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Morphology, cytology and taxonomic remarks of four species of *Stigeoclonium* (Chaetophorales, Chlorophyceae) from Argentina

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SUMMARY

Four species of *Stigeoclonium* from Argentina were studied by means of transmission electron microscopy and light microscopy. For species identification, we collected data related to the prostrate system and zoospore germination. We also determined the chromosome number for each species. *Stigeoclonium aestivale* showed a more developed erect system than the prostrate one, zoospore germination was predominantly of erect type and the chromosome number was 8. *Stigeoclonium tenue* presented well developed, erect and prostrate systems, zoospore germination was initially of the prostrate type and the chromosome number was 5. In *Stigeoclonium variabile* the prostrate system predominated over the erect one, zoospore germination was strictly of the prostrate type and the chromosome number was 3. *Stigeoclonium farctum* presented a more developed prostrate system than the erect one, zoospore germination was strictly of the prostrate type and the chromosome number was 8. The ontogeny of the zoospore germination was related to the final relative development of the prostrate and erect portions of adult thalli.

Key words: Chaetophoraceae, karyology, *Stigeoclonium*, taxonomy, ultrastructure.

INTRODUCTION

The order Chaetophorales includes epiphytic or epilithic freshwater algae composed of either branched or unbranched filaments, and occasionally, parenchymatous thalli of a heterotrichous nature, consisting of prostrate and erect systems. Cells are uninucleate, with a parietal chloroplast having one or more pyrenoids (Mattox & Stewart 1984). This order is currently circumscribed mainly by ultrastructural traits such as cytokinesis resulting from the formation of a cell plate in a phycoplast and the presence of plasmodesmata (John 1984; Mattox & Stewart 1984).

The family Chaetophoraceae is a well-defined taxon in terms of its ultrastructural traits. Typical specimens of this family show pyrenoids with appressed thylakoids. The quadriflagellate zoospores possess upper basal bodies that lie directly opposite, whereas the lower pair of basal bodies is rotated clockwise. Each pair of adjacent basal bodies is joined by means of peripheral and proximal fibers (John 1984; Watanabe & Floyd 1996).

The genus *Stigeoclonium* includes algae that form bright green tufts consisting of uniseriate branched heterotrichous filaments. It is an extremely polymorphic genus, which makes species delimitation difficult (Francke & Simons 1984). The genus was established by Kützing (1843), who described 29 species (Kützing 1845, 1849, 1853) based on several vegetative traits: cellular dimensions, degree of branching, presence/absence of hairs, thallus color, and habitat. Islam (1963) reviewed the genus critically recognizing 28 species, and then 42 taxa were described by Printz (1964). Cox and Bold (1966) were the first to propose a species concept that was based on the morphology of the prostrate system. They concluded that, under uniform culture conditions, the morphological attributes of the prostrate system were considerably more stable than those of the erect system and that these attributes provided a better basis for the identification of the seven species from North America. This approach was adopted by Francke and Simons (1984) and Simons *et al.* (1986) who also evaluated the significance of zoospore germination as a taxonomic character as first noted by Berthold (1878). Francke and Simons (1984) recognized four morphological groups as species. Simons *et al.* (1986) provided more detail on the types of germination and recognized three species from the

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Netherlands based on this criterion. Branco *et al.* (2002) identified six species from southeastern Brazil, combining characters of the prostrate and erect systems, and Skinner and Entwisle (2004) described three species from Australia following the species concept of Simons *et al.* (1986).

In the present study, four isolates of *Stigeoclonium*-like thalli from Argentina were studied using transmission electron microscopy (TEM) to assess whether they belong to the family Chaetophoraceae. To identify the isolates at the species level, we studied thallus development from zoospores using light microscopy. Isolates were also submitted to karyological studies.

MATERIALS AND METHODS

Stigeoclonium specimens were collected in the south of Buenos Aires province, Argentina, in streams and water pools located between 38°8′–38°43′ S and 61°47′–62°16′ W. Specimens were cultured under unialgal conditions in Bold's Basal Medium (Stein 1973) at 10°C and 16/8 hs photoperiod provided by cool-white fluorescent lamps. Zoospore formation was induced by transferring filaments to Petri dishes filled with fresh culture medium after which zoospore release occurred within 24 h. The filaments were removed and zoospore germination and thallus development were studied for 6 weeks. Sterile coverslips were floated on the medium to provide a surface for the filaments to attach themselves. Coverslips were removed at subsequent intervals to observe the development of the prostrate system. Taxonomic identification was based on zoospore germination type and thallus development features according to Cox and Bold (1966), Francke and Simons (1984), and Simons *et al.* (1986). Light microscopy was carried out with a Carl Zeiss Axiolab (Jena, Germany) microscope with anoptral phase-contrast. Karyological observations were carried out on cultured samples fixed in 3:1 absolute alcohol: glacial acetic acid for about 24 h and stained with iron-acetic carmin (Johansen 1940) and acetic orcein (La Cour 1941). For TEM observation, thalli were fixed at 4°C in 2% glutaraldehyde in filtered culture medium, postfixed in 1% OsO_4, dehydrated through a graded acetone series, and embedded in Spurr's low-viscosity resin (Spurr 1969) using a flat embedding technique (Reymond & Pickett-Heaps 1983). Sections were cut with a diamond knife, mounted on Formvar-coated grids and stained with uranyl acetate and lead citrate. Sections were observed with a Jeol 100 CX-II electron microscope at the Centro Científico Tecnológico Bahía Blanca.

Strains were deposited at the UTEX Culture Collection (accessions LB 2921, LB 2922, LB 2923, LB 2924).

RESULTS

Ultrastructure

Filaments from the four isolates possessed transverse cell walls with uniformly distributed plasmodesmata without desmotubules (Figs 1, 2, arrowheads). Both longitudinal and transverse cell walls had fibrillar or homogenous structure or slight stratification, and in some cases, the filaments were covered by a mucilaginous layer (Fig. 2). The nucleus had a central position and two polar vacuoles were always present (Fig. 3). The chloroplast contained one or two pyrenoids surrounded by a thylakoidal membrane (inset Fig. 3, arrowheads) and starch plates of different sizes.

Species account

Stigeoclonium aestivale (Hazen) Collins, Tufts Coll. Stud. Sci. 2: 221 (1909)

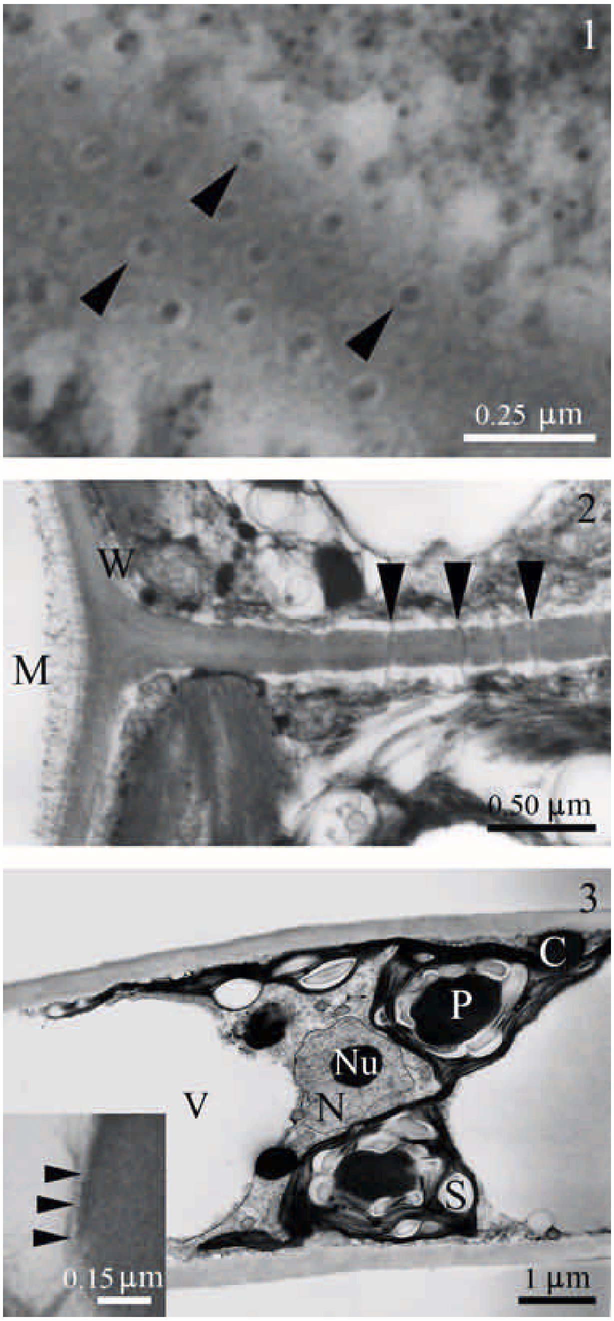
Thallus development. Zoospore germination was predominantly erect. Zoospores elongated and the first division gave rise either to an erect filament or to a prostrate cell that grew bilaterally (Fig. 4). In the latter case, the erect filament appeared almost simultaneously and continued developing while the incipient prostrate system was reduced (Fig. 5). During the second week of development the erect system continued to grow while the prostrate system was unbranched (Fig. 6). In the fourth week, the development of the prostrate system lagged behind that of the erect system and it consisted of an unbranched or sparsely branched filament that was discernible during the whole development (Fig. 7). The erect system of the mature thallus was more developed than the prostrate one. Cells of the prostrate system were doliiform, 4–6 μm in diameter, and 2.3–2.5 times longer than wide. Cells of the erect system were cylindrical, 4 μm in diameter, and 1.8–3.7 times longer than wide (Fig. 7). The branching was either alternate or opposite.

Karyology. A chromosome number of 8 was determined (Figs 8,9).

Habitat. Either epiphytic on shoots and roots of aquatic angiosperms or epilithic.

Distribution. Cosmopolitan. In Argentina this species was reported from the province of Buenos Aires (El Burro lagoon) (Tell 1972).

Material examined. Napostá Chico stream, 38°28′ 60″ S, 61°53′ 60″ W, *K.M. Michetti S4,* 11.xi.1998 (BBB). UTEX Culture Collection (LB 2924).



Figs 1–3. Transmission electron micrographs of *Stigeoclonium.* C, chloroplast; M, mucilage; N, nucleus; Nu, nucleolus; P, pyrenoid; S, starch; V, vacuole; W, cell wall. 1. *Stigeoclonium tenue*. Transverse section through a cell wall showing plasmodesmata (arrowheads). 2. *Stigeoclonium variabile*. Details of the mucilage layer of a longitudinal cell wall and of the plasmodesmata in a transverse cell wall (arrowheads). 3. *Stigeoclonium aestivale*. Vegetative cell showing cytoplasmic organelles. Scale bar, 1 µm. Inset: Detail of the pyrenoid matrix surrounded by a thylakoidal membrane (arrowheads).

Stigeoclonium tenue (C. Agardh) (Kützing, Phyc. Gen. 253 (1845)

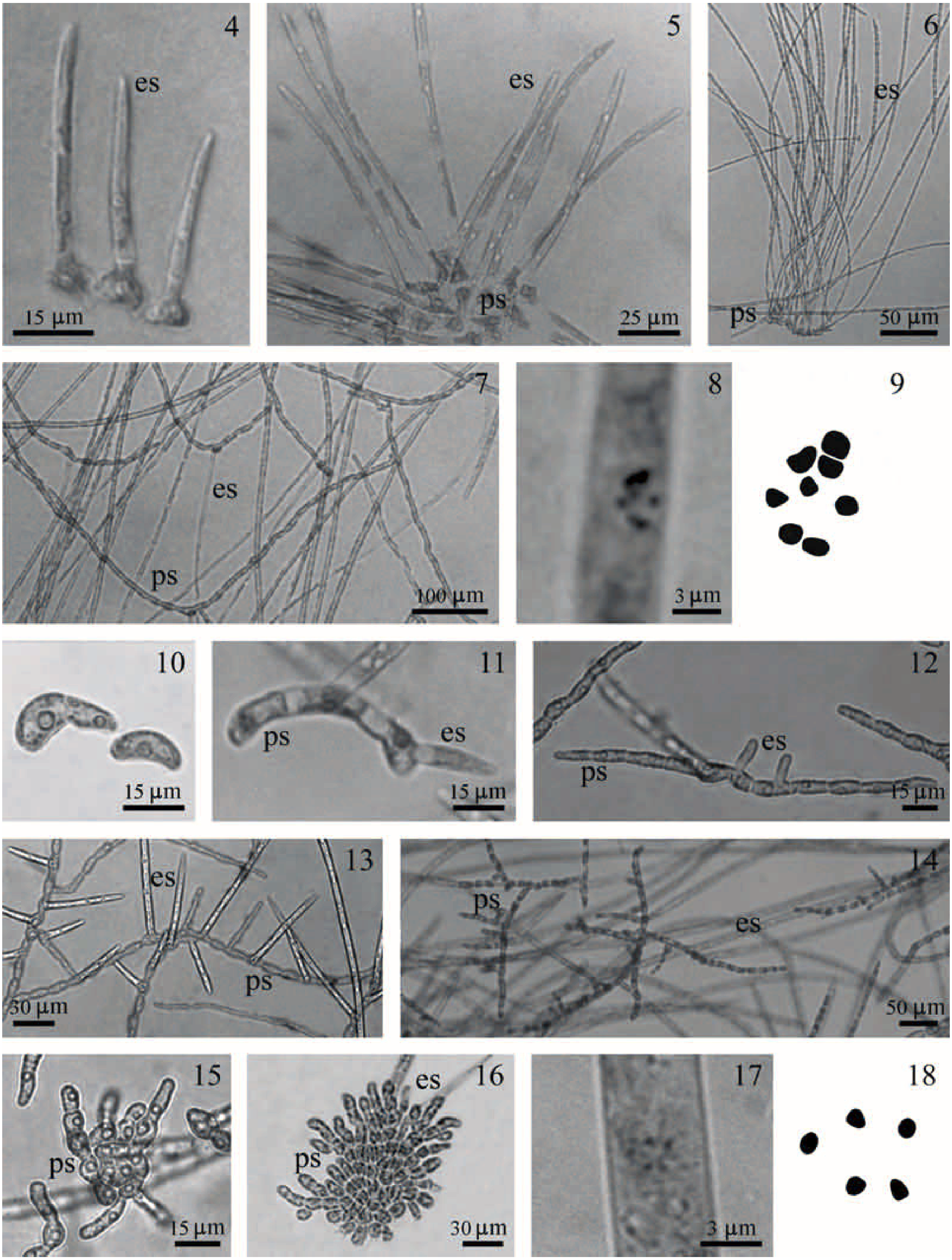
Thallus development. Zoospore germination was initially prostrate, and was immediately followed by the formation of the erect system. The zoospore elongated slightly, acquiring an ovoid shape with its main axis parallel to the substrate, and divided in a unipolar or bipolar fashion to develop into a short prostrate filament (Fig. 10). This stage persisted for a couple of days after which an erect filament with a pointed apex developed from the central region of the germling (Fig. 11). During the first 2 weeks of development of the prostrate system, a principal axis or primary filament became apparent, from which lateral branches arose (Fig. 12) subsequently becoming branched (Fig. 13). During the third and fourth week of development, a mature, extensively branched prostrate system had developed, rendering the principal axis no longer distinguishable (Fig. 14). In some instances, zoospores germinated in groups (Fig. 15) giving rise to a compact prostrate system with isodiametric central cells (Fig. 16). In the mature thallus, both the prostrate and erect system were well developed. Cells of the prostrate system were doliiform with marked constrictions at the level of the transverse cell walls, 4–8 µm in diameter, and 1.5–2.0 times longer than wide. Cells in the erect system were cylindrical, rarely constricted at the level of the transverse cell walls. The principal axis consisted of cells 6–8 µm in diameter and 1.3–3.5 times longer than wide. The branching pattern was alternate or opposite, rarely dichotomous or verticillated. Lateral branches consisted of cells 4–5 µm in diameter, varying from isodiametric to 1.7–6.5 times longer than wide (Fig. 16). Occasionally the erect filaments bore uni- or multicellular hyaline apical hairs. Rhizoids extended from the filaments of the prostrate portion.

Karyology. A chromosome number of 5 was determined (Figs 17,18).

Habitat. Thalli epilithic or epiphytic on shoots and roots of aquatic angiosperms or other algae such as *Oedogonium* Link and *Cladophora* Kütz.

Distribution. Cosmopolitan. In Argentina *S. tenue* was reported from the province of Córdoba (Primero River) by Seckt (1931), from the province of Santa Cruz (Santa Cruz River and Calafate River) by Seckt (1931, 1950–1956), and from the province of Buenos Aires (San Miguel del Monte lagoon) by Guarrera (1962).

Observations. Thalli with more compact prostrate systems were also observed in nature.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Material examined. Paso de las Piedras dam, 38°22′ S, 62°12′ W, *K.M.Michetti S3*, 10.iii.1996 (BBB). UTEX Culture Collection (LB 2921).

Stigeoclonium variabile (Nägeli) Islam, Nova Hedwigia, 10: 55 (1963)

Thallus development. Zoospore germination was strictly prostrate with a delayed development of the erect system. Germlings gave rise to a prostrate primary filament which, after 2 weeks in culture, remained unbranched (Figs 19,20) and then became scarcely branched (Fig. 21). The erect system developed in the course of the third week (Fig. 21). During the fourth week the mature prostrate system was an openly branched filament. The prostrate system of the mature thallus was far more prominent than the erect system (Fig. 22). Cells of the prostrate system were doliiform with marked constrictions at the level of the transverse walls, 6–10 μm in diameter and ranged from isodiametric to 1.2 times longer than wide. Terminal rhizoidal filaments were 3–4 μm in diameter. Cylindrical cells of the erect system were 4–5 μm in diameter and 1.8–2.6 (–5) times longer than wide (Fig. 22), and only rarely possessed constrictions at the level of the transverse walls. Both alternate and opposite branches were observed.

Karyology. A chromosome number of 3 was determined (Figs 23,24).

Habitat. Epiphytic thalli on artificial substrates.

Distribution. Cosmopolitan. In Argentina *S. variabile* has been reported from the province of Tucumán (Los Aguirre Lagoon) by Tracanna (1979).

Material examined. Effluent pools, Bahía Blanca, 38°43′ 0″ S, 62°16′ 60″ W, *P.I. Leonardi S2*, 4.xii.1995 (BBB). UTEX Culture Collection (LB 2923).

Stigeoclonium farctum Berthold, Nova Acta Kgl Leopold. Carol. Dt. Akad. Naturforscher 40: 201 (1878)

Thallus development. Zoospore germination was strictly prostrate with delayed development of the erect system. Germlings gave rise to a primary prostrate filament (Fig. 25), which, after 2 weeks under culture, developed branches (Fig. 26). Eventually, the prostrate system had a feather-like appearance with dense branches (Fig. 27). The erect system developed during the course of the third week (Fig. 27). During the fourth week, a compact, mature prostrate system had developed with a central pseudoparenchymatous area composed of filaments laterally trapped with branches occupying the spaces between the principal filament and the open margins (Fig. 28). The prostrate system of the mature thallus was far more prominent than the erect system. Cells of the prostrate system ranged from polyhedrical and isodiametric to cylindrical, 3–6 μm in diameter, and 1.6–2.0 times longer than wide. Cylindrical cells of the erect system were 4–5 μm in diameter and 2.5–3.0 times longer than wide (Fig. 28). Both alternate and opposite branches were noted.

Karyology. A chromosome number of 8 was determined (Figs 29,30).

Habitat. Epiphytic on *Rorippa* sp.

Distribution. Cosmopolitan. In Argentina *S. farctum* has been reported from the Calchaquí River (province of Salta) by Seckt (1931) and from the Salada lagoon (province of Santa Cruz) by Seckt (1950–1956).

Material examined. El Divisorio stream, 38°28′ 60″ S, 61°53′ 60″ W, *M.V. Sánchez Puerta S5*, 20.vi.2001 (BBB). UTEX Culture Collection (LB 2922).

DISCUSSION

The presence of plasmodesmata and pyrenoids lacking traversing thylakoidal membranes in the vegetative

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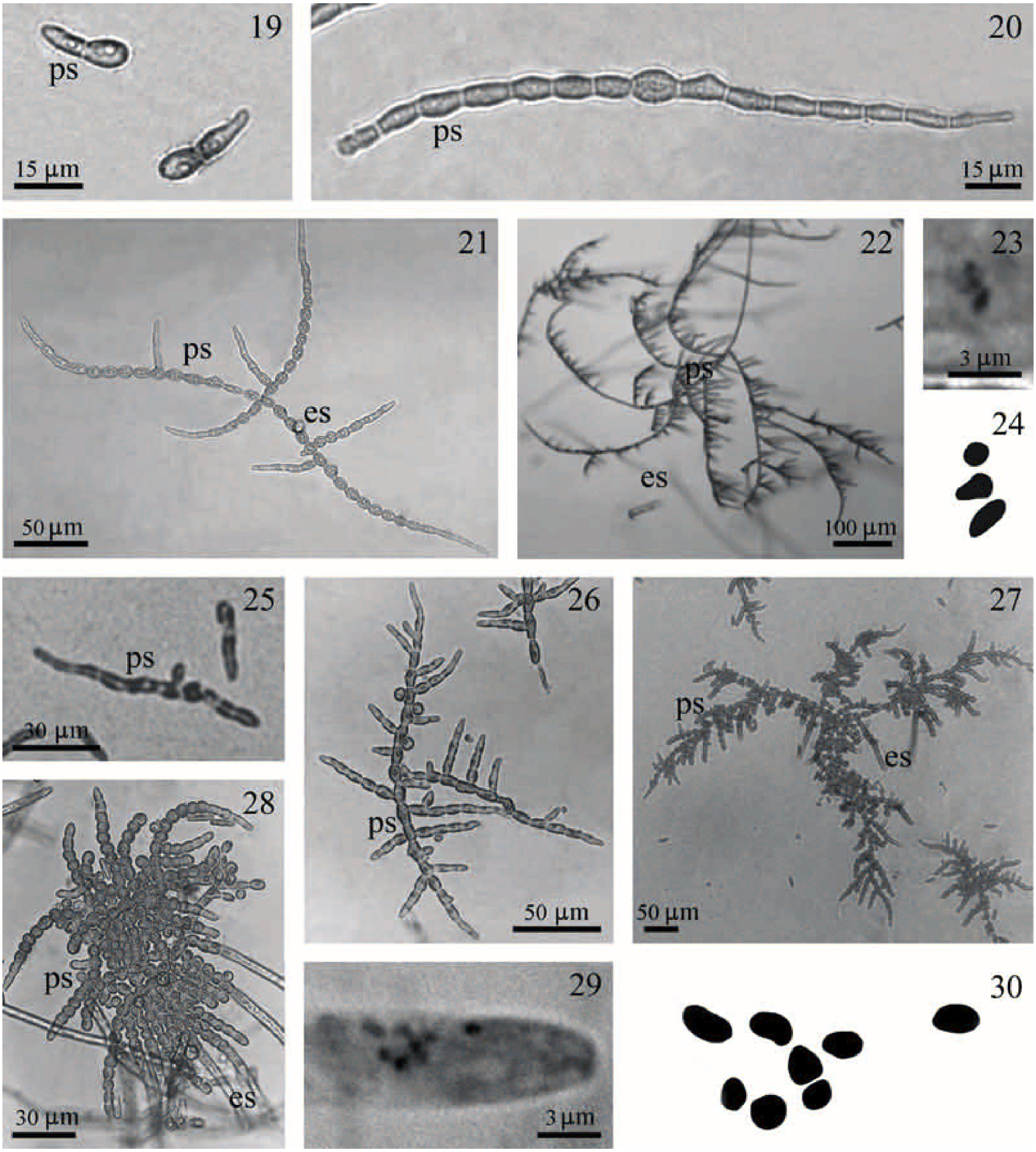
Figs 4–18. Thallus development and karyology of *Stigeoclonium*. es, erect system; ps, prostrate system. 4–9. *Stigeoclonium aestivale*. 4. Predominantly erect zoospore germination. 5. One-week-old culture. Note the reduced prostrate system. 6. Two-week-old culture showing profuse development of an erect system and a prostrate system composed of an unbranched filament. 7. Four-week-old culture showing a mature thallus. A poorly developed prostrate system made up of branched filaments with a distinct principal axis is observed. Well developed erect system. 8–9. Metaphase ($n = 8$) and corresponding camera lucida drawing, respectively. 10–18. *Stigeoclonium tenue*. 10. Initially prostrate zoospore germination. 11. Formation of the erect system. 12–13. One-to-two week-old culture. The prostrate system is made up of a primary filament with unilateral branches. 14. Three-to-four week-old culture. Mature thallus showing an extensively branched prostrate system and a well developed erect system. 15. Cluster of prostrate systems resulting from germination of a group of zoospores. 16. Compound thallus resulting from germination of a group of zoospores. 17–18. Metaphase ($n = 5$) and corresponding camera lucida drawing, respectively.



Figs 19–30. Thallus development and karyology of *Stigeoclonium.* es, erect system; ps, prostrate system. 19–25. *Stigeoclonium variabile.* 19. Strictly prostrate zoospore germination. 20. Two-week-old culture. Unbranched prostrate primary filament. 21. Three-week-old culture. Note the loosely branched prostrate system and the poorly developed erect system. 22. Four-week-old culture. Mature thallus composed of an open prostrate system profusely developed with respect to the erect system. 23–24. Metaphase ($n = 3$) and corresponding camera lucida drawing, respectively. 25–30. *Stigeoclonium farctum.* 25. Strictly prostrate zoospore germination. 26. Two-week-old culture. The prostrate system is extensively branched. 27. Three-week-old culture. A prostrate system with a feather-like pattern with dense branches is observed. Note the early formation of the erect system. 28. Four-week-old culture. Mature thallus consisting of a pseudoparenchymatous prostrate system that is more developed than the erect system. 29–30. Metaphase ($n = 8$) and corresponding camera lucida drawing, respectively.

cells suggests that the algal isolates belong to the Chaetophoraceae (Manton 1964; McBride 1970; Floyd *et al.* 1972; Stewart *et al.* 1973; Stewart & Mattox 1975; Lokhorst *et al.* 1984; Ducher *et al.* 1988).

A relationship between the degree of development of the prostrate and erect systems in adult thalli and the type of zoospore germination has been previously documented for *Stigeoclonium* (Cox & Bold 1966). Adult thalli with well-developed erect systems and reduced prostrate systems had an erect type of zoospore germination, whereas those with extensive prostrate systems were mainly characterized by a prostrate type of germination (Cox & Bold 1966). The present study provides further support for this relationship. We observed a prostrate type of germination in *S. farctum* and *S. variabile*, which show a predominant adult prostrate system over the erect one. In *S. tenue*, where there was a balanced development between the two systems, the zoospore germination was initially prostrate. In *S. aestivale*, the erect system prevailed over the prostrate system and zoospore germination was predominantly of the erect type. These observations on *Stigeoclonium* and previous studies on *Chaetophora elegans* (Michetti *et al.* 2004) indicate that zoospore germination pattern in Chaetophoraceae appears to be a good indicator of the relative development of the prostrate and erect portions in the adult thalli.

Taxonomic remarks

The morphology of the prostrate system of *Stigeoclonium aestivale* was similar to previous reports (Cox & Bold 1966; Francke & Simons 1984). However, the present study is the first, to our knowledge, to report a predominantly erect germination type in *Stigeoclonium.*

The morphological description of *Stigeoclonium tenue* agrees with previous descriptions (Cox & Bold 1966; Francke & Simons 1984; Simons *et al.* 1986; Skinner & Entwisle 2004). Adult thalli developed a prostrate system with an indistinct principal axis, similar to the *aestivale* morphology of *S. tenue* described by Simons *et al.* (1986). In the present study, the prostrate system of *S. tenue* was sometimes more compact, similar to the morphotype *pascheri* of *S. tenue* (Francke & Simons 1984). In nature, compact prostrate systems have been found only occasionally and could be interpreted as resulting from the germination of groups of zoospores, since coalescence was confirmed in culture through periodic observation of the same coverslips over which thalli developed. Cienkowski (1876) observed that the zoospores of *S. stellare* fused with one another giving rise to filaments that formed a compact prostrate system. However, Cox and Bold (1966) claimed that, although zoospores germinated in groups in several of the species they studied; it was always possible to distinguish individual growths of zoospores, with no evidence of coalescence.

Our morphological description of the adult prostrate system of *Stigeoclonium variabile* was comparable to one of the three forms identified by Printz (1964); that is, form *Gayanum* Heering. The description also matched one of the first developmental stages of the prostrate system reported by Cox and Bold (1966). A bilateral prostrate germination pattern was also observed by Printz (1964) and Cox and Bold (1966). Some authors have considered *S. variabile* as a morphological and/or ecological type of *S. farctum* with a more open prostrate system (Francke 1982; Francke & Simons 1984; Simons *et al.* 1986). However, according to our taxonomic criterion – morphology of the prostrate system and zoospore germination – we consider *S. variabile* to be a separate species.

Similarities between the adult forms of *S. tenue* and those of *S. variabile* observed in the present study are worth noting. The only difference between the two species was the type of zoospore germination.

The mature prostrate system of *Stigeoclonium farctum* was comparable to the young forms of *S. farctum* observed by Printz (1964) and Cox and Bold (1966) and to the morphology *variabile* in *S. farctum* (Francke & Simons 1984; Simons *et al.* 1986). It also matched the description given by Branco *et al.* (2002). The strictly prostrate germination type observed in our isolates was similar to drawings of *S. farctum* in Printz (1964) and to those presented by Francke and Simons (1984) and Simons *et al.* (1986). In contrast, Cox and Bold (1966) described two types of germination for this species: a more frequent, prostrate type giving rise to a filament that branched several times, and an erect type with subsequent development of the prostrate system occurring less frequently.

Karyology

Karyological data can only be compared among species that have been identified using the same taxonomic criteria (Francke & Simons 1984). For that reason, we only included comparisons with those species recognized by Cox and Bold (1966). As shown in Table 1, it is extremely difficult to unambiguously determine the chromosome number for each species of the genus *Stigeoclonium*. The observed chromosome number of 8 in *S. aestivale* agrees with that observed in the same species by Francke and Simons (1984); however, 10 chromosomes were also reported for *S. aestivale* (Sarma & Shashikala 1980; Francke & Simons 1984). The chromosome number of 5 repeatedly found in *S. tenue* differed from those previously reported (Sarma & Shashikala 1980; Francke & Simons 1984). The chromosome number of 3 observed in *S. variabile* did not correspond to the numbers observed by Sarma and

Table 1. Chromosome number (*n*) recorded in the present and previous studies

Species	Chromosome numbers (*n*) Present study	Early studies
Stigeoclonium aestivale	8	8, 10 (Francke & Simons (1984)
		10 (Sarma & Shashikala 1980)
Stigeoclonium tenue	5	11 (Sarma & Shashikala 1980)
		6, 8, 12 (Francke & Simons 1984)
Stigeoclonium variabile	3	6 (Sarma & Shashikala 1980)
		8 (Francke & Simons 1984)
Stigeoclonium farctum	8	8 (Francke & Simons 1984)

Shashikala (1980) and Francke and Simons (1984). It is conceivable that Sarma and Shashikala's count referred to the diploid condition of *S. variabile*. Instead, no correspondence was found between our count and Francke and Simons (1984). The chromosome number of 8 observed in *S. farctum* agrees with that obtained by Francke and Simons (1984).

In his review on the karyology of the order Chaetophorales, Sarma (1982) stated that aneuploidy is the principal factor involved in the highly variable chromosome counts. Francke and Simons (1984) suggested that polyploidy could also contribute to such variability, particularly in the genus *Stigeoclonium*.

Stigeoclonium genus in Argentina

Eleven species of *Stigeoclonium* have been reported for Argentina (Tell 1972; Tracanna 1979, 1985; Luque & Martínez de Fabricius 2002). However, these studies based their taxonomic identification on morphological traits, such as the morphology of erect system. This is the first contribution to the identification of *Stigeoclonium* species based on the characteristics of the prostrate system and zoospore germination, along with ultrastructural traits and karyological data. Further studies based on this updated taxonomic understanding of the genus will allow for a better delimitation of the species and for an appropriate validation of the seven remaining species reported for Argentina.

Key to the studied species of *Stigeoclonium*

1. Zoospore germination predominantly erect. Prostrate system made up of branched filaments with a distinct principal axis. *Stigeoclonium aestivale*
   Zoospore germination initially prostrate. 2
2. Germination initially prostrate with early formation of erect portion. Prostrate system composed of branched filaments, usually open, but occasionally closed *Stigeoclonium tenue*
   Germination strictly prostrate, with delayed formation of the erect system 3
3. Prostrate system open *Stigeoclonium variabile*
   Prostrate system closed *Stigeoclonium farctum*

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