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New insights into postfertilization in Dudresnaya (Gigartinales, Rhodophyta) and Acrosymphyton (Acrosymphytales, Rhodophyta)

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Abstract:	Dudresnaya P.Crouan & H.Crouan (Dumontiaceae, Gigartinales) and Acrosymphyton Sjöstedt (Acrosymphytaceae, Acrosymphytales) are florideophycean genera (Rhodophyta) traditionally described as presenting a typical non-procarpic behaviour. The postfertilization stages of their corresponding generitypes, D. verticillata (Withering) Le Jolis and A. purpuriferum (J.Agardh) G.Sjöstedt, have been described on many occasions. The main objective of this study was to investigate the development of the carpogonial and auxiliary fusion cells in the generitypes. Main results include a) the description of the carpogonial fusion cell development by secondary connection (via fusion in D. verticillata and via conjunctor cells in A. purpuriferum); b) the assessment of the existence, prior to diploidization, of preliminary auxiliary fusion cells in both species, and the description of their development; and c) the observation of occasional procarpic behaviour in both species, with gonimoblast developing from the secondary connecting filament initials in D. verticillata and from the carpogonial fusion complex in A. purpuriferum. The study has allowed to increase the understanding of the postfertilization processes and phylogeny of procarpic and non-procarpic taxonomic groups, and supports the hypothesis that the procarpic behaviour is secondarily derived from non-procarpic ancestors.

New insights into postfertilization in *Dudresnaya* (Gigartinales, Rhodophyta) and *Acrosymphyton* (Acrosymphytales, Rhodophyta)

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ABSTRACT

Dudresnaya P.Crouan & H.Crouan (Dumontiaceae, Gigartinales) and *Acrosymphyton* Sjöstedt (Acrosymphytaceae, Acrosymphytales) are florideophycean genera (Rhodophyta) traditionally described as presenting a typical non-procarpic behaviour. The postfertilization stages of their corresponding generitypes, *D. verticillata* (Withering) Le Jolis and *A. purpuriferum* (J.Agardh) G.Sjöstedt, have been described on many occasions. The main objective of this study was to investigate the development of the carpogonial and auxiliary fusion cells in the generitypes. Main results include a) the description of the carpogonial fusion cell development by secondary connection (via fusion in *D. verticillata* and via conjunctor cells in *A. purpuriferum*); b) the assessment of the existence, prior to diploidization, of preliminary auxiliary fusion cells in both species, and the description of their development; and c) the observation of occasional procarpic behaviour in both species, with gonimoblast developing from the secondary connecting filament initials in *D. verticillata* and from the carpogonial fusion complex in *A. purpuriferum*. The study has allowed to increase the understanding of the postfertilization processes and phylogeny of procarpic and non-procarpic taxonomic groups, and supports the hypothesis that the procarpic behaviour is secondarily derived from non-procarpic ancestors.

KEYWORDS

Acrosymphyton purpuriferum, *Dudresnaya verticillata*, Non-procarpic behaviour; Reproduction

INTRODUCTION

In the most primitive Florideophyceae (Rhodophyta), gonimoblast filaments develop directly from the carpogonium or from the so called carpogonial fusion cell, while in the more advanced groups their development takes place through the involvement of an auxiliary cell (see e.g., Bornet & Thuret 1867; Schmitz 1883; Fritsch 1945; Kylin 1956; Dixon 1973; Hommersand & Fredericq 1990). Auxiliary cells, besides allowing the initiation of the gonimoblast, function as a mechanism to rule out incompatible or discordant fertilizations (Boo & Lee 1983). They can be located in the same branch system that suspends the carpogonium (= *procarpic behaviour*) or in an independent and distant branch system (= *non-procarpic behaviour*) (Fritsch 1945; Dixon 1973; Hommersand & Fredericq 1990). In procarpic genera, the transfer of the diploid nucleus from the carpogonium to the auxiliary cell is accomplished by connecting cells or by direct fusion of the carpogonium with the auxiliary cell. On the other hand, in non-procarpic genera, due to the distance between the abovementioned branch systems, the development of secondary connecting filaments (term coined by Berthold 1884) is necessary.

From the currently recognized orders inside the Rhodophyta (see Guiry & Guiry 2022), only six contain genera with a non-procarpic behaviour, namely: Acrosymphytales, Halymeniales, Nemastomatales, Peyssonneliales, Sebdeniales, and Gigartinales. As far it is known, the first five are entirely non-procarpic (Bornet & Thuret 1876; Kylin 1923, 1956; Sjöstedt 1926; Womersley 1994), but within the Gigartinales, only some families are non-procarpic: Areschougiaceae, Blinksiaceae, Calosiphonaceae, Caulacanthaceae, Cruoriaceae, Cubiculosporaceae, Furcellariaceae, Gainiaceae, Placentophoraceae, Rhabdoniaceae, Rhizophyllidaceae and Solieriaceae (Sjöstedt 1926; Kylin 1923, 1956; Dixon & Irvine 1977; Kraft 1973, 1976; Min-Thein & Womersley 1976; Gabrielson & Hommersand 1982; Irvine

1983; Hommersand & Fredericq 1990; Womersley 1994). In addition, the Dicranemataceae and the Kallymeniaceae (Gigartinales) includes both procarpic and non-procarpic genera (Kylin 1928; Norris 1957; Womersley 1994). In the case of the Kallymeniaceae, the procarpic behaviour is probably secondarily derived (Hommersand & Fredericq 1990). Related to this, *Dumontia contorta* (S.G.Gmelin) Ruprecht, the type species of Dumontiaceae, is considered non-procarpic, but in the species of *Weeksia* Setchell (Dumontiaceae) the auxiliary cell branches are non-functional and gonimoblast develop from a nutritive cell of the carpogonial branch (Abbot 1968). On the other hand, the type species *Polyides rotunda* (Polyidaceae, Gigartinales) (Joly & Ugadim 1966) and *Ptilocladiopsis horrida* (Ptilocladiopsidaceae, Gigartinales) (Rodríguez-Prieto *et al*. 2014) can simultaneously present procarpic and non-procarpic behaviours. The same is true for the type of Gloiosiphonaceae (Gigartinales), *Gloiosiphonia capillaris* (Hudson) Carmichael *in* Berkeley, that has been described as procarpic, but it can develop secondary connecting filaments that can diploidize auxiliary cells that are distant from the carpogonial branch (Sjöstedt 1926).

Secondary connecting filaments (*sensu* Fritsch 1945 and Robins & Kraft 1985), whose role is to allow the diploidization of the auxiliary cell, somehow 'transporting' the diploid nucleus from the carpogonium to the auxiliary cell, originate from the carpogonium itself in the type species of the Areschougiaceae (Min-Thein & Womersley 1976), Blinksiaceae (Hollenberg & Abbott 1968), Cubiculosporaceae (Kraft 1973), Gloiosiphonaceae (Sjöstedt 1926), Placentophoraceae (Kraft 1975), Solieriaceae (Gabrielson & Hommersand 1982), Nemastomatales (Berthold 1884), Rhabdoniaceae (Womersley 1994), Sebdeniales (Kylin 1923), and possibly, in the Cruoriaceae (Kylin 1928). Contrasting, in Acrosymphytales (Bornet & Thuret 1867, 1876), Calosiphonaceae (Berthold 1884), Dumontiaceae (Kylin 1923), Gainiaceae (Moe 1985), and Ptilocladiopsidaceae (Rodríguez-

Prieto *et al.* 2014), the secondary connecting filaments arise from a carpogonial fusion cell. The origin of secondary connecting filaments in the rest of non-procarpic groups is unknown. The carpogonial fusion cell develops from the fusion of the carpogonium with one or more cells of the carpogonial branch, but in Dumontiaceae and Acrosymphytales, the carpogonium can cut off one or more primary connecting filaments which can participate too in the development of the carpogonial fusion cell (Bornet & Thuret 1867, 1876; Oltmanns 1898; Kylin 1923). In the rest of the taxonomic non-procarpic orders (or families inside the Gigartinales), the origin of the secondary connecting filaments is unknown in the type species, except for the Kallymeniaceae, where these filaments arise from a fusion cell (Kylin 1928) that, taking in account that it includes most cells of the carpogonial branch system, could be called a 'carpogonial fusion system'.

In the type species of Acrosymphytales (Bornet & Thuret 1867), Blinksiaceae (Hollenberg & Abbott, 1968), Calosiphonaceae (Berthold 1884), Dumontiaceae (Kylin 1923), Polyidaceae (Kylin 1923), Ptilocladiopsidaceae (Rodríguez-Prieto *et al.* 2014), Halymeniaceae (Womersley 1994), and Sebdeniales (Kylin 1923), the presence of tertiary connecting filaments has been described. These kinds of filaments originate from the diploidized auxiliary cell and transfer the diploid nucleus to another auxiliary cell to diploidize it (Hommersand & Fredericq 1990). In the other groups these filaments are either absent, as in Gainiaceae (Moe 1985), or its presence has been not reported.

Secondary and, where present, tertiary connecting filaments can either be simple and grow by elongation, or be branched and grow by division. The later can diploidize several auxiliary cells, thus increasing the success of the fertilization which is quite difficult because of nonflagellate male gametes (Bornet & Thuret 1876; Drew 1954, 1955; Searles 1980; Kraft 1981).

Studies on postfertilization events in red algae (Rhodophyta) began at the end of the 19th century (Bornet & Thuret 1867, 1876, 1880), as a culmination of the previous advances on the knowledge of female reproductive structures performed mainly by Pringsheim (1855, 1856) and Bornet (1859). In this account, Bornet & Thuret (1867, 1876), provided the first description of female reproductive structures, fertilization and postfertilization stages in *Dudresnaya verticillata* (Withering) Le Jolis and *Acrosymphyton purpuriferum* (J.Agardh) G.Sjöstedt, the target species of the present study, and the type species of their respective genera: *Dudresnaya* P.Crouan & H.Crouan and *Acrosymphyton* Sjöstedt, respectively. Following these studies, a number of authors contributed to the knowledge of the reproduction of both *D. verticillata* (Bornet & Thuret 1867 1876; Agardh 1879; Schmitz 1883; Oltmanns 1898; Kylin 1928; Lindstrom 1985; Robins & Kraft 1985; Athanasiadis 1987) and *A. purpuriferum* (Bornet & Thuret 1867, 1876; Ardissone 1883; Schmitz 1883; Oltmanns 1898; Sjöstedt 1926; Kylin 1930; Feldmann 1939). Most female reproductive structures and postfertilization processes of these typically non-procarpic species were already described in those works and reproduced later in others. However, some aspects, which are incomplete or totally lacking have been the goal of the present study: the origin and development of the carpogonial fusion cells, and that of th primary, secondary and tertiary connecting filaments.

MATERIAL AND METHODS

Specimens were collected subtidally in the Mediterranean continental coast of Spain at depths between 27-48 m by SCUBA diving, and preserved in 3-5% Formalin in seawater or preserved as herbarium sheets. Preparations were stained with 1% aniline blue acidified with 1% HCl or treated with Wittmann's aceto-iron-haematoxylin-chloral hydrate (Wittmann 1965) and

mounted in 50% Hoyer's mounting medium modified by Rodríguez-Prieto & Hommersand (2009). Habit images were taken with an Epson scanner (Tokyo, Japan) and a Canon EOS 350D (Canon, Tokyo, Japan), and photomicrographs were made with an AxioCam MRc attached to an Axioskop 2 plus microscope (Carl Zeiss, Oberkochen, Germany). Voucher specimens were deposited at the Herbarium of University of Girona, Spain (HGI). Herbarium abbreviations follow Thiers (2022).

RESULTS

Dudresnaya verticillata

Figs 1-32

TAXONOMIC STATUS: *Dudresnaya verticillata* (Withering) Le Jolis 1863: 117 (Dumontiaceae, Gigartinales) was described by Withering (1796: 127) as *Ulva verticillata* Withering, from a specimen collected by Thomas Velley. This specimen is accepted provisionally as lectotype but it is unlocalized. The species was also described later under several different names: *Ulva coccinea* Poiret, nom. illeg. 1808: 165, *Rivularia verticillata* (Withering) J.E.Smith 1813: pl. 2466 and *Borrichius gelatinosus* S.F.Gray, nom. illeg. 1821: 330, and finally placed inside *Dudresnaya* as *D. coccinea* (C.Agardh) P.Crouan & H.Crouan 1835: 98. The current name, *D. verticillata*, was proposed by Le Jolis (1863), considering the priority of the epithet proposed by Withering (1796).

GEOGRAPHIC DISTRIBUTION: Mediterranean, eastern Atlantic (from Norway to Portugal), Macaronesian archipelagos and Russian Arctic (Guiry & Guiry 2022). It has also been reported from the Red Sea (Osman & Mohammed 2016; Einav *et al*. 2021).

HABITAT: It grows usually epilithically or epiphytically at the infralittoral and circalittoral levels, often in areas with strong currents (Rodríguez-Prieto *et al*. 2013). Occasionally, it has been described as epizoic (Robins & Kraft 1985).

SPECIMENS EXAMINED: Catalunya: Cap Norfeu, Roses, Girona, 7/8/1998, 30 m, C. Rodríguez-Prieto, HGI-A 2203 female; Es Ricard, Tossa de Mar, Girona, 27/8/2001, 48 m, C. Rodríguez-Prieto, HGI-A 7054 female; Illes Formigues, Palamós, Girona, 20/7/2003, 30 m, C. Rodríguez-Prieto, HGI-A 6192 female; Illes Formigues, Palamós, Girona, 8/8/2010, 37 m, C. Rodríguez-Prieto, HGI-A 9283 female; Illes Formigues, Palamós, Girona, 5/9/2010, 37 m, C. Rodríguez-Prieto, HGI-A 9315 female; Palamós, Girona, 20/12/2009, 27 m, C. Rodríguez-Prieto, HGI-A 9369 female; Mont de Fora, Palamós, Girona, 07/08/2022, 24 m, C. Rodríguez-Prieto, HGI-A 21513 female, HGI-A 21546 female (Fig. 1). Valencian Community: Piedra Joaquín, Illes Columbrets, Castelló de la Plana, Castelló, 9/8/2011, 45 m, E. Ballesteros, HGI-A 14329 female.

FEMALE REPRODUCTIVE STRUCTURES AND POSTFERTILIZATION STAGES: Carpogonial and auxiliary cell branches (Figs 2-6) usually developed adventitiously in a bifurcation of the inner cortex and were always outwardly directed. Exceptionally, they were found laterally on cortical filaments. Auxiliary cell branches were much more abundant than carpogonial branches (Fig.

2). Carpogonial branches were simple and (8-) 8-10 (-18) uninucleate cells long, comprising: the carpogonium, the hypogynous cell, 3-4 darkly-stained subhypogenous cells (being the first one the located right below the hypogynous cell), 2-11 lighter cells, and a small supporting cell (Fig. 3). The hypogenous cell and the subhypogenous cells presented a big haploid nucleus probably containing amplified levels of DNA, and occupying nearly the half of the cell (Fig. 3), a big vacuole and a few parietal plastids, although the later were not easy to observe due to the strong stain of those cells. A big nucleolus was seen inside the nucleus when using haematoxylin stain. The lighter cells and the supporting cell had a big vacuole, a small haploid nucleus and several well distinguishable parietal plastids (Fig. 3). The carpogonium had also some parietal plastids. Young carpogonial branches were straight, but mature ones were distally reflexed (Fig. 3) because the division plane of mitosis was oblique in the carpogonium, just as it occurred, but to a lesser degree, in the hypogynous cell and in the first subhypogenous cell. The trichogynes were simple but occasionally bifurcate, commonly traversing the outer cortex and even extending well beyond the surface of the thallus (Fig. 3). Trichogynes were covered by a thick mucilaginous coat and had a narrow neck at the base (Fig. 3, arrow).

Auxiliary cell branches were distant from the carpogonial branches. They were usually simple, up to 10-23 cells long (Figs 4-6), although some branches were observed with one or more long laterals. The auxiliary cell was intercalary and situated between two bigger cells (Fig. 5) that presumably had a nutritional role during the development of the gonimoblast. In the early stages of development, it was not possible to distinguish either the auxiliary cell or the nutritive cells, because most cells of the branch had a similar size (Fig. 4). In long and mature auxiliary cell branches, the auxiliary cell and the nutritive cells were perfectly distinguishable (Fig. 5) because they had a bigger haploid nucleus occupying

mostly a half of the cell, while the distal and proximal cells of the branch were lighter(Fig. 6), with a smaller nucleus, and with distinguishable parietal plastids.

On many occasions, numerous spermatia were observed adhered to the surface of the trichogyne (Fig. 7, arrows). Attachment of a spermatium to a carpogonium appears to require the presence of binding substances secreted by exocytosis from the tip of the trichogyne (Broadwater & Scott 1982). One (Fig. 8) or several (Fig. 9) male nucleus were observed migrating through the trichogyne towards the carpogonium and swelling the trichogyne. The neck present at the base of the trichogyne (Fig. 3, arrow) avoided the supernumerary nuclei to reach the carpogonium, closing and effectively preventing possible cases of polyfertilization, as it occurs in many other species (Broadwater & Scott 1982).

After fertilization, the diploid nucleus inside the carpogonium divided one to several times, and the carpogonium developed some small protuberances (Fig. 10). One of these protuberances elongated towards the subhypogenous cells (Fig. 10, arrow). The other protuberances elongated too to develope a number of secondary connecting filaments where the aforementioned diploid nuclei were distally located (Fig. 10). The secondary connecting filaments were initially unseptate (Fig. 10) but began to septate later (Fig. 11, white arrow). At this point, the trichogyne, that did not degrade, occasionally branched at the base (Fig. 11, arrowhead). Then, the carpogonium fused with the first (Fig. 12) or the second subhypogenous cell (Fig. 13) forming the first carpogonial fusion cell. The subhypogenous cells have a nutritive role and were named ‘nutritive auxiliary cells’ by Robins & Kraft (1985).

When mature, the first carpogonial fusion cell divided to give rise to a primary connecting filament (Fig. 13) which usually fused with the third (Fig. 14) or/and the fourth subhypogenous cell (Fig. 15), giving rise to a second carpogonial fusion cell that remained

connected to the first through a primary pit connection. During the postfertilization process both carpogonial fusion cells increased in size (Fig. 15). The set composed of the first and second carpogonial fusion cells constituted a 'carpogonial fusion complex' (Fig. 16). Occasionally, only one carpogonial fusion cell developed (Fig. 17).

Mature carpogonial fusion cells gave rise to several secondary connecting filaments (Figs 17-19) that could reach lengths greater than 700 µm. Up to seven secondary connecting filaments could develop from a carpogonial fusion complex (Fig. 20). These filaments remained connected to the carpogonial fusion cell by a basal primary pit connection (Fig. 17, arrowhead). Initially, the secondary connecting filaments were one-celled, dense and had a conspicuous nucleus (Fig. 21), but, as they grew, their cells divided (Fig.18), elongate and became hyaline, and their nucleus became less conspicuous (Fig. 19). Some of the secondary connecting filaments branched at maturity. In more advanced postfertilization phases, the cells that made up the carpogonial fusion complex increased in size and could even become lobbed (Fig. 20), while became progressively thinner at maturity (Fig. 22). The subsidiary cells maintained always their big haploid nucleus (Fig. 21) although be a part of the carpogonial fusion cell.

When a secondary connecting filament approached an auxiliary cell, a hormonal response occurred, and the auxiliary cell formed a process that extended towards the secondary connecting filament and fused with it. In our opinion, this process, that was very well described, for instance, by Hommersand & Fredericq (1990) for *D. crassa* M. Howe, is not that simple. We believe that in fact the auxiliary cell divided, cutting off a daughter cell. The latter, remained fused (not pit-connected) with the auxiliary cell, constituting a preliminary auxiliary fusion cell, where both constituents (the auxiliary cell and its daughter cell) were always distinguishable but united by a thick channel (Fig. 23, arrow). When the

secondary connecting filament contacted the preliminary auxiliary fusion cell, it fused with it forming the true auxiliary fusion cell (Fig. 23). The fusion was always performed in the part of the auxiliary fusion cell corresponding to the daughter cell of the auxiliary cell (Figs 24, 25). The original haploid nucleus of the auxiliary cell was always distinguishable inside the original auxiliary cell, although this was fused with its daughter cell (Fig. 23).

The diploid nucleus inside the auxiliary fusion cell divided one or several times, and the auxiliary fusion cell cut off 1-3 tertiary connecting filaments (Figs 23-25) where a diploid nucleus migrated. These filaments were basally pit-connected to the auxiliary fusion cell (Fig. 24, arrowhead). Tertiary connecting filaments raised always from the part of the daughter cell in the auxiliary fusion cell. The young tertiary connecting filaments were dense and had a large diploid nucleus inside them, and as they grew, their cells divided, elongated, and became more hyaline (Figs 24, 25). The diploid nucleus in growing connecting filaments was situated distally, thus, the distal ends of these filaments were darker (Fig. 25). In most cases, the auxiliary fusion cell cut off the tertiary connecting filaments before cutting off a gonimoblast initial (Fig. 26). Gonimoblast filaments developed initially to the upper part (Fig. 27) and then to the lower one (Figs 28, 29). During the gonimoblast development, the haploid nucleus was still distinguishable inside both components of the auxiliary fusion cell (Fig. 29). The mature gonimoblast could be made up of up to three gonimolobes (Fig. 30) and measured 115-264 µm in diameter. All the cells of the gonimoblast filaments were transformed into carposporangia 6.5-15.2 µm in diameter. When tertiary connecting filaments fused with other auxiliary fusion cells, they could diploidize them and new tertiary connecting filaments could be generated again (Fig. 31), allowing the development of several gonimoblast from a single initial fecundation.

Exceptionally, a procarpic behaviour was observed (Fig. 32). In this case, gonimoblast filaments were seen developing directly on secondary connecting filaments initials (Fig. 32).

Acrosymphyton purpuriferum

Figs 33-68

TAXONOMIC STATUS: *Acrosymphyton purpuriferum* (J.Agardh) G.Sjöstedt was described by J.Agardh (1842: 85) from a specimen collected at Pozzuoli and Amalfi (Italia), as *Dudresnaya purpurifera* J.Agardh (Dumontiaceae, Gigartinales). The species has two heterotypic synonyms: *Nemalion purpuriferum* (J.Agardh) Kützing 1849: 713 and *Helminthiopsis purpurifera* (J.Agardh) Papenfuss 1958: 105. It was placed into *Acrosymphyton* Sjöstedt, a new genus created by Sjöstedt (1926) inside the Dumontiaceae, as *A. purpuriferum* (J.Agardh) G.Sjöstedt. Sjöstedt (1926) created the genus mainly because the structure of the carpogonial branches and auxiliary cell branches. Then, the genus was transferred to a new family, the Acrosymphytaceae (Lindstrom 1987), mainly on the basis of pinnately branched carpogonial branches, with filaments from the fertilized carpogonium contacting terminal cells of the laterals, and terminal auxiliary cells. And finally, Withall & Saunders (2007 ‘2006’) raised the family to the order rang.

GEOGRAPHIC DISTRIBUTION: Mediterranean, Macaronesian archipelagos and Atlantic Morocco (Guiry & Guiry 2022).

HABITAT: It grows epilithic or epiphytic in infralittoral and circalittoral levels, often in areas with strong marine currents (Rodríguez-Prieto *et al.* 2013).

SPECIMENS EXAMINED: Catalunya: Illes Formigues, Palamós, Girona, 20/7/2003, 30 m, C. Rodríguez-Prieto, HGI-A 6048, male and female, HGI-A 6050 female, HGI-A 6242, female; Illes Formigues, Palamós, Girona, 1/9/2019, 35 m, C. Rodríguez-Prieto, HGI-A 20477 female, HGI-A 20479 female; Illes Formigues, Palamós, Girona, 5/9/2010, 37 m, C. Rodríguez-Prieto, HGI-A 9319 male and female; Illes Formigues, Palamós, Girona, 30/9/2018, 35 m, C. Rodríguez-Prieto, HGI-A 19941 female, HGI-A 19942 female; La Llosa, Palamós, Girona, 7/9/2020, 37 m, C. Rodríguez-Prieto, HGI-A 20789 female; S'Agulla, Blanes, Girona, 12/6/2012, E. Ballesteros, HGI-A 15566 female (Fig. 33).

FEMALE REPRODUCTIVE STRUCTURES AND POSTFERTILIZATION STAGES: Carpogonial and auxiliary cell branches were extremely abundant in the examined specimens (Fig. 34). They developed adventitiously in a bifurcation of the inner cortex and were always outwardly directed (Fig. 35). Carpogonial branches were branched, with a main axis (5-) 7-11 (-15) cells long, comprising: the carpogonium, the hypogynous cell, 3-4 darkly-stained subhypogenous cells, 2-11 lighter cells, and a supporting cell (Fig. 36). The hypogenous, the subhypogenous cells and the laterals of the subhypogenous cells presented a big haploid nucleus and were stained intensely with haematoxylin and aniline blue (Fig. 36). Plastids in those cells were difficult to see because the size of the nucleus. In the lighter cells, the nucleus was smaller, and several parietal discoid plastids were distinguishable (Fig. 36). The carpogonium was devoid of plastids, as it occurs in most Florideophyceae (Fritsch 1945). Branching was irregularly

pinnate and distichous from the subhypogenous cells 2-4 (Figs 35-38). Proximal cells presented secund branches or were unbranched (Figs 35-38). Branches were usually longer in the proximal cells than in the distal ones giving the carpogonial branch a pyramidal appearance (Figs 35-38). Carpogonial branches were distally reflexed (Figs 35-38), due to oblique plane of division of the mitosis experienced by the carpogonium, the hypogynous cell and the first subhypogenous cell. The trichogynes were simple and spirally twisted near the base (Fig. 38, arrowhead), usually very long (Fig. 37), and even extending beyond the surface of the thallus (Fig. 35). Trichogynes were covered by a thick mucilaginous coat and had a slight neck at the base (Fig. 38, arrow).

Auxiliary cell branches (Figs 39-41) were usually simple, up to (3-) 5-9 (-11) cells long, and distant from the carpogonial branches, although occasionally they might branch once or several times (Fig. 41). The auxiliary cell was located terminally on the auxiliary cell branches (Figs 39-41). In the first stages of development the auxiliary cell was not very conspicuous (Fig. 39), but at maturity it was perfectly distinguishable from the rest of the cells of the auxiliary cell branch (Figs 40, 41). Auxiliary cells presented a big nucleus, with amplified DNA, and they stained intensely, while the rest of cells of the auxiliary cell branch were lighter and it was possible to see their parietal plastids (Fig. 40). In addition, the auxiliary cell was subspherical, while the other cells were cylindrical and gradually shorten towards the apex (Fig. 40). In branched auxiliary cell branches, each branch presented an auxiliary cell in a distal position (Fig. 41).

Numerous spermatia could adhere to the surface of the trichogyne (Fig. 37, arrows) and, occasionally, it was possible to observe one (Fig. 38) or more (Fig. 42) male nuclei inside the trichogyne, slightly thickening it. The trichogyne contracted immediately after the

first male nucleus entered the base of the carpogonium; closing and avoiding possible cases of polyfertilization.

Presumably after fertilization, the diploid nucleus inside the carpogonium divided once and the carpogonium itself slightly swelled and began to develop one primary connecting filament (Fig. 43, arrow) were one of the sun diploid nuclei migrated. When mature, this filament was pit-connected to the carpogonium (Fig. 44, arrow), and elongated towards the terminal cell of one of the laterals of the carpogonial branch. This lateral was often the derivative of the third subhypogenous cell (Fig. 45, arrowhead), although it may also be the one of the fourth or fifth subhypogenous cells. During the postfertilization process, the nucleus of the carpogonium might divide again, and the carpogonium might generate another primary connecting filament (Fig. 46, arrow), usually directed in an opposite direction, whose cell begin to divide (Fig. 47, arrow).

The development of the first carpogonial fusion cell took place thanks to the involvement of a conjunctor cell (Figs 47-52, cc) cut off from the terminal cell of a lateral of the carpogonial branch (Figs 47-52, arrowhead), with which it remained pit-connected (Fig. 48, arrow). The conjunctor cell fused with the primary connecting filament to form the first carpogonial fusion cell, while initially preserving its haploid nucleus (Fig. 49). Next, the first carpogonial fusion cell increased considerably in size and gave rise to up to three long secondary connecting filaments (Fig. 50). Occasionally, the first carpogonial fusion cell could secondarily connect with two subhypogenous cells, always by conjunctor cells (Fig. 49). Then, the first carpogonial fusion cell could divide and gave rise to a second carpogonial fusion cell (Fig. 51), which remained pit-connected to the first one (Fig. 51, arrow). The second carpogonial fusion cell was also secondarily connected with the distal cell of one of the laterals of the carpogonial branch (Fig. 51, double arrowhead) via a second conjunctor

cell. In advanced stages, the nucleus of the conjunctor cell usually moved to the fusion cell and the conjunctor cell appeared empty (Fig. 51). The set composed of the first and the second carpogonial fusion cells constituted a carpogonial fusion complex (Fig. 51). In later stages of development, the carpogonial fusion cell fused too with the carpogonium (Fig. 52, white arrow). Besides, in very advanced stages, two first carpogonial fusion cells were observed on both sides of the carpogonial branch (Fig. 53), product of the connexion of two primary connecting filaments with terminal cells of laterals of both side of the carpogonial branch.

Both the first and second mature carpogonial fusion cells gave rise to several secondary connecting filaments (Fig. 54) that remained pit-connected to the carpogonial fusion cell (Fig. 54). Initially, the secondary connecting filaments were simple and unicelled, (Fig. 49), but as they mature, their cells divided, and mature filaments branched (Fig. 54). The apical cell of the filament was shorter and denser than the rest, and had the diploid nucleus situated distally (Fig. 54, black arrowheads), while the rest of the cells of the secondary connecting filament were hyaline and had the nucleus in the intermediate zone (Fig. 54, white arrowheads). In young stages, the carpogonial fusion cells were big and swelled (Fig. 53), but in the more advanced stages, where the secondary connecting filaments were already well developed, the carpogonial fusion cells became thinner (Fig. 54). The longer secondary connecting filaments observed where about 200 µm long (Fig. 54).

Prior to its diploidization, and in the proximity of a secondary connecting filament, the auxiliary cell developed a protrusion (Fig. 55, arrowhead) and then divided giving rise to a daughter cell with which it was connected by a wide fusion (not by a primary pit connection) forming a preliminary auxiliary fusion cell (Fig. 56). In this fusion cell the two components were always well distinguishable, and each bore a big haploid nucleus inside

(Fig. 57). Secondary connecting filaments connected with the preliminary auxiliary fusion cell, fusing to form a true auxiliary fusion cell (Fig. 58). Thus, the diploidized auxiliary fusion cell presented two haploid nuclei and one diploid nucleus (Fig. 58). Then, the diploid nucleus divided twice, and the auxiliary fusion cell gave rise to a single gonimoblast initial that bore one of the diploids son nuclei (Fig. 59), and one non-basally septated tertiary connecting filament that bore another of the son nuclei (Fig. 60). The gonimoblast initial cell then divided to generate the gonimoblast filaments (Fig. 61) and ultimately the gonimoblast (Fig. 62), which could reach 51-90 µm in diameter and, once mature, could be made up of up to three gonimolobes (Fig. 63). All the cells of the gonimoblast filaments were transformed into carposporangia, which measured 10.7-15.6 µm in diameter (Fig. 63). Carpospores had often been observed germinating *in situ* on the mother plant (Fig. 64, arrowheads. Secondary connecting filaments could branch and simultaneously diploidize several auxiliary fusion cells (Fig. 65). Tertiary connecting filaments were observed allowing the in series diploidization of several auxiliary fusion cells (Fig. 66). Tertiary connecting presented a similar development than secondary connecting filaments and, as this ones, they could branch (Fig. 67).

A procarpic behaviour was observed exceptionally. In these cases, gonimoblasts developed on the terminal cell of laterals of carpogonial branches (Fig. 68), which acted as auxiliary cells.

DISCUSSION

The present study represents a significant progress in the knowledge of non-procarpic genera and it allows to increase understanding of the postfertilization processes and phylogeny of non-procarpic taxonomic groups. Main results for both species studied include a) the

description of the carpogonial fusion cell development by secondary connection (via fusion in *D. verticillata* and via conjunctor cells in *A. purpuriferum*); b) the assessment of the existence, prior to diploidization, of preliminary auxiliary fusion cells in both species, and the description of their development; and c) the observation of rare and occasional procarpic behaviour, with gonimoblast developing from the secondary connecting filament initial in *D. verticillata* and from the carpogonial fusion complex in *A. purpuriferum*.

Development of the carpogonial fusion cells and the carpogonial fusion complex

According to Bornet & Thuret (1876), Oltmanns (1898) and Kylin (1928) illustrations, there are two carpogonial fusion cells in *D. verticillata*. Following Oltmanns (1898) and Kylin (1928), they develop as follows: a) after fertilization, the carpogonium grows elongating to the subsidiary cell; b) the carpogonium divides giving rise to a primary connecting filament that contains a diploid nucleus; b) the carpogonium fuses with the second subhypogenous cell forming the first carpogonial fusion cell; c) the primary connecting filament fuses with the third subhypogenous cell forming the second carpogonial fusion cell; d) both carpogonial fusion cells remain connected by a primary pit connection and then grow and lobe. Bornet & Thuret (1876) differed from Oltmanns (1898) and Kylin (1928) in the fact that the carpogonium does never divide before fusing with the subhypogenous cell, what implies that the primary connecting filament is cut off from the first carpogonial fusion cell. Our understanding of the processes is similar at the one described by Bornet & Thuret (1876). Robins & Kraft (1985) interpreted the protrusion of the carpogonium as a primary connecting filament, and their interpretation was followed by Afonso-Carrillo & Tabares (2004) in the description of the postfertilization stages in *D. abbottiae* Afonso-Carrillo & Tabares.

However, as this extension lacks a nucleus, in our opinion it cannot be interpreted as a primary connecting filament. On the other hand, Kylin (1928), who paid special attention to nuclear behaviour, drew only one nucleus in the first carpogonial fusion cell. However, we observed that the diploid nucleus in the first carpogonial fusion cell divides several times before cutting off the secondary connecting filaments, what is compatible with the development of several secondary connecting filaments. We observed too that the big haploid nucleus of the subsidiary cells remains in the body cell of the subsidiary cell after the fusion, as was shown by Kylin (1928). This is interesting because it shows that the secondary connection is mediated in this species by fusion.

With respect to *A. purpuriferum*, Schmitz (1883), Oltmanns (1898), Kylin (1930) and Lindstrom (1987) postulate that the development of the carpogonial fusion cell is accomplished by secondary connection of a primary connecting filament with a terminal cell of a lateral of the carpogonial branch, although according to the last three authors they can also do this with subterminal cells. Oltmanns (1898) specifies that almost all cells of the carpogonial branch are susceptible to undergo such processes. However, Oltmanns (1898) point out that the secondary connection between primary connecting filaments and terminal cells of laterals is made by fusion, and the rest of authors followed his criteria. Our results suggest that the secondary connection does not occur by fusion, but via conjunctor cells. Thus, according to our descriptions, the development of the carpogonial fusion cells happens as follows: 1) the terminal cell of a lateral of the carpogonial branch divides cutting of a conjunctor cell to which it remains subsequently connected through a primary pit connection; 2) the primary connecting filament fuses with the conjunctor cell, constituting the first carpogonial fusion cell. The nucleus of the conjunctor cell (initially in the centre of the cell) has been observed to move towards the carpogonial fusion cell. The migration of the nucleus shows that the subsidiary itself is not the cell that connects with the primary connecting

filament, as it occurs in *D. verticillata*, where the nucleus of the subsidiary cell does not migrate.

Kraft (1981) pointed out that some families as Dumontiaceae (including at that time *Acrosymphyton*) or Calosiphonaceae, lack secondary pit connections. However, the carpogonial fusion cells are formed by secondary connections both in *D. verticillata* and *A. purpuriferum*. Besides, Pueschel (2021) have recently ruled out secondary connections via fusion in Florideophyceae, except for the Hildenbrandiales, where more studies are needed. Nevertheless, our results points that this is true for the carpogonial fusion cell in *A. purpuriferum*, but the one in *D. verticillata* is formed by fusion.

The term ‘carpogonial fusion complex’ is applied here to a structure composed of two carpogonial fusion cells that initially are pit-connected. In *A. purpuriferum,* the carpogonial fusion complex can ultimately fuse with the carpogonium, whereas in *D. verticillata*, the carpogonium was already initially participating in the first carpogonial fusion cell. At maturity, both cells in the carpogonial fusion complex are very swelled and lobed, but they progressively decrease in size, as secondary connecting filaments develop. Occasionally, in *D. verticillata*, only one carpogonial fusion cell is formed and, consequently, the carpogonial fusion complex is absent. The term ‘fusion complex’ was applied by Robins & Kraft (1985) in *D. verticillata*, without explaining the detailed development. The term ‘carpogonial fusion complex’ was applied previously by Afonso-Carrillo & Tabares (2004) in *D. abbottiae*, for a structure composed of the carpogonium, the primary connecting filament and the subhypogenous cells. Kylin (1928) illustrated a true carpogonial fusion complex in *D. verticillata*, and Littler (1974) in *D. littleri* I.A. Abbott (Littler, 1974).

Assessment of the existence of preliminary auxiliary fusion cells and the description of their development

Both in *D. verticillata* (Bornet & Thuret 1867, 1876; Agardh 1879; Schmitz 1883; Oltmanns 1898; Kylin 1928; Lindstrom 1985; Robins & Kraft 1985; Athanasiadis 1987) and *A. purpuriferum* (Bornet & Thuret 1867, 1876; Ardissone 1883; Schmitz 1883; Oltmanns 1898; Sjöstedt 1926; Kylin 1930; Feldmann 1939), the secondary connecting filament were considered to directly fuse either with the auxiliary cell or with a process of the auxiliary cell. Then, the part of the secondary connecting filament that fused with the auxiliary cell swelled and developed a sort of ampulla where all the cell content of the auxiliary fusion cell was transferred. This was followed by other authors in the description of other species, as for example Lee (1963) in *D. hawaiiensis* Lee, Littler (1974) in *D. littleri*, and Afonso-Carrillo *et al.* (2002) in *D. multiramosa* Afonso-Carrillo, Sansón & Reyes. Our results suggest that before contacting with the secondary connecting filament, the auxiliary cell cuts off a daughter cell with which it remains fused (not pit-connected), thus forming what we have called a 'preliminary auxiliary fusion cell', which boars two haploid nuclei. The nuclear behaviour is here again crucial, because the original nucleus in the auxiliary cell never migrates to the fusion cell and it is distinguishable during all the postfertilization process, contrasting with literature data. Later on, the secondary connecting filament fuse with the primary auxiliary fusion cell forming the true auxiliary fusion cell, that bears two haploid nuclei plus one diploid one. The secondary connecting filament fuses always in the side of the daughter cell, and the tertiary connecting filaments and the gonimoblast initial are cut off also from this side.

Rare and occasional procarpic behaviour

Most descriptions of the procarpic/non-procarpic behaviour of a taxonomic group in the literature calls for the existence of just one of those behaviours in each group. This has traditionally been so in Acrosymphytales and Dumontiaceae (Gigartinales), except in the case of *Weeksia* (Dumontiaceae), where the auxiliary cell branches are non-functional and gonimoblast develop from a nutritive cell of the carpogonial branch (Abbot 1968). In this study, we have shown that in *A. purpuriferum* and *D. verticillata* a procarpic behaviour can be occasionally present, with gonimoblast developing from the secondary connecting filament initial in *D. verticillata*, and from the carpogonial fusion complex in *A. purpuriferum*. Our opinion is that the existence of both behaviours in the same species can be more common than postulated before. The incidence of the presence of procarpic and non-procarpic behaviour in the same specimen is very occasional in *D. verticillata* and *A. purpuriferum*, while in Ptilocladiopsidaceae (C. Rodríguez-Prieto *observations*) the character is more common.

Molecular analyses from Tai *et al.* (2001), Verbruggen *et al.* (2010) and D'Archino & Sutherland (2013), showed a strong relationship among Polyipodiaceae, Dumontiaceae, Kallymeniaceae and Rhizophyllidaceae, and Rodríguez-Prieto *et al.* (2014) included in this group the Ptilocladiopsidaceae. All this families (except by now the Rhizophyllidaceae), although being essentially non-procarpic can either present a procarpic behaviour in some of their representatives, as in the Kallymeniaceae (Kylin 1928; Norris 1957), or some species with both procarpic and non-procarpic behaviour, as in the Polypodiaceae and Ptilocladiopsidaceae (Joly & Ugadim 1966; Rodríguez-Prieto *et al*. 2014). On the other hand, the Acrosymphytales, that we have shown that can be occasionally non procarpic, is a basal group for a large set of both procarpic and non procarpic groups (Le Gall & Saunders

2007; Verbruggen *et al.* 2010; Yang *et al.* 2016), including the cluster of Dumontiaceae, Kallymeniaceae and Rhizophyllidaceae (Verbruggen *et al.* 2010). Hommersand & Fredericq (1990) pointed that in Kallymeniaceae the procarpic behaviour is probably secondarily derived, and Chiang (1970) concurred with Norris (1957) and Balakrishnan (1960) that most families of the uncient Cryptonemiales, probably evolved from a polycarpogonial, nonprocarpic ancestor. Our results support the hypothesis that the procarpic behaviour is secondarily derived.

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LEGENDS FOR FIGURES

Figs 1–12. *Dudresnaya verticillata*. Habit, carpogonial branch, auxiliary cell branch, fecundation and first postfertilization stages. No stain (Fig. 1); aniline blue (Fig. 2); haematoxylin (Figs 3–12).

Fig. 1. Habit of a female gametophyte (HGI–A 21513). Scale bar = 1 cm.

Fig. 2. Situation of carpogonial branches (black arrows) and auxiliary cell branches (white arrows) (HGI–A 21546). Scale bar = 50 µm.

Fig. 3. A mature and distally reflexed carpogonial branch adventitiously developing on the inner cortex. Note that the hypogenous and subhypogenous cells present a conspicuous haploid nucleus whose DNA has amplified, and that the trichogyne presents a basal neck (arrow) (HGI–A 14329). Scale bar = 50 µm.

Fig. 4. Young auxiliary cell branch. Note that all cells of the branch (arrowheads) are uninucleate (HGI–A 2203). Scale bar = 20 µm.

Fig. 5. Mature auxiliary cell branch showing its adventitious origin. Note that the auxiliary cell is intercalary in the branch and it is slightly smaller than the nutritive cells, which are situated immediately above and below it. Note that both the auxiliary cell and the nutritive cells present a conspicuous haploid nucleus with amplified DNA (HGI–A 14329). Scale bar = 20 µm.

Fig. 6. An especially long auxiliary cell branch, composed of up to 22 cells (HGI–A 14329). Scale bar = 50 µm.

Fig. 7. Carpogonial branch with several spermatia (arrows) attached to the trichogyne surface (HGI–A 9283). Scale bar = 20 µm.

Fig. 8. Fertilized carpogonial branch where the carpogonium is beginning to develop a protrusion (black arrow) that is contacting the first subhypogenous cell. Note that the trichogyne is surrounded by a thick mucilaginous coat (white arrow) and that a male nucleus can be seen moving through the inside, causing the trichogyne to swell. Note too that plastids are visible in the basal unmodified cells of the carpogonial branch (HGI–A 9315). Scale bar = 20 µm.

Fig. 9. Postfertilization stage were several male nuclei can be observed inside the trichogyne and that the carpogonium presents both a female and a male nucleus (HGI–A 9283). Scale bar = 20 µm.

Fig. 10. Fertilized carpogonium where the diploid nucleus has divided twice, originating two son nuclei that are located at the distal part of two developing secondary connecting filaments. Note that the protrusion of the hypogenous cell has contacted (arrow) with the first subhypogenous cell (HGI–A 9315). Scale bar = 20 µm.

Fig. 11. A later stage in Fig. 10, where the carpogonium is swelled and the secondary connecting filament is beginning to divide (white arrow). Note that trichogyne is branching (arrowhead) (HGI–A 14329). Scale bar = 20 µm.

Fig. 12. Stage where the protrusion of the carpogonium has already fused with the first subhypogenous cell forming the first carpogonial fusion cell (HGI–A 14329). Scale bar = 20 µm.

Abbreviations: auxiliary cell (ac); carpogonium (cp); diploid nucleus (dn); female nucleus (fm); first carpogonial fusion cell (cpfc1); haploid nucleus (hn); hypogenous cell (hy); male nucleus (mn); nutritive cell (nc); plastid (pl); secondary connecting filament (cf2); subhypogenous cell (shy1–4); supporting cell (sc); trichogyne (tr), vegetative cell (vc).

Figs 13–19. *Dudresnaya verticillata*. Development of the carpogonial fusion cells and the secondary connecting filaments. Haematoxylin (Figs 13–15, 17–19); aniline blue (Fig. 16).

Fig. 13. A first carpogonial fusion cell originated by fusion (arrowhead) between the carpogonium and the second subhypogenous cell. This carpogonial fusion cell has divided and has cut off a primary connecting filament (HGI–A 7054). Scale bar = 20 µm.

Fig. 14. A later stage in Fig. 13 where the primary connecting filament has already fused with the third subhypogenous cell forming a second carpogonial fusion cell which begins to elongate (arrow) to connect with the fourth subhypogenous cell (HGI–A 7054). Scale bar = 10 µm.

Fig. 15. A detail of a later stage in Fig. 14, where the two carpogonial fusion cells have conspicuously swelled. Note that the nucleus of the subsidiary cells is not transferred to the carpogonial fusion cell (HGI–A 6192). Scale bar = 20 µm.

Fig. 16. Unusual case where the first carpogonial fusion cell was developed from fusion between the carpogonium and the first and second subhypogenous cells (HGI–A 9315). Scale bar = 20 µm.

Fig. 17. First carpogonial fusion cell developing a secondary connecting filament which is basally septate (arrowhead) (HGI–A 9315). Scale bar = 20 µm.

Fig. 18. Two-celled secondary connecting filament developing from the second carpogonial fusion cell (HGI–A 9315). Scale bar = 20 µm.

Fig. 19. A later stage in Fig. 18 where the secondary connecting filaments developed from the second carpogonial fusion cell are notoriously elongated (HGI–A 9315). Scale bar = 20 µm.

Abbreviations: carpogonial fusion cell (cpfc1–2); connecting filament (cf1–2); hypogenous cell (hy); subhypogenous cell (shy1–4); supporting cell (sc); trichogyne (tr).

Figs 20–25. *Dudresnaya verticillata*. Carpogonial fusion complex, secondary connecting filaments, and development of the auxiliary fusion cell and tertiary connecting filaments. Haematoxylin (Figs 20–24); aniline blue (Fig. 25).

Fig. 20. A mature carpogonial fusion complex where both carpogonial fusion cells have conspicuously increased in size, and the second one is lobbing. Note that each carpogonial fusion cell is cutting off several secondary connecting filaments, which are basally pit-connected (arrowhead) to the carpogonial fusion cell (HGI–A 9315). Scale bar = 20 µm.

Fig. 21. Detail of a postfertilization stage, in which both carpogonial fusion cells have developed from fusion with the second subhypogenous cell (HGI–A 14329). Scale bar = 50 µm.

Fig. 22. Postfertilization stage where several spermatia (arrows) are still attached to the distal part of the trichogyne. Both carpogonial fusion cells are giving rise to several secondary connecting filaments, the younger ones are short, dense and composed of small cells, and the older ones are hyaline and composed of very elongated cells (HGI–A 14329). Scale bar = 50 µm.

Fig. 23, 24. Diploidization of the auxiliary cell at different focus. Note that the auxiliary cell has cut off a lateral cell with which it is partially fused (Fig. 23, arrow), and that the last segment of the secondary connecting filament has fused with the preliminary auxiliary fusion cell diploidizing it and forming a true auxiliary fusion cell (Fig. 24). Then, from the auxiliary fusion cell, two tertiary connecting filaments originate, directed to the opposite side of where the secondary connecting filament arrived. Note that the fusion cell is lobated and that the tertiary connecting filaments are formed by septation, remaining connected to the fusion cell by a primary pit connection (Fig. 24, arrowhead). Note also that young tertiary connecting filaments have a very dense cytoplasmic content and that, as they lengthen, they become hyaline (HGI–A 14329, 7054). Scale bars = 20 and 10 µm.

Fig. 25. Lateral view of an auxiliary cell branch where the auxiliary fusion cell has received a secondary connecting filament and has originated a branched and septate tertiary connecting filament (HGI–A 7054). Scale bar = 20 µm.

Abbreviations: auxiliary fusion cell (afc); carpogonial fusion cell (cpfc1–2); connecting filament (cf2–3); diploid nucleus (dn); haploid nucleus (hn); hypogenous cell (hy); nutritive cell (nc), subhypogenous cell (shy1–3); trichogyne (tr).

Figs 26–33. *Dudresnaya verticillata*. Development of tertiary connecting filaments, gonimoblast, and in series diploidization. Aniline blue (Figs 26, 31); hematoxylin (Figs 27–30, 32).

Fig. 26. Detail of a fully developed auxiliary fusion cell producing three tertiary connecting filaments. Note that the last segment (arrow) of the secondary connecting filament has fused with the auxiliary fusion cell, and that the tertiary connecting filaments are basally septate (HGI–A 9369). Scale bar = 10 µm.

Fig. 27. Diploidized auxiliary fusion cell cutting off a gonimoblast initial (HGI–A 14329). Scale bar = 50 µm.

Fig. 28. A later stage on the development of the gonimoblast where the gonimoblast filaments grow to both sides of the auxiliary fusion cell (HGI–A 14329). Scale bar = 20 µm.

Fig. 29. Developing gonimoblast on an auxiliary fusion cell where one haploid nuclei is situated inside each of the components of the auxiliary fusion cell. Note the diploid nuclei inside one of the tertiary connecting filament (HGI–A 9283). Scale bar = 20 µm.

Fig. 30. Gonimoblast composed of three gonimolobes (HGI–A 9315). Scale bar = 20 µm.

Fig. 31. In series diploidization of three auxiliary fusion cells by tertiary connecting filaments (HGI–A 7054). Scale bar = 50 µm.

Fig. 32. Procarpic behaviour where the first carpogonial fusion cell has cut off several secondary connecting filaments, one of which is producing gonimoblast filaments (HGI–A 9315). Scale bar = 20 µm.

Abbreviations: auxiliary fusion cell (afc); carpogonial fusion cell (cpfc1–2); diploid nucleus (dn); haploid nucleus (hn); gonimoblast (g); gonimoblast initial (gi); gonimolobe (gl1–3); hypogenous cell (hy); nutritive cell (nc); secondary connecting filament (cf2); tertiary connecting filaments which ensure successive diploidizations (cf3, cf3', cf3'', cf3'''); subhypogenous cell (shy1–3); trichogyne (tr).

Figs 33–42. *Acrosymphyton purpuriferum*. Habit, carpogonial branch, auxiliary cell branch, and fertilization. No stain (Fig. 33); haematoxylin (Figs 34–36, 38, 40–42); aniline blue (Figs 37, 39).

Fig. 33. Habit of a female gametophyte (HGI–A 15566). Scale bar = 1 cm.

Fig. 34. Situation of carpogonial branches (black arrows) and auxiliary cell branches (white arrows) (HGI–A 6242). Scale bar = 50 µm.

Fig. 35. Mature adventitious carpogonial branch showing a trichogyne that far exceeds the surface of the thallus (HGI–A 20477). Scale bar = 50 µm.

Fig. 36. Detail of a distally reflexed carpogonial branch. Note the discoid plastids (arrows) inside the lighter cells, and that the hypogenous cell, the subhypogenous cells, and the cells of the laterals are strongly stained, due to the considerable size of their nucleus (HGI–A 19941). Scale bar = 20 µm.

Fig. 37. Carpogonial branch with numerous spermatia (arrows) attached to the trichogyne (arrowhead) (HGI–A 20789). Scale bar = 20 µm.

Fig. 38. Fertilization stage where the male nucleus has penetrated the trichogyne causing it to swell. Note that the trichogyne is twisted near the base (arrowhead) and has a narrow neck basally (arrow) (HGI–A 6048). Scale bar = 50 µm.

Fig. 39. Developing auxiliary cell branch, situated adventitiously on the bifurcation point of cortical filaments (HGI–A 9319). Scale bar = 20 µm.

Fig. 40. Mature auxiliary cell branch where proximal cells are cylindrical and gradually shorten (HGI–A 20477). Scale bar = 20 µm.

Fig. 41. Branched auxiliary cell branch with an auxiliary cell situated distally in each branch (HGI–A 20477). Scale bar = 20 µm.

Fig. 42. Fertilization stage where the trichogyne has several male nuclei inside and begins to branch (arrowhead) (HGI–A 20477). Scale bar = 20 µm.

Abbreviations: auxiliary cell (ac); carpogonium (cp); female nucleus (fn); hypogenous cell (hy); male nucleus (mn); plastid (pl); subhypogenous cell (shy1–4); supporting cell (sc); trichogyne (tr); vegetative cell (vc).

Figs 43–52. *Acrosymphyton purpuriferum*. Development of the carpogonial fusion cells and the secondary connecting filaments. Haematoxylin. Scale bars = 20 µm.

Fig. 43. Fertilized carpogonium which is giving rise to a primary connecting filament (arrow) (HGI–A 19942).

Fig. 44. A later stage in Fig. 44 where the primary connecting filament is elongating and it is already basally septated (arrow) (HGI–A 20479).

Fig. 45. Primary connecting filament close to connect with the terminal cell of a lateral (arrowhead) derived from the third subhypogenous cell (HGI–A 20477).

Fig. 46. Later stage in Fig. 45, where the carpogonium gives rise to a second primary connecting filament (arrow), growing into the opposite direction of the first one (cf1) (HGI–A 20477).

Fig. 47. Stage similar to the previous one where the second primary connecting filament that is growing by division (arrow). Note that the terminal cell of one of the laterals of the carpogonial branch (arrowhead) has cut off a conjunctor cell which has fused with the primary connecting filament and has transferred its nucleus to the carpogonial fusion cell (HGI–A 20477).

Fig. 48. Postfertilization stage where the terminal cell of one of the laterals of the carpogonial branch (arrowhead) has divided (arrow) giving rise to a conjunctor cell which remain pit-connected (arrow) to its mother cell. Note that walls of the conjunctor cell and the primary connecting filament are already in contact but not fused (HGI–A 20479).

Fig. 49. A later stage where the first carpogonial fusion cell has secondary connected with two subsidiary cells via conjunctor cells. Note that the upper conjunctor cells has totally fused with the primary connecting filament forming the first carpogonial fusion cell, and has transferred its nucleus to the fusion cell, while the second conjunctor cell, although already

fused preserves its nucleus Note also that the first carpogonial fusion cell has noticeably increased in size and has given rise to three secondary connecting filaments (HGI–A 20477).

Fig. 50. The same as in Fig. 49, but the secondary connecting filaments have considerably elongated and the carpogonial fusion cell is lobbing (HGI–A 20477).

Fig. 51. Mature postfertilization stage where the first carpogonial fusion cell – which is fused with the conjunctor cell cut off from the terminal cell (arrowhead) of a lateral of the third subhypogenous cell – has cut off a second carpogonial fusion cell, which remained pit-connected to the first one (arrow) forming a carpogonial fusion complex. The later, in turn, has also secondarily connected with a lateral (double arrowhead) of the fourth subhypogenous cell and is generating two secondary connecting filaments. Note that the first conjunctor cell is totally fused with the first carpogonial fusion cell, and the second conjunctor cell has already transferred the nucleus to the second carpogonial fusion cell but it is still distinguishable (HGI–A 20479).

Fig. 52. Mature postfertilization stage where the first carpogonial fusion cell has fused (white arrow) with the carpogonium and is giving rise to numerous secondary connecting filaments, some of them branched (black arrow) (HGI– A 20477).

Abbreviations: conjunctor cell (cc); connecting filament (cf1–2); carpogonial fusion cell (cpfc1–2); carpogonium (cp); diploid nucleus (dn); hypogenous cell (hy); subhypogenous cell (shy3–5); supporting cell (sc); trichogyne (tr).

Figs 53–62. *Acrosymphyton purpuriferum*. Advanced postfertilitzation stages, auxiliary cell branch and gonimoblast development. Haematoxylin.

Fig. 53. Mature postfertilization stage were two first carpogonial fusion cells are developing, one in each side of the carpogonial branch (HGI–A 6242). Scale bar = 20 µm.

Fig. 54. Mature secondary connecting filaments. Note that the apical cell of these filaments (black arrowhead) is short and dense, presenting a nucleus situated near the apex, while the rest of cells are hyaline and very elongated, with the nucleus situated in the median part of the cell (white arrowheads). Note also that, at this stage, the carpogonial fusion complex has already thinner and that some spermatia (arrows) are still attached to the trichogyne (HGI–A 6050). Scale bar = 50 µm.

Fig. 55. Stage prior to the diploidization of the auxiliary cell where a protrusion (arrowhead) can be seen in the auxiliary cell (HGI–A 19942). Scale bar = 20 µm.

Fig. 56. Later stage than in figure 55 where the auxiliary cell has already divided giving rise to a daughter cell, with which it remains fused to form the primary auxiliary fusion cell. Note that both components of the primary auxiliary fusion cell bear a big haploid nucleus (HGI–A 19942). Scale bar = 20 µm.

Fig. 57. The same as in the Fig. 46, but the protrusion is here lateral (HGI-A 19942). Scale bar = 20 µm.

Fig. 58. Auxiliary fusion cell where the auxiliary cell and its daughter cell are still distinguishable and each possess a haploid nucleus. Note that the secondary connecting filament has already transferred the diploid nucleus to the auxiliary fusion cell (HGI–A 20477). Scale bar = 20 µm.

Fig. 59. Diploidization of the auxiliary cell via a branched and septate secondary connecting filament arising from the second carpogonial fusion cell (HGI–A 20477). Scale bar = 20 µm.

Fig. 60. Auxiliary fusion cell that has cut off a gonimoblast initial. Note that the auxiliary fusion cell is still connected to a secondary connecting filament, which in the last segment does not have a nucleus as it has spilled it inside the fusion cell when merging with it. Note also that the tertiary connecting filament is developing by elongation of the auxiliary fusion cell and presents a duplicate of the diploid nucleus at the base (HGI–A 19942). Scale bar = 10 µm.

Fig. 61. Stage similar to that in Fig. 60, but the secondary connecting filament has disintegrated and the diploid nucleus is advancing through the interior of the tertiary connecting filament. Note the gonimoblast is beginning to develop (HGI–A 20477). Scale bar = 20 µm.

Fig. 62. Auxiliary fusion cell with a secondary connecting filament and a tertiary one (HGI–A 20477). Scale bar = 20 µm.

Abbreviations: auxiliary cell (ac); auxiliary fusion cell (afc); connecting filament (cf2–3); carpogonial fusion cell (cpfc1–2); diploid nucleus (dn); gonimoblast (g); gonimoblast initial (gi); haploid nucleus (hn); primary auxiliary fusion cell (pafc); supporting cell (sc); trichogyne (tr).

Figs 63–68. *Acrosymphyton purpuriferum*. Gonimoblast development and in series diploidization process. Aniline blue (Fig. 62); no stain (Fig. 63); haematoxylin (Figs 64–67).

Fig. 63. Gonimoblast made up of three gonimolobes arising from a single gonimoblast initial (HGI–A 9319). Scale bar = 20 µm.

Fig. 64. Carpospores germinating *in situ* (arrowheads) (HGI–A 15566). Scale bar = 20 µm.

Fig. 65. Simultaneous diploidization of two auxiliary fusion cells in a squashed preparation. Note that the first carpogonial fusion cell cuts off a secondary connecting filament that branches (arrowhead), allowing each of the branches to diploidize a different auxiliary fusion cell (HGI–A 19941). Scale bar = 20 µm.

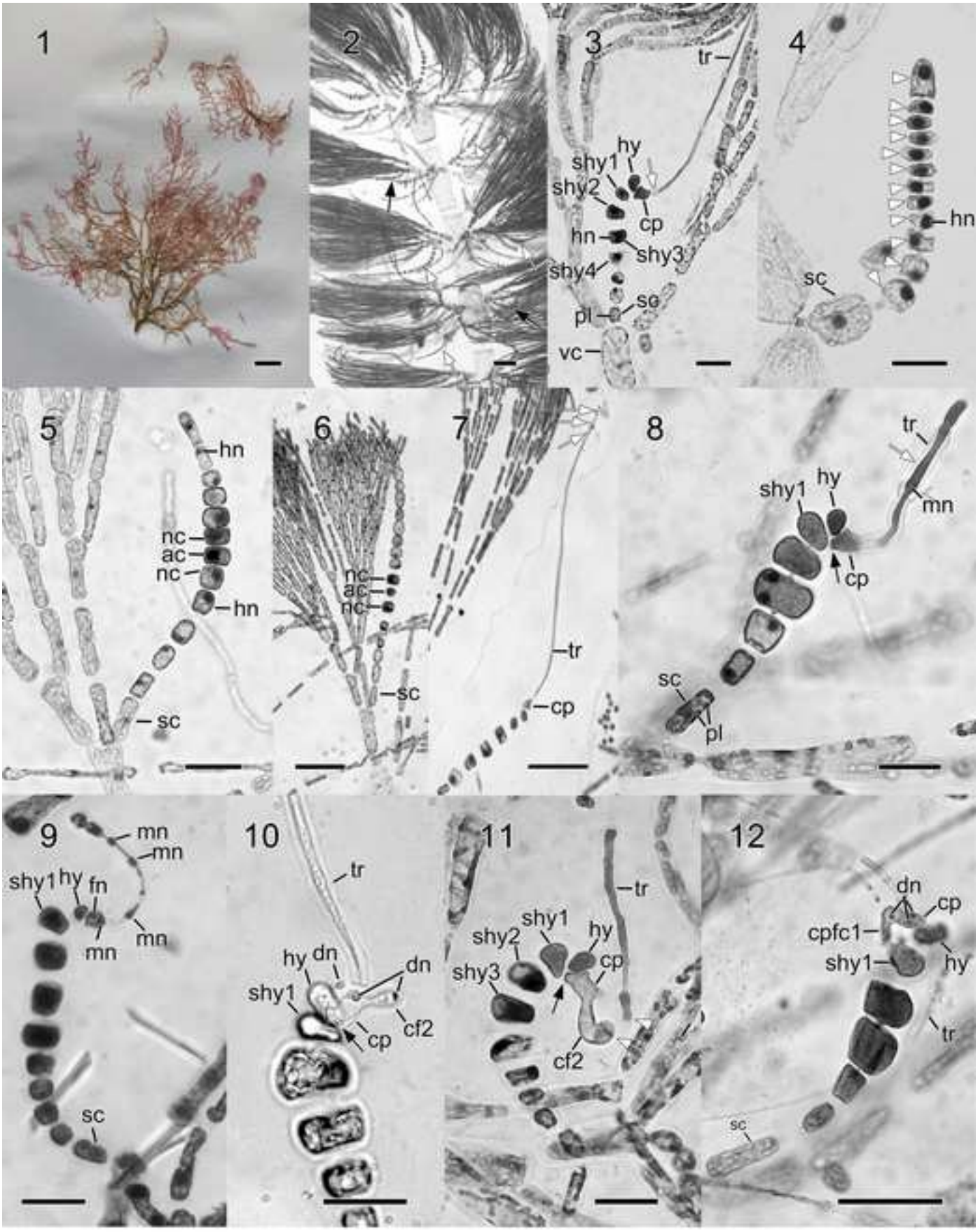
Fig. 66. Reproductive process showing, from left to right, the secondary connecting filament generated by the first carpogonial fusion cell connecting with a first auxiliary fusion cell, diploidizing it and allowing the gonimoblast to develop. This auxiliary fusion cell cut off at the same time a tertiary connecting filament that connects with a second auxiliary fusion cell and performs the same process. This is repeated successively, causing the in series diploidization of several auxiliary fusion cells (HGI–A 19941). Scale bar = 20 µm.

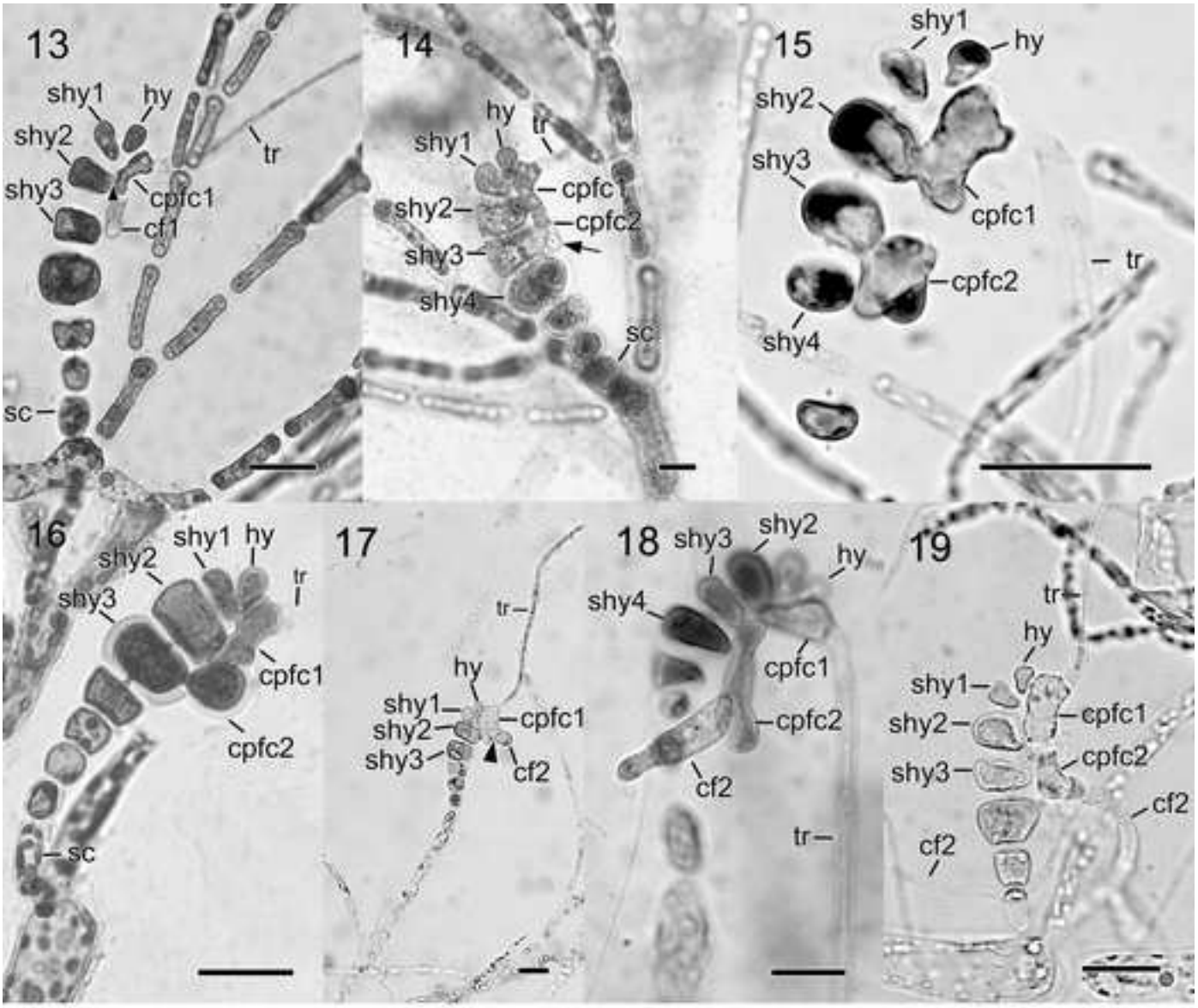
Fig. 67. Detail of a branched tertiary connecting filament (arrowhead) (HGI–A 20477). Scale bar = 50 µm.

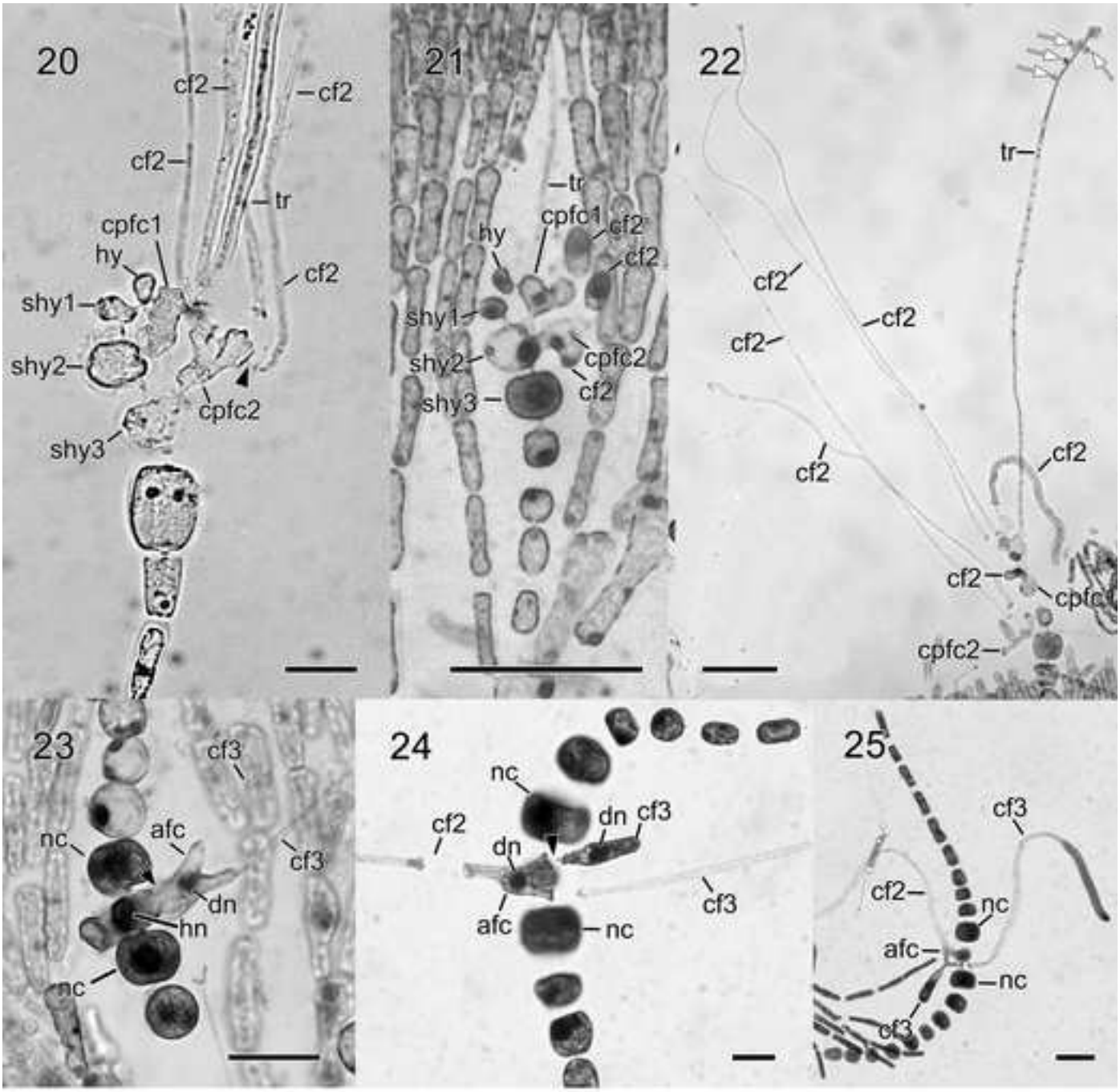
Fig. 68. Procarpic case where a terminal cell of a lateral of the carpogonial branch is acting as an auxiliary cell (HGI–A 19942). Scale bar = 20 µm.

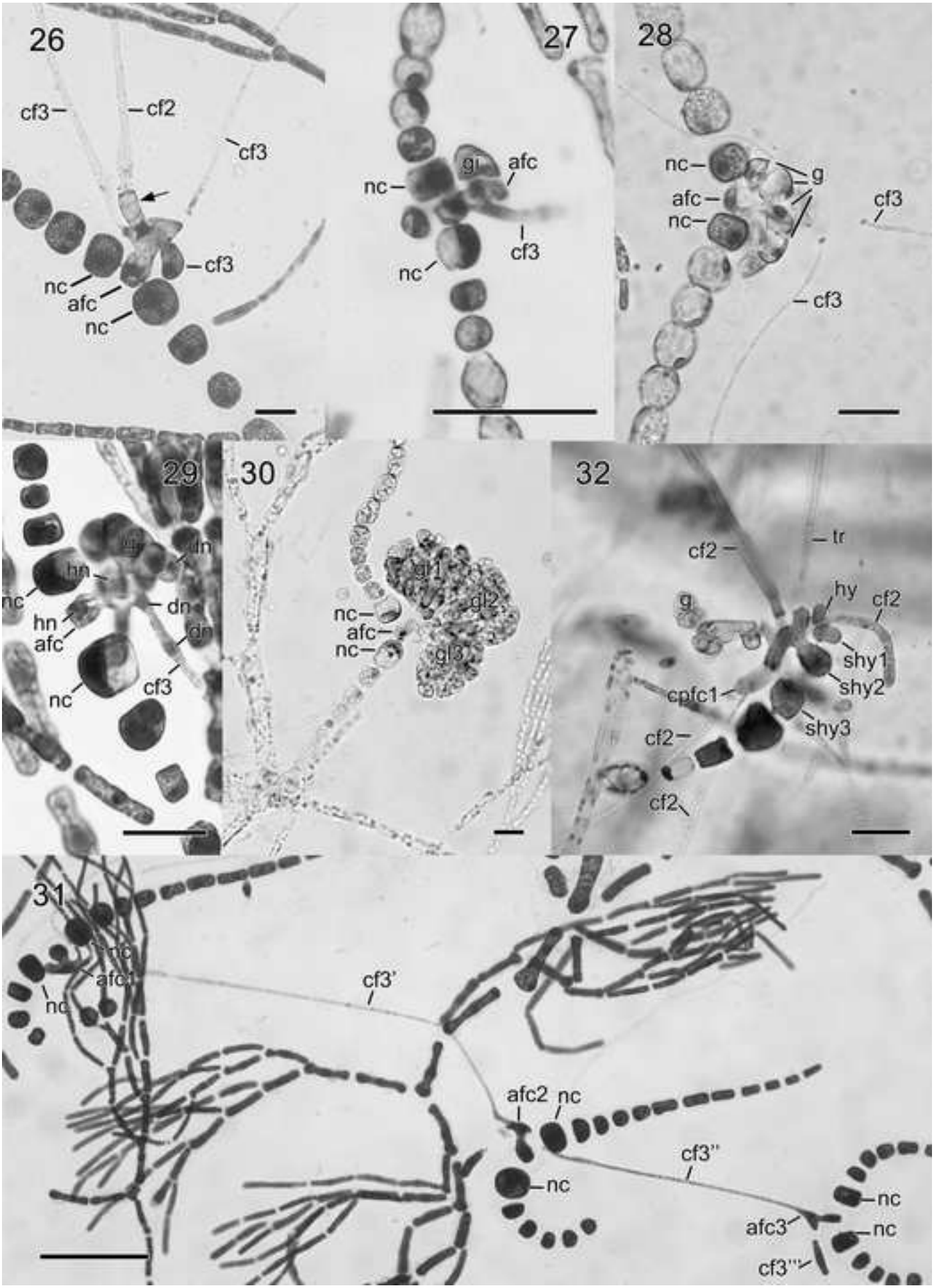
Abbreviations: auxiliary fusion cell (afc1–3); secondary connecting filament (cf2); tertiary connecting filament that ensure successive diploidizations (cf3, cf3’, cf3’’); first carpogonial

fusion cell (cpfc1); gonimoblast (g); gonimoblast initial (gi); supporting cell (sc); trichogyne (tr).









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