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RESEARCH ARTICLE

Phylogenetic position of parabasalid symbionts from the termite Calotermes flavicollis based on small subunit rRNA sequences

Delphine Gerbod[1]
Virginia P. Edgcomb[2]
Christophe Noël[1,3]
Pilar Delgado-Viscogliosi[4,5]
Eric Viscogliosi[1,3]

[1]Laboratoire de Biologie Comparée des Protistes, UPRESA CNRS 6023, Aubière, France
[2]Center for Molecular Evolution, Marine Biological Laboratory, Woods Hole, USA
[3]Institut Pasteur, INSERM U167, Lille, France
[4]Laboratoire d'Oncologie Moléculaire, Centre Jean Perrin, Clermont-Ferrand, France
[5]Institut Pasteur, Eaux et Environnement, Lille, France

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Correspondence to:
Eric Viscogliosi. Institut Pasteur. INSERM U167.
1, Rue du Professeur Calmette.
59019 Lille. France
Tel.: +33-3-20877960
Fax: +33-3-20877888
E-mail: eric.viscogliosi@pasteur–lille.fr

Summary Small subunit rDNA genes were amplified by polymerase chain reaction using specific primers from mixed-population DNA obtained from the whole hindgut of the termite *Calotermes flavicollis*. Comparative sequence analysis of the clones revealed two kinds of sequences that were both from parabasalid symbionts. In a molecular tree inferred by distance, parsimony and likelihood methods, and including 27 parabasalid sequences retrieved from the data bases, the sequences of the group II (clones Cf5 and Cf6) were closely related to the Devescovinidae/Calonymphidae species and thus were assigned to the Devescovinidae *Foaina*. The sequence of the group I (clone Cf1) emerged within the Trichomonadinae and strongly clustered with *Tetratrichomonas gallinarum*. On the basis of morphological data, the Monocercomonadidae *Hexamastix termitis* might be the most likely origin of this sequence.

Key words Parabasalid protists · Termites · Small subunit rRNA · Phylogeny · Molecular evolution

Introduction

Parabasalids (phylum Parabasalia) are anaerobic flagellated protists which contain at least one parabasal apparatus consisting of a parabasal body (Golgi complex) and a parabasal filament [4, 22]. They also can be distinguished by the presence of the microtubular axostyle-pelta complex, composed of a sheet of cross-linked microtubules that are the longitudinal axis of the cell. All parabasalid genera studied to date lack mitochondria and peroxisomes, but have specialized organelles, called hydrogenosomes, in which anaerobic metabolism takes place [27, 31]. These microorganisms carry out a special type of closed mitosis called cryptopleuromitosis, characterized by the persistence of the nuclear envelope and the presence of an extra-nuclear spindle [3, 42]. Moreover, ribosomal RNAs from parabasalids have the molecular sizes typical of large subunit (LSU) and small subunit (SSU) prokaryotic ribosomal RNAs [16, 32], which may be consistent with the postulated early evolutionary divergence of these protists [7]. This was confirmed by phylogenetic analyses inferred from the comparison of SSU rRNA sequences, in which the amitochondriate protist groups, microsporidia, diplomonads and parabasalids, represent the earliest eukaryotic lineages [29, 38].

On the basis of cytological studies, parabasalid taxa were classified into two orders: Trichomonadida and Hypermastigida [6]. Species of the trichomonads (order Trichomonadida) were separated into five families [4, 21, 28, 35]: Monocercomonadidae, Trichomonadidae (divided into two main subfamilies: the Trichomonadinae, including the human parasite *Trichomonas vaginalis*, and the Tritrichomonadinae, including the bovine parasite *Tritrichomonas fœtus*), Cochlosomatidae (only one genus recently studied [35]), Devescovinidae and Calonymphidae. Most Monocercomonadidae, Trichomonadidae and Cochlosomatidae species are associated with the respiratory, digestive and reproductive systems of vertebrates, including mammals, and many of them have been cultured. Some Monocercomonadidae species are known to be free-living and have been isolated from sediments [10, 11]. Devescovinidae, Calonymphidae and hypermastigids (order Hypermastigida), are all found exclusively in association with the digestive tract, especially in the hindgut of termites and wood-eating cockroaches [13, 14, 47]. The relationships of the parabasalids with their hosts and with other symbionts are complex and in most cases not well understood. However, parabasalids are

thought to play a major role in digestion of ingested cellulose and wood [15, 48].

The termite *Calotermes flavicollis* Fabricius is found mainly in Southern France. Aside from a number of bacteria, it harbors at least five parabasalids in the hindgut [13, 14, 47], one of which is the hypermastigid *Joenia annectens* Grassi. It contains also trichomonad species of the family Monocercomonadidae, *Hexamastix termitis* Grassi and *Tricercomitus divergens* Kirby. Finally, it has also two further trichomonads, *Foaina dogieli* Duboscq and Grassé and *Foaina grassii* Duboscq and Grassé, which are assigned to the family Devescovinidae. In addition, two oxymonad flagellates, *Microrhopalodina inflata* Grassi and Foa and *Opisthomitus avicularis* Duboscq and Grassé, can also be found in the hindgut of this termite. None of these microorganisms has been cultivated yet. These parabasalid taxa have been characterized only on the basis of their morphology, and their exact phylogenetic position remains uncertain. Molecular sequences, especially SSU rRNA sequences, are frequently used to analyze the phylogeny of microorganisms (for review see Olsen and Woese [34]). Specific primers allow the amplification of SSU rRNA coding regions from selected eukaryotes such as the parabasalids, and can be used to amplify species which are difficult or even impossible to culture in laboratories [30]. Several authors obtained sequences from parabasalids including trichomonads [1, 2, 9, 10, 12, 17, 23, 37] and hypermastigids [8, 23, 33]. These have allowed for the phylogenetic analyses of a large number of parabasalid species. This approach has enhanced our ability to investigate the molecular diversity of parabasalid symbionts in the hindgut of termites. In this paper, SSU rRNA genes were amplified from whole-hindguts of *Calotermes flavicollis*. The phylogeny of parabasalids was inferred using these new sequences and we present the position of the yet uncultivated trichomonad genus *Foaina*.

Materials and methods

Collection of termites and DNA extraction Wood-eating termites *Calotermes flavicollis* (Isoptera, Kalotermitidae) were collected in wood of vines in the vicinity of Banyuls-sur-mer, France. Termites-infested wood, moistened with distilled water, was kept in plastic boxes at room temperature. The hindguts of five to ten *Calotermes flavicollis* were removed by forceps, transferred to cavity slides and the contents released by dicing them in Ringer's solution (0.12 M NaCl, 3.5 mM KCl, 2 mM $CaCl_2$, 2.5 mM $NaHCO_3$, pH 7.2–7.4). The solution was centrifuged for 4 min at 1300 ×*g*, and the pellet resuspensed in Ringer's. For micrographs, a small part of the cell suspension was fixed in 4% paraformaldehyde in Ringer, kept overnight at 4ºC, washed twice in phosphate buffered saline (PBS) pH 7.2, and allowed to adhere to the surface of polysine microslides (Menzel-Glaser, Germany). Parabasalid cells were viewed using a Leitz Fluovert FU microscope, and video recordings were made in real time onto Sony Digital Still Recorder. Single images were captured and analyzed by the Leica Qwin System. DNA was purified from the cell suspension by standard phenol/chloroform extraction and precipitation with ethanol and acetate.

PCR amplification, cloning and sequencing SSUrRNA genes were amplified from the purified DNA by PCR using EurobioTaq II DNA polymerase (Eurobio, Les Ulis, France) according to the manufacturer's instructions. The PCR primers, specific for trichomonad SSU rRNA genes [17, 30] were: the forward primer Euk5 (5´-TTATTAGCGGCCGCAYTTGGT TGATYCTGCC-3´) and the reverse primer Euk3 (5´-ATATG CGGCCGCTTACGACTTTTSCTTCC-3´) where Y represents C or T and S represents C or G. After the denaturation step at 94ºC for 5 min, 40 cycles of amplification (1 min at 94ºC, 1 min at 52ºC and 2 min at 72ºC) were performed, followed by an extension step at 72ºC for 15 min. A prominent band of approximately 1.5 kb was electroeluted after fractionation by agarose gel electrophoresis and cloned into the T/A plasmid vector pGEM-T Easy (Promega Biotech, Madison, WI). The standard alkaline lysis method was used for minipreparation of plasmid DNA. The resulting plasmids were sequenced on both strands with the dideoxy termination method using T7 DNA sequencing kits (Pharmacia Biotech, Piscataway, NJ). Problem areas and compressions were solved using the Deaza G/A T7 sequencing kit (Pharmacia Biotech).

Table 1 Organisms and accession numbers used for phylogenetic analyses

Organism	Accession number
Achyla bisexualis	M32705
Diaphanoeca grandis	L10824
Oryza sativa	X00755
Oxytricha granulifera	X53486
Toxoplasma gondii	M97703
Parabasalids	
Calonympha sp.	X97976
Coronympha octonaria	U17504
Devescovina sp.	X97974
Dientamoeba fragilis	U37461
Ditrichomonas honigbergii	U17505
Hypotrichomonas acosta	AF076959
Metadevescovina extranea	X87132
Metadevescovina polyspira	U17506
Monocercomonas sp.	U17507
Monotrichomonas carabina	AF072906
Monotrichomonas sp.	AF072905
Pentatrichomonas hominis	AF124609
Pentatrichomonoides scroa	X87131
Porotermes adamsoni symbiont	AF052702
Pseudotrichomonas keilini	U17511
Pseudotrypanosoma giganteum	AF052706
Reticulitermes flavipes gut symbiont 1	U17508
Tetratrichomonas gallinarum	AF124608
Trichomitus batrachorum strain BUB	AF076958
Trichomitus batrachorum strain R105	AF124610
Trichomitus trypanoides	X79559
Trichomonas tenax	U37711
Trichomonas vaginalis	U17510
Trichonympha agilis	AB003920
Trichonympha cf. collaris	AF023622
Trichonympha magna	AF052713
Tritrichomonas foetus ATCC 30924	U17509

Phylogenetic analysis The SSU rDNA gene sequences obtained in this study were aligned with a set of eukaryotic sequences including 27 other parabasalids retrieved from data bases (Table 1). We restricted the phylogenetic inferences to 1403 sites that could be unambiguously aligned according to conservation of primary and secondary structures. Full-length alignment and sites used in analyses are available upon request. Phylogenetic trees were constructed using maximum-likelihood (ML), maximum parsimony and distance methods implemented in PAUP* version 4.0.0d63 [40]. Parsimony analyses were performed with TBR branch-swapping and 1000 bootstrap replicates. Heuristic searches under likelihood criteria were performed using 1000 random-addition replicates with TBR branch-swapping. Models for use in likelihood searches were evaluated using the likelihood ratio test (LRT). Likelihood ratio tests indicated that all models tested simpler than the GTR+I+gamma model had significantly poorer fit to the data for the parsimony-based reference topology. Therefore, maximum likelihood and minimum evolution distance methods were performed using a GTR model with six classes of substitutions, unequal base frequencies, proportion of invariant sites of 0.16702, and the evolutionary rate of the remaining portion of sites varying according to a gamma distribution with a shape parameter of 0.422994. Distance trees were calculated with 1000 bootstrap replicates. Maximum likelihood bootstrap analyses were done with 100 replicates. After parameter optimization, heuristic searches under maximum likelihood found a single best tree (ln L = –13766.89578).

Results and Discussion

Identification of parabasalid cells In the vicinity of Banyuls-sur-mer (Southern France), two genera of termites can be found: *Calotermes flavicollis*, easily recognizable by its larger size, and *Reticulitermes lucifugus* (Isoptera, Rhinotermitidae). Although the exact composition of hindgut microbiota can change, several authors have identified five parabasalid species in the *Calotermes flavicollis* hindgut [13, 14, 47]. We found all these taxa in our cell suspensions and each of them can be recognized from their size and number of flagella. Two species of the genus *Foaina* have been identified: *Foaina dogieli* (ca. 30 μm, Fig. 1A) and *Foaina grassii* (ca. 12 μm). Both species have three anterior flagella and a recurrent one that adheres to the cell-body membrane for a variable length. Among the Monocercomonadidae family, *Hexamastix termitis* (around 20 μm) is

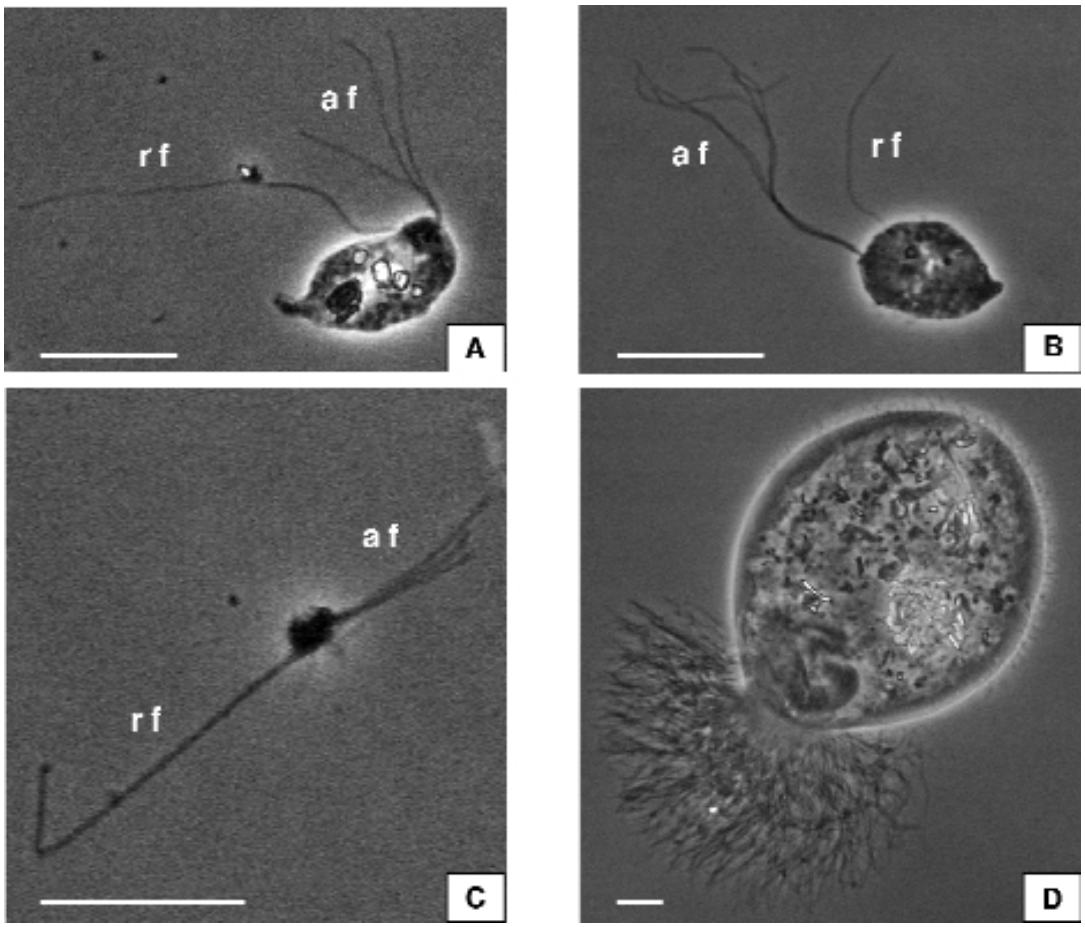

Fig. 1 Micrographs of the parabasalid symbionts identified in the hindgut of *Calotermes flavicollis*. A: *Foaina dogieli*; B: *Hexamastix termitis*; C: *Tricercomitus divergens*; D: *Joenia annectens*. Abbreviations: **rf** = recurrent flagellum; **af** = anterior flagella. Scale bars each represent 20 μm

the only genus in which fully developed individuals have five anterior flagella (Fig. 1B). The recurrent flagellum does not adhere to the body for an appreciable distance. The second Monocercomonadidae species is *Tricercomitus divergens* (Fig. 1C), one of the smallest flagellated protists identified so far (ca. 1 to 5 μm). It has three anterior flagella and a recurrent one which adheres to the whole length of the cell-body membrane. The recurrent flagellum is typically many times longer than the body. Finally, the last parabasalid species found in our preparations correspond to large cells (variable size but generally more than 100 μm) identified as *Joenia annectens* (Fig. 1D). In hypermastigids, certain characters have undergone an exaggerated development such as the multiplication of flagella (many thousands in *Joenia* [19]) and parabasal fibers, each supporting a Golgi complex [20].

Analysis of the SSU rDNA sequences obtained from whole-hindgut of *Calotermes flavicollis* In parabasalids, the SSU rRNA is much smaller than in most eukaryotes, characteristically less than 1600 bp. The amplification of the SSU rDNA coding region of parabasalids analyzed in this study produced a DNA fragment of about 1500 bp as determined by gel electrophoresis. This fragment was eluted and then cloned into the pGEM-T vector. Six clones containing full length PCR products were selected (Cf1 to Cf6). To evaluate sequence heterogeneity in this set of clones, we initially sequenced about 300 bp from the 5´ and 3´ end of the clones which includes the rapidly evolving variable domains V1, V2 and V5 (ca. *Escherichia coli* positions 77–109, 187–265, and 1439–1463 from 5´ end [39]). Sequenced clones were found to fall into two different groups. The first group included clones Cf1 to Cf4, whose sequences differed at only a few positions, and thus one clone (Cf1) was sequenced completely. The dissimilarity observed between individual clones of group I may be due to amplification errors or micro-heterogeneity in the SSU rDNA coding regions. The group II included clones Cf5 and Cf6. The 5´ and 3´ sequences of both clones differed at thirteen positions, many more differences than those observed between individual clones of the group I. Since the clones Cf5 and Cf6 could correspond to distinct species, the entire SSU rDNA sequences of both clones have been obtained. The nucleotide sequences obtained in this study are available in the GenBank data base under the accession numbers: Cf1-AF215856; Cf5-AF215857; Cf6-AF215858. Sequencing of the cloned fragments showed that the exact length excluding the amplification primers is 1516 bp for the clone Cf1 and 1494 bp for both clones Cf5 and Cf6. The G+C contents of the sequenced regions were 47.9% for the clone Cf1 and 49.3% for both clones Cf5 and Cf6. These lengths and compositions are comparable to the SSU rRNA genes in other trichomonads in the data base, with the known exception of *Dientamoeba fragilis* [37].

Phylogenetic analysis of the parabasalids The sequences of clones Cf1, Cf5 and Cf6 were added to an existing database of 27 parabasalid SSU rDNA genes. The SSU rDNA sequences of *Oxytricha granulifera*, *Toxoplasma gondii*, *Oryza sativa*, *Diaphonoeca grandis* and *Achyla bisexualis* were used as outgroups for rooting the parabasalid tree. This data set included 1,403 nucleotides which we analyzed with maximum-likelihood (ML), distance matrix and maximum parsimony methods. The ML tree (Fig. 2) shows that parabasalids compose a robust monophyletic group. As described in our previous studies [9, 10, 46], four well-supported clusters are identified. A first clade consists of nearly all species from the subfamily Trichomonadinae (*Tetratrichomonas gallinarum*, *Trichomonas tenax*, *Trichomonas vaginalis*, *Trichomitus trypanoides*, *Pentatrichomonoides scroa*, *Pentatrichomonas hominis* and *Pseudotrypanosoma giganteum*), including the new sequence Cf1, and all the free-living genera belonging to the Monocercomonadidae (*Monotrichomonas*, *Ditrichomonas* and *Pseudotrichomonas*). A second clade unites the Devescovinidae (*Metadevescovina polyspira*, *Metadevescovina extranea* and *Devescovina* sp.) and the Calonymphidae (*Calonympha* sp. and *Coronympha octonaria*). This group also includes the *Porotermes adamsoni* symbiont sequences as shown by Keeling et al. [23] and the new sequences Cf5 and Cf6. The third clade is a small group that contains *Trichomitus batrachorum* and *Hypotrichomonas acosta* whereas the last clade comprises the three hypermastigid species of the genus *Trichonympha*. Most of the salient points of the SSU rRNA tree have been already discussed (for review see Viscogliosi et al. [46]), such as (i) the late emergence of the free-living trichomonads, suggesting that they descend from endobiotic ancestors and thus that they are secondarily free-living, (ii) the polyphyly of the Monocercomonadidae and of the genus *Trichomitus*, indicating the need for a revision of the parabasalid taxonomy; and (iii) the earliest emergence of the hypermastigids (that have a complex cytoskeleton) whereas the Monocercomonadidae (that have a rudimentary cytoskeleton) were thought to be the most ancient parabasalid lineage, suggesting that the traditional polarization of cytoskeleton complexity from simple to complex must be turned upside down.

Identification of the new SSU rDNA gene sequences As stated above, the sequences Cf5 and Cf6 are closely related to the large group including Devescovinidae and Calonymphidae taxa. This grouping is supported by bootstrap resampling in ML (72%), distance matrix (93%) and parsimony (53%) methods, and is consistent with the morphological similarity between these taxa as identified by earlier light- and electron-microscopy studies [4, 21]. Devescovinidae and Calonymphidae are mainly characterized by the presence of a specialized organelle called the cresta by Kirby [24]. This organelle may be homologous with the undulating membrane of Trichomonadidae. In the latter family, the recurrent flagellum is attached to the cell body. The undulating membrane, which is probably the principal means of locomotion in these genera [26] consists of the recurrent flagellum and microfibrillar structures located below the membrane of the cell along the adherent zone with the recurrent flagellum [4]. In Devescovinidae and Calonymphidae, the recurrent flagellum

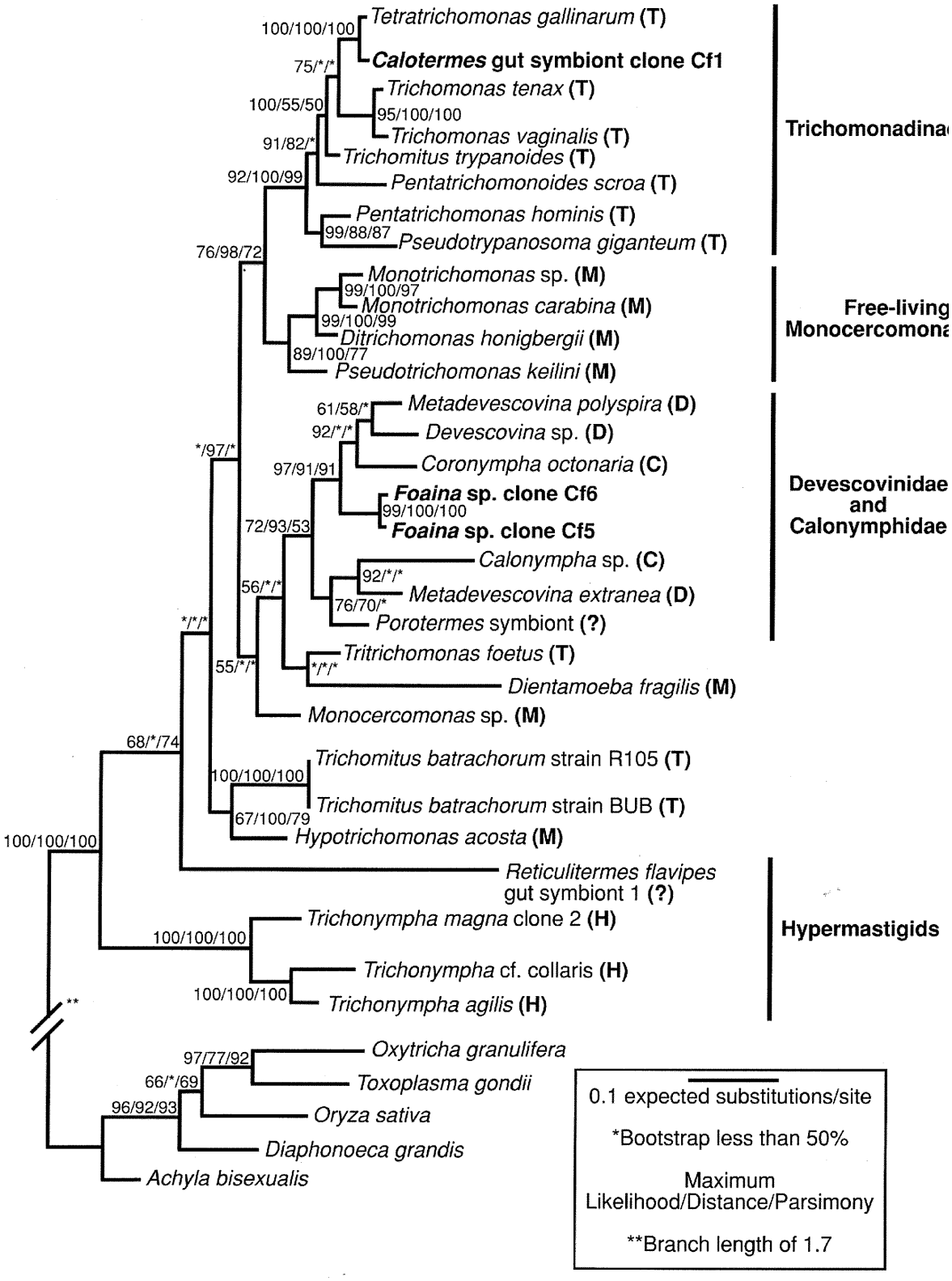

Fig. 2 Phylogeny of parabasalids based on the comparison of SSU rDNA sequences. The tree was constructed with PAUP using the maximum likelihood/distance/parsimony. Asterisks designate nodes with bootstrap values below 50%. The systematic of each parabasalid taxon is indicated: (M) = Monocercomonadidae; (T) = Trichomonadidae; (D) = Devescovinidae; (C) = Calonymphidae; (H) = Hypermastigid; (?) = Unidentified. New sequences obtained in this study (clones Cf1, Cf5 and Cf6) are indicated in bold

adheres to the plasma membrane of the cell; below there is a complex system of fibers which make up the cresta. Calonymphidae differ from Devescovinidae in the polymonad organization of the calonymphid genera, characterized by multiplication of the nuclei and of their mastigont systems, producing many karyomastigonts per cell. Both karyomastigonts and akaryomastigonts are present in some genera.

Differentiation of the genera in these two groups is in no way dependent on the number of flagella. This number is constant in the individual mastigonts of all genera, including 1 recurrent and 3 anterior flagella. Consequently, taxonomy among Devescovinidae has been mainly based on differences in structure and development of other mastigont organelles. Additional characters have been found for taxonomic purposes among the Calonymphidae, such as the presence of akaryomastigonts and their numerical proportion to the karyomastigonts. The genus *Foaina* is the only member of Devescovinidae (or possibly Calonymphidae) reported in *Calotermes flavicollis* [47] and thus is a very likely candidate for the origin of Cf5 and Cf6 sequences. Additional observations strengthen this assignment. Few electron microscopy data are available in *Foaina* [4] but it has been clearly shown that this genus shares some characteristics with all other devescovinid taxa such as the presence of a cresta. The cresta's basic structure appeared to be related to that of *Devescovina,* the type genus for this family. However, several ultrastructural differences have been described between these two genera regarding the length of the cresta, the development of the axostyle-pelta complex, and the form of the parabasal body. In our tree, the Cf5 and Cf6 sequences effectively emerged in a clade including *Devescovina* sp. (but also *Metadevescovina polyspira* and *Coronympha octonaria*). This clustering was supported by high bootstrap values in ML (97%), distance methods (91%) and parsimony (91%). In addition, *Foaina* has been proposed to be a primitive genus among the Devescovinidae on the basis of several morphological characters [4, 13, 18, 25]. In this regard we have noted that Cf5 and Cf6 sequences emerged early in the Devescovinidae/Calonymphidae group in our phylogenetic tree, which is in agreement with previous cytological studies. Finally, Cf5 and Cf6 sequences have 98.2% identity (1503 positions compared), comparable to the percentage observed, for instance, between the two *Trichomonas* species, *Trichomonas vaginalis* and *Trichomonas tenax* (97.7% similarity and 1584 positions compared). In *Calotermes flavicollis*, two distinct species are solely identified in the genus *Foaina*, which suggests that Cf5 and Cf6 sequences may correspond to *Foaina dogieli* and *Foaina grassii*. For all this, we claim that the Cf5 and Cf6 sequences correspond to the genus *Foaina*. Since two species of this genus are described and we are not able to distinguish these by our molecular approach, we assign our Cf5 and Cf6 sequences to *Foaina* sp.

Other aspects that address the origin and taxonomy of the Devescovinidae/Calonymphidae group, including *Foaina,* can also be noted. First, the grouping of *Tritrichomonas fœtus* and *Monocercomonas* sp. is very tenuous in our phylogenetic tree, as observed in previous analyses [9, 17, 23, 33]. However, *Tritrichomonas* and *Monocercomonas* sp. are strongly clustered in phylogenies based on large subunit rRNA [41], iron-containing superoxide dismutase [44], glyceraldehyde-3-phosphate dehydrogenase [45] and fumarase (Gerbod et al., in preparation) sequences, in correlation with cytological studies [4, 21]. Note that *Tritrichomonas* and *Monocercomonas* sp. emerged as sister groups of the Devescovinidae/Calonymphidae clade in our tree. It has been shown that *Tritrichomonas* and *Monocercomonas* sp. share morphological characters with the Devescovinidae/Calonymphidae taxa [4, 21]. In the light of these data, it has been suggested that the Devescovinidae/Calonymphidae might have evolved from the genera *Tritrichomonas* and *Monocercomonas* sp., which is in agreement with our molecular data. It must be taken into account that a taxon is considered to be natural when it appears on phylogenetic trees as a monophyletic clade. However, the Devescovinidae do not form a monophyletic group (*sensu* Hennig) in our phylogenetic tree. We also note the polyphyly of the genus *Metadevescovina*; one of the two species, either *Metadevescovina extranea* or *Metadevescovina polyspira* should be reclassified. Pending further studies, it seems advisable to retain all of the members of the Devescovinidae/Calonymphidae group in a single family since this clustering should render this new group monophyletic. Such observations underline the extreme difficulty to identify homologous morphological features, and to define polarization of character states for systematic purposes in parabasalids.

The last unidentified sequence that we analyzed was that of clone Cf1. In our phylogenetic tree, the Cf1 sequence is closely related to the Trichomonadinae and, particularly, to *Tetratrichomonas gallinarum* with high bootstrap support. According to our observations and to previous studies [13, 47], none of the Trichomonadidae taxa (Trichomonadinae or, possibly, Tritrichomonadinae) has been identified in the hindgut of *Calotermes flavicollis.* As discussed above, we have assigned the Cf5 and Cf6 sequences to *Foaina*. In addition, *Joenia annectens* shares some characteristics with all other hypermastigid taxa. The hypermastigid sequences available so far from the genus *Trichonympha* and possibly *Reticulitermes flavipes* gut symbiont 1 show a basal emergence that is very distantly related to the sequence Cf1. This means that the unidentified Cf1 sequence can be assigned to either the species *Tricercomitus divergens* or *Hexamastix termitis*, although both species belong to the Monocercomonadidae and not to the Trichomonadidae. The Trichomonadidae are characterized by a complex cytoskeleton. These taxa possess an undulating membrane and a broad striated root, the costa, which is connected to the basal bodies [4, 43]. The costa extends immediately below the undulating membrane within the cytoplasm and it is assumed that it serves as a mechanical support for the undulating membrane. In contrast, the Monocercomonadidae have no costa, and the undulating membrane

present in some members is weakly developed. However, we have already shown that the presence or absence of the costa and undulating membrane are not valid taxonomic criteria to classify species in one of these two families [9, 10, 46]. In our phylogenetic tree, the Monocercomonadidae form a polyphyletic group and all Monocercomonadidae studied to date are sister groups of Trichomonadidae. This suggests that Monocercomonadidae have lost secondarily some cytoskeletal structures, such as the costa and the undulating membrane. Thus, the assignment of the Cf1 sequence to Monocercomonadidae species in the Trichomonadinae is consistent. Some electron microscopy data are available for *Tricercomitus divergens* [5] and *Hexamastix termitis* [3, 4]. A comparative morphological analysis can be done between these genera and *Tetratrichomonas gallinarum*. The structure of *Tetratrichomonas gallinarum* has not been published yet, but is very similar to that of *Tetratrichomonas limacis* [36]. Unfortunately, the number of characters that can be analyzed is very restricted. However, *Hexamastix termitis* has similar features with *Tetratrichomonas gallinarum* in the length and development of the axostyle-pelta complex and in the shape of the parabasal body. In contrast, the ultrastructure of *Tricercomitus divergens* seems more closely related to that of the genus *Monocercomonas*, as already suggested [4, 5]. Thus our Cf1 sequence probably corresponds to *Hexamastix termitis*. This assignment could be confirmed by whole-cell hybridization using fluorescently labeled oligonucleotides specific for the rDNA sequence in question.

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