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Somatic meiosis and development of the juvenile gametophyte in members of the Batrachospermales *sensu lato* (Rhodophyta)

ORLANDO NECCHI JR.[1]* AND JAVIER CARMONA JIMÉNEZ[2]

[1]*São Paulo State University, Zoology and Botany Department, Rua Cristóvão Colombo, 2265-15054-000 São José do Rio Preto, SP, Brazil*
[2]*Phycology Laboratory, Faculty of Sciences, National Autonomous University of Mexico, Ciudad Universitaria, A.P. 70-620, Coyoacán 04510, México, D.F.*

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Seven populations (six in culture and one sampled directly from nature) of the freshwater red algal families Batrachospermaceae, Lemaneaceae and Thoreaceae were examined, involving three species of *Batrachospermum*, two of *Paralemanea* and one of *Thorea*. All '*Chantransia*' stages ultimately produced juvenile gametophytes. The production of juvenile gametophytes in the three populations of *Batrachospermum* was generally most abundant at 15°C and low irradiances (47–68 μmol photons m^{-2} s^{-1}). The most abundant gametophyte development in the *Paralemanea* species was observed at 10°C and low or high irradiances (47–142 μmol photons m^{-2} s^{-1}). Gametophyte production in Thoreaceae occurred at higher temperatures (20°C) and also at low irradiances. In species of the Batrachospermaceae and Lemaneaceae, the 'elimination cells' can be situated on the basal or suprabasal cell of the juvenile gametophyte, but the position is usually fixed in individual species. The presence and position of the elimination cells remain to be established in Thoreaceae. Our results corroborate a previous study suggesting that the position of elimination cells is such a constant feature that it is of potential diagnostic value at the generic or infrageneric (sectional or specific) level. The characteristics observed in the development of the juvenile gametophytes in species of Batrachospermaceae and Lemaneaceae essentially agreed with general descriptions in the previous studies. The characteristics of the Thoreaceae, with a distinctive developmental pattern of the juvenile gametophyte and the occurrence of two morphological types in the '*Chantransia*' stage, support the proposal to elevate it to the ordinal level. Two remarkable observations in *Batrachospermum* species were the production of numerous juvenile gametophytes from filaments of the same plant of the '*Chantransia*' stage and the formation of a system of rhizoidal filaments or cell agglomeration of the juvenile gametophytes, which produced new gametophytes. These two characteristics potentially increase the formation of additional gametophytes under favourable conditions.

INTRODUCTION

The life history pattern of the Batrachospermales is designated as the '*Lemanea*' type and involves only one spore type, the carpospore (Dixon 1982; Sheath 1984). Tetraspores are not formed by the '*Chantransia*' stage, which instead directly produces the juvenile gametophytes on the apical cells (Sheath 1984). Such division occurs in the diploid vegetative cells of the '*Chantransia*' stage and gives rise to haploid axes by a process known as somatic meiosis, which is unique to the Batrachospermales *sensu lato* in the red algae (Magne 1967; Balakrishnan & Chaugule 1975, 1980; Pueschel & Cole 1982). This order of red algae originally embraced three families – Batrachospermaceae, Lemaneaceae and Thoreaceae – but recently it has been proposed that the Thoraceae be elevated to the ordinal status (Sheath *et al.* 2000).

The whole process of somatic meiosis involves unequal meiotic divisions and results in one large viable cell (the first cell of the juvenile gametophyte) and two smaller nonfunctional cells, each with reduced cytoplasm and a pycnotic nucleus (Magne 1967; Balakrishnan & Chaugule 1975, 1980; von Stosch & Theil 1979). The latter cells are designated as 'polar bodies' (von Stosch & Theil 1979) or 'elimination cells' (Magne 1967; Balakrishnan & Chaugule 1975, 1980).

* Corresponding author (orlando@bot.ibilce.unesp.br).

The development of juvenile gametophytes from the '*Chantransia*' stage has been observed in some species of *Batrachospermum* Roth, *Lemanea* Bory, *Paralemanea* (P.C. Silva) Vis & Sheath and *Thorea* Bory, either in culture or in natural populations (Swale 1962; Magne 1967; Balakrishnan & Chaugule 1975, 1980; Huth 1979, 1981; von Stosch & Theil 1979; Chesnick & O'Flaherty 1986; Necchi 1987; Coomans & Hommersand 1990; Necchi & Zucchi 1997). However, only a few investigations have described the conditions favouring this development (Huth 1979, 1981; Chesnick & O'Flaherty 1986) and few have provided cytological details of the unique type of meiotic cell division that accompanies it (Magne 1967; Balakrishnan & Chaugule 1975, 1980; Huth 1979, 1981; von Stosch & Theil 1979; Necchi 1987).

The '*Lemanea*' type of life history has some features that could be interpreted as adaptive advantages (e.g. conveying a greater probability of success to the one product of meiosis because it remains attached to the diploid '*Chantransia*' phase in a favourable habitat), but this is offset by the fact that three of the meiotic products are eliminated, reducing the potential diversity of genotypes produced by meiosis (Sheath 1984). Furthermore, the number of potential gametophytes is reduced by 75% compared with that of a life history employing tetrasporic meiosis. The '*Lemanea*' life history strategy appears to be an adaptation for growth in the unidirectional flow pattern of streams (Sheath 1984, p. 141). The '*Chantransia*' stage

Table 1. Location and date of collection of the Batrachospermales *sensu lato* populations ('*Chantransia*' stage and respective gametophyte) analysed in this study.

Species and populations[1]	Location and date of collection
Batrachospermum delicatum (Skuja) Necchi & Entwisle (#1)	Córrego do Feijão, Itirapina, Visconde do Rio Claro, Route SP-310., São Paulo, Brazil. 22°09′S, 47°47′W. 29 May 1995
B. cf. *antipodites* Entwisle (#70)	Waterfall, Lamington National Park, Queensland, Australia. 28°15′S 153°12′E. 10 Jul. 1997
Batrachospermum sp. (#94)	River Tone, Kurihashi-Machi, Saitama-Ken, Japan. 36°08′N, 139°42′E. 07 Aug. 1971
Paralemanea catenata (Kützing) Vis & Sheath (#96)	Knoxville, Mountain Home, Tennessee, USA, 35°58′N, 83°56′W. 17 Mar. 1995
Paralemanea sp. (#104)	Huichihuayan, Huehuetlán, San Luis Potosí, Mexico. 21°27′N, 98°58′W. 11 Nov. 1999
P. mexicana (Kützing) Vis & Sheath (BALE1942)[2]	Arroyo Meyuca, Coatepec de las Harinas, State of Mexico, Mexico. 19°56′N, 99°55′W. 08 Sep. 1990
Thorea hispida (Thore) Desvaux (#64)[3]	Jumirim, tributary of Sorocaba River, São Paulo, Brazil. 23°06′S, 47°48′W. 19 Sep. 1997

[1] Isolate number in culture collection; populations preserved in Carnoy's solution.
[2] Herbarium number; population preserved in formaldehyde. This species was not grown in culture.
[3] '*Chantransia*' stage produced from carpospore germination.

is often perennial, whereas the attached gametophytes are formed seasonally. In addition, the '*Chantransia*' stage has been reported under a wider range of environmental conditions than the gametophytes (Hambrook & Sheath 1991; Necchi 1993, 1997) and plays an important role in population maintenance in lotic habitats.

The present investigation was carried out to document the cytological details of somatic meiosis, as well as the temperature and irradiance favouring its occurrence, and also the early stages of the juvenile gametophyte development in various members of the Batrachospermales *sensu lato* under culture and field conditions.

Table 2. Juvenile gametophyte production from the '*Chantransia*' stage in Batrachospermales *sensu lato* populations in various combinations of temperature and irradiance (after 120 d). Photoperiod 12 : 12 h light–dark (+ = gametophytes present but in low number, ++ = gametophytes abundant, — = not observed).

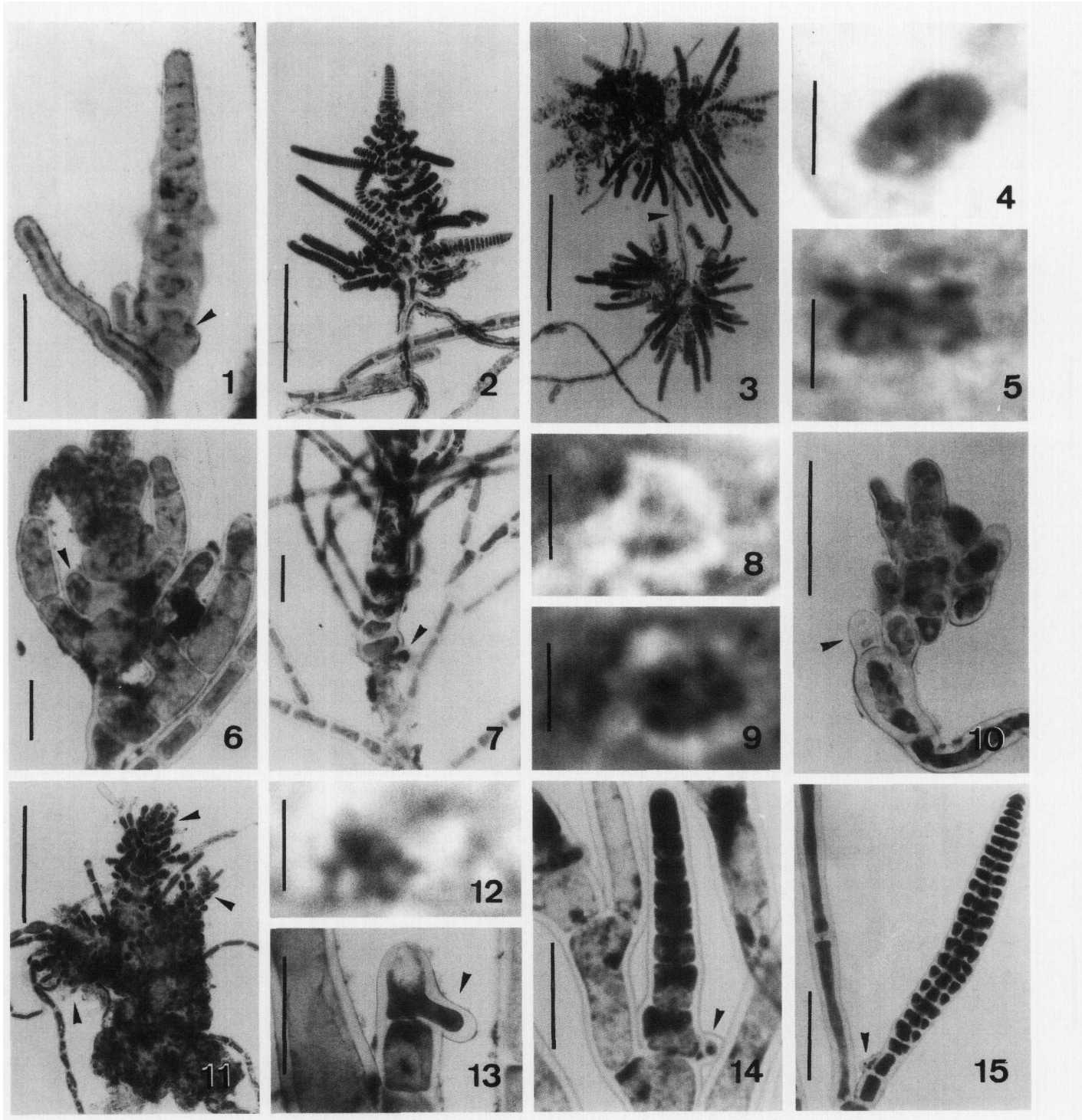
Species and populations	Irradiance (μmol photons m^{-2} s^{-1})	Gametophyte production		
		10°C	15°C	20°C
Batrachospermum delicatulum (#1)	47–50	none	+	—
	66–68[1]	—	—	++
	137–142	+	+	—
B. cf. *antipodites* (#70)	47–50	none	+	none
	66–68[1]	—	—	none
	137–142	none	+	none
Batrachospermum sp. (#94)	47–50	none	+	+
	66–68[1]	—	—	+
	137–142	none	none	—
Paralemanea catenata (#96)	47–50	++	+	—
	66–68[1]	—	—	none
	137–142	++	+	—
Paralemanea sp. (#104)	47–50	++	none	none
	66–68[1]	—	—	none
	137–142	++	none	—
Thorea hispida (#64)	47–50	none	none	++
	66–68[1]	—	—	++
	137–142	none	none	+
	164–179[1]	—	—	+

[1] Culture conditions before experiments.

MATERIAL AND METHODS

Seven populations of freshwater red algae belonging to the Batrachospermaceae, Lemaneaceae and Thoreaceae, involving three species of *Batrachospermum,* two of *Paralemanea* and one of *Thorea,* were examined in this study: six were studied in culture and one under field conditions (Table 1). The species identification was made on the basis of the co-occurrence of the '*Chantransia*' stages with identifiable gametophytic phases in the same stream or by germinating carpospores in culture from known species. The *Paralemanea* sp. was grown in culture from a '*Chantransia*' stage collected from a site with no mature gametophyte and thus it could not be identified to species level. Identification to generic level was based on the gross morphology of the juvenile gametophytes and on the fact that *Paralemanea* is the only genus in the family with confirmed occurrence in Mexico. Isolate #70 was tentatively associated with *B. antipodites,* on the basis of the shape of the fascicle cells (typically cylindrical) in juvenile gametophytes, because two species occurred in the stream where the '*Chantransia*' stage was collected (T. Entwisle, unpublished observations). We circumscribed the order Batrachospermales to include a member of the Thoreaceae (*T. hispida*) that it has recently been suggested (Sheath *et al.* 2000) should be transferred to a new order, the Thoreales. The reason for our decision to study it alongside other members of the Batrachospermales *sensu lato* is that it shares the essential characteristics of somatic meiosis and early development of juvenile gametophytes (Swale 1962), as well as other features (Pueschel & Cole 1982), with the remaining families (Batrachospermaceae and Lemaneaceae). The order Thoreales has not yet been formalized (R. Sheath, unpublished observations).

Isolation into culture followed the procedures described in a previous study (Necchi & Zucchi 1997). The isolates were kept in a 20 : 1 water–soil culture medium inside RI 12-555 Revco incubators, with illumination from above supplied by cool-white fluorescent lamps (Phillips 15 W), under the following conditions: temperature 15 or 20°C; irradiance 66–68 μmol photons m^{-2} s^{-1} or 164–179 μmol photons m^{-2} s^{-1}; photoperiod 12 : 12 h light–dark. The culture populations were



Figs 1–15. Different stages in the development of the juvenile gametophytes in *Batrachospermum* and *Paralemanea.* Scale bars = 5 μm (Figs 4, 5, 8, 9, 12), 20 μm (Figs 1, 6, 7, 10, 13–15) or 50 μm (Figs 2, 3, 11).

Figs 1–5. *Batrachospermum delicatulum* (isolate #1).

Fig. 1. Juvenile gametophyte, showing probable elimination cells (arrowhead).

Fig. 2. Profusely branched juvenile gametophyte.

Fig. 3. Two juvenile gametophytes linked by a rhizoidal filament (arrowhead).

Fig. 4. Fascicle cell of juvenile gametophyte with chromosome number $n = 3$ (cf. Fig. 29).

Fig. 5. '*Chantransia*' stage cell with chromosome number $2n = 6$ (cf. Fig. 30).

Figs 6–9. *Batrachospermum* cf. *antipodites* (isolate #70).

Fig. 6. Juvenile gametophyte, showing elimination cells (arrowhead).

Fig. 7. Later stage of the juvenile gametophyte, showing elimination cells (arrowhead).

Fig. 8. Fascicle cell of juvenile gametophyte with chromosome number $n = 4$ (cf. Fig. 31).

Fig. 9. '*Chantransia*' stage cell with chromosome number $2n = 8$ (cf. Fig. 32).

subjected to various experimental conditions, involving two temperatures (10 and 15°C), combined with two irradiances: low (47–50 μmol photons m^{-2} s^{-1}) and high (137–142 μmol photons m^{-2} s^{-1}) under the same photoperiod (Table 2). Isolates were examined biweekly for the production of the juvenile gametophytes. For microscopical observations, plants were preserved in Carnoy's solution and stained by Wittmann's haematoxylin technique, according to procedures described by Necchi & Sheath (1992) and Sheath & Cole (1993). Chromosome counts were made for all populations in culture on the basis of a minimum of 10 observations for each cell type. The sample from natural conditions was preserved in 4% formaldehyde and deposited in the herbarium FCME (Holmgren *et al.* 1990). We analysed all the cytological characters previously considered to be important in the relevant studies on somatic meiosis and development of juvenile gametophytes in the Batrachospermales (Magne 1967; Balakrishnan & Chaugule 1975; Huth 1979, 1981; von Stosch & Theil 1979). We adopted the term elimination cell, originally proposed by Magne (1967), instead of polar bodies (von Stosch & Theil 1979) or 'residual cells' (Sheath 1984), because it more accurately describes the nature of such cells. For the position of the elimination cells, we used the terms basal or suprabasal cell to indicate the occurrence on the first or second cell of the juvenile gametophyte, respectively, according to Balakrishnan & Chaugule (1975).

RESULTS

All populations with the '*Chantransia*' stage examined in culture (Table 1) ultimately produced juvenile gametophytes (Figs 1–28; Table 2) under at least some of the combinations of temperature and irradiance tested. The population of *P. mexicana,* which was sampled from nature, also produced juvenile gametophytes. The '*Chantransia*' stage of all species analysed grew and reproduced by monospores under the experimental conditions tested, even when they simultaneously produced juvenile gametophytes. The characteristics of the somatic meiosis and the development of juvenile gametophytes are described separately for each family.

Batrachospermaceae

The '*Chantransia*' stage of *B. delicatulum* (isolate #1) produced juvenile gametophytes at 10–20°C under the full range of irradiances tested, but most abundantly at 20°C and 66–68 μmol photons m^{-2} s^{-1} (Table 2). The gametophytes of *B.* cf. *antipodites* (isolate #70) were present only at 15°C, but under a range of irradiances of 47–142 μmol photons m^{-2} s^{-1} (Table 2). The '*Chantransia*' stage of *Batrachospermum* sp. (isolate #94) produced gametophytes at 15–20°C, but exclusively under low irradiances of 47–68 μmol photons m^{-2} s^{-1} (Table 2).

In all three populations of *Batrachospermum,* elimination cells could be detected, but we were not able to observe the initial stages of their formation. The first elimination cell is characterized by an extreme reduction in cytoplasm, and the cell is clearly distinguishable by its pycnotic nucleus. The second elimination cell is slightly larger, the amount of cytoplasm is not so reduced, and the nucleus does not stain as strongly as that of the first elimination cell (Figs 1, 6, 10). The later stages in gametophyte development show the initial fascicle cells and the axial cells of young thalli, including some cells that are abundantly branched (Figs 2, 3, 7, 11). The site of meiosis in all species of Batrachospermaceae analysed was generally situated one or two cells above a unilateral branching of the '*Chantransia*' filament (Figs 1, 2, 6, 7). The elimination cells were situated on the basal cell of the juvenile gametophytes in *B. delicatulum* (isolate #1, Fig. 1) and *Batrachospermum* sp. (isolate #94, Fig. 10), whereas they were on the suprabasal cell (Figs 6, 7) in *B.* cf. *antipodites* (isolate #70).

The chromosome numbers in cells of the '*Chantransia*' stage and of juvenile gametophyte fascicle cells (Figs 4, 5, 8, 9, 12, 29–33; Table 3) showed unequivocally that a new life-cycle phase had been formed. The juvenile gametophytes in isolates #1 and #94 formed a system of rhizoidal filaments or a cluster of cells, which produced new gametophytes (Figs 3, 11). In isolate #1 we observed the simultaneous formation of numerous juvenile gametophytes on several filaments of the same '*Chantransia*' plant.

Lemaneaceae

The '*Chantransia*' stage of *P. catenata* (isolate #96) produced juvenile gametophytes at 10–15°C and 47–142 μmol photons m^{-2} s^{-1} (Table 2), whereas in the isolate of the *Paralemanea* sp. (isolate #104), gametophyte development was observed only at 10°C, although under the same range of irradiances (Table 2). The field population of *P. mexicana* was collected during summer in a cold (12°C) , shallow (depth 1–60 cm), and shaded or partly shaded stretch of stream.

Elimination cells were observed in the three populations of *Paralemanea* examined, both in culture (isolates #96 and #104) and natural conditions (BALE1942); early developmental stages were present. After the first meiotic division, one of the two resulting nuclei degenerates and is pushed out into a small lateral protuberance (Figs 13, 17, 20), which is later cut off as an elimination cell (Figs 14, 18, 21). The elimination cells are preserved during gametophyte development and can be observed even during the later stages (Figs 15, 16, 22). As in Batrachospermaceae, the first elimination cell is smaller and

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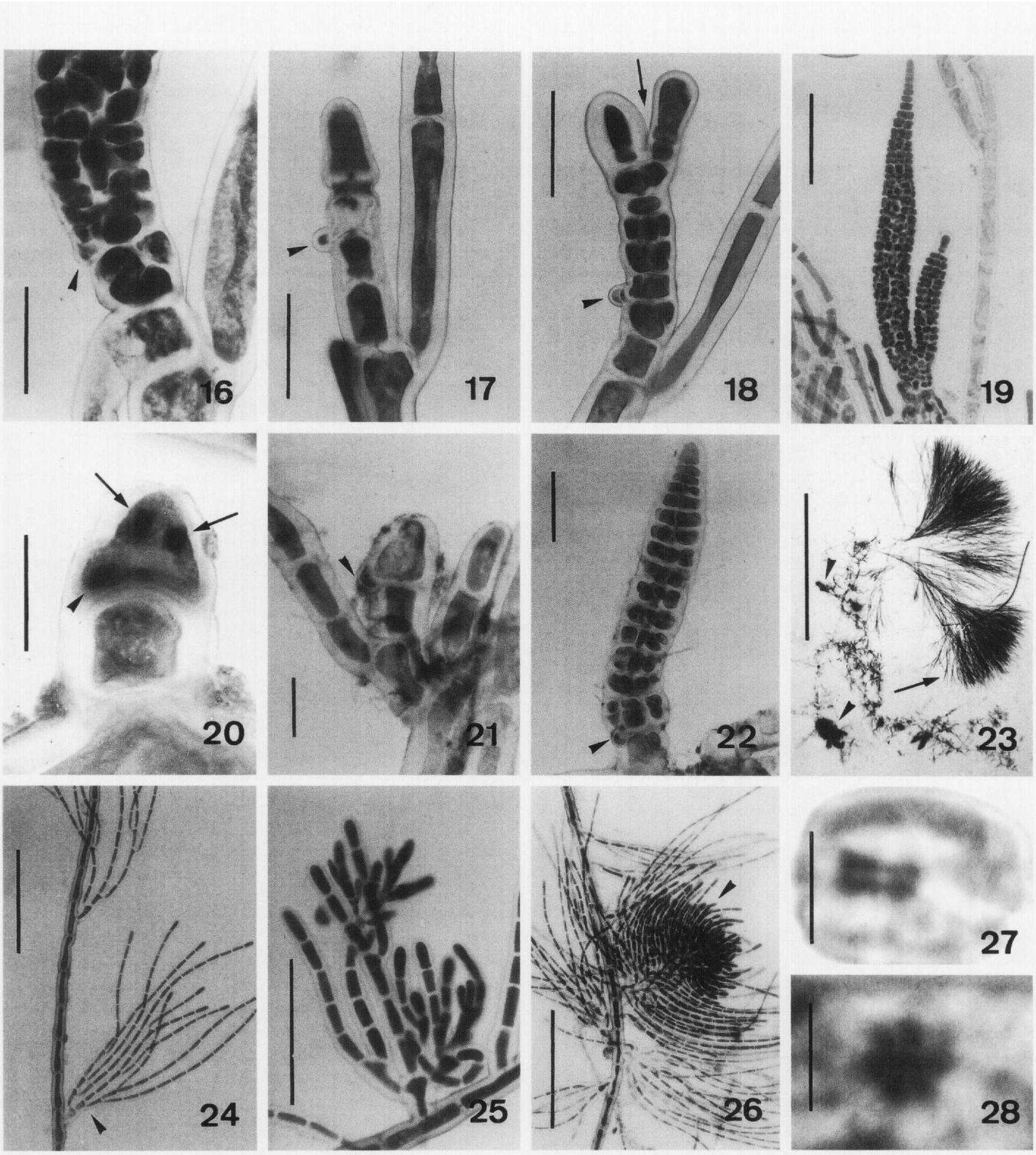
Figs 10–12. *Batrachospermum* sp. (isolate #94).
Fig. 10. Juvenile gametophyte, showing elimination cells (arrowhead).
Fig. 11. Cell aggregation with three juvenile gametophytes (arrowheads).
Fig. 12. '*Chantransia*' stage cell with chromosome number $2n = 8$ (cf. Fig. 33).
Figs 13–15. *Paralemanea catenata* (isolate #96).
Fig. 13. Early stage of the juvenile gametophyte, showing the formation of the first elimination cell (arrowhead).
Fig. 14. Juvenile gametophyte, showing elimination cells (arrowhead).
Fig. 15. Later stage of the juvenile gametophyte, showing elimination cells (arrowhead).



Figs 16–28. Different stages in the development of the juvenile gametophytes in *Paralemanea* and *Thorea*. Scale bars = 5 μm (Figs 27, 28), 20 μm (Figs 16–18, 20, 21), 50 μm (Figs 19, 22, 24–26) or 1 cm (Fig. 23).

Fig. 16. *Paralemanea catenata* (isolate #96). Late stage of the gametophyte development, showing elimination cells (arrowhead).

Figs 17–19. *Paralemanea* sp.(isolate #104).

Fig. 17. Early stage of the juvenile gametophyte, showing the formation of the first elimination cell (arrowhead).

Fig. 18. Juvenile gametophyte, showing two elimination cells (arrowhead) and branched apex (arrow).

Fig. 19. Later stage of the juvenile gametophyte, showing branched gametophyte.

Figs 20–22. *Paralemanea mexicana* (BALE1942).

Fig. 20. Initial stage of meiosis in a '*Chantransia*' apical cell, showing two nuclei (arrows) and protrusion of the first elimination cell (arrowhead).

Fig. 21. Early stage of the juvenile gametophyte, showing two elimination cells (arrowhead).

Fig. 22. Juvenile gametophyte, showing two elimination cells (arrowhead).

has a more highly pycnotic nucleus than the second one (Figs 14–16, 21). The site of meiosis in all species of Lemaneaceae analysed was generally situated one or two cells above a unilateral branching of the '*Chantransia*' filament (Figs 13–15, 17, 18, 21).

The juvenile gametophytes of *P. catenata* (isolate #96) and *P. mexicana* (BALE1942) were unbranched (Figs 15, 22), whereas those of the *Paralemanea* sp. (isolate #104) were branched during the early and late stages (Figs 18, 19). Later stages of the juvenile gametophytes revealed the cortication typical of the Lemaneaceae (Figs 16, 19). Several filaments of the same plant of the '*Chantransia*' stage simultaneously produced numerous juvenile gametophytes in all populations examined.

The elimination cells were always formed on the suprabasal cell of the juvenile gametophytes in all populations of the *Paralemanea* examined (Figs 13–18, 20–22).

Thoreaceae

The '*Chantransia*' stage of *T. hispida* (isolate #64) produced juvenile gametophytes only at 20°C, but under a wide range of irradiances (47–179 μmol photons m^{-2} s^{-1}); however, they were more abundant at low irradiances (47–68 μmol photons m^{-2} s^{-1}; Table 2). Two types of morphology were observed in the '*Chantransia*' stage: (1) typical tuft-like plants, with erect and densely branched filaments, and cylindrical cells, reproducing by monosporangia; and (2) modified plants, with an irregular arrangement of loosely clustered and sparsely branched filaments, producing juvenile gametophytes (Fig. 23). The latter plants form axial filaments, from which the juvenile gametophytes are produced as lateral fascicles (Figs 24, 25). This isolate formed numerous juvenile gametophytes simultaneously on several filaments of the same '*Chantransia*' plant.

We were not able to observe elimination cells, despite many attempts. The juvenile gametophyte development is rapid, and initial cells soon become obscured by later growth (Fig. 26). Thus, the precise site of meiosis could not be determined. Rhizoids were formed on initial branches of the haploid fascicles. Later stages of the juvenile gametophytes showed the typical multiaxial construction of the Thoreaceae (Fig. 26). An unequivocal indication that the lateral fascicles on the axial filaments of the modified '*Chantransia*' stage represent a new phase is that the chromosome number in cells of the '*Chantransia*' stage ($2n = 8$) is double that of the assimilatory filaments of the juvenile gametophytes ($n = 4$) (Figs 27, 28, 34, 35; Table 3).

DISCUSSION

This study reinforces the conclusion that there is a tendency for the '*Chantransia*' stage (vegetative and reproductive) to occur over a wider range of environmental conditions (in this case temperature and irradiance), than the gametophytes, which has previously been reported from field populations (Hambrook & Sheath 1991; Necchi 1993, 1997), although we did not study mature gametophytes. The production of juvenile gametophytes in the three populations of *Batrachospermum* occurred at a similar temperature (15°C) to that reported by Huth (1979) for *B. gelatinosum* (Linnaeus) de Candolle (as *B. moniliforme*), but at a different photoperiod (14 : 10 h light–dark) and irradiance (9–18 μmol photons m^{-2} s^{-1}). Chesnick & O'Flaherty (1986) found that the development of gametophytes in *Batrachospermum* sp. was most abundant at 15°C, an irradiance of 46–93 μmol photons m^{-2} s^{-1}, and a variable photoperiod (8 : 16, 12 : 12 and 16 : 8 h light–dark). Thus, available information from culture studies suggests that temperatures of *c.* 15°C and low irradiances are generally favourable for gametophyte induction in the Batrachospermaceae, and that the photoperiod does not seem to be critical, although photoperiod effects were not tested in our study. However, additional information is still necessary and species-specific responses to temperature, irradiance and photoperiod should also be considered.

Seasonal studies (Dillard 1966; Rider & Wagner 1972; Hambrook & Sheath 1991; Necchi 1993; Necchi & Branco 1999) have indicated that interactions between light and temperature influence the appearance of the macroscopic gametophytic phase in several species of *Batrachospermum*. Generally, gametophyte development is promoted by the lower temperatures and higher irradiances occurring during the dry season in the tropics or between late autumn and early summer in temperate regions. It is hard to distinguish the single factors acting on plant growth from the field data, but lower temperatures have been indicated as favourable to gametophyte development both in natural and culture conditions. Thirb & Benson-Evans (1984) observed that growth, carpospore germination and the formation of juvenile gametophytes in *Lemanea fluviatilis* C. Agardh were higher at low temperatures (10–15°C). Similar results were obtained in the present study with abundant gametophyte development at 10°C. This is also in accord with the field data, because low temperatures (12–16°C) have been reported as typical for the occurrence of *Paralemanea* species in North America (Vis & Sheath 1992; J.J. Carmona & O. Necchi, unpublished observations). There are no records of environmental factors favouring gametophyte production in the Thoreaceae, and our results suggest that it occurs under higher temperatures (20°C) than in the Batrachospermaceae and the Lemaneaceae. This agrees with its geographical distribution, because it tends to be more common in tropical and subtropical or warm temperate regions (Sheath *et al.* 1993; Carmona & Necchi 2001).

The elimination cells in the Batrachospermaceae can be located either on the basal or on the suprabasal cell of the ju-

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Figs 23–28. *Thorea hispida* (isolate #64).
Fig. 23. Typical (arrow) and modified '*Chantransia*' stage with juvenile gametophytes (arrowheads).
Fig. 24. Juvenile gametophyte (arrowhead) from an axial filament of the '*Chantransia*' stage.
Fig. 25. Detail of an early stage of the juvenile gametophyte.
Fig. 26. Later stage of the juvenile gametophyte (arrowhead) borne from an axial filament of the '*Chantransia*' stage.
Fig. 27. Assimilatory filament cell of a juvenile gametophyte with chromosome number $n = 4$ (cf. Fig. 34).
Fig. 28. '*Chantransia*' stage cell with chromosome $2n = 8$ (cf. Fig. 35).

Table 3. Chromosome numbers of Batrachospermales *sensu lato* populations.

Species and population	Cell type	Chromosome number
Batrachospermum delicatulum (#1)	fascicle of juvenile gametophyte	$n = 3$
	vegetative cells of '*Chantransia*' stage	$2n = 6$
B. cf. *antipodites* (#70)	fascicle of juvenile gametophyte	$n = 4$
	vegetative cells of '*Chantransia*' stage	$2n = 8$
Batrachospermum sp. (#94)	vegetative cells of '*Chantransia*' stage	$2n = 8$
Paralemanea catenata (#96)	vegetative cells of '*Chantransia*' stage	$2n = c.\ 30–36$
Paralemanea sp. (#104)	vegetative cells of '*Chantransia*' stage	$2n = c.\ 28–32$
Thorea hispida (#64)	assimilatory filaments of juvenile gametophyte	$n = 4$
	vegetative cells of '*Chantransia*' stage	$2n = 8$

venile gametophytes (Balakrishnan & Chaugule 1975, 1980), but the position is usually fixed in individual species. Thus, elimination cells are located on the suprabasal cell in *Batrachospermum* sp., *B. ceylanicum* Balakrishnan & Chaugule, *B. mahabaleshwarense* Balakrishnan & Chaugule and *B. huillense* (Skuja) Necchi & Entwisle (as *Sirodotia huillensis* (West & West) Skuja), whereas in *B. gelatinosum* and *B. brasiliense* they are situated on the basal cell (Balakrishnan & Chaugule 1975, 1980; Necchi 1987). In the Lemaneaceae, elimination cells have been reported to occur on the suprabasal cell (Magne 1967; this study), but Huth (1981) found them in variable positions on the juvenile gametophytes of *L. fluviatilis*. However, in Huth's material, the juvenile gametophytes appeared to start their differentiation before the occurrence of meiosis, thus leading to the formation of elimination cells on distinct positions on the gametophyte only after meiosis had occurred. Balakrishnan & Chaugule (1980) argued that the position of the elimination cells is such a constant feature that it is of potential diagnostic value. Our results corroborate the usefulness of the position of the elimination cells as an additional diagnostic character at the generic and infrageneric (sectional or specific) levels. However, the precise observation of elimination cells is not always practical and may be variable in some conditions.

The characteristics observed during the development of the juvenile gametophytes in species of Lemaneaceae were similar to those described for several species of *Lemanea* and *Paralemanea* by Magne (1967), Huth (1981) and Coomans & Hommersand (1990). The general features observed in species of Batrachospermaceae were also very similar to those in previous reports, e.g. Balakrishnan & Chaugule (1975, 1980), Huth (1979), von Stosch & Theil (1979) and Necchi (1987). A remarkable observation was the formation of numerous juvenile gametophytes from filaments of the same '*Chantransia*' plant, which has already been reported by Huth (1979, 1981) in *Batrachospermum* and *Lemanea*. Another interesting characteristic was the formation of a system of rhizoidal filaments or the aggregation of cells in the juvenile gametophytes of some species of *Batrachospermum*, from which new gametophytes are produced. These two characters can increase considerably the formation of new gametophytes from the same plant of the '*Chantransia*' stage and enhance the production of gametophytes under favourable conditions.

The development of juvenile gametophytes in the Thoreaceae was similar to that observed under culture conditions in *T. hispida* (as *T. ramossisima* Bory) by Swale (1962). However, the two morphological types of the '*Chantransia*' stage (typical and modified) that we observed have apparently not been described before. The presence and position of the elimination cells remain to be determined in *Thorea*. The characteristics of the Thoreaceae, with a distinct developmental pattern of the juvenile gametophytes and the occurrence of two morphological types in the '*Chantransia*' stage, are consistent with the proposal of Sheath *et al.* (2000) to elevate it to the ordinal level.

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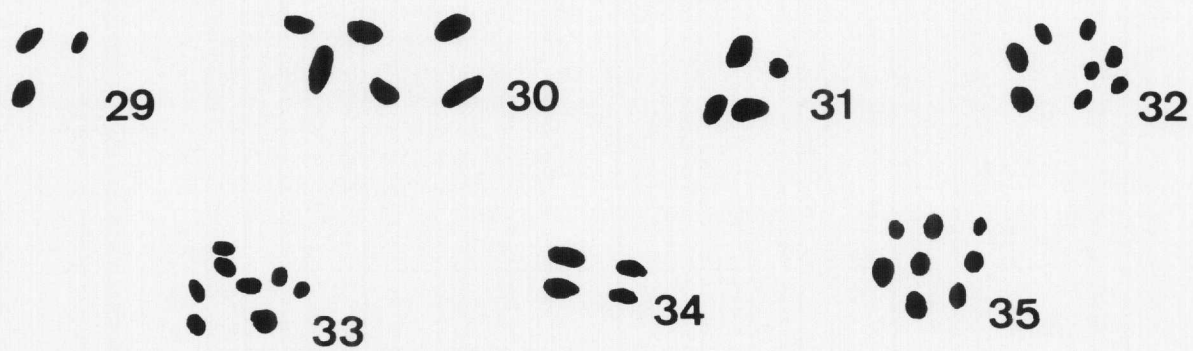

Figs 29–35. Drawings clarifying respective chromosome photomicrographs.
Figs 29, 30. *Batrachospermum delicatulum:* haploid and diploid nuclei (cf. Figs 4, 5).
Figs 31, 32. *Batrachospermum* cf. *antipodites:* haploid and diploid nuclei (cf. Figs 8, 9).
Fig. 33. *Batrachospermum* sp.: diploid nucleus of '*Chantransia*' phase (cf. Fig. 12).
Figs 34, 35. *Thorea hispida:* haploid and diploid nuclei (cf. Figs 27, 28).

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