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Ultrastructure of *Joenina pulchella* Grassi, 1917 (Protista, Parabasalia), a reassessment of evolutionary trends in the parabasalids, and a new order Cristamonadida for devescovinid, calonymphid and lophomonad flagellates

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Abstract

The parabasalids include parasites (e.g. trichomonads) as well as many hypermastigid flagellates which live in termites and other wood-eating insects and contribute to the cellulose-digesting capacity of those animals. A hypermastigid, *Joenina pulchella* Grassi, is shown to have a "flagellar area" composed of 1300 flagella, including three privileged basal bodies which have homologues in the trichomonads. The cytoskeleton includes preaxostylar fibres, two parabasal fibres and two atractophores with the parabasal fibres subdividing to form many parabasals. The microtubular rows of the pelta-axostyle system surround the flagellar area and converge towards a multispiralled axostylar trunk. On the basis of similarities of ultrastructure, joeniids and devescovinids are argued to be members of the same clade. *Projoenia* Lavette is in the sister group to *Devescovina* Foa and gives rise to the series *Placojoenia* Radek, *Joenia* Grassi, *Joenina* Grassi. *Projoenia* has a "flagellar area" as in the joeniids, but also a recurrent flagellum with a paraxonemal fibre and a cresta as does *Devescovina. Projoenia* has a parabasal fibre twisted around the axostyle, as well as a multispiralled axostyle. In *Placojoenia, Joenia* and *Joenina* the recurrent flagellum is absent or reduced to the basal body as is the cresta; the parabasal apparatus becomes multibranched. The classical Hypermastigida is in need of major revision. Parabasalids such as Lophomonadidae, Joeniidae, Deltotrichonymphidae, and possibly Rhizonymphidae and Kofoidiidae, collectively the lophomonads, have conserved the trichomonad/devescovinid organization and have a trichomonad-like morphogenesis involving only the privileged basal bodies and attached fibres. They can be distinguished from the rest of hypermastigids and should be classified with the Devescovinidae and Calonymphidae in a large clade – the Cristamonadida (new order). The remaining hypermastigids (the Trichonymphina and Spirotrichonymphina) have a rostrum which separates in two hemi-rostra at division, form a sister group to all other parabasalids and are not closely related to the remainder of the hypermastigids.

Key words: joeniids, devescovinids, hypermastigids, parabasalids, phylogeny, protist

Introduction

The parabasalids comprise about 80 genera of secondarily amitochondriate flagellates (protists) (see review Brugerolle & Müller, 2000). Almost all are endobiotic. They are important as parasites, including some genera (e.g. *Trichomonas* Donné) which cause health problems for humans (Krieger, 1990) or affect livestock (Bon-Durant & Honigberg, 1994). Others with large numbers of flagella, and referred to as hypermastigids, are abundant in the intestines of wood-eating termites and cockroaches. Some of these major, sometimes dominant, xylophagous insect members of terrestrial ecosystems have endogenous cellulase enzymes (Watanabe et al., 1998), but the flagellates form part of the microbial community that provides them with the ability to digest wood (Brugerolle & Müller, 2000).

The parabasalids can be distinguished by a variety of ultrastructural features – including the arrangement of the basal bodies of the flagella, the associated cytoskele-

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tal elements, the pleuromitotic type of division and other unusual organelles such as hydrogenosomes which contribute to energy metabolism (Brugerolle, 1991; Brugerolle & Müller, 2000).

Our traditional understanding of evolutionary relationships in this group is based on similarities evident from light microscopic studies (Cleveland et al., 1934; Kirby, 1941; Grassé, 1952; Honigberg, 1963; Lee, 1985a, b), supplemented with electron microscopy (see review Brugerolle, 1975–1976; and Hollande & Carruette-Valentin, 1971; Brugerolle & Taylor, 1977; Brugerolle & Müller, 2000). Despite the recent expansion of ultrastructural accounts to genera such as *Ditrichomonas* Farmer (Farmer, 1993), *Pentatrichomonoides* Kirby (Brugerolle, 1994), *Holomastigotoides* Grassi & Foa (Lingle & Salisbury, 1995), *Cochlosoma* Kotlan (Pecka et al., 1996), *Stephanonympha* Janicki (Rösel et al., 1996), *Placojoenia* Radek (Radek, 1997), *Pseudotrypanosoma* Grassi (Brugerolle, 1999), *Caduceia* Franca (d'Ambrosio et al., 1999), *Foaina* Janicki (Brugerolle, 2000), *Snyderella* Korby (Dolan et al., 2000a), *Metacoronympha* Kirby (Dolan et al., 2000b), *Microjoenia* Grassi and *Spirotrichonymphella* Grassi (Brugerolle, 2001), the descriptions of the parabasalid genera are still incomplete. For several families, particularly those symbiotic in termites and wood-eating cockroaches, no representatives have been studied by electron microscopy.

On the basis of information to date, it has been argued that there are two subgroups (orders): the Trichomonadida with up to six flagella, a parabasal apparatus and an axostyle (Grassé, 1952; Honigberg, 1963; Brugerolle, 1975–1976): and the Hypermastigida with many flagella, many parabasals and axostyles (Grassé, 1952; Hollande & Carruette-Valentin, 1971). Two major subsets of hypermastigids can be distinguished on the basis of organization and morphogenesis: the lophomonads (lophomonadines, joeniids and kofoidiids) and trichonymphids (Hollande, 1979, 1986; Hollande & Carruette-Valentin, 1971, 1972; Hollande & Valentin, 1969b). Several key issues remain unresolved: (1) the particular nature of the relationship between trichomonads and hypermastigids, and (2) how to rationalise the two patterns of organization within the hypermastigids.

More recently, phylogenetic studies based primarily on the comparative analysis of SSU rRNA sequences have identified several incongruities in the classical evolutionary hypothetical trees (Berchtold & König, 1995; Dacks & Redfield, 1998; Delgado et al., 2000; Edgcomb et al., 1998; Fröhlich & König, 1999; Gerbod et al., 2000; Gunderson et al., 1995; Keeling et al., 1998; Ohkuma et al., 1998, 2000; Silberman et al., 1996; Viscogliosi et al., 1993, 2000). These studies confirm the heterogeneity of hypermastigids, with the Joeniidae and Deltotrichonymphidae (*Koruga* Cleveland) being close to the devescovinid trichomonads. The other group of hypermastigids, the trichonymphids, branch from the base of the parabasalid evolutionary lineage. This prompted a reinvestigation of the joeniids, and we here confirm similarities with the devescovinids – thereby eliminating the initial incongruity. This is corroborated by further results of molecular phylogeny (Keeling et al., 1998; Fröhlich & König, 1999) and suggests that the classification of the parabasalids is now overdue for change.

This article includes the first account of the ultrastructure of the joeniid *Joenina pulchella* Grassi, 1917. We compare this organism with other joeniids and the devescovinid trichomonads now presumed to be their closest relatives. We extend this comparison to discuss the evolutionary relationships with other genera or families.

Material and methods

Termites of the species *Porotermes grandis* Holmgren, 1912 were collected in dump wood in the Blue Mountains area near Sydney, Australia, and stored in plastic bags for one month in the laboratory. The hindgut of a termite was opened into a few drops of Ringer's solution and the contents observed by phase contrast microscopy using a Zeiss Axioplan microscope. Smears made using rapid Giemsa stain were also examined. For transmission electron microscopy, the entire gut biota was fixed in a solution of 1% glutaraldehyde in 0.1 M phosphate buffer for 1 hour at room temperature. After a buffer wash, cells were post-fixed in 1% osmium tetroxide in the buffer for 1 hour. After a water rinse, cells were embedded in 1% agar, stained "en bloc" with saturated uranyl acetate in 70% ethanol for 1 hour, then completely dehydrated in an alcohol series and finally embedded in Spurr's or Epon resins. Sections were cut on a Reichert Ultracut S microtome, stained with lead citrate for 15 minutes, carbon-coated, and observed using a JEOL 1200 EX electron microscope at 80 KV.

Cells of *Joenia annectens* Grassi, 1885 from the termite *Kalotermes flavicollis* Fabricius, 1793 collected in France, cells of *Devescovina striata* Foa, 1905 from the termite *Neotermes castaneus* Burmeister, 1839 (Brugerolle, 2000), and cells of *Deltotrichonympha operculata* Sutherland, 1933 or *Koruga bonita* Cleveland, 1966 from *Mastotermes darwiniensis* Froggatt, 1897 collected in Australia, were fixed and treated as described above.

Observations

General organization

Joenina pulchella is large, about 100 µm long, and easy to distinguish from other flagellates symbiotic with this termite due to its anterior crista or panache of flagella (Figs 1a, 2a, b). The nucleus is situated below the flagellar area and is partly covered and surrounded by parabasal bodies. The axostyle has an anterior capitulum

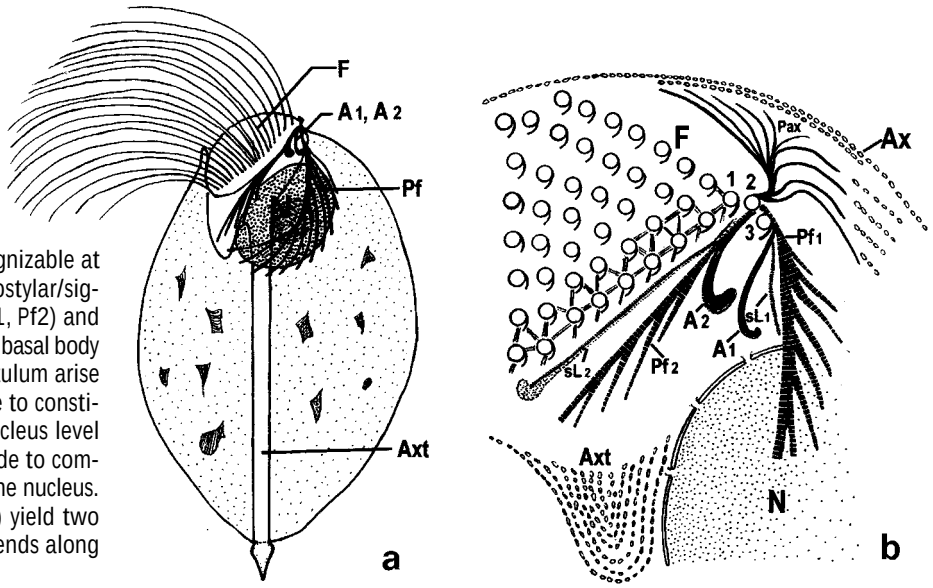

Fig. 1. Diagrammatic representation of the entire cell of *Joenina pulchella* (a) and details of the region of privileged basal bodies (b). The flagellar area (F) is constituted by regularly arranged and interlinked basal bodies. Three privileged basal bodies are recognizable at the corner of the flagellar area. Preaxostylar/sigmoid fibres (Pax), parabasal fibres (Pf1, Pf2) and atractophores (A1, A2) converge toward basal body #2. Microtubules of the axostylar capitulum arise along preaxostylar fibres and converge to constitute the axostyle trunk (Axt) at the nucleus level (N). Parabasal fibres (Pf1, Pf2) subdivide to compose the parabasal apparatus around the nucleus. Atractophore bodies/batachii (A1, A2) yield two striated laminae (sL) one of which extends along one side of the flagellar area.

that surrounds the flagellar area, and the axostylar trunk traverses the cell axially, protruding at the posterior end (Fig. 1a). Usually, the cytoplasm is filled with wood particles.

The general organization of the cell is shown in several longitudinal sections (Figs 2c, d). The flagellar area is situated anteriorly and is obliquely oriented toward the ventral side of the cell. The nucleus is located under the flagellar area and is surrounded by numerous Golgi bodies which collectively form the parabasal apparatus. The two parabasal fibres (Pf) are attached to basal body #2 of the privileged basal bodies (see below). This basal body is situated at the dorsal corner of the flagellar area (Figs 1b, 2c, d, e). The two parabasal fibres subdivide and each supports a Golgi body, the whole forming several parabasals which extend over and around the nucleus (Fig. 1b). The microtubular ribbon that forms the axostylar capitulum surrounds the flagellar area and, together with additional microtubular rows, makes up the axostylar trunk (Figs 1, 2c). This widens posteriorly where it terminates (not shown).

The flagellar area

About 1300 flagella are inserted in a rather triangular zone which is referred to as the "flagellar area" (Fig. 3a). Three privileged basal bodies are located at one corner of the flagellar area (Figs 1b, 2d, 3a). They are arranged in the same way as in trichomonads, with basal body #2 bearing preaxostylar/sigmoid fibres, and with basal bodies #1 and #3 situated on each side of basal body #2 and bearing a hook-shaped fibre (Figs 1b, 2d, e). Basal body #1 is closest to the basal bodies of the flagellar area. We did not observe a fourth basal body (R) and presume that it is absent in *Joenina* Grassi. Two parabasal fibres (Pf1 and Pf2) and two atractophores (A1 and A2) attach to basal body #2 (Figs 1b, 2c, d, e, f). The basal bodies in the flagellar area are arranged in lines and inserted at a regular distance from each other (Fig. 3a). All basal bodies are oriented in the same direction, and each bears a hook-shaped fibre which attaches to the proximal side of triplets 3 and 4 of the basal body. The hook-shaped fibres are finely striated as in their trichomonad homologues (Figs 3c, e). Another microfibrillar bundle is attached on the distal part of triplets 6, 7, 8 and connects basal bodies of the same line (Figs 3b, c, d, e). A fibrous meshwork composed of short striated fibres, converging on the proximal section of each basal body, interconnects the basal bodies of the flagellar area (Figs 1b, 3a inset, b, d, e).

The parabasal apparatus

Two major parabasal fibres attach to the privileged basal body #2 (Figs 1b, 4a). One (Pf1) follows a curving path and subdivides in several branches which radiate over the surface of the nucleus (Fig. 4c). The other (Pf2) lines the flagellar area and also subdivides into several branches which bear Golgi bodies (Figs 4a, b, c, d). The section of these fibres is not smooth, but irregular (Fig. 4e) exactly like those observed in devescovinids (not shown). In longitudinal section parabasal fibres are cross-striated with a pattern and a period of about 40 nm (Fig. 4f), similar to that of parabasal fibres of de-

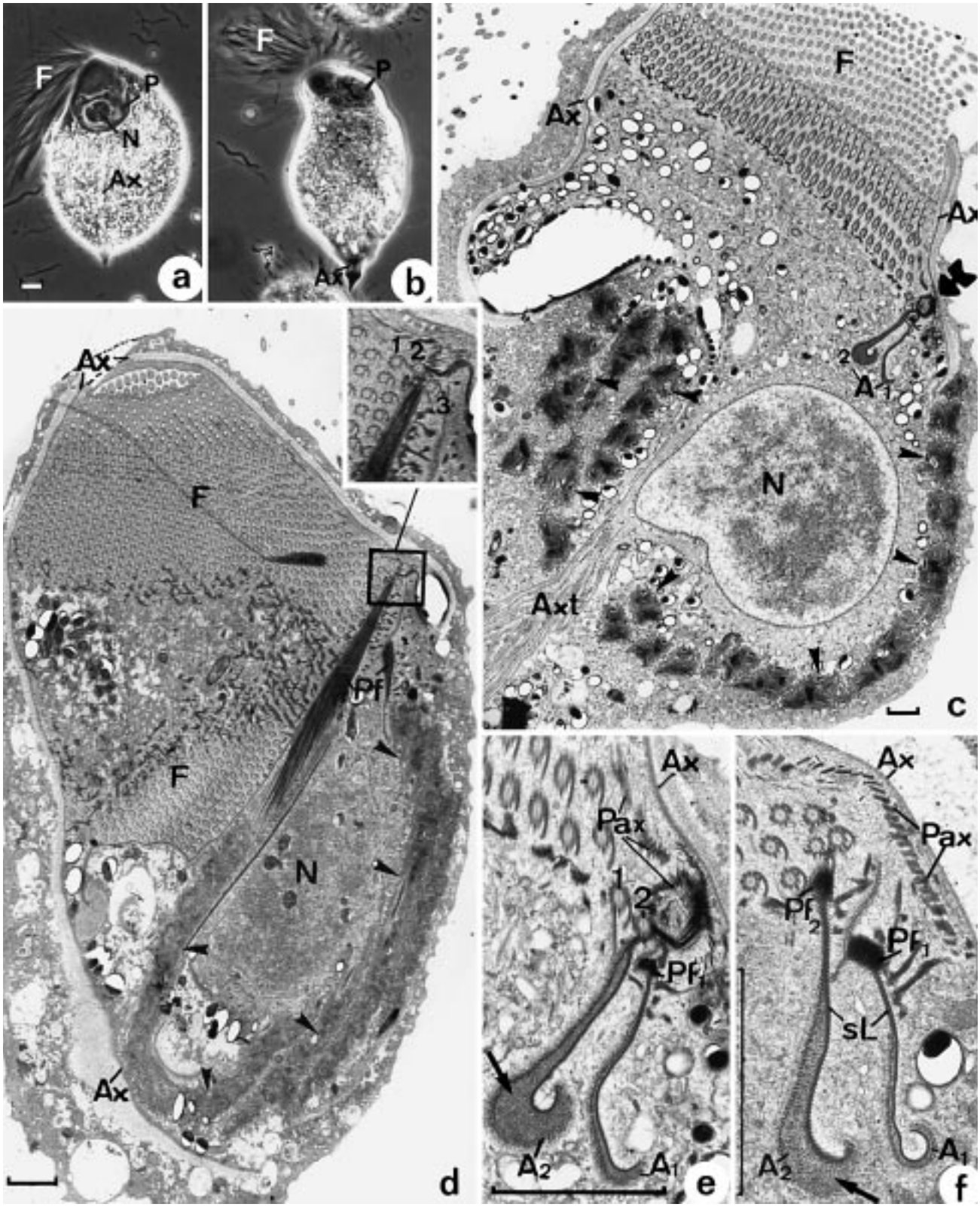

Fig. 2. General morphology of *Joenina pulchella* by light microscopy (a, b) and electron microscopy (c, d, e, f). **a, b.** Anterior panache of flagella (F) above the nucleus (N) neighbored by the parabasal organelle (P) and axostylar trunk (Ax) protruding posteriorly. **c.** Longitudinal section showing the anterior flagellar area (F) with privileged basal bodies (arrow) and attached atractophores (A1, 2) on one side, the nucleus (N) sur-

vescovinids. Two organelles, referred to as "batachii" by Grassi (1917) and as "atractophores" by Hollande (Hollande & Valentin, 1969b), are attached to the base of each parabasal fibre (Figs 1, 2c, e, f). The one (A1) that is linked to parabasal fibre Pf1 is thinner than the other (A2) which is attached to parabasal fibre Pf2. They have a compound substructure, being composed of a curved striated lamina that is associated with concentrically organized dense material (Fig. 2f). Each striated lamina extends beyond the atractophores (Figs 1, 4b, d). The lamina from A2 lines the internal side of the flagellar area and is associated with amorphous material at each margin (Fig. 4d). Atractophores act as the nucleating site or poles of the mitotic division spindle.

The pelta-axostyle system

The pelta-axostyle complex includes the axial cytoskeletal structure that is composed of an anterior capitulum and the axostylar trunk. The capitulum surrounds the flagellar area on the side away from the nucleus (Figs 3a, 5a). In the region near the privileged basal bodies and the preaxostylar/sigmoid fibres, there are two microtubular rows as in the pelta-axostyle junction of trichomonads (Fig. 5a, inset). Preaxostylar fibres are more numerous and longer than in trichomonads (Fig. 5a). The zone where preaxostylar fibres disperse (Fig. 5b) is also where many additional thin microtubular rows arise. These converge with the peripheral row of the capitulum to form the axostylar trunk (Figs 5a, c). The axostylar trunk is composed of many microtubular rows of varying widths. They are rolled up, forming patterns resembling wood grain in longitudinal and transverse sections (Figs 5c, d). The component microtubules of the microtubular rows are linked together as are those of trichomonads (Fig. 5e).

Other organelles

The nucleus is attached to axostylar microtubular rows by microfilamentous material (not shown). The nuclear envelope displays numerous nuclear pores (Fig. 4c). The cytoplasm contains hydrogenosomes, many symbiotic bacteria (Fig. 5a) and food vacuoles including pieces of wood.

Discussion

Flagellar apparatus of devescovinids and joeniids

Ultrastructural studies of the flagellar apparatus of five devescovinid genera – *Calonympha* Foa and *Devescovina* Foa (Joyon et al., 1969), *Foaina* (Brugerolle, 2000), *Macrotrichomonas* Grassi (Hollande & Valentin, 1969a), and *Stephanonympha* (Rösel et al., 1996) – have revealed common features in this family. Similarly, ultrastructural results are available for the joeniid genera, *Joenia* Grassi (Hollande & Valentin, 1969b; Hollande, 1979), *Placojoenia* (Radek, 1997), *Projoenia* Lavette (Lavette, 1970), and now for *Joenina*.

All joeniids have almost the same organization: a flagellar area composed of multiple basal bodies and including privileged basal bodies (Figs 1, 6). These basal bodies of the flagellar area bear hook-shaped fibres like those of basal bodies #1 or #3. The number of basal bodies is estimated at 500 in *Projoenia* (Lavette, 1970), 1000 in *Placojoenia* (Radek, 1997), 1300 in *Joenina*, and up to 1500 in *Joenia annectens* (Hollande & Valentin, 1969b). The number seems to increase with cell size. All genera have privileged basal bodies #1, #2, #3, as do trichomonads sensu stricto (s. s.) and devescovinids, but not all have a recurrent flagellum (R): it is present in *Projoenia*, is reduced to the basal body in *Joenia*, and absent in *Joenina*. This is reminiscent of the reduction of basal bodies and fibres in the trichomonad genera *Protrichomonas* Alexeieff, *Parahistomonas* Honigberg & Kuldova, *Histomonas* Smith and *Dientamoeba* Jepps & Dobell (Brugerolle, 1980).

The most striking similarity between devescovinids and joeniids is the presence of a modified recurrent flagellum (Fig. 6). This has a similar paraxonemal rod and there is an association with the cresta in *Devescovina* (Joyon et al., 1969; Brugerolle, 1975–1976) and *Projoenia* (Lavette, 1970). These studies now suggest that *Projoenia* is a sister taxon to the joeniids, sharing some derived characters with that group and some of the ancestral characters of *Devescovina* and devescovinids. The ultrastructure of *Projoenia* was described incompletely by Lavette (1970). Its devescovinid characters were not recognized at that time, nor by authors who have studied other joeniid genera such as *Joenia* and *Placojoenia*.

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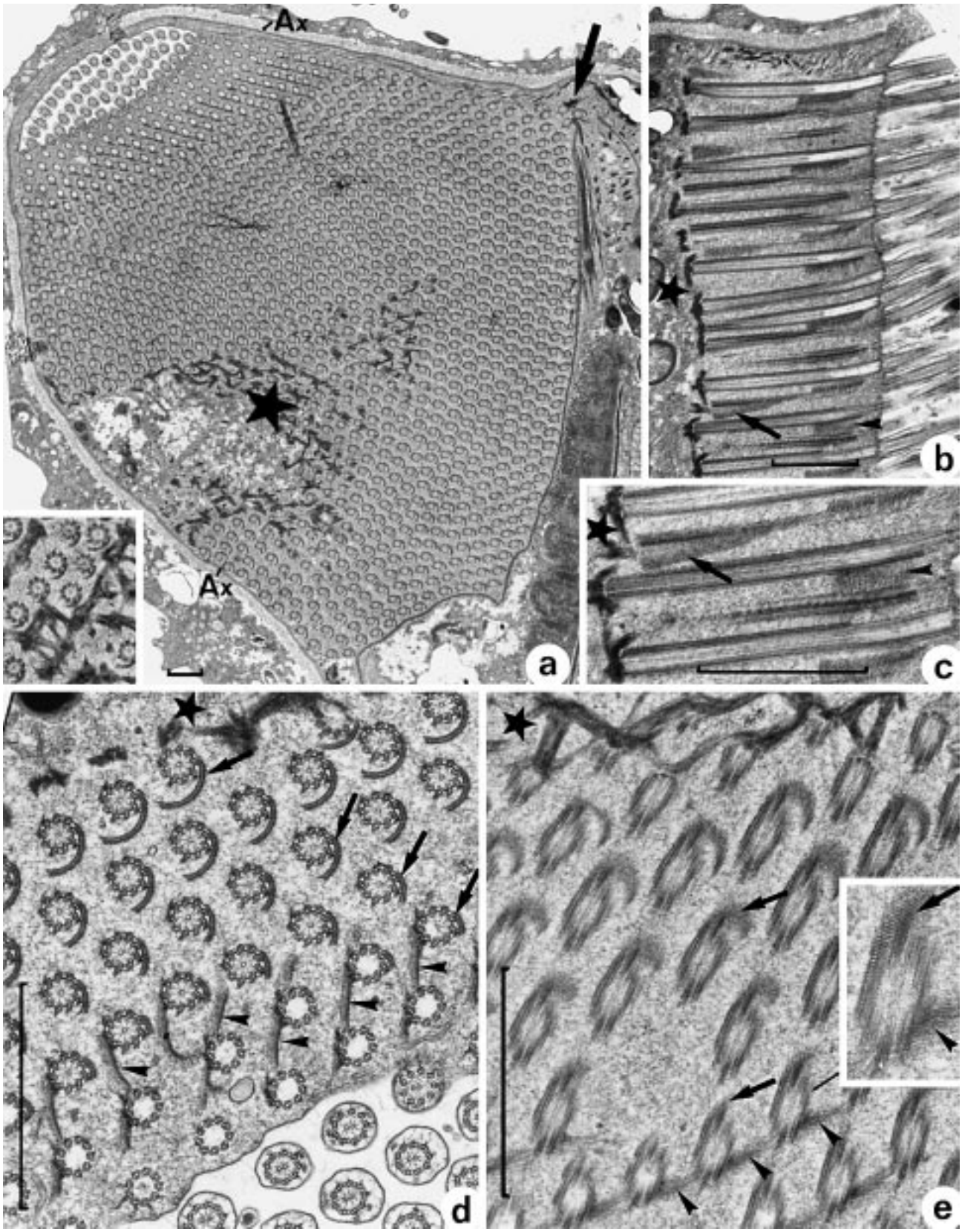
rounded by parabasal bodies (arrowheads), the axostylar capitulum (Ax) around the flagellar area, and the axostylar trunk (Axt). **d.** Anterior oblique section across the flagellar area (F) with privileged basal bodies #1, #2, #3 on a corner (inset), the parabasal fibres (Pf) with parabasal bodies (arrowheads) around the nucleus (N), and the axostylar capitulum (Ax) surrounding the flagellar area. **e, f.** In the zone of privileged basal bodies, the two atractophores (A1, A2) and the two parabasal fibres (Pf1, Pf2) are attached to basal body #2 bearing preaxostylar fibres (Pax). Atractophore body is composed of a striated lamina (sL) and of associated striated material (arrow). Bars = 10 µm in Figs a, b, and 1 µm in others.


Ax
Ax
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The arguments presented here link two groups which were previously regarded as distinct.

In *Projoenia,* the parabasal apparatus is composed of two main branches converging towards the basal body #2, as in trichomonads s. s. and devescovinids (Fig. 6) (Brugerolle, 1975–1976, 2000; Joyon et al., 1969). One branch is twisted around the axostyle both in *Devescovina* and *Projoenia*. In both, the pelta-axostyle microtubules originate close to privileged basal bodies in contact with preaxostylar/sigmoid fibres. The axostyle structure is similarly rolled up in all genera, but the genera differ in the number of microtubules and in the diameter of the bundle which increases in large joeniid genera such as *Joenia* (G. B. unpublished data). The atractophores are larger in joeniids than in devescovinids and the striated lamina that forms part of the atractophore expands under the flagellar area in *Joenia* and *Joenina*.

The comparison of devescovinids and joeniids allows the development of hypotheses as to the sequence of character transformations (Fig. 6). The privileged basal bodies 1, 2, 3, R and their associated structures are identical in *Devescovina* and *Projoenia*. In *Joenia* and in *Placojoenia* the recurrent flagellum (R) is reduced to its basal body, and in *Joenina* it seems to have disappeared. The recurrent flagellum of *Projoenia* is complete and possesses a paraxonemal rod very similar to that of *Devescovina* and other devescovinids. A cresta, a microfibrillar structure under the adhesion zone of the recurrent flagellum of *Devescovina,* is also present in *Projoenia*. In *Joenia, Placojoenia* and *Joenina* there is no recurrent flagellum nor accompanying cresta. Basal bodies of the flagellar area of all joeniids, except *Placojoenia*, have the hook-shaped fibre similar to that of privileged basal bodies #1 and #3 of devescovinids and trichomonads s. s. There are two main parabasal fibres in devescovinids and in joeniids, one of which is twisted around the axostyle in *Devescovina*, *Projoenia* and in *Placojoenia*. These fibres have the same striation pattern and in transverse section they have an irregular appearance in devescovinids and joeniids, but not in trichomonads s. s. The axostyle is composed of a rolled microtubular row in devescovinids, and this structure is very similar in all joeniid genera. Devescovinids and joeniids have no costa nor an undulating membrane.

An evolutionary sequence can now be suggested: *Projoenia* is the sister taxon to the devescovinids, and has retained the recurrent flagellum and the cresta, but the development of the flagellar area is the evolutionary innovation that distinguishes the joeniid lineage (Fig. 6). We presume that the flagellar area formed by multiplication of one privileged basal body bearing a hook-shaped fibre. We suggest that the next taxa on this lineage include *Joenia* and probably *Placojoenia* and these have lost the recurrent flagellum and the associated cresta. They have retained the basal body of the recurrent flagellum. Subsequently, *Joenina* seems to have lost the basal body of the recurrent flagellum. *Projoenia* and *Placojoenia* still have a major parabasal fibre twisted around the axostyle, as in *Devescovina*. In *Joenia* and *Joenina* the two parabasal fibres have changed to subdivide into many branches which extend over and around the nucleus. The basic spiral pattern of the axostyle of *Devescovina* is retained in the joeniids, but contains additional microtubular rows and becomes multispiralled. Other joeniid genera such as *Joenopsis* Cutler, *Joenoides* Grassé, *Cyclojoenia* Nurse (Grassé, 1952; Yamin, 1979; Lee, 1985a) not studied by EM yet might complete this sequence.

The morphological studies of devescovinids and joeniids show that these two groups are very close and belong to the same evolutionary line. This is consistent with molecular comparisons of SSU rRNA (Berchtold & König, 1995; Gunderson et al., 1995; Keeling et al., 1998; Fröhlich & König, 1999; Gerbod et al., 2000; Ohkuma et al., 2000; Viscogliosi et al., 2000). It also agrees with the pattern in morphogenesis, because the flagellar area of joeniids regresses, and only the privileged basal bodies and their attached fibres participate in division and are partitioned (Grassé, 1952; Hollande & Valentin, 1969b; Hollande & Carruette-Valentin, 1972; Hollande, 1979). The division is very similar to that of trichomonads s. s. or devescovinids (Grassé, 1952; Brugerolle, 1975) and dissimilar to the pattern in trichonymphids which involves division of the rostral region (Cleveland et al., 1934; Grassé, 1952; Hollande & Carruette-Valentin, 1971).

There is little molecular information explicitly relating to the placement of the joeniids. However, Keeling

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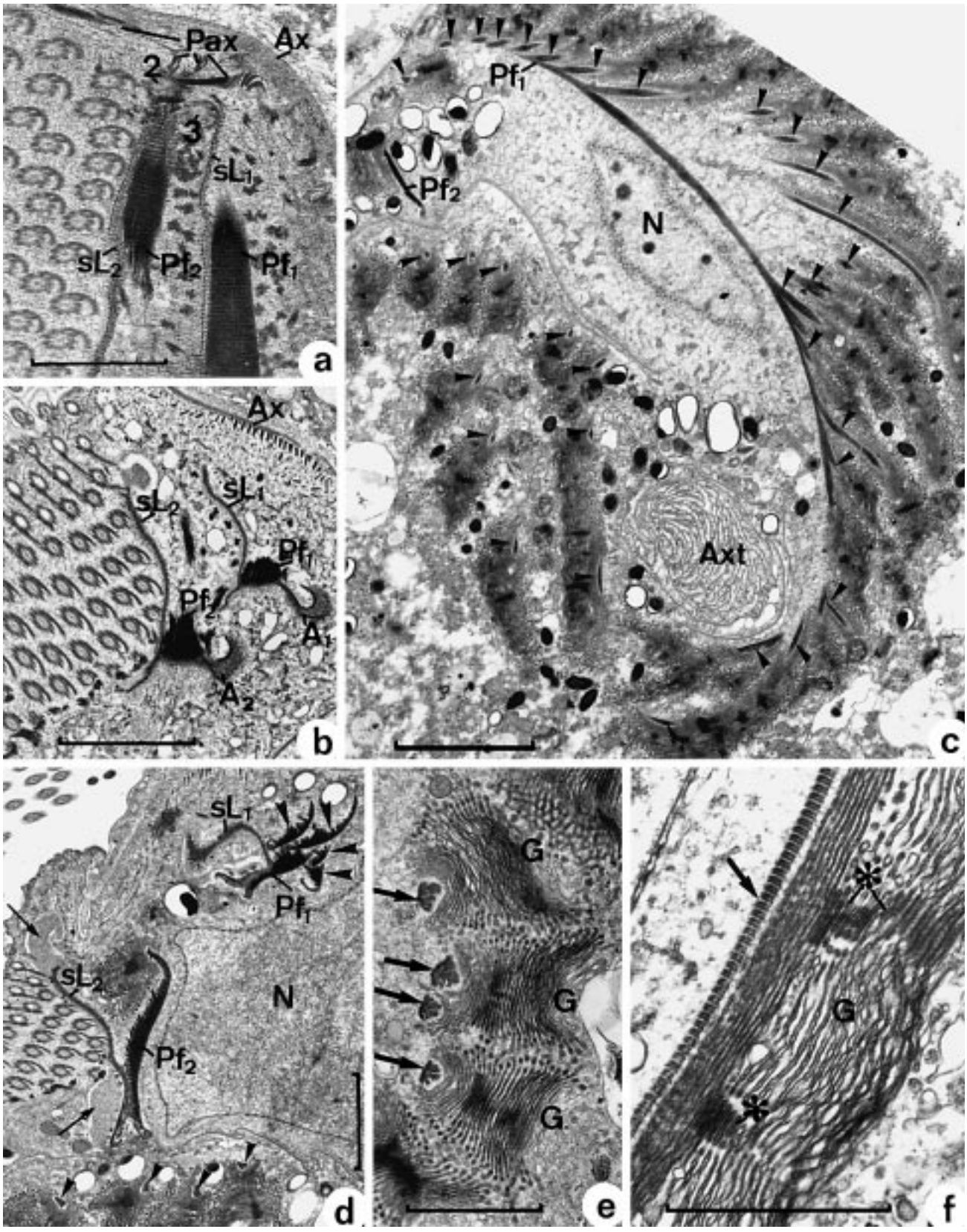
Fig. 3. Electron micrographs of the flagellar area in *Joenina pulchella*. **a.** Transverse section showing the entire flagellar area with privileged basal bodies on the corner (arrow) partially surrounded by the axostylar capitulum (Ax). Basal bodies are similarly oriented and connected by a fibrous network at their bases (arrowheads and inset). **b, c, d, e.** Sections of the flagellar area parallel (b, c), transverse (d) and oblique (e) to the basal bodies. Each basal body, on its proximal side, bears a hook-shaped fibre which is finely striated (arrow) (Fig. e and inset), basal bodies are linked by microfibrillar bundles attached on their distal sides (arrowheads). The underlying network (*) is present on every figure. Bars = 1 µm.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et al. (1998) compared SSU rRNA sequences of the symbionts of *Porotermes* Hagen. The source of one sequence which grouped with the devescovinids was not identified. We think it most likely comes from *Joenina pulchella* because no devescovinid species have been described in *Porotermes*. If our suggestions are right, we can predict that the SSU rDNA sequences of other joeniids will also group with the devescovinids, for example with that of *Joenia annectens* from *Kalotermes flavicollis*, which is not difficult to isolate by hand-screening.

There are other genera and families which have an organization and morphogenetic patterns similar to joeniids, and which are also related. The first is *Lophomonas* Janicki which has a flagellar area comprising privileged basal bodies and a pelta-axostyle similar to that of joeniids (Hollande & Carruette-Valentin, 1972). The second is the Deltotrichonymphidae, a family that contains the genera *Deltotrichonympha* Sutherland and *Koruga*, symbionts of the termite *Mastotermes darwiniensis* (Cleveland, 1966a, b, c; Tamm & Tamm, 1973a, b, 1980; Hollande, 1986). These large cells have arrays of several thousand flagella forming a dome-shaped flagellar area at the anterior end. Ultrastructural studies show that the privileged basal bodies are present and retained during division as in joeniids, whereas the rest of the flagellar area disintegrates before its reestablishment in each daughter cell.

Molecular comparisons show that the SSU rDNA sequence of *Koruga* is grouped with those of devescovinid genera (Fröhlich & König, 1999). Other genera and families yet to be studied by electron microscopy, with a flagellar area and a morphogenesis similar to that of joeniids, such as Rhizonymphidae (Grassé, 1952) and Kofoidiidae (Grassé, 1952), might also belong to this group.

Ultrastructural studies also show that the calonymphids are best regarded as polymonad devescovinids (Joyon et al., 1969; Brugerolle, 1975–1976; Rösel et al., 1996; Dolan et al., 2000a, b). SSU rRNA of the calonymphids *Coronympha* Kirby and *Calonympha* group with devescovinid genera (Gunderson et al., 1995; Fröhlich & König, 1999).

Previous ultrastructural studies revealed similarities of the body part of the undulating membrane structures between the genus *Tritrichomonas* Kofoid and devescovinids and led to the argument that this was the trichomonad genus closest to devescovinids (Brugerolle, 1975–1976). This relationship seems to be confirmed by several recent molecular phylogenetic trees (Silberman et al., 1996; Edgcomb et al., 1998; Keeling et al., 1998; Delgado-Viscogliosi et al., 2000; Gerbod et al., 2000; Ohkuma et al., 2000; Viscogliosi et al., 2000).

The phylogeny of parabasalids

The classic/intuitive phylogeny, based on comparisons of morphological characters of the flagellar apparatus and of morphogenesis, contends that the simple trichomonads could be at the base of the parabasalid lineage, and that the more complex hypermastigids with many flagella and multiple fibres are derived. This view is challenged by molecular phylogeneticists who argue, based primarily on SSU rRNA sequence comparisons, that the trichonymphid and spirotrichonymphid hypermastigids are at the base of the parabasalid lineage. The same studies also indicate that the hypermastigids are polyphyletic and that the joeniids and deltotrichonymphids have derived independently from simple trichomonads (Fröhlich & König, 1999; Ohkuma et al., 2000).

Comparative morphology and molecular phylogeny support the case for increasing size and complexity in the evolutionary line of devescovinids, joeniids, deltotrichonymphids. The traditional perspective which placed trichonymphids and spirotrichonymphids with the lophomonadids/joeniids (Hollande & Carruette-Valentin, 1971; Lee, 1985a, b; Brugerolle & Lee, 2001) is not supported. Despite much scrutiny, no convincing missing links have been found between these two groups (Hollande, 1979). Also, there is no support for an argument that there has been a regressive evolution from complex trichonymphids toward simple trichomonads. These two evolutionary lines have a common origin/ancestor but have subsequently become independent. If trichonymphids are really at the base of the tree they must have been preceded by organisms less complex which probably have become extinct (Philippe et al., 2000), or which have not yet been identified.

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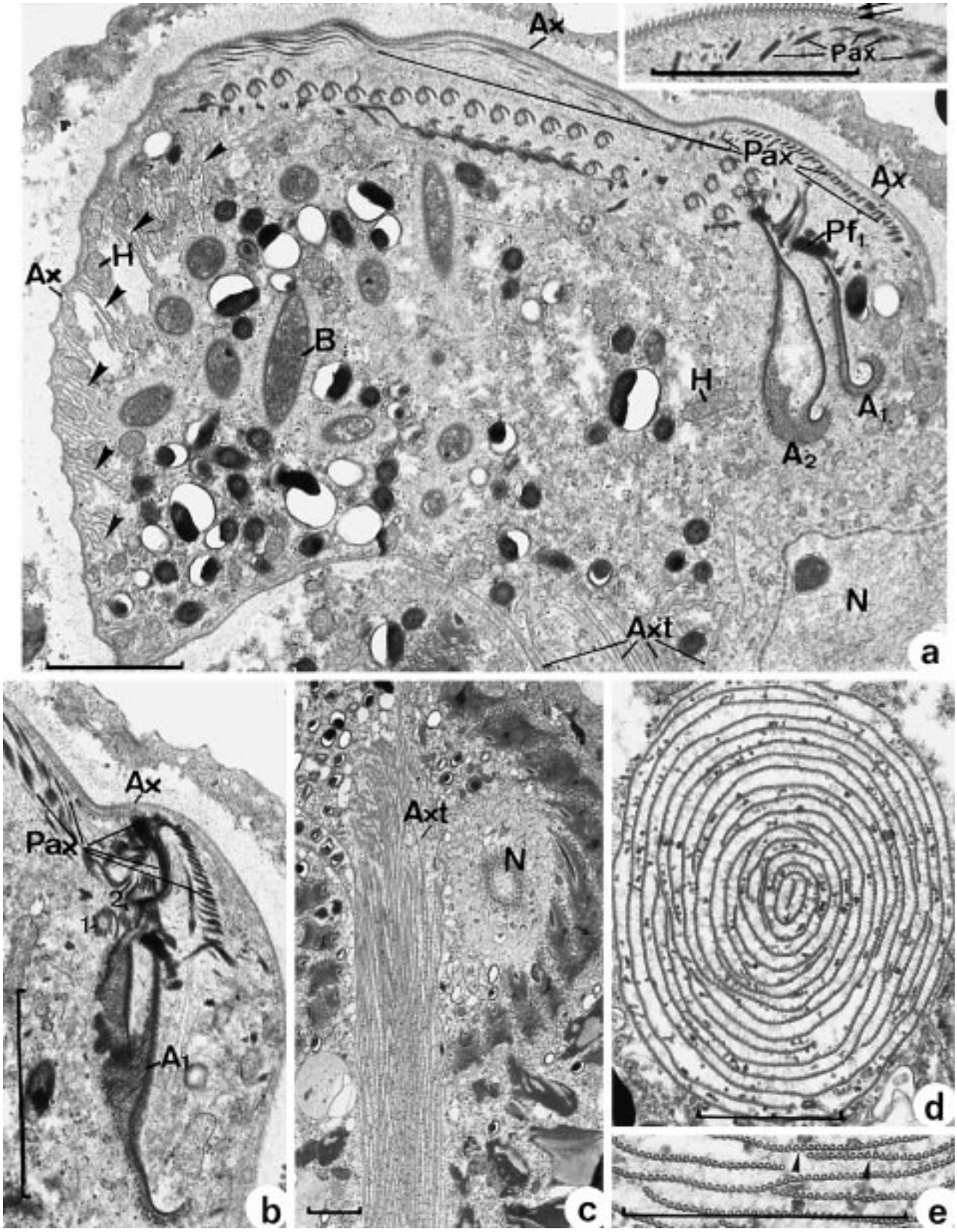
Fig. 4. Electron micrographs of the parabasal apparatus in *Joenina pulchella*. **a, b.** The two parabasal fibres (Pf1, Pf2) and the striated laminae (sL1, sL2) of the atractophores (A1, A2) are attached to basal body #2 which bears preaxostylar fibres (Pax); (Ax) capitulum of axostyle. **c, d.** The two primary parabasal fibres (Pf1, Pf2) subdivide (arrowheads), yielding several parabasals around the nucleus (N) and the axostylar trunk (Axt). **e, f.** Parabasal fibres (arrows) supporting the Golgi bodies (G) are irregular in shape (Fig. e) and cross-striated (Fig. f); Golgi stacks of vesicles show dense areas (*). Bars = 1 µm.


Ax
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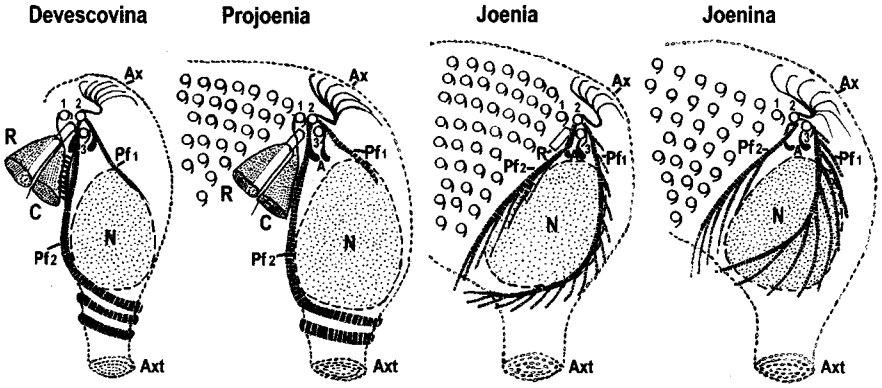

Fig. 6. Morphological evolution in the devescovinid/joeniid line. Privileged basal bodies #1, #2, #3 and R comprise a complete recurrent flagellum (R) with a paraxonemal rod and a cresta structure (C) in *Devescovina* and in *Projoenia*, whereas in *Joenia* and *Joenina* the recurrent flagellum and the accompanying cresta are lost. *Projoenia*, *Joenia* and *Joenina* have developed a flagellar area but have retained privileged basal bodies. Two parabasal fibres are present in genera of this evolutionary line, in *Devescovina* and *Projoenia* one fibre is preponderant and twisted around the axostyle. The latter has nearly the same ultrastructural organization in all genera.

Modification of parabasalid systematics

If the classification of protists is to reflect their phylogeny (Patterson, 1999), the joeniids and their close relatives, such as the lophomonads, can no longer be grouped with trichonymphids, because we are of the view that the Hypermastigida grouping is polyphyletic. The devescovinid/joeniid/deltotrichonymphid grouping differs from the trichonymphid/spirotrichonymphid group in the organization of the flagellar apparatus, in morphogenesis and ribosomal RNA genes. Devescovinids/joeniids/deltotrichonymphids resemble trichomonads s. s. in having privileged basal bodies, two attached parabasal fibres, and an axostyle. They divide in the same way as trichomonads s. s., with only the privileged basal bodies and attached striated fibres participating in the division. The other basal bodies which make up the flagellar area regress and are rebuilt in the daughter cells. In contrast, trichonymphids have a bilaterally symmetrical flagellar apparatus composed of two semi-circular flagellar areas (hemi-rostra) which include parabasal fibres and multiple axostyles. At division, the rostrum separates in two hemi-rostra and then a complete rostrum is rebuilt from each of the hemi-rostra in the daughter cells. There is no regression of basal bodies in trichonymphids (Cleveland et al., 1934; Grassé, 1952; Hollande & Carruette-Valentin, 1971). Spirotrichonymphids have a flagellar apparatus that differs from that of trichonymphids because there is a radial symmetry superimposed on the bilateral symmetry revealed at division (Cleveland, 1938; Grassé, 1952; Hollande & Carruette-Valentin, 1971; Lingle & Salisbury, 1995). Spirotrichonymphids and trichonymphids have a similar morphogenesis in which the flagellar bands separate into two parts that are subsequently brought back together in the daughter cells.

Despite the recurring criticisms from molecular phylogeneticists that the traditional systematics does not follow the conventions of phylogenetic systematics, it is still not possible to rebuild a complete and durable new systematics. Many genera and representatives of families have not yet been studied with electron microscopy, and/or there are no rRNA sequences or other molecular data. Positions of several genera or families in molecular trees are unstable, and groupings continue to change as more sequences are analyzed. Many sequences from termite symbionts were not assigned to a species by in situ

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Fig. 5. Electron micrographs of the pelta-axostyle system. **a, b.** The flagellar area is circumscribed by the microtubular row of the pelta-axostyle capitulum (Ax). This peripheral row and additional rows of microtubules (arrowheads) converge to form the axostylar trunk (Axt) at the nucleus level (N). From privileged basal body #2 arise the preaxostylar/sigmoid fibres (Pax) which fan along the double row of the pelta-axostyle junction (Ax) (Fig. a and inset, b); hydrogenosomes (H) and numerous bacteria (B) are present in this zone (Fig. a). **c, d, e.** Longitudinal (c) and transverse (d, e) sections of the axostylar trunk. The encased rows of microtubules resemble wood grain; notice that microtubules are linked by intra-row bridges (Fig. e). Bars = 1 µm.

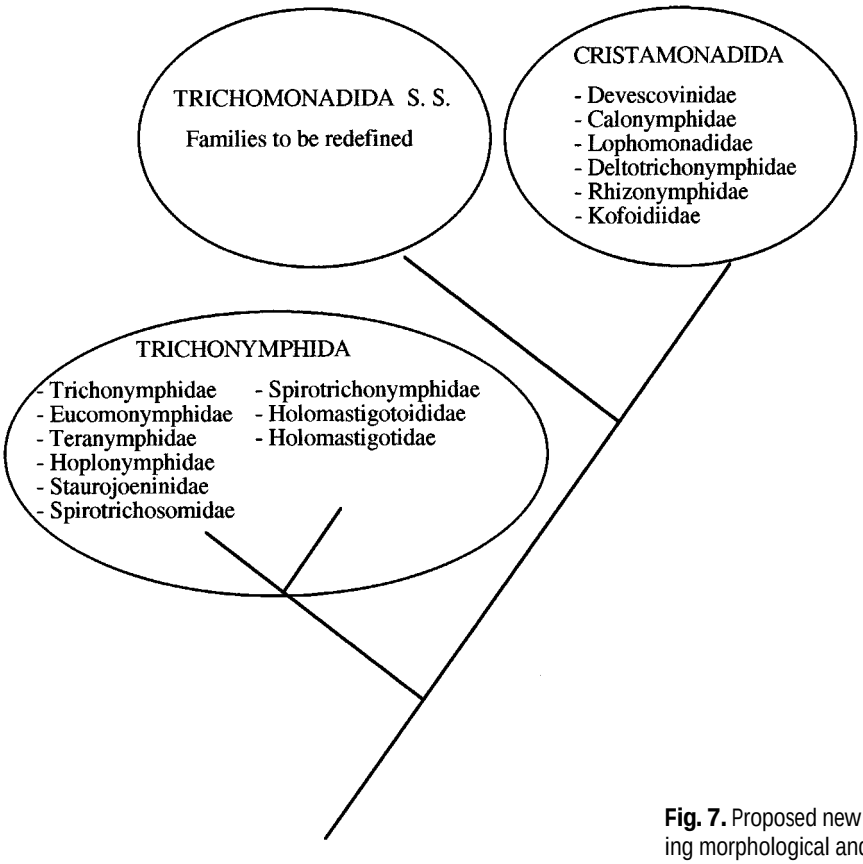

Fig. 7. Proposed new system at the order level in Parabasalia, including morphological and molecular data.

hybridization (Gunderson et al., 1995; Keeling et al., 1998; Gerbod et al., 2000; Ohkuma et al., 2000); hand isolation cannot be applied to small species, but isolation with a modern micromanipulator has been used with success (Fröhlich & König, 1999). Some genera have been wrongly identified. The trichomonad isolated from *Reticulitermes santonensis* Feytaud under the name *Trichomitus trypanoides* Berchtold, Breunig & König (Berchtold et al., 1995) corresponds to *Trichomonas trypanoides* Duboscq & Grassé (Kirby, 1931) which is probably best assigned to the genus *Trichomitopsis* Kofoid & Swezy (Honigberg, 1963). Misidentification probably also applies to the two distant sequences attributed to two species of the genus *Metadevescovina* Light (Berchtold & König, 1995; Gunderson et al., 1995).

We propose that the classic hypermastigid and trichomonad groupings be replaced by three orders: 1) the order Cristamonadida comprising Devescovinidae, Calonymphidae, Joeniidae, Lophomonadidae, Deltotrichonymphidae, Rhizonymphidae and Kofoidiidae; 2) the order Trichonymphida with the remaining hypermastigid families of the Trichonymphina sub-order from Hollande & Carruette-Valentin (1971), Hoplonymphidae, Staurojoenidae, Trichonymphidae, Eucomonymphidae, Teranymphidae, Spirotrichosomidae, and families of the Spirotrichonymphina sub-order from Hollande & Carruette-Valentin (1971), Spirotrichonymphidae, Holomastigotoididae, Holomastigotidae; and 3) the order Trichomonadida s. s. comprising families to be redefined according to the results given by molecular phylogeny (Fig. 7).

Diagnosis

Cristamonadida – parabasalids with a *crista* (= an egret or a panache of flagella). Parabasalids with four privileged basal bodies/flagella up to a thousand forming a flagellar area. Privileged basal bodies and attached fibres only participate in the division, the others are discarded. No costa and no undulating membrane. When present, recurrent flagellum is enlarged, containing paraaxonemal microfibrils; microfibrillar cresta along the adhesion zone of the recurrent flagellum. Pelta-axostyle system with one or several spiralled row(s) of microtubules in the axostylar trunk. Parabasal apparatus with two main branches which can be further subdivided, one main branch often twisted around the axostylar trunk. Multimastigont genera with several to hundreds of karyo- and/or akaryomastigonts in one or two families.

All symbiotic in termites or wood roaches.

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