

Incorporation of elements in foraminiferal shells provides another means to constrain physico-chemical parameters of the marine (paleo-)environment. Important proxies are stable isotopes of oxygen and carbon, which are often measured on *Uvigerina*. Since *Uvigerina* taxa incorporate stable oxygen isotopes in their shell in near-equilibrium with ambient sea water (e.g. Shackleton, 1974; Woodruff et al., 1980; McCorkle et al., 1997), marine oxygen isotope records have been based on these species (e.g. Mix et al., 1995; Zachos et al., 2001). Many *Uvigerina* species occupy a shallow infaunal habitat (e.g. Corliss, 1985; Jorissen et al., 1998; De Stigter et al., 1998). Effort has been invested in studies to establish effects of microhabitat and calcification depth (e.g. McCorkle et al., 1997; Schmiedl et al., 2004) on the carbon isotope signature of *Uvigerina* (e.g. Grossman, 1984; Wilson-Finelli et al., 1998; Tachikawa & Elderfield, 2002; Mackensen & Licari, 2004).

The genus *Uvigerina* was first recorded in sediments of lower Eocene age (Loeblich & Tappan, 1988). Galloway (1933) proposed *Bulimina* as its ancestor, giving rise first to *Uvigerinella* and then to *Uvigerina*, of which juvenile stages have a *Bulimina*-like aperture. According to Haynes (1981), *Uvigerina* and *Trifarina* may have evolved from *Praebulimina* in two independent lineages since the late Cretaceous.

In current classification systems, *Uvigerina* belongs to the family Uvigerinidae Haeckel, 1894,

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which is placed in the superfamily Buliminacea Jones, 1975 (Loeblich and Tappan, 1988). The family includes the Recent genera *Uvigerina*, *Euuvigerina*, *Neouuvigerina* and *Siphouvigerina*, grouped in the subfamily Uvigerininae Haeckel, 1894 and the Recent genera *Angulogerina* and *Trifarina*, grouped in the subfamily Angulogerininae Galloway, 1933. Members of Uvigerinidae are characterized by a triserial test tending to biseriality or uniseriality, a terminal aperture with a neck, a phyaline lip and an internal toothplate (Loeblich & Tappan, 1988). Distinctive features of Uvigerininae are rounded and inflated chambers, while Angulogerininae are characterized by triangular sections of their tests. Another morphologically similar genus, *Rectuvigerina*, which

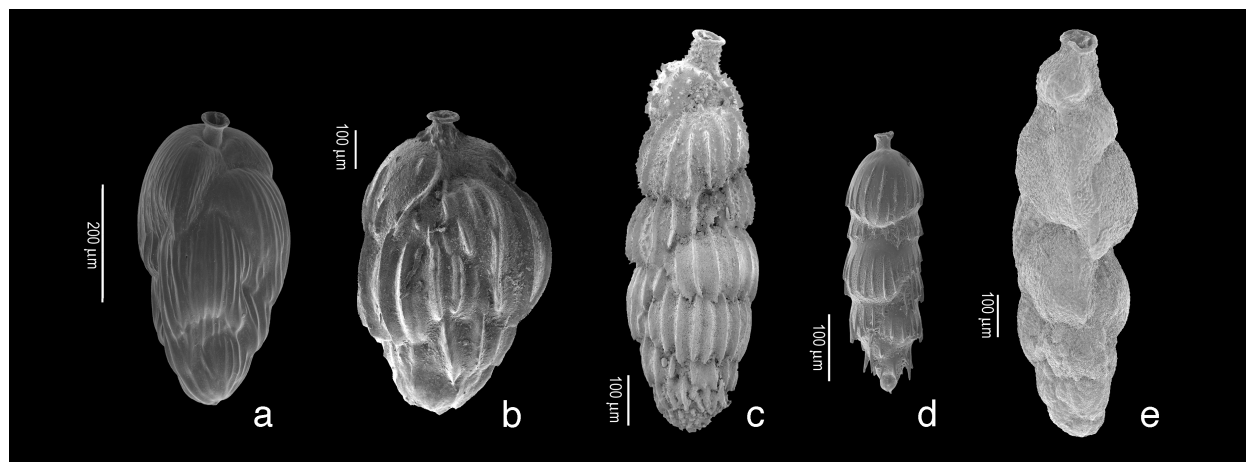


Figure 4.1. SEM pictures of the examined uvigerinids: a) *U. elongatastriata* (U273), b) *U. mediterranea*, c) *U. peregrina* (U67), d) *R. phlegeri* (U239), e) *T. earlandi*. For specimens from which DNA was extracted and sequenced, the DNA number is indicated in brackets.

was examined in this study, has been classified in the family Siphogenerinoididae Saidova, 1981. Specimens belonging to this family have triserial or biserial tests, showing a tendency to develop uniseriality, and an aperture with a toothplate (Loeblich & Tappan, 1988).

The genus *Uvigerina* has been divided by Van der Zwaan et al. (1986a) in three morphological groups. The *U. semiornata* group is characterized by a test that is triserial throughout, a short apertural neck standing in a depression, broad and high chambers strongly overlapping the previous ones, and pores with an elongated shape. The *U. peregrina* group shows a frequent tendency to reduced seriality. The relatively long apertural neck is not in a depression, the chambers are more or less inflated and not strongly overlapping the previous ones. The pores are rounded, the sutures are straight and often the basal chamber sutures are depressed. The ornamentation is variable and can be either hispid or costate, or a combination of both. In the *U. bononiensis* group the seriality is reduced during ontogeny. This group is further characterized by a neck that is not standing in a depression, a costate ornamentation, “en crochet” sutures, and rounded pores. In our material, two species are classified inside the *U. semiornata* group (*U. elongatastriata* and *U. mediterranea*), one in the *U. peregrina* group (*U. peregrina*) and one in the *U. bononiensis* group (*R. phlegeri*) (Fig. 4.1).

The present classification and phylogeny of *Uvigerina* is based exclusively on morphological features but recently ribosomal DNA sequences of several *Uvigerina* species were published (Ertan et al., 2004). Here, we report 61 new sequences of *Uvigerina* and other rotaliids (GenBank accession numbers AY914562-AY914600 and AY934735-AY934756), which we used for phylogenetic analyses together with previously published sequences.

Our goals were to infer the phylogenetic position of *Uvigerina* among rotaliid foraminiferans, to analyse its intrageneric relationships, and to examine intraspecific variation in a population of *U. peregrina*. Our results are compared to existing molecular data on rotaliid foraminifera. We discuss the position of *Uvigerina* in the rotaliid tree, and the possible differences between genetically and morphologically based taxonomies.

4.2. Material and methods

4.2.1. Sampling and SEM identification

Live specimens of *Uvigerina* and *Rectuvigerina* were collected during three cruises: in May 2002 with the R/V Trygve Braarud (University of Oslo, Norway) in the Oslo Fjord, in May 2003 with the R/V Arne Tiselius (Kristineberg Marine Research Station, Sweden) in the Skagerrak and the Kattegat, and in October 2003 with the R/V Pelagia (Royal Netherlands Institute for Sea Research, The Netherlands) on the Portuguese coast of the Atlantic (Fig. 4.2). The specimens of *Trifarina earlandi* were collected in November 1998 and 1999 in Antarctica (Explorer Cove, McMurdo Sound).



Figure 4.2. Map of Europe indicating the three areas sampled during the cruises.

1) Oslo Fjord (Norway); 2) Swedish coast of Skagerrak; 3) Portuguese coast of Atlantic.

Sediment samples were collected by boxcoring and multicoring. The top few centimeters were sampled with a spoon and immediately sieved using cold bottom water (fractions 500/250/125 μ m). The different fractions were stored in the refrigerator at 4°C.

Specimens were cleaned and picked under a dissection microscope within hours to a few days. Living individuals were distinguished from dead ones by their natural coloration (e.g. greenish-brownish for *U. peregrina* and *R. phlegeri*, orange for *U. elongatastriata*), lack of cytoplasm in the last

chamber, good preservation of the test (not eroded or broken), and presence of debris around the aperture. Whenever possible, specimens were transferred to Petri dishes containing clean sea water and observed a few hours after picking, to check whether they were alive. Putatively living specimens were dried on Chapman slides; later the dried specimens were coated with gold and pictured with scanning electron microscope (SEM). All the SEM pictured specimens were extracted for DNA, however the percentages of positive results were variable.

4.2.2. Morphometrical analysis

Uvigerina peregrina was very abundant in samples from the Oslo Fjord and showed a wide range of morphologies. SEM pictures of individuals from this population were used to perform morphometrical analyses. A general view of the specimens and a view of the pores at a higher magnification were used. Eight characteristics were measured or observed. Three of them are metrical criteria: the maximal length (maxL) without the neck, the maximum transversal diameter (MTD) and the number of chambers (nc). Two ratios were calculated from the metrical criteria: $MTD/\max L * 100$ and $nc/\max L * 100$. Five of the measured characteristics are non-metrical: the shape of the chambers (inflated, marginate, standard (not inflated nor marginate)), the number of costae (small, medium, large), the number of pores (small, medium, large), the spinosity (absent, between costae, on the last chamber), and the position of the neck (terminal, inclined, in a depression, with spines).

Bivariate graphs were made using the three metrical criteria. The software employed was Statview 4.5 (Abacus Concepts). Metrical as well as non-metrical criteria were used for multivariate analyses: Detrended Correspondence Analysis (DCA) and Canonical Variates Analysis (CVA, alias Fisher's linear discriminant analysis), using the program CANOCO (Ter Braak and Smilauer, 1998). In order to incorporate nominal variables in our analyses, they were transformed into

Table 4.1. List of new SSU sequences and origin of DNA samples.

Access #	Species	DNA #	Collection site	Cells	Cloning
AY934735	<i>Bolivina</i> sp.	JPM99	Mediterranean		direct
AY934736	<i>Bolivina</i> sp.	170	Tahiti		direct
AY934737	<i>Cassidulinoides porrectus</i>	3924	Terranova Bay, Antarctica		2
AY934738	<i>Cassidulina laevigata</i>	2508	Oslo Fjord, Norway	1	2
AY934744	<i>Stainforthia fusiformis</i>	3965	Skagerrak, Sweden	150	2
AY934745	<i>Stainforthia fusiformis</i>	3979	Dunstaffnage, Scotland	50	1
AY934743	<i>Stainforthia</i> sp.	2641	Svalbard, Norway		direct
AY934746	<i>Virgulina concava</i>	3991	Dunstaffnage, Scotland	5	1
AY934747	<i>Bulimina marginata</i>	3599	Oslo Fjord, Norway	130	1
AY934748	<i>Bulimina marginata</i>	523	Kosterfjord, Sweden	3	direct
AY914562	<i>Globobulimina turgida</i>	3601	Oslo Fjord, Norway	20	2
AY914563, AY914564	<i>Rectuvigerina phlegeri</i>	U239	Nazaré Canyon, Portugal	1	4
AY914566, AY914567	<i>Trifarina earlandi</i>	1145	McMurdo, Antarctica	5	2
AY914568	<i>Trifarina earlandi</i>	1994	NH-Ice Hut, Antarctica	10	direct
AY914565	<i>Trifarina earlandi</i>	2187	McMurdo, Antarctica	5	3
AY914577, AY914578	<i>Uvigerina elongatastriata</i>	U273	Nazaré Canyon, Portugal	1	2
AY914569, AY914570	<i>Uvigerina peregrina</i>	U26	Oslo Fjord, Norway	1	3
AY914571	<i>Uvigerina peregrina</i>	U32	Oslo Fjord, Norway	2	direct
AY914572	<i>Uvigerina peregrina</i>	U67	Oslo Fjord, Norway	1	direct
AY914573	<i>Uvigerina peregrina</i>	U169	Skagerrak, Sweden	1	2
AY914574, AY914575	<i>Uvigerina peregrina</i>	U184	Skagerrak, Sweden	1	3
AY914576	<i>Uvigerina peregrina</i>	U195	Skagerrak, Sweden	1	3
AY934739	<i>Discorbis rosea</i>	753	Florida, USA	1	2
AY934749	<i>Epistominella</i> sp.	286	Channel, France	10	direct
AY934750	<i>Epistominella vitrea</i>	2060	Cape Evans, Antarctica	4	3
AY934741	<i>Cibicides wuellerstorfi</i>	C184	Setubal Canyon, Portugal	1	3
AY934742	<i>Cibicides lobatulus</i>	C39	Oslo Fjord, Norway	1	2
AY934740	<i>Planorbulinella</i> sp.	358	Elat, Israel	4	2
AY934751	<i>Nonionella labradorica</i>	3600	Oslo Fjord, Norway	60	5
AY934752	<i>Nonionella labradorica</i>	3966	Skagerrak, Sweden	20	1
AY934753	<i>Melonis pompilioides</i>	1400	Skagerrak, Sweden		3
AY934756	<i>Pullenia subcarinata</i>	1087	NH-Ice Hut, Antarctica	2	2
AY934755	<i>Pullenia subcarinata</i>	1148	McMurdo, Antarctica	1	3
AY934754	<i>Pullenia subcarinata</i>	1850	McMurdo, Antarctica	1	2

'dummy variables', e.g. a nominal variable with three categories was split into three separate variables with values of 0 or 1.

DCA is a unimodal ordination technique (Ter Braak, 1995). In our analysis, individual specimens were treated as 'samples' (as defined in CANOCO) and their morphological characteristics as 'species'. Thus, the specimens were arranged on DCA axes, maximizing the spread of their corresponding characteristics along the axes. The method of detrending was by 2nd order polynomials. To obtain

Table 4.2. List of new ITS sequences and origin of DNA samples.

Access #	Species	DNA #	Collection site	Depth	Cells	Cloning
AY914579, AY914580, AY914581	<i>Uvigerina peregrina</i>	U37	Oslo Fjord Norway	195m	1	3
AY914582, AY914583	<i>Uvigerina peregrina</i>	U42	Oslo Fjord Norway	195m	1	2
AY914584, AY914585, AY914586	<i>Uvigerina peregrina</i>	U51	Oslo Fjord Norway	54m	1	3
AY914587, AY914588	<i>Uvigerina peregrina</i>	U66	Oslo Fjord Norway	87m	1	2
AY914589	<i>Uvigerina peregrina</i>	U67	Oslo Fjord Norway	87m	1	1
AY914590, AY914591, AY914592	<i>Uvigerina peregrina</i>	U72	Oslo Fjord Norway	87m	1	3
AY914593, AY914594, AY914595	<i>Uvigerina peregrina</i>	U86	Oslo Fjord Norway	87m	1	3
AY914596, AY914597	<i>Uvigerina peregrina</i>	U87	Oslo Fjord Norway	87m	1	2
AY914598, AY914599, AY914600	<i>Uvigerina peregrina</i>	U194	Skagerrak Sweden	60m	1	3

a CVA, we performed a special kind of canonical correspondence analysis (CCA) as explained by Ter Braak and Smilauer (1998, pp. 60-62). Again the specimens were 'samples'. The *Uvigerina* types were 'species' with an abundance of either 0 or 1, so each sample consisted of only one 'species'. The morphological characteristics are included as 'environmental parameters' (in CANOCO terminology). Thus, in CCA the axes were defined as linear combinations of the values of the morphological characteristics. The linear combination of characteristics that gave the best separation of the *Uvigerina* types was the first axis, the second best (independent of the first) was the second axis, etc. The eigenvalues in CVA (θ) could be derived from the eigenvalues in CCA (λ): $\theta = \lambda / (1 - \lambda)$. Because type 4 specimens appeared to be outliers in the analyses, we eliminated them in the CVA in order to improve the separation of the other three types.

4.2.3. DNA extraction, PCR amplification, cloning and sequencing

DNA was extracted from single specimens using DOC lysis buffer (Pawlowski et al., 1994). For the extraction of multiple specimens, DNeasy Plant Mini Kit (Qiagen) was used. Two regions of ribosomal DNA (rDNA) were examined here: a fragment of the SSU (small subunit) rDNA situated at the 3' end of approximately 1,000 base pairs, and the ITS (Internal Transcribed Spacer) region (ITS1 + 5.8S + ITS2) with a length of about 1,000-1,100 base pairs. The SSU fragment was amplified using the primer pair s14F3-sB and reamplified using the primers s14F1-sB. The ITS region was amplified with s20-2TAIC and reamplified with sBr-2TAIC. The sequences of these primers can be found in Pawlowski (2000) except s14F3 (5' ACG CAM GTG TGA AAC TTG 3') and sBr (5' GTA GGT GAA CCT GCA GAA GG 3'). SSU and ITS were amplified by PCR using a total volume of 50 μ l. The thermal cycle parameters consisted of 40 cycles of 30 s at 94°C, 30 s at 50°C and 120 s at 72°C, followed by 5 min at 72°C for final extension. Reamplification was carried out using 35 cycles of 30 s at 52°C instead of 50°C, all other parameters remaining

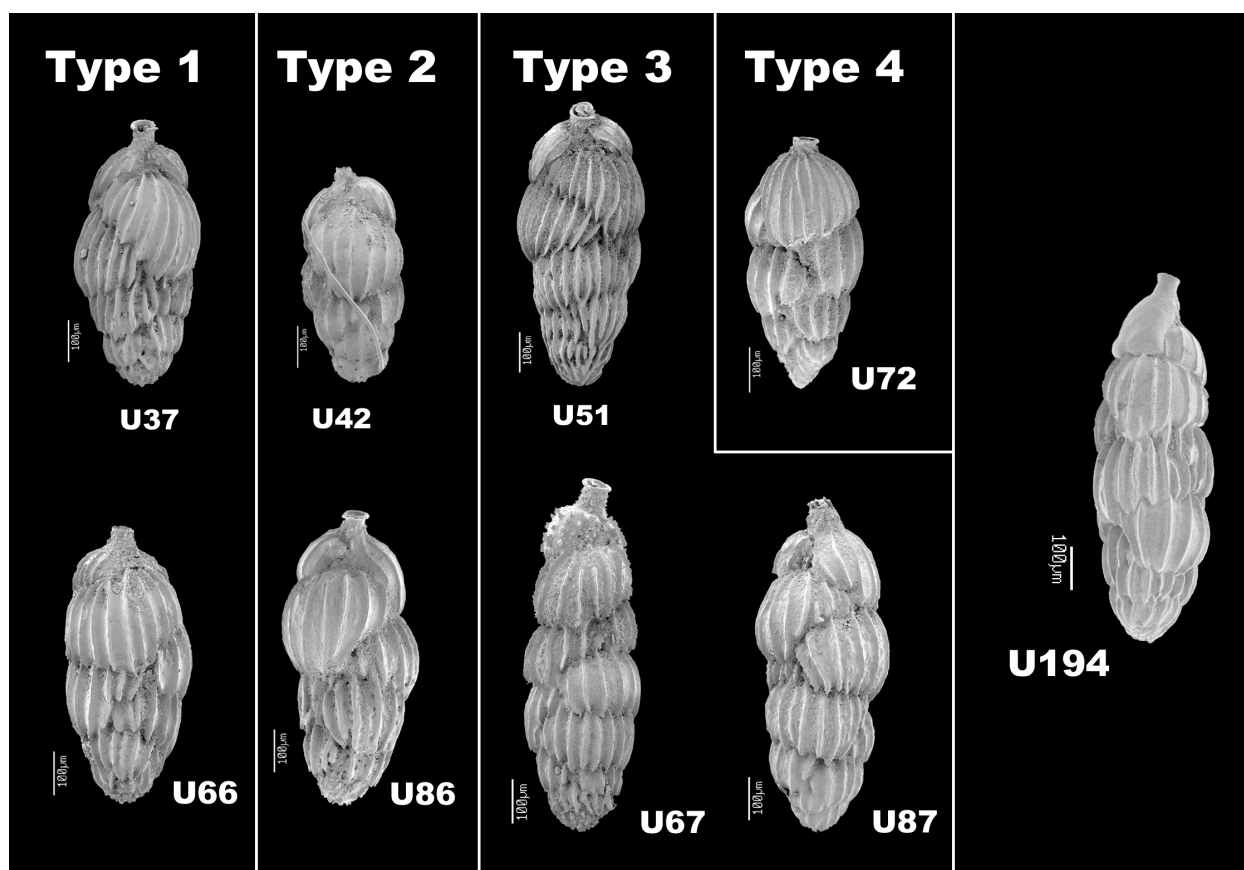


Figure 4.3. SEM pictures of the *U. peregrina* specimens used for the ITS. Morphologically, U37 and U66 belong to type 1, U42 and U86 to type 2, U51, U67 and U87 to type 3, and U72 to type 4. All these specimens were sampled in Oslo Fjord. U194 was collected on the Swedish coast of Skagerrak and was excluded from the morphometrical study.

unchanged. Positive PCR products were purified using High Pure PCR Purification Kit (Roche Diagnostics). PCR products obtained from DNA samples 170, 523, 1994, 2641, U32, and U67 were sequenced directly, while the remaining PCR products (see Tables 1 and 2) were ligated in the pGEM-T Vector (Promega) and cloned using ultracompetent cells XL2-Blue MRF' (Stratagene). Sequencing reactions were prepared using ABI-PRISM Big Dye Terminator Cycle Sequencing Kit and analysed with DNA sequencers ABI-377 or ABI-PRISM 3100 (Perkin-Elmer), all according to the manufacturer's instructions.

4.2.4. Phylogenetic analysis

The new SSU and ITS sequences of rotaliids presented here have been deposited in the EMBL/GenBank data base, their accession numbers are reported in Tables 4.1 and 4.2. To extend our data set, we used rotaliid sequences deposited in the GenBank data base.

For the partial SSU, 52 sequences of Rotaliida and four sequences of Textulariida, used as outgroup, were analysed. We excluded from our analyses the sequences of *Ammonia*, *Elphidium*, *Haynesina* and the Glabratellidae available in the Genbank, because their very rapid rates of evolution reduce the number of unambiguously aligned sites and bias the analyses. Sequences were aligned manually employing Seaview (Galtier et al., 1996). Of the ~1,000 base-pair fragment of the SSU, 695 unambiguously aligned sites were used for the phylogenetic analysis of rotaliids and 781 for the analysis of uvigerinids. The maximum likelihood (ML) trees were obtained using the PhyML program (Guindon and Gascuel, 2003), with the HKY model (Hasegawa et al., 1985) allowing transitions and transversions to have potentially different rates and the General Time Reversible (GTR) model allowing all these rates to be different (Lanave et al., 1984; Rodriguez et

al., 1990). To correct the among-site rate variations, the proportion of invariable sites (I) and the α parameter of γ distribution (G), with eight rate categories, were estimated by the program and taken into account in all analyses. Non-parametric ML bootstraps (with 100 replicates) were calculated using PhyML. Bayesian inferences (BI) were obtained with MrBayes v.3.0 (Huelsenbeck and Ronquist, 2001), using the same models of DNA evolution as for the ML analyses. The program was run for 1,000,000 generations, sampled every 100 generations, with four simultaneous chains. 10,000 trees were sampled, of which the first 1,000 were discarded as burn-in.

For the constrained tree topology, we used TreeView (Page, 1996) to build the constrained tree and PAUP* version 4.0b10 (Swofford, 1998) for the K-H (Kishino and Hasegawa, 1989) and the S-H (Shimodaira and Hasegawa, 1999) tests.

In addition, the ITS region of 22 clones belonging to nine different specimens of *Uvigerina peregrina* was analysed with PhyML (871 unambiguously aligned sites).

4.3. Results

4.3.1. Morphometrical study

In the Oslo Fjord population of *Uvigerina peregrina*, four different morphological types can be distinguished with morphometrical analyses. The different morphotypes are shown in Figure 4.3. The type 1 (30 specimens) generally has a standard shape of the chambers, a large number of costae, spines between the costae or no spines, a small number of pores and the neck is positioned at the top. The type 2 (15 specimens) is characterized by inflated chambers and a low number of costae; it often has no spines or spines between the costae, a large number of pores and the neck is inclined and/or spinose. The type 3 (12 specimens) is characterized by an elongated shape; it usually has a standard shape of the chambers, a large number of costae, spines between the costae and a terminal neck. The type 4 (two specimens) is essentially defined by a marginate shape of the chambers, with no spines and a terminal neck. No relation was found between the morphotypes and the different sampling locations.

The type 3, with a more elongated shape, can be separated from the other specimens in a bivariate graph (Fig. 4.4a). Calculation of the ratio $MTD/\max L * 100$ also allows to separate the type 3 (except 2 specimens), with values below 42, from the other types (Fig. 4.4b). DCA enables us to graphically link the criteria with the groups they characterize and the morphological variability of the specimens (Fig. 4.5). There is a good separation between types 2 and 3 on the first (horizontal) axis (except one specimen from type 3) and between type 4 and the other types on the second

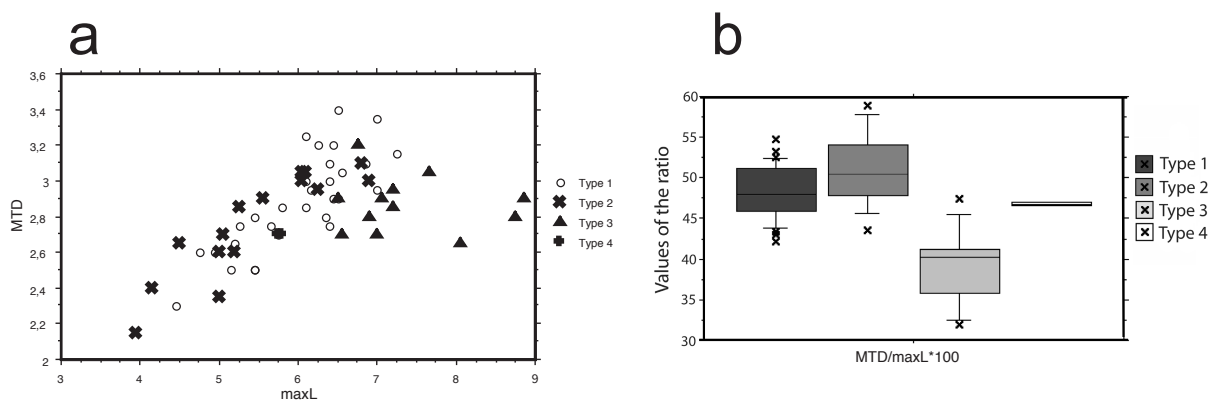


Figure 4.4.

a) Bivariate graph comparing the maximal length (maxL) and the maximum transversal diameter (MTD).
 b) Box-plots of the ratio $MTD/\max L * 100$ for each morphotype. The box and the vertical lines coming from it (the "whiskers") represent 100% of the values. The box is delimited by the first quartile (Q1, 25%) at the bottom and the third one (Q3, 75%) at the top. Inside the box, the horizontal line represents the median (50%). Crosses indicate values outside the limits of the "whiskers" (the "outliers").

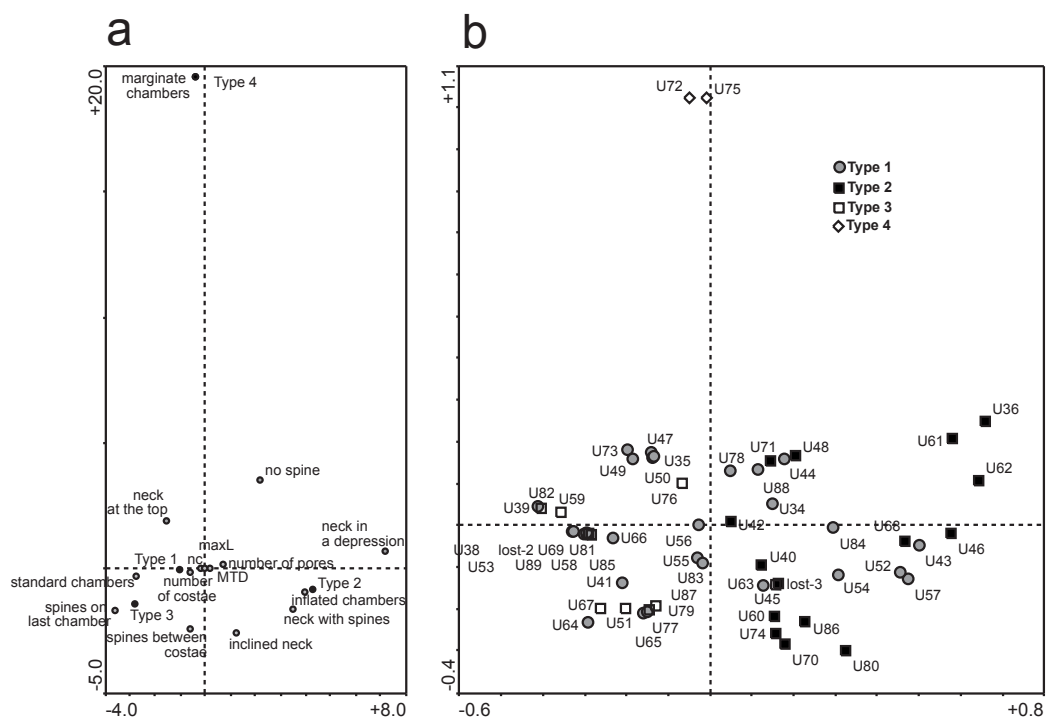


Figure 4.5. DCA graphs:

a) Position of the criteria and center of each group.

b) Position of the specimens, labelled after the analysis.

Eigenvalues of the 1st and 2nd axis were 0.194 and 0.143, respectively. The percentage of variance in the 'species' data accounted for by the 1st axis was 23.8%, and 41.4% for both axes together.

axis (vertical). Type 1 specimens form a cloud within the groups formed by types 2 and 3 (Fig. 4.5). Because type 4 specimens are strong outliers in the analyses, they were excluded from the CVA in order to improve the separation of the other three morphotypes. CVA maximizes the separation between the types based on the morphological characteristics on the first axis: type 2 is well separated from type 3; type 1, in the middle of the graph, overlaps on the left hand side with type 2 and with type 3 on the right hand side (Fig. 4.6). The second axis scores emphasize morphological differences between type 1 and the other two types.

4.3.2. Molecular phylogeny

The phylogeny of Rotaliida was inferred by using the ML method with the HKY model and an estimation of the parameters I and G (HKY+I+G model), and reveals three major groups of sequences, each one composed of several families (Fig. 4.7). The first group comprises the families Rosalinidae, Discorbidae, Planulinidae, Planorbulinidae, Calcarinidae and Nummulitidae. The second distinctive group includes the families Bolivinidae, Cassidulinidae, Uvigerinidae and Buliminidae (*Globobulimina*). The third group is composed of the families Nonionidae, Cibicididae, Pseudoparrellidae, Chilostomellidae, Virguliniellidae, Stainforthidae and Buliminidae (*Bulimina*). The first two groups appeared in all analyses, albeit without strong ML support (45% bootstrap (BS), 0.97 posterior probabilities (PP) and 41% BS, 0.98 PP, respectively). The third group is not stable and it appears only in ML analysis with HKY+I+G model, with very weak support (23% BS). In other analyses, the sequences forming this group appeared as a series of independent lineages branching at the base of other Rotaliida. This is probably due to the insufficient phylogenetic signal related to the slow rates of evolution of these sequences.

Despite the poor resolution of relationships at the base of the Rotaliida, there is a relatively good support for the majority of morphologically recognized families. Among eight families that are represented in our data by at least two genera, five (Nummulitidae, Calcarinidae, Cassidulinidae,

Bolivinidae and Uvigerinidae) are supported by more than 95% BS and 0.97 PP, and only three families appeared as polyphyletic (Planorbulinidae, Nonionidae and Buliminidae). The polyphyly of the three genera *Melonis*, *Pullenia* and *Nonionella*, representing Nonionidae in our analyses, can be an artefact given the fact that the relationships between their slowly evolving sequences are not well resolved. In the case of Buliminidae, the independent origin of *Bulimina* and *Globobulimina* seems more strongly supported. However, the Kishino-Hasegawa test used to compare our tree (Fig. 4.7) with a tree having a constrained topology imposing the monophyly of Buliminidae, show that the difference of likelihood between the forced ($-\ln L = 15,418.0$) and non-forced ($-\ln L = 15,373.4$) topologies is not significant.

The phylogenetic position of the genus *Uvigerina* among the Rotaliida is relatively stable. In all analyses, *Uvigerina* branches together with *Trifarina* and *Rectuvigerina* in the highly supported clade of Uvigerinidae (97% BS, 0.97 PP). This clade appears either as sister to the group Cassidulinidae + Bolivinidae (in ML analyses with HKY+I+G model, cf. Fig. 4.7) or more rarely as sister to the genus *Globobulimina* (in ML analysis with GTR+I+G model, supported by 33%). In the latter case, Bolivinidae and Cassidulinidae form a sister group to Rosalinidae and Planulinidae (data not shown).

Relationships within the clade of Uvigerinidae were analysed using the ML method with the HKY+I+G model, including 28 sequences of *Uvigerina*, *Rectuvigerina* and *Trifarina* as well as 4 sequences of *Globobulimina* used as outgroup. Our analyses (Fig. 4.8a) show the presence of two main clades: one containing *U. peregrina*, *R. phlegeri* and *T. earlandi* and the other comprising *U. elongatastriata* and *U. mediterranea*. There is a good support (95% BS, 1.0 PP) for the clade *elongatastriata* + *mediterranea*, but much weaker for the clade *peregrina* + *phlegeri* + *earlandi* (63% BS, 0.53 PP). Within this second clade, *R. phlegeri* branches as sister group to *T. earlandi* although their grouping is not strongly supported (81% BS, 0.94 PP). All five species form highly supported (91-100% BS, 0.95-1.0 PP) monophyletic clades. Two sequences obtained from GenBank database and identified as *U. akitaensis*, branch independently, one appears as sister

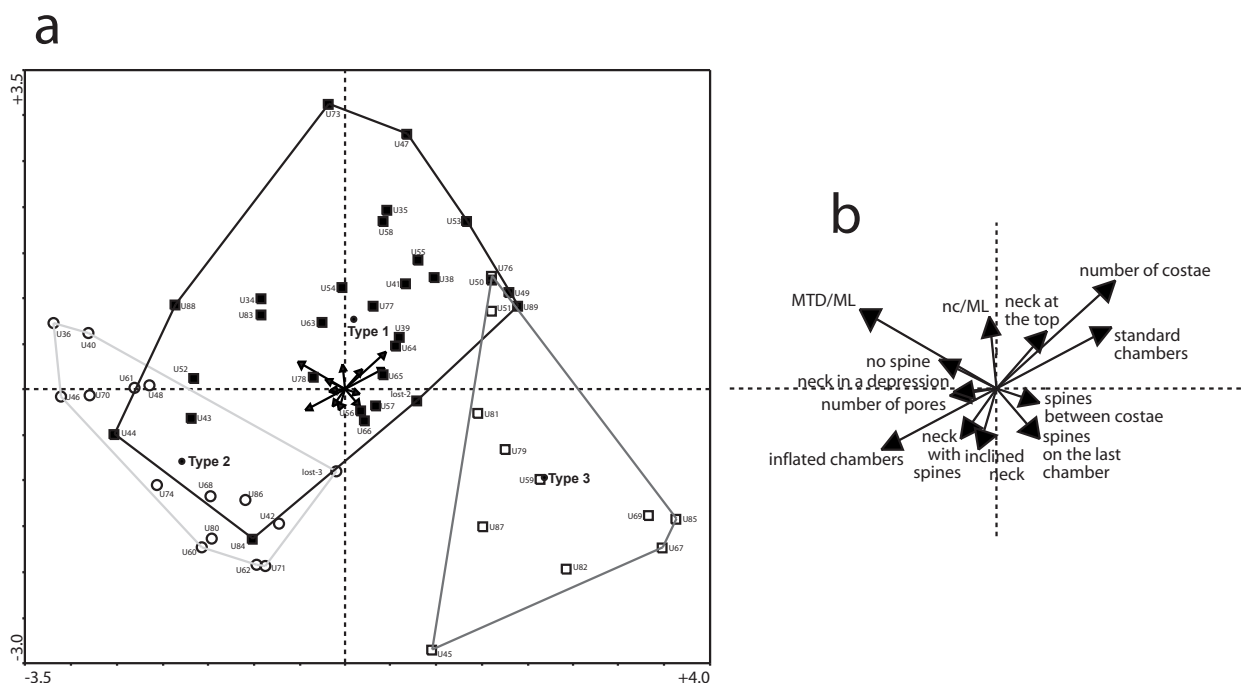


Figure 4.6. CVA triplot:

a) Complete triplot.

b) Detail of the morphological characteristics in the ordination.

CVA eigenvalues q were 1.817 and 0.667 for the 1st and 2nd axis, respectively. The percentage of variance in the 'species' data accounted for by the 1st axis was 32.2%, and 52.2% for both axes together. CVA did not include type 4.

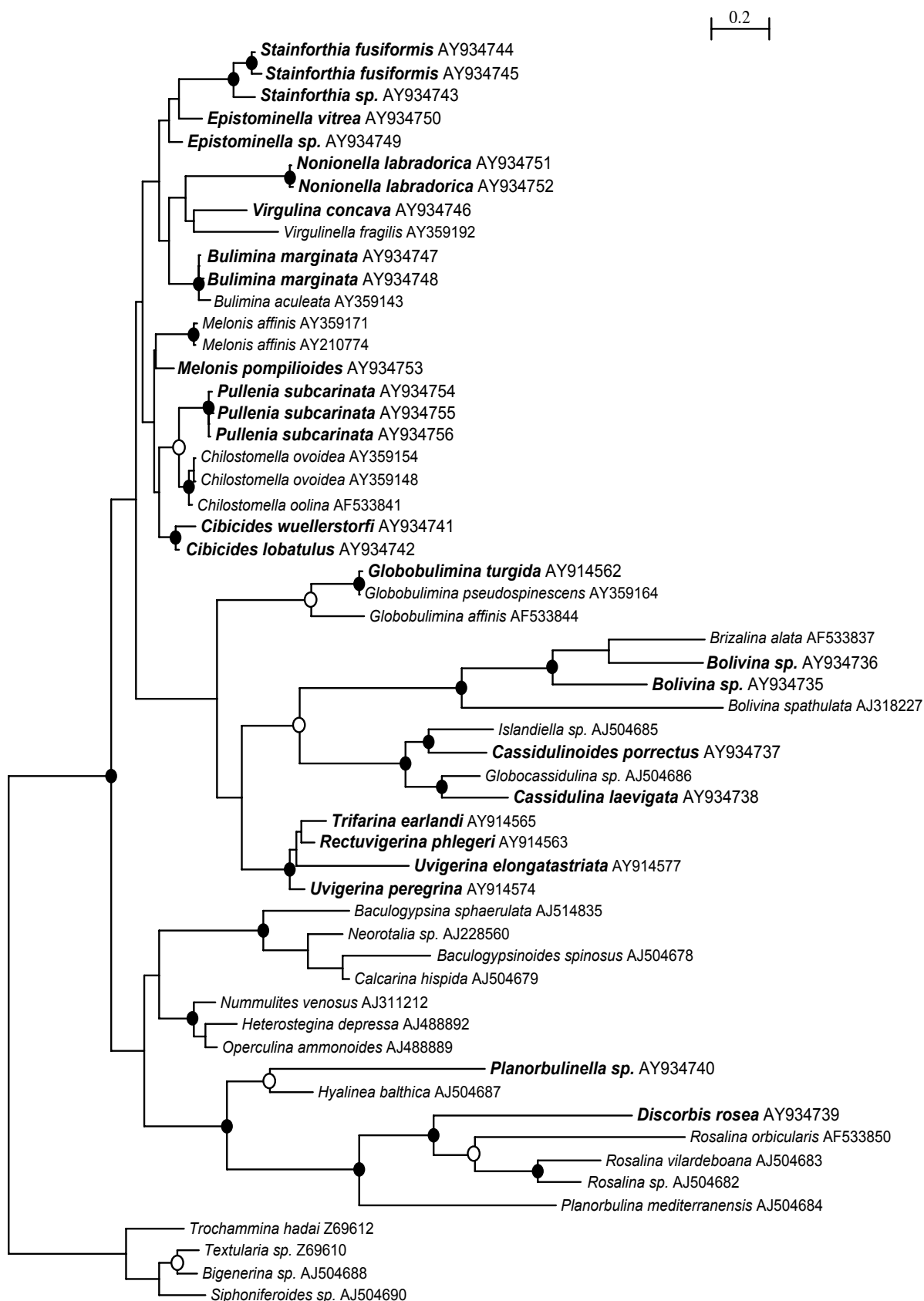


Figure 4.7. Phylogeny of Rotaliida inferred from partial SSU rDNA sequences (695 unambiguously aligned sites) using the ML (HKY+I+G) method. Tree rooted on textulariids. Black dots indicate the internal nodes supported by BS higher than 95% and PP higher than 0.95. White dots indicate the internal nodes supported by BS between 75% and 95%. Species names written in bold designate new sequences, the other ones were taken from GenBank (accession numbers are added).

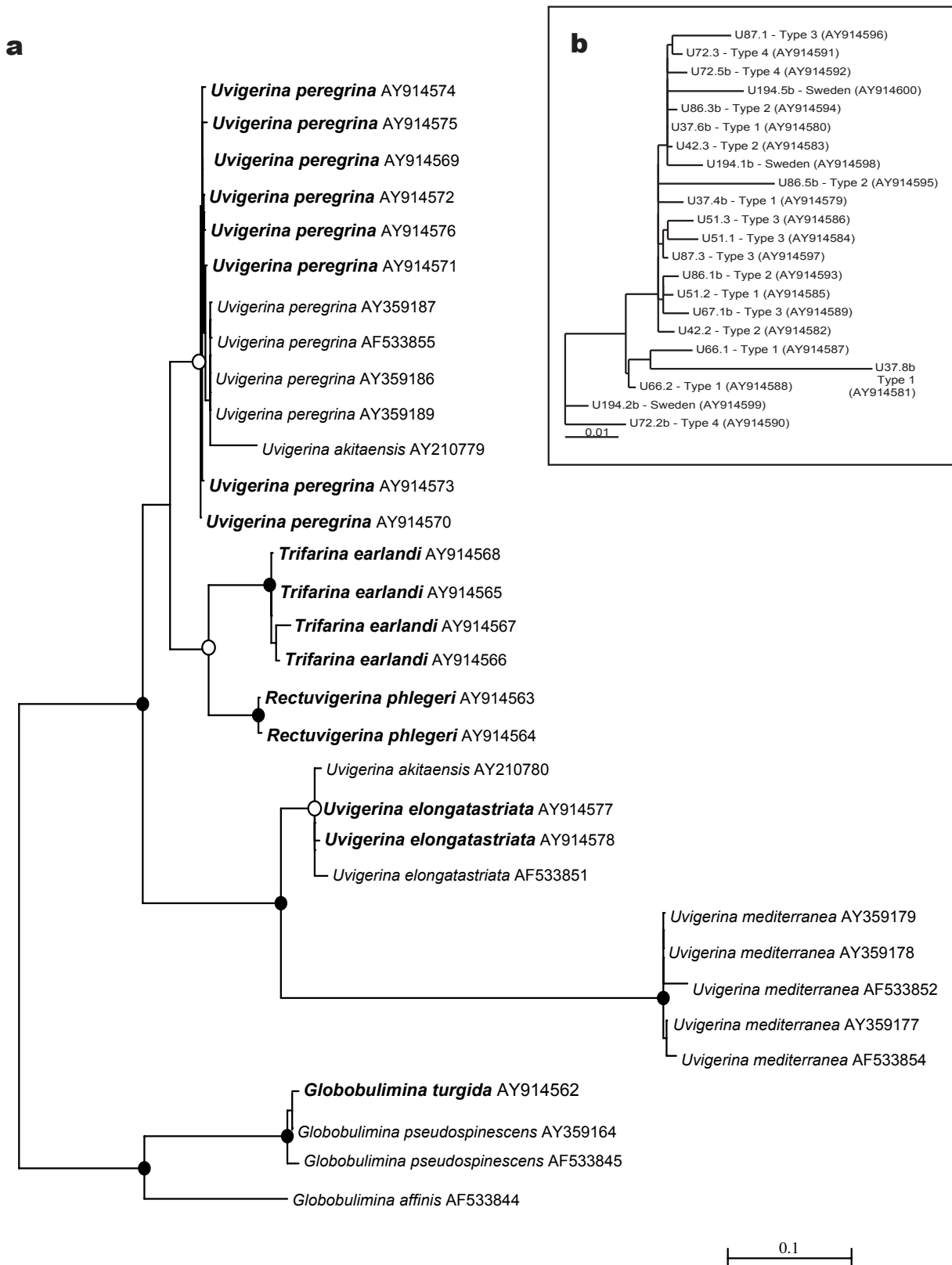


Figure 4.8.

a) Phylogeny of Uvigerinidae inferred from partial SSU rDNA sequences (781 unambiguously aligned sites) using the ML (HKY+I+G) method. Tree rooted on *Globobulimina*. Black dots indicate the internal nodes supported by BS higher than 95% and PP higher than 0.95. White dots indicate the internal nodes supported by BS between 75% and 95%. Species names written in bold designate new sequences, the other ones were taken from GenBank (accession numbers are added).

b) Phylogeny of *U. peregrina* inferred from the ITS sequences (871 unambiguously aligned sites). Accession numbers are added.

to *U. peregrina*, while the other is almost identical to the sequences of *U. elongatastriata*. This suggests that the determination of this species needs to be revised. It is interesting to note that D. B. Scott (Scott et al., 2000) considers *U. akitaensis* as a variant of *U. peregrina*, which seems to be confirmed by the molecular data.

Because a large morphological variation was observed within the population of *U. peregrina* sampled at Oslo Fjord (see previous section), we examined its genetic diversity by sequencing the ITS region, a part of the ribosomal DNA which is much more variable than the SSU. We analysed 22 sequences from 9 different specimens of which the PCR products were cloned. With the exception of a few rapidly evolving sequences (below 5% of divergence), the divergence of most of them is below 1%. The variations in ITS are mainly limited to single nucleotide substitutions and few differences in length of repetitive regions. The phylogenetic analysis of these sequences (Fig. 4.8b) does not reveal any particular grouping, neither according to the origin of the specimens (Oslo Fjord versus Swedish coast of Skagerrak) nor according to their morphology. The sequences of different clones originating from the same specimen often branch separately, suggesting that the range of intra- and interindividual ribosomal variation is about the same.

4.4. Discussion

4.4.1. Molecular phylogeny of Rotaliida

In the first attempt to establish the phylogeny of Rotaliida based on molecular data, Ertan et al. (2004, Fig. 7) distinguished two major groups: the buliminids and the rotaliids. The buliminids were composed of the genera *Bolivina*, *Globobulimina*, *Uvigerina*, *Bulimina* and *Virgulinea*, while the rotaliids included *Ammonia*, *Elphidium*, *Haynesina* and *Rosalina*, as well as an independent group comprising *Chilostomella* and *Melonis*. The distinction of these two rotaliid groups agrees with morphology-based classifications (cf. Haynes, 1981). However, the limited number of rotaliid species (11) used in the study of Ertan et al. (2004) and a very weak support for these two groups shed some doubts on the validity of such a distinction.

In our study we have examined the same fragment of the SSU rRNA gene, but we significantly increased the number of examined taxa by adding 21 new genera to our analyses. The general structure of our tree is similar to that obtained by Ertan et al. (2004). Although we did not include *Ammonia*, *Elphidium* and *Haynesina* in our dataset, independent analyses confirm their branching close to *Rosalina* (data not shown). The main difference between our results and those of Ertan et al. (2004) consists in the position of *Bulimina aculeata* and *Virgulinea fragilis*. These two species appeared at the base of buliminids in Ertan et al. (2004), while they branch close to *Stainforthia*, *Epistominella* and *Nonionella* in all our trees.

The independent branching of *Bulimina* in our data is surprising given the fact that the position of this genus together with *Globobulimina* in the family Buliminidae, and the placement of this family together with Uvigerinidae in the superfamily Buliminacea, has never been questioned (Galloway, 1933; Cushman 1959; Loeblich & Tappan 1964, 1988). The characteristic features of Buliminacea are a high trochospiral coil and an internal toothplate which connects the aperture with the previous chamber foramen (Loeblich & Tappan, 1988). The internal toothplate is generally considered a very important taxonomic character and was used by some authors to group all foraminifera possessing this feature in higher rank categories, such as the orders Dentata (Hofker, 1956) and Buliminida (Haynes, 1981), or the superorder Buliminoida (Mikhalevich & Debenay, 2001). In view of our data the internal toothplates could have appeared independently several times in the evolution of foraminifera. However, we cannot exclude that the independent branching of *Bulimina* is an artefact of partial single gene phylogeny. Indeed, the support for the basic groups of Rotaliida is rather weak in all our analyses (Fig. 4.7). As shown by statistical tests, the relations between slowly evolving groups of sequences are not resolved and closer relationships between *Bulimina* and *Globobulimina* cannot be completely excluded. Moreover, an independent analysis of actin-coding gene sequences shows that although *Bulimina* and *Globobulimina* branch separately, both

genera lack an intron characteristic for rotaliids, which could suggest that they are not so distantly related (Flakowski et al., 2005). Clearly, additional sequence data on complete SSU rRNA and protein-coding genes are necessary to resolve this problem.

4.4.2. *Uvigerina*, *Rectuvigerina* and *Trifarina* are closely related

Phylogenetic analysis of our data suggests that besides the genus *Uvigerina*, the clade of Uvigerinidae also includes at least some members of the genera *Rectuvigerina* and *Trifarina* (Fig. 4.8a). In all analyses, both genera group with *U. peregrina* and although this grouping is not very well supported (63% BS, 0.53 PP), it is highly unlikely that the three *Uvigerina* species (*U. peregrina*, *U. elongatastriata* and *U. mediterranea*) form a monophyletic group. The morphological criteria used to separate the three genera are not very solid. For *Trifarina* the discriminating character is the triangular cross section: all other criteria are the same as for *Uvigerina* (Haynes, 1981; Loeblich & Tappan, 1988). *Rectuvigerina* differs from *Uvigerina* by one or more uniserial chambers and by an internal siphonlike toothplate (Mathews, 1945). However, a tendency to uniseriality is also observed in other species belonging to *Uvigerina*. Van der Zwaan et al. (1986a) argued that the tendency to reduced seriality is characteristic of more advanced, geologically younger morphologies. Moreover, toothplate morphology is extremely variable, even between populations. Separate classification of *Rectuvigerina* in the family Siphogenerinoididae Saidova, 1981 is not supported by our data. Indeed, *R. phlegeri* was included in the genus *Uvigerina*, as a member of the *U. bononiensis* group by Van der Zwaan et al. (1986a). Although we could not examine any other representatives of this group, the division of *Uvigerina* proposed by these authors is congruent with our molecular data, that show a separation between the *U. peregrina* and *U. elongatastriata* + *U. mediterranea* clades.

4.4.3. Skagerrak *U. peregrina* is genetically homogeneous

The statistical analyses of the morphology of the Oslo Fjord population of *Uvigerina* show a separation between four different morphological types (Figs. 4.4-4.6). Although overlaps were observed between type 1 and types 2 and 3, these different morphotypes could, in theory, be described as separate morphospecies.

Several recent studies revealed cryptic diversity of well established morphospecies in planktonic (Huber et al., 1997; de Vargas et al., 1999, 2001, 2002; Darling et al., 2000) and benthic (Pawlowski et al., 1995; Holzmann et al., 1996; Holzmann and Pawlowski, 1997, 2000; Tsuchiya et al., 2000, 2003; Hayward et al., 2004) foraminifera. Given the high morphological variability observed in *U. peregrina* from Oslo Fjord, we expected to find a high genetic divergence within this population. The ITS sequences of nine specimens representing the different morphotypes of *U. peregrina* we examined did not confirm our expectations (Fig. 4.8b). The divergence observed in these sequences corresponds to the level of intraspecific variations, because the difference observed between sequences of distinct specimens is comparable to the one between clones from one individual. This confirms a certain homogeneity also detected in other North Sea foraminifera studied in our laboratory, especially *Ammonia* sp. and *Elphidium williamsoni* (unpublished data), although ITS sequencing was not carried out for these species. The morphological variability of *U. peregrina* noticed in our samples seems to be within the range of variability characteristic for a single species. To accurately define species in *Uvigerina* in terms of morphological and genetic variations, more precise studies on morphometry and genetic variations in other species of this genus would be necessary. The high morphological plasticity of *Uvigerina* species observed in this population could theoretically allow to distinguish separate morphospecies. However, the low genetic diversity obtained here shows that the origin of the variation could be ecological rather than genetic in nature and this should be taken into consideration when using this genus as a proxy in paleoecological reconstructions.