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# *Vischeria stellata* (Eustigmatophyceae): ultrastructure of the zoospores, with special reference to the flagellar apparatus

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**Summary.** Ultrastructure of the zoospores of *Vischeria stellata* (R. Chodat ex Poulton) Pascher is investigated, with particular reference to the system of flagellar roots. Microtubular roots and a rhizoplast are present and a model showing their distribution is proposed. Four microtubular roots attach to the basal bodies in a system basically similar to that displayed by the heterokont algae and fungi. The rhizoplast is also similar to that of other heterokont algae. We conclude from these observations that the class Eustigmatophyceae should be placed within the division Heterokontophyta.

**Keywords:** Flagellar roots; Eustigmatophyceae; Heterokontophyta; *Vischeria*; Zoospores.

**Abbreviations:** C chloroplast; B′ basal body of the emergent flagellum; B″ second basal body; E eyespot; F emergent flagellum; FS flagellar swelling; LV lamellate vesicle; M mastigonemes; MTs microtubules; N nucleus; R 1–R 4 microtubular roots; Rh rhizoplast; SB striated band; SV spiral vesicle.

## Introduction

The separation of the Eustigmatophyceae from the Tribophyceae as a new class of yellow-green algae was based, essentially, on the unique ultrastructure displayed by the zoospores (Hibberd and Leedale 1970, 1971 a, 1972). However, despite the general structure of the uniflagellate and biflagellate zoospores being relatively well known (Hibberd 1980, Hibberd and Leedale 1972, Lee and Bold 1973), the system of flagellar roots has not yet been documented in detail.

Ultrastructural aspects of the flagellar apparatus have been considered to be of taxonomic and phylogenetic significance in green algae (for reviews, see Mattox and Stewart 1984, Melkonian 1984, O'Kelly and Floyd 1984) and are also receiving increased attention in phylogenetic considerations of other algal groups and heterokont protists (for reviews, see Hibberd 1979, Moestrup 1982, Preisig 1989). This paper presents a recon-

**Figs. 1–10.** Transmission electron micrographs of *Vischeria stellata.*

**Fig. 1.** Longitudinal section of the zoospore, showing its general structure. View from left side of the zoospore. × 23,000

**Fig. 2.** Mastigonemes on the emergent flagellum. × 8,500

**Fig. 3.** Longitudinal section of the base of the emergent flagellum, showing the transitional helix. Note the number of gyres in section (arrow), four on one side and five on the other. *E* Eyespot, *FS* flagellar swelling. × 42,000

**Figs. 4–10.** Seven selected transverse sections of two different series (Figs. 4–7 and Figs. 8–10) through the anterior end of the cell, to show the transition region of the long flagellum, the two flagellar basal bodies and their associated roots. Dorsal side of the zoospore to the top of the page. × 72,000

**Fig. 4.** Axonemal MTs, transitional helix and roots R 1 and R 2

**Fig. 5.** Transitional fibres connecting axoneme with the plasmalemma (arrows), roots R 1 and R 2

**Fig. 6.** Triplet MTs in basal body B′, roots R 1, R 2, and one MT of root R 4

**Fig. 7.** Basal bodies, roots R 1 and R 2. Note a striated fibrous band connecting both basal bodies

**Fig. 8.** Roots R 1, R 4, and striated band

**Fig. 9.** Four MTs of root R 3 between the basal bodies. Root R 1 is still visible

**Fig. 10.** Cartwheel pattern in basal body B′

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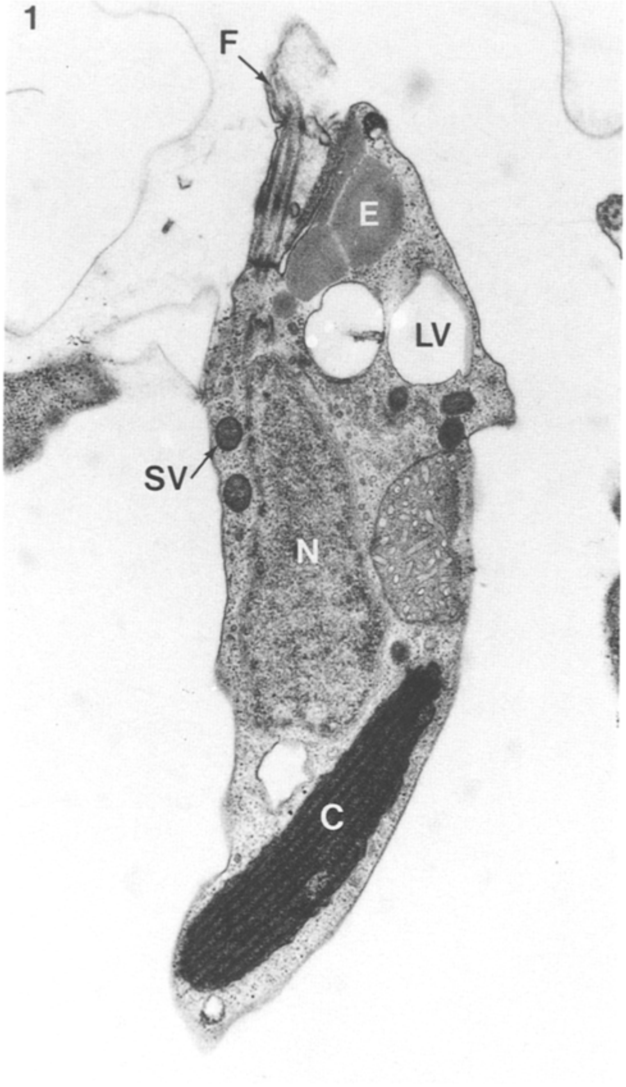
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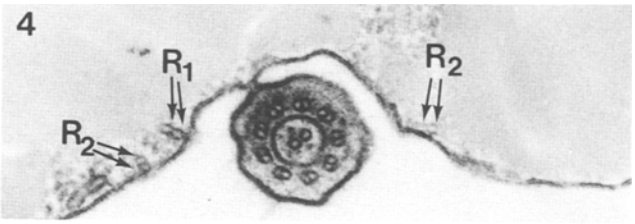
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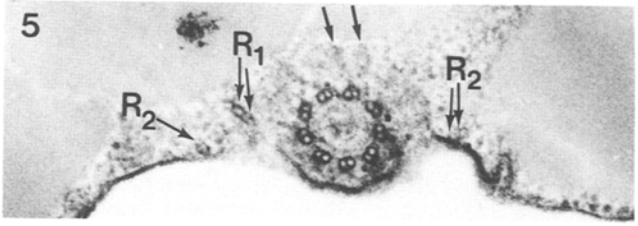
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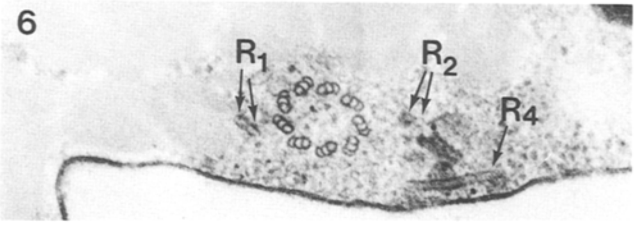
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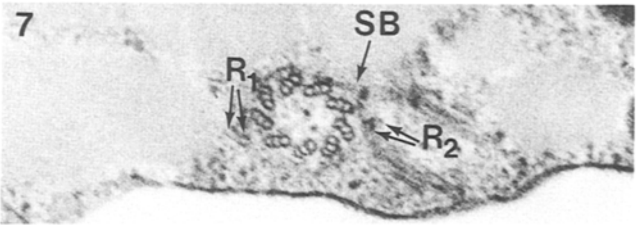
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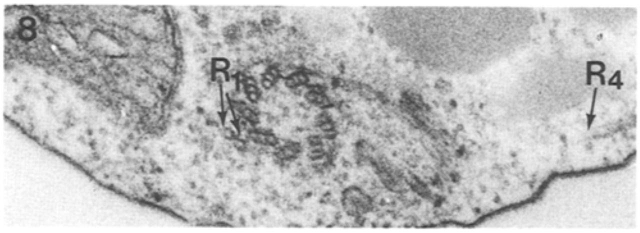
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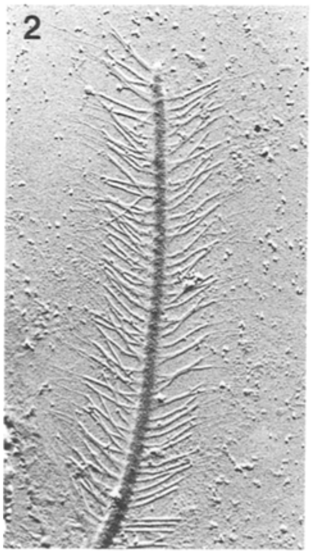
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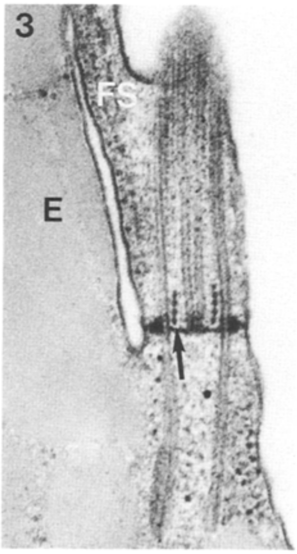
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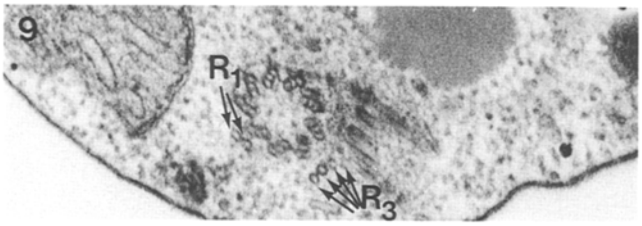
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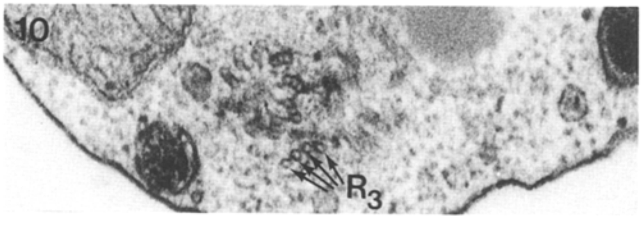
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struction from serial sections of zoospores from the eustigmatophycean species *Vischeria stellata* (R. Chodat ex Poulton) Pascher of a model showing the system of flagellar roots. So far, data available on other representatives of this class (Hibberd 1969, Hibberd and Leedale 1972, Santos 1990) agree with the model proposed.

## Materials and methods

Unialgal cultures of *Vischeria stellata* (strains 33.83 and 887.2) were obtained from the culture collection of algae at Göttingen (Schlösser 1982). Cultures were mantained at 12 °C in constant light, at an irradiance of 5,000 lux on medium 1 or 1 b (for chemical composition, see Schlösser 1982). Induction of zoospores was effected by addition of fresh liquid medium to cultures on solidified medium (more than 1 month old), followed by subjecting the culture to a long period of darkness (2–3 days). Direct preparations of zoospores were obtained by fixing a drop of material on a formvar/carbon-coated grid in 2% osmium tetroxide vapour, drying at room temperature and shadow-casting with gold/palladium. For sectioning, zoospores were fixed in a mixture of equal parts of 5% glutaraldehyde and 4% osmium tetroxide in 0.2 M cacodylate buffer, pH 7.2, for 1–2 h at 4 °C. After three 5–10 °C min rinses in buffer, the material was dehydrated in a graded ethanol series, embedded in Spurr's resin and sectioned on a Reichert OMU 4 ultramicrotome. Sections were stained with 0.5% uranyl acetate in 20% ethanol and Reynolds' lead citrate. Observation of direct preparations and sections was carried out on a JEOL 1200 EX electron microscope.

## Results

### *General structure of the zoospores*

The general morphology of the zoospores of *Vischeria stellata* (Fig. 1) has been previously described (Hibberd 1969, Hibberd and Leedale 1972). The zoospores are flask-shaped, usually 10–15 µm long and about 5 µm at maximum width. They are naked cells, with a single emergent flagellum, almost as long as the cell body, inserted subapically. A large extraplastidial eyespot and a pyriform nucleus occupy the anterior part of the cell, while a single chloroplast lacking a pyrenoid is situated at the posterior end. The rest of the cell is filled with the other organelles and different sorts of vesicles, in particular the lamellate and spiral vesicles typical of eustigmatophycean zoospores.

### *Flagellum, transition region, and basal bodies*

The flagellum (F) has tripartite tubular hairs (mastigonemes) on its surface (Fig. 2), a swelling with electron-dense material close to the cell surface and to the eyespot (Fig. 3), and consists of the usual 9 + 2 pattern of MTs in the axoneme (Fig. 4). The transition region, between the axoneme and the basal body (Figs. 3–5), consists of a transverse partition slightly above the cell surface and a transitional helix with 4–5 gyres (Fig. 3). Faint transitional fibres connect the axonemal MTs with the plasmalemma in this region (Fig. 5).

The basal body (B') consists of nine triplet MTs (Fig. 6) also connected to the plasmalemma by transitional fi-

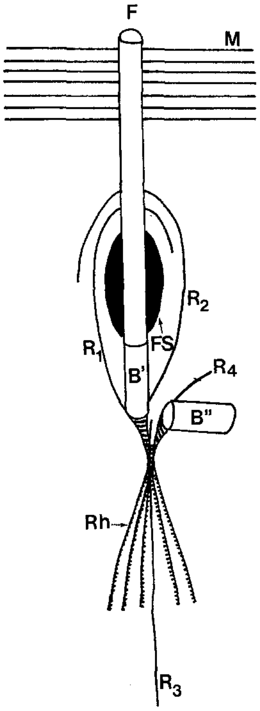

**Fig. 11.** Schematic drawing showing the relative positions of basal bodies (*B', B''*), flagellar roots (*R 1–R 4*), and flagellar swelling (*FS*) in zoospores of the eustigmatophycean species *Vischeria stellata*. Root R 4 possibly runs around the eyespot (not shown). View from the ventral to the dorsal side of the zoospore. *F* Emergent flagellum, *M* mastigonemes, *Rh* rhizoplast

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**Figs. 12–20.** *Vischeria stellata*. Selected longitudinal and transverse sections of the flagellar apparatus of the zoospores, showing the different microtubular roots. View from the ventral side of the zoospore, except for Figs. 17–20 where specific orientation is given

**Fig. 12.** Roots R 1, R 3, and R 4 shown in relation to the basal bodies. × 42,000

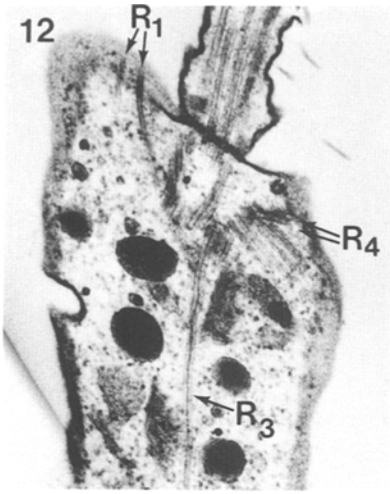
**Fig. 13.** Roots R 1 and R 4. *N* Nucleus, *SV* spiral vesicle. × 70,000

**Fig. 14.** Flagellar swelling (*FS*) in oblique section. Note the three MTs of root R 1, passing around it. × 48,000

**Figs. 15** and **16.** Two consecutive sections of a series, showing root R 2 (Fig. 15), dense material connecting the basal bodies and root R 3 with basal body B' (arrows, Fig. 16) and root R 4. *E* Eyespot. Fig. 15, × 54,000; Fig. 16, × 60,000

**Figs. 17** and **18.** Sections showing the five MTs of root R 3 in a semicircular arc near the basal bodies (Fig. 17) and lower in the cell when the basal bodies are no longer seen and the tip of the nucleus (*N*) appears. Dorsal side of the zoospore to the top of the page. × 95,000

**Figs. 19** and **20.** Two consecutive sections, showing root R 4. View from the left side of the zoospore. × 37,000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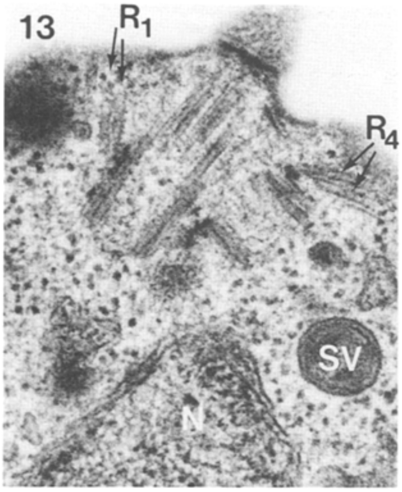
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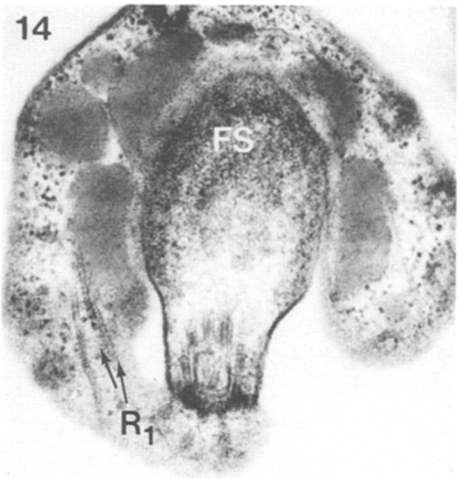
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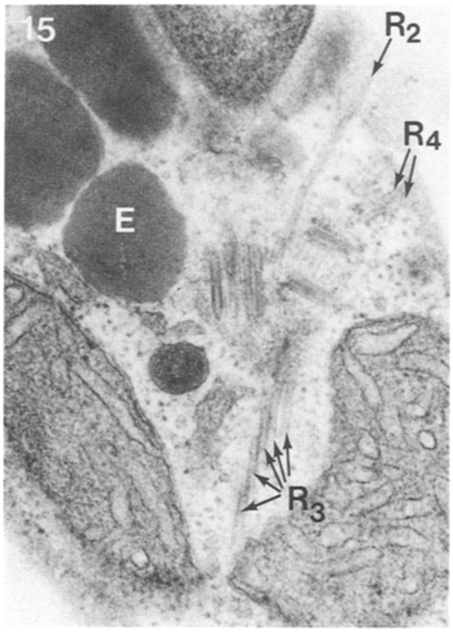
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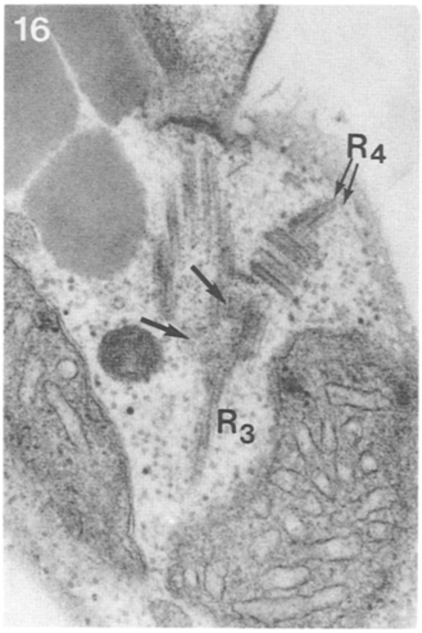
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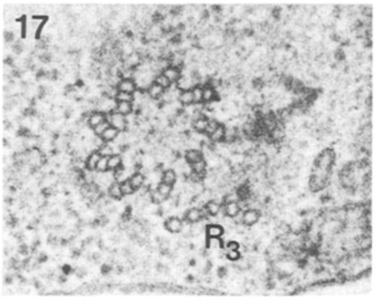
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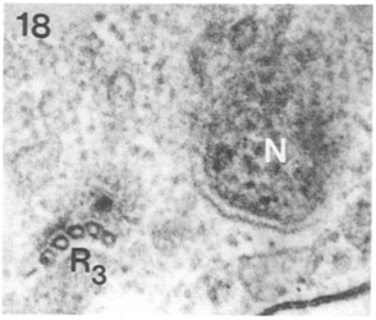
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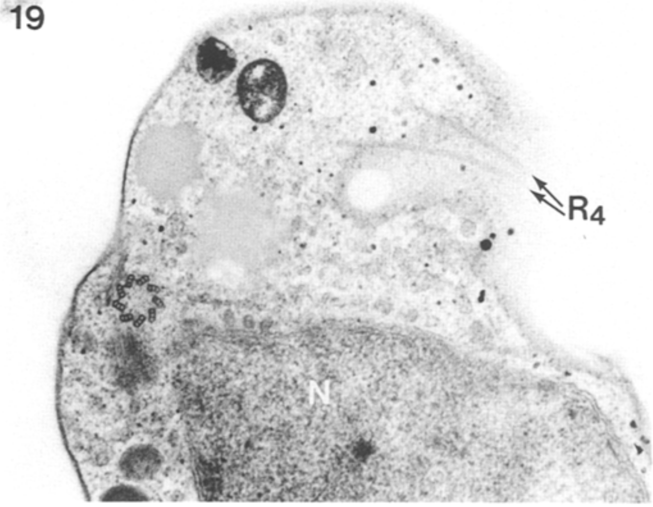
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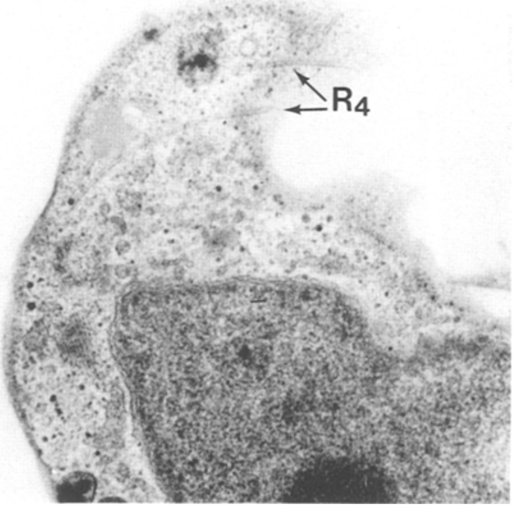
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bres. A second basal body (B″) lies more or less perpendicular to the first and is connected to it by a striated band (Figs. 7–9). There is a cartwheel structure at the proximal end of basal body B′ (Fig. 10).

### *Flagellar roots*

A system of four microtubular roots and one rhizoplast is present and is diagrammatically illustrated in Fig. 11. Because cytoskeletal microtubules were not clearly observed associated with the R 1 root of *Vischeria*, as occurs in many other heterokonts (Andersen 1989), and because the tripartite tubular hairs and flagellar swelling occur on the same flagellum in *Vischeria*, unlike most heterokonts (Preisig 1989), the labeling of microtubular roots R 1–R 4 for *Vischeria* is not as certain as for many other heterokonts.

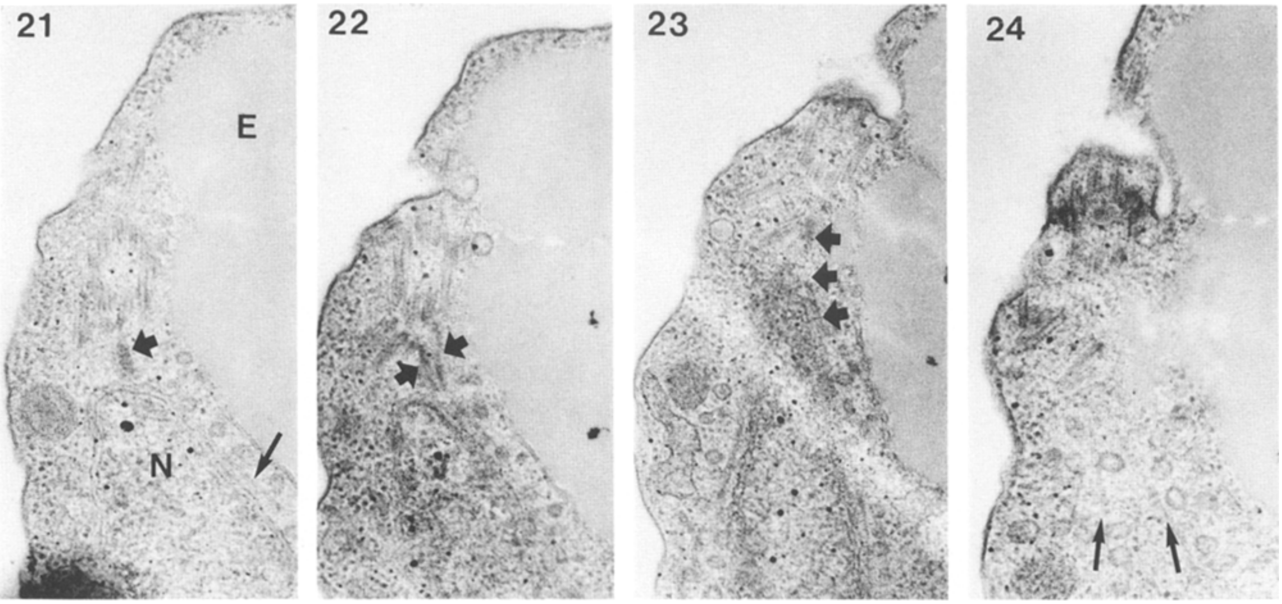
Root R 1 originates at basal body B′ (Figs. 12 and 13) and runs anteriorly, close to the plasmalemma and around the flagellar swelling for a considerable extent, almost completing a loop around it (Fig. 14). Transverse sections of the zoospores show this root to consist of two MTs (Figs. 4–9) but in longitudinal sections three MTs are evident (Figs. 13 and 14); the leftmost in Figs. 13 and 14 is widely separated from the other two MTs and is probably part of root R 2.

Root R 2 extends from the opposite side of basal body B′ (Fig. 15), runs close to the plasmalemma around the swelling and consists of two MTs at the origin, reducing to one (Figs. 4–7).

Root R 3 is associated with dense material between the basal bodies (Figs. 12, 15, and 16) and runs close to the plasmalemma down to the posterior end of the cell (not shown). It is composed of a band of five MTs which appear in cross-section as a semicircular arc (Figs. 17 and 18). It originates between the two basal bodies (Fig. 17; see also Figs. 9 and 10) and, in some sections (Fig. 12 and 16), it seems to be connected to basal body B′ by dense material.

Root R 4 consists of two MTs and extends from basal body B″ to run close to the plasmalemma (Figs. 19 and 20; see also Figs. 12, 13, 15, and 16) and possibly around the eyespot (see Figs. 6 and 8).

The only fibrous root seen in these zoospores is the rhizoplast (Figs. 21–24). It originates near the basal bodies and passes deeply into the cell, forming a characteristic association with the nucleus (Figs. 21 and 22). Near the nucleus, the rhizoplast splits into several branches spreading over the nuclear surface (Figs. 23 and 24). Near the basal bodies it appears also branched and divided into two parts that connect with the basal bodies (Fig. 22).



**Figs. 21–24.** *Vischeria stellata.* Selected longitudinal sections of a series, showing the rhizoplast in the zoospores. View from the left side of the zoospore

**Fig. 21.** Cross-banded structure (large arrow) and a fibre (small arrows) near the nuclear envelope. *E* Eyespot, *N* nucleus. × 50,000

**Fig. 22.** Two branches that connect with the basal bodies (arrows). × 50,000

**Fig. 23.** The rhizoplast divides into several parts (arrow) near the nucleus. × 47,500

**Fig. 24.** Fibres (arrows) immediately above the plane of the nuclear surface. × 60,000

## Discussion

### *The flagellar roots*

Four basic types of microtubular roots are now recognized in heterokont algae and fungi, referred to as roots R 1, R 2, R 3, and R 4 (for reviews, see Andersen 1987, 1989; Preisig 1989). These roots are also present in the zoospores of *Vischeria stellata* and other eustigmatophycean species observed (Santos 1990) in a system that, so far, seems typical for the class and similar to that displayed by the oomycete fungi (compare Fig. 11 with Fig. 1 d of Andersen 1987).

Root R 1 has the same origin and consists of two or three MTs as it does in the heterokont fungi (Barr and Désaulniers 1989) and most of the other heterokont algae (Andersen 1987, Preisig 1989) but the evidence that it also nucleates cytoskeletal MTs is confined to an occasional "whiskery" appearance in *Vischeria* (not shown). A difference is that this root R 1, together with root R 2, seems to loop around the anterior flagellum, as do roots R 3 and R 4 around the posterior flagellum in the Chrysophyceae. The roots perhaps have a similar function of maintaining a close association between the flagellar swelling and the eyespot in both groups and, if this is so, it may be a feature common to other organisms with related types of photoreceptors (e.g., species of Tribophyceae and Paeophyceae). Root R 1 in the Synurophyceae loops around both flagella but the photoreceptor consists only of paired flagellar swellings that are not associated with the cell membrane or chloroplast envelope, and an eyespot is absent (Andersen 1985, 1987). The loop may therefore help to maintain the parallel position of the flagella and swellings, possibly important in the photoreception of these organisms.

Root R 3 is associated with dense material between the two basal bodies and runs directly to the posterior end of the cell whereas in many heterokonts it extends laterally to the ventral surface. Transverse sections of the root show that the five MTs are arranged in a semicircular manner as in *Thraustochytrium aureum* Goldstein (Barr and Allan 1985) but it was not possible to determine with certainty if it originates from basal body B″, basal body B′, or both; in some sections (Fig. 16) it seems to be connected to basal body B′ by two bands of dense material. A function in maintaining the flask-shaped form typical of the eustigmatophycean zoospores seems likely since some of the MTs are seen running to the posterior end of the cell.

Finally, root R 4 also originates from basal body B″ but seems to curve around the bulging eyespot when entire transverse sections are examined (shown partially in Figs. 6 and 8); if this is so, it presumably maintains the characteristic shape of the eyespot and also of the anterior end of the cell, together with roots R 1 and R 2.

The rhizoplast seems basically similar to that described for other heterokont algae (Chrysophyceae, Synurophyceae, and Raphidophyceae; for reviews, see Moestrup 1982, Preisig 1989).

### *Division level classification*

The class Eustigmatophyceae has been generally accepted by phycologists but its proposed elevation to a division as the Eustigmatophyta has been controversial. Several authors place the class in this separate division (e.g., Leedale, 1974, 1980; Loeblich and Loeblich 1978; Hibberd 1981) whereas some include it in the same division as other heterokont algal classes and heterokont fungi (e.g., Cavalier-Smith 1986).

Flagellar differences have long been used for taxonomic and phylogenetic purposes and the ultrastructure of the motile cell, in particular the flagellar apparatus, has recently become the most useful indication of relationships within the protists in general and the algae in particular (Hibberd 1979, Moestrup 1982, Melkonian 1984, O'Kelly and Floyd 1984, Preisig 1989). Hibberd considers the most consistent classification to be that of van den Hoek (1978), where the eustigmatophytes are raised to the level of division; conversely, Preisig (1989) maintains the Eustigmatophyceae within the Heterokonta sensu Cavalier-Smith (1986). The main ultrastructural features shared by the Eustigmatophyceae and several of the classes included in this division are as follows.

1. Heterokont flagellation, with tripartite tubular hairs (mastigonemes) on the anteriorly directed flagellum.
2. Transition region, with a transitional helix in both flagella of the eustigmatophycean species having biflagellate zoospores [e.g., *Pseudocharaciopsis minuta* (Braun) Hibberd (Santos 1990); *Pseudocharaciopsis ovalis* (Chodat) Hibberd (Hibberd 1969)].
3. Basal bodies more or less perpendicular to one another.
4. Similar patterns of flagellar roots.
5. Photoreceptor system involving a flagellar swelling.
6. Chloroplast surrounded by endoplasmic reticulum, sometimes connected with the nuclear envelope.
7. Chloroplast lamellae consisting of three thylakoids.

The main differences of cell ultrastructure that separate the Eustigmatophyceae from those same groups are the following.

1. Unique type of photoreceptor system, consisting of a flagellar swelling located at the base of the anteriorly directed, mastigoneme-bearing long flagellum (even if the species is biflagellate) in association with an eyespot that is very large, extraplastidial and not membrane-bounded. In other heterokont algae where a photoreceptor is present, it is of the chrysophycean or synurophycean types (Andersen 1987); the first includes a swelling on the smooth short flagellum associated with an intraplastidial eyespot and the second consists of paired flagellar swellings.
2. Absence of a girdle lamella in the Eustigmatophyceae, though some rapidophycean (see Heywood 1989), tribophycean (Massalski and Leedale 1969, Hibberd and Leedale 1971 b), synurophycean and chrysophycean species (see Andersen 1986) also lack the girdle lamella.

In our opinion, the new information presented on the system of flagellar roots displayed by *Vischeria stellata* and other eustigmatophycean species (Santos 1990) supports its relationship to the heterokont algal classes Raphidophyceae, Tribophyceae, Chrysophyceae, Synurophyceae, Phaeophyceae, Bacillariophyceae, and the fungal classes Oomycetes and Hyphochytriomycetes. The Eustigmatophyceae should therefore be placed with these classes in a common division, the Heterokontophyta (van den Hoek 1978, Leedale 1980).

## Acknowledgements

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