Cryptogamie, Algol., 2003, 24 (2): 117-131

Blue-greenish acrochaetioid algae in freshwater habitats are "Chantransia" stages of Batrachospermales *sensu lato* (Rhodophyta)

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(Received 25 February 2002, accepted 15 September 2002)

Abstract — Fourteen culture isolates of freshwater acrochaetioid algae from distinct regions around the world were analysed, including the reddish species *Audouinella hermannii*, the dubious blue-greenish species *A. pygmaea*, and "Chantransia" stages from distinct taxonomic origins in the Batrachospermales *sensu lato* (Batrachospermaceae, Lemaneaceae and Thoreaceae). Four isolates (two 'Chantransia' stages and two species of *Audouinella, A. hermannii* and *A. pygmaea*) were tested under experimental conditions of temperature (10-25 °C), irradiance (65 and 300 µmol photons m^{-2} s^{-1}) and photoperiod (16:8 h and 8:16 h light/dark cycles). Plant colour is proposed as the only vegetative character that can be unequivocally applied to distinguish *Audouinella* from 'Chantransia', blue-greenish representing "Chantransia" stages and reddish applying to true *Audouinella* species (also forming reproductive structures other than monosporangia, e.g. tetrasporangia). Some isolates of *A. pygmaea* were proven to be unequivocally 'Chantransia" stages owing either to production of juvenile gametophytes or to derivation from carpospores. No association of the morphology of *A. pygmaea* was found with any particular species, thus it should be regarded as a complex involving many species of the Batrachospermales *sensu lato*, as is also the case with *A. macrospora*. We therefore recommend that all blue-greenish acrochaetioid algae in freshwater habitats be considered as "Chantransia" stages of members of the Batrachospermales, and that the informal descriptors "pygmaea" and "macrospora" be used to distinguish the two discernable morphologies. Induction of gametophytes occurred under much wider conditions than previously reported, reinforcing the conclusion that requirements are probably species-specific. Although phenotypic plasticity was in evidence, with temperature, irradiance and photoperiod affecting morphology, no alga showed variation outside the limits based on traditional taxonomic studies. No overall trend was observed for vegetative or reproductive characters in response to temperature, irradiance and photoperiod for all the algae tested, only for specific algae or characters. Effects of temperature and irradiance on morphological characters were more evident, as well as strong interactions between these variables, whereas few differences were generally found in response to photoperiod and irradiance.

Acrochaetiales / Batrachospermales / culture / irradiance / morphology / photoperiod / temperature

Résumé — Les algues acrochétioïdes bleu vert des eaux douces sont des stades « Chantransia » des Batrachospermales *sensu lato* (Rhoduphyta). Quatorze souches d'algues acrochétioïdes d'eau douce provenant de diverses régions du monde ont été

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analysées, notamment l'espèce à thalle rouge *Audouinella hermannii*, l'espèce douteuse à thalle bleu vert *A. pygmaea* et des stades "Chantransia" de diverses espèces appartenant aux Batrachospermales *sensu lato* (Batrachospermaceae, Lemaneaceae et Thoreaceae). Quatre souches (deux stades "Chantransia" et deux espèces d'*Audouinella*, *A. hermannii* et *A. pygmaea*) ont été testées dans des conditions expérimentales de température (10-25 °C), d'irradiance (65 et 300 µm photons m^{-2} s^{-1}) et de photopériode (cycles lumière/obscurité de 16/8 h et 8/16 h). La couleur des thalles est proposée comme le seul caractère végétatif permettant de distinguer *Audouinella* des stades "Chantransia", ces derniers étant bleu verdâtre tandis que les thalles rougeâtres sont de vrais *Audouinella* (aussi capables de former des structures reproductrices autres que des monosporocystes, par exemple des tétrasporcystes). On prouve que quelques souches de *A. pygmaea* sont en fait des stades "Chantransia", soit qu'elles produisent de jeunes gamétophytes, soit qu'elles dérivent de carpospores. On n'a pas pu associer la morphologie d'*A. pygmaea* à une espèce particulière ; il doit donc être considéré comme un complexe impliquant de nombreuses espèces des Batrachospermales *s.l.*, tout comme *A. macrospora*. En conséquence, nous recommandons que toutes les algues acrochétioïdes d'eau douce à thalle bleu vert soient considérées comme des stades "Chantransia" de diverses Batrachospermales *s.l.* et que les descripteurs informels "pygmaea" et "macrospora" soient utilisés pour désigner ces deux morphologies distinctes. L'induction de gamétophytes a eu lieu dans des conditions beaucoup plus larges que lors des observations antérieures, ce qui implique que les exigences dans ce domaine sont propres à chaque espèce. Bien qu'une certaine plasticité phénotypique ait été mise en évidence, la température, l'irradiance et la photopériode affectant divers caractères morphologiques, aucune des algues n'a montré de variation dépassant les limites données par les études taxinomiques traditionelles. Chez les algues étudiées, aucune tendance générale n'a été observée liant des caractères végétatifs ou reproductifs en réponse à la température, à l'irradiance ou à la photopériode ; on note seulement des réponses spécifiques pour certaines algues ou certains caractères. Les effets de la température et de l'irradiance sur les caractères morphologiques ont été plus évidents, de même que de fortes interactions entre ces variables, tandis que peu de différences on été généralement trouvées en réponse à la photopériode et le rayonnement.

Acrochaetiales / Batrachospermales / culture / rayonnement / morphologie / photopériode / température

INTRODUCTION

Freshwater acrochaetioid algae include species of the genus *Audouinella* (Acrochaetiales) and also "Chantransia" stages of members of the Batrachospermales (Necchi *et al.*, 1993a, 1993b; Necchi & Zucchi, 1995, 1997). Besides the long-standing controversy regarding generic delimitation within the Acrochaetiaceae, there is an additional problem in identifying freshwater taxa because the "Chantransia" stage can be misinterpreted as species of *Audouinella* (Necchi & Zucchi, 1997).

Skuja (1934) proposed some criteria to distinguish freshwater *Audouinella* from "Chantransia" stages, including plant colour (reddish vs. blue-greenish), type of branching (regular vs. irregular) and abundance of monosporangia (abundant vs. few). In addition, *Audouinella* can potentially produce gametangia, carposporophytes and tetrasporangia (Drew, 1935; Korch & Sheath, 1989; Necchi *et al.*, 1993a; Carmona & Necchi, 2001), whereas the only reproductive mode reported for the bluish species is asexual monosporangia (Necchi *et al.*, 1993b; Necchi & Zucchi, 1997). Although the distinction between *Audouinella* and "Chantransia" is a major difficulty in the systematics of the freshwater red algae, experimental analyses to test the validity of existing criteria are still scarce. Necchi & Zucchi (1997) made a comparative analysis of field and culture populations of the blue-greenish species *A. macrospora* with the "Chantransia" stage of *Batrachospermum* and concluded that this species represents the "Chantransia" stage. In addition, they raised the possibility that all blue-greenish freshwater acrochaetioid algae are "Chantransia" stages and that all reddish forms are true *Audouinella* species.

Pueschel *et al*. (2000) investigated SSU rDNA gene sequences and pit plug ultrastructure of blue-greenish forms ("Chantransia" stage and *A. macrospora*-like culture isolates) to ascertain whether they represent life history stages of the Batrachospermales or blue-green coloured members of the Acrochaetiales. Sequence analyses unequivocally placed the *A. macrospora* and 'Chantransia' isolates in clades with distinct species of *Batrachospermum*, revealing affinities with the Batrachospermales. Pit plugs of *A. macrospora* had thickened plug caps and no cap membranes, showing batrachospermalean rather than acrochaetialean affinities. The pit plugs of "Chantransia" were essentially similar in structure but the plug cores were smaller in diameter than those of *A. macrospora*.

Garbary (1979) investigated the morphological plasticity in six marine species of *Audouinella* as a response to different temperatures. He found that all characteristics evaluated showed variation attributable to temperature, but with species-specific responses in particular characters. No similar study has been conducted with freshwater species, either for temperature or other environmental factors (e.g. irradiance and photoperiod). Glazer *et al*. (1997) described a new type of phycoerythrin in blue-greenish freshwater acrochaetioid ("Chantransia" stages and *A. macrospora*-like), suggesting affinities between the two. Zucchi & Necchi (2001) tested the responses of growth and pigment content (chlorophyll *a*, phycocyanin and phycoerythrin) to temperature, irradiance and photoperiod in some freshwater red algae, including *Audouinella hermannii* (Roth) Duby and *A. pygmaea* (Kützing) Weber-van Bosse and two "Chantransia" stages.

The status of the blue-greenish species *A. pygmaea* has not been evaluated, despite some suggestions (Israelson, 1942; Necchi & Zucchi, 1997; Pueschel *et al*., 2000) that it might represent a "Chantransia" stage. Thus, this investigation was carried out to compare this species with a reddish *Audouinella* (*A. hermannii*) and several isolates of the "Chantransia" stage from distinct taxonomic origins in the Batrachospermales *sensu lato* (Batrachospermaceae, Lemaneaceae and Thoreaceae). In addition, we evaluated the responses of morphological features to temperature, irradiance and photoperiod in freshwater acrochaetioid algae under culture conditions to test the stability of criteria applied to distinguish *Audouinella* from "Chantransia".

MATERIALS AND METHODS

Fourteen isolates of freshwater acrochaetioid algae, from distinct regions around the world, were analysed in this study (Tab. 1). These included two isolates of the reddish species *Audouinella hermannii*, five of the dubious blue-greenish species *A. pygmaea*, and seven of "Chantransia" stages from known taxonomic origins. The identification of *A. pygmaea* followed Necchi *et al.* (1993b) and Necchi & Zucchi (1995), who had examined the type specimen as part of a recircumscription of the taxon. The species identifications of the "Chantransia" stages were made on the basis of co-occurrence of the respective gametophytic phase with the "Chantransia" stages in the same stream (isolates 1 and 70) or the germination from carpospores into

Tab. 1. Location, time and maintenance conditions in culture of the freshwater acrochaetioid algae isolates analyzed. Photoperiod 12:12 h.

Location	*Time in culture (days)*	*Temperature (°C)*	*Irradiance (μmol m^{-2} s^{-1})*	*Species and isolate number**
Brazil: São Paulo, Itirapina, Route SP-310, km 213-214, Córrego do Feijão, 22°09'S, 47°47'W	412	20	140	"Chantransia" stage of *Batrachospermum delicatulum* (Skuja) Necchi & Entwisle Isolate no. 1
Brazil: São Paulo, São José do Rio Preto, Córrego da Lagoa, 20°49'S, 49°20'W	405	20	140	"Chantransia" stage of *Batrachospermum macrosporum* Montagne - Isolate no. 13
Brazil: Santa Catarina, São Pedro de Alcântara, Salto, 27°34'S, 48°50'W	785	20	140	"Chantransia" stage of *Batrachospermum ambiguum* Montagne - Isolate no. 35
Australia: Victoria, Melbourne, near Warburton, Yarra River, 37°49'S, 144°58'E	360	15	45	*Audouinella hermannii* (Roth) Duby Isolate no. 38
USA: Massachusetts, Amherst, Univ. of Massachusetts, Botany Department, 42°23'N, 72°31'W	807	20	125-160	*Audouinella pygmaea* (Kützing) Weber–van Bosse - Isolate no. 55
Brazil: São Paulo State, Pindamonhangaba, near Forest Reserve, 22°43'S, 45°27'W	170	20	165-175	"Chantransia" stage of *Batrachospermum* a*trum* (Hudson) Harvey - Isolate no. 56
Brazil: São Paulo State, Jumirim, tributary of Sorocaba River, 23°06'S, 47°48'W	830	20	65-85	"Chantransia" stage of *Thorea violacea* Bory emend. Sheath, Vis et Cole - Isolate no. 64
Australia: Queensland, Lamington National Park, waterfall, 28°15'S, 153°12'E	598	20	140	"Chantransia'" stage of *Batrachospermum* cf. *antipodites* Entwisle et Foard - Isolate no. 70
USA: Texas, Comal Springs, Landa Park, New Braunfels, 29°42'S, 98°08'W	203	20	85	*A. pygmae*a - Isolate no. 90
USA: North Carolina, Wake County, Lower Barton Creek, 35°30'S, 80°00'W	203	20	40	*A. pygmaea* - Isolate no. 92
USA: Kansas, Douglas County, Lone Star Lake, 37°31'S, 97°01'W	203	20	85	*A. pygmaea* - Isolate no. 93
Japan: Saitama-Ken, Kurihashi-Machi, Tone River, 36°08'S, 139°42'E	203	20	140	*A. pygmaea* - Isolate no. 94
USA: Missouri, Rockbridge Spring, 38°30'S, 93°30'W	111	15	45	*A. hermannii* - Isolate no. 95
USA: Tennessee, Mountain Home, Knoxvillle, 35°58'S, 83°56'W	111	20	40	"Chantransia" stage of *Paralemanea catenata* (Kützing) Vis et Sheath - Isolate no. 96

* Numbers in private culture collection.

culture from known species (all others). We recognise the taxonomic category Batrachospermales *sensu lato*, including a member of the Thoreaceae (*Thorea hispida*) that has been recently transferred to a new order (Thoreales, Sheath *et al.* 2000; see also Harper & Saunders, 1998), because its members share the common feature of having the "Chantransia" stage in their life history.

Isolation into culture followed the procedures described in a previous study (Necchi & Zucchi 1997). The isolates were kept in 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). All isolates were examined in optimal culture conditions (i.e. those with best growth for each isolate), specified in Tab. 1. In addition, four isolates (1, 13, 38 and 55) were tested under experimental conditions of temperature, irradiance and photoperiod. These included two "Chantransia" stages with distinct origins (monosporic field populations and culture isolate from carpospore germination of *B. macrosporum*) and two species of *Audouinella*, one reddish (*A. hermannii*) and one blue-greenish (*A. pygmaea*).

Irradiances within the incubators were adjusted by moving the shelves or using a neutral black filter. Measurements were made (in distilled water) with a Li-Cor LI-189 quantameter with a spherical quantum sensor LI-193 SA. Temperature and irradiance experiments followed a 4×2 factorial design, with temperatures of 10, 15, 20 and 25 °C (± 0.3 °C) at low (65 ± 2 µmol photons m^{-2} s^{-1}) and high (300 ± 5 µmol photons m^{-2} s^{-1}) irradiances (see Tab. 2). In photoperiod and irradiance experiments a 2 x 2 factorial design was applied: long day (16:8 h light/dark cycle) and short day (8:16 h) and the two irradiances specified above, all at 20 °C (see Tab. 2).

The experiments ran for 45 days, this period determined from preliminary growth curves (Zucchi & Necchi, 2001). Plant segments were grown from initial fresh weights of 1.0-3.5 mg and three replicates were used in experiments, each consisting of a test tube (16×150 mm) with 10 ml of culture medium. Position of tubes within each set was changed at 5 day intervals to minimize irradiance differences among the replicates. For microscopic observations, plants were preserved in 4 % formaldehyde and processed as described in Necchi *et al.* (1993a, 1993b) and Necchi & Zucchi (1995, 1997). The morphological characters previously considered to be important in those studies and those used to distinguish *Audouinella* from "Chantransia" (Skuja, 1934) were analysed. In addition, macroscopic plant colour was evaluated under living conditions at the end of experiments by comparison with standardized colour codes described by Biesalski (1957).

Numerical data were submitted to Analysis of Variance (ANOVA – one way) and Newman-Keuls test (Zar, 1999). ANOVA – two way was applied to test simultaneously the effect of two variables (temperature + irradiance or photoperiod + irradiance) on morphological characters of each alga exposed to distinct treatments. Analyses were performed with the Statistica package (version 6.0).

RESULTS

Morphology under optimal culture conditions

Three general patterns of plant colour, with nuances within each one, were observed in the isolates studied: blue-green, brown and red. The blue-green pattern varied from a more bluish colour (codes 21, 22 and 23 according

Tab. 2. Results for selected vegetative and reproductive characters of the freshwater acrochaetioid algae tested under distinct treatments of temperature, irradiance and photoperiod. Isolate numbers according to Table 1. LD – long day (16:8 light/dark); SD – short day (8:16 light/dark). Low and high irradiances correspond to 65 and 300 µmol photons m^{-2} s^{-1}, respectively. Photoperiodic responses tested under constant temperature (20 °C).

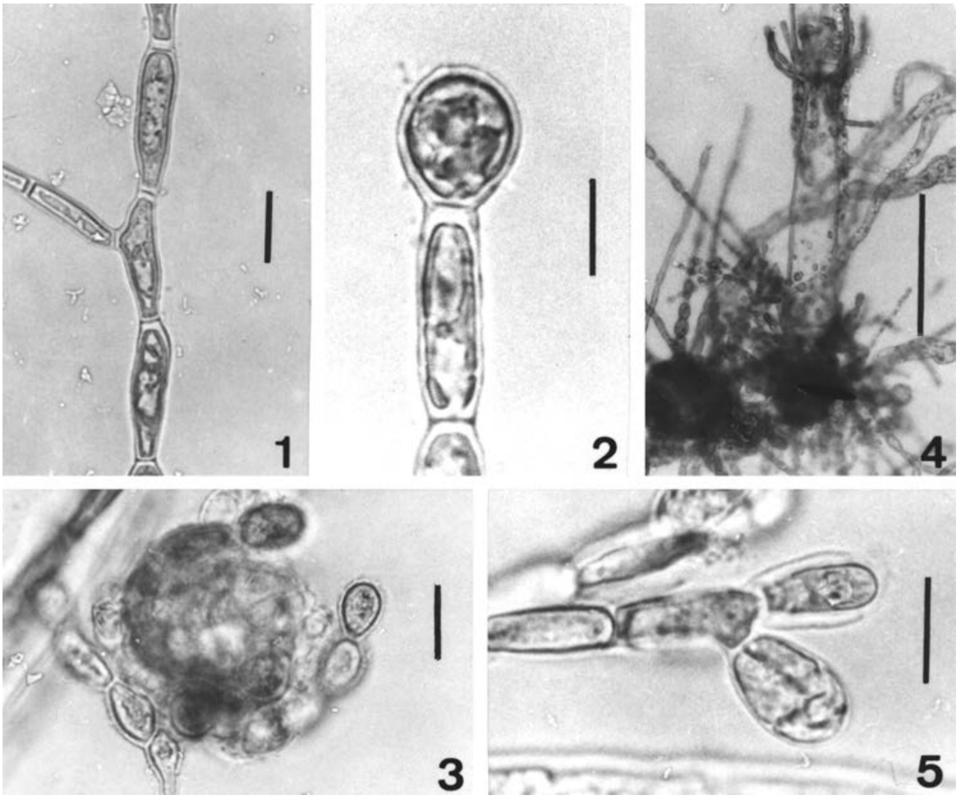
Alga/characters*	*Low irradiance*				*High irradiance*				*Low irradiance*		*High irradiance*	
	10°	*15°*	*20°*	*25°*	*10°*	*15°*	*20°*	*25°*	*SD*	*LD*	*SD*	*LD*
"Chantransia" (1)												
Plant colour[1]	24E	23K	23E	24R	Pale	Pale	24L	1E	23W	23Q	23L	24E
Juvenile gametophyte[2]	+	+	++	++	+	+	++	++	++	++	++	++
Monosporangia[2]	+	++	+	-	-	+	++	+	++	++	++	+++
"Chantransia" (13)												
Plant colour	24L	22K	22K	22W	Pale	24F	23K	24L	22Q	22Q	22Q	24L
Juvenile gametophyte	–	–	–	–	–	–	–	–	–	–	–	–
Monosporangia	++	++	++	-	++	+	++	++	++	++	+	+++
A. hermannii (38)												
Plant colour	5Q	5R	5R	5Q	4A	4Q	5Q	4Q	5W	4Q	5Q	3K
Juvenile gametophyte	–	–	–	–	–	–	–	–	–	–	–	–
Monosporangia	++	++	+	++	++	+++	+	+	–	++	+	–
A. pygmaea (55)												
Plant colour	22Q	22L	21L	21R	23E	23K	21E	21L	22W	22W	22L	22Q
Juvenile gametophyte	–	–	–	–	+	–	–	–	–	–	–	–
Monosporangia	++	++	++	++	++	+++	++	++	++	++	++	++

1. colour codes according to Biesalski (1957).
2. – absent; + = < 20 per plant; ++ = 25-50; +++ = > 50.
* according to Table 1.

to Biesalski, 1957) in *A. pygmaea* (55, 93 and 94) and some "Chantransia" stages (35 and 56) to green nuances (code 24) in three isolates of "Chantransia" (1, 13 and 96) and one of *A. pygmaea* (92). Brown nuances (codes 1 and 3) were observed in two isolates of "Chantransia" (64 and 70) and one of *A. pygmaea* (90), whereas the red patterns (codes 5 and 7) were found in the two isolates of *A. hermannii*.

The shape of vegetative cells ranged from exclusively cylindrical in *A. hermannii* (95) and "Chantransia" stages (1, 13, 70 and 96), to cylindrical or elliptical in *A. hermannii* (38), to cylindrical, elliptical or barrel-shaped (in variable proportions), rarely spherical or irregular-shaped in *A. pygmaea* (55, 90, 92, 93 and 94, Fig. 1) and "Chantransia" stages (35, 56 and 64). The chloroplasts were parietal and irregularly lobed in all isolates. Hairs were observed in two isolates of "Chantransia" (1 and 13, long and sparse) and *A. hermannii* (95, short or long and abundant).

The shape of monosporangia ranged from spherical, subspherical or obovoid in "Chantransia" stages (1, 13, 35, 64), to spherical, subspherical, obovoid or elliptical in *A. pygmaea* (55, 70, 90, 92, 93, 94, Fig. 2), *A. hermannii* (38) and some "Chantransia" stages (56 and 64). The quantity of monosporangia ranged from abundant (> 50 per plant) in some isolates of *A. pygmaea* (55, 92 and 94) and "Chantransia" stages (1, 13 and 64), to moderate (25-50) in *A. hermannii* (38), *A. pygmaea* (93) and "Chantransia" (56), to few (< 20) in *A. pygmaea* (90) and "Chantransia" (35, 70). One isolate of "Chantransia" stage (96) did not produce



Figs 1-5. Relevant morphological characters of some freshwater acrochaetioid algae. Figs 1-4. *A. pygmaea.* Fig. 1. Vegetative filament with an open branch (isolate 55). Fig. 2. Detail of a monosporangium (isolate 94). Figs 3-4. Different stages in the development of juvenile gametophyte of *Batrachospermum* (isolate 94). Fig. 3. Early stage with no definite shape. Fig. 4. Late stage showing a typical morphology. Fig. 5. *A. hermannii* (isolate 95). Detail of a mature and an immature tetrasporangium. Scale bars: 250 µm for Fig. 4; 25 µm for Figs 1, 3; 10 µm for Figs 2-5.

monosporangia, whereas in *A. hermannii* (95, Fig. 5) tetrasporangia were observed instead of monosporangia. Gametangia and carposporangia were not observed in any of the isolates analysed. Juvenile gametophytes of *Batrachospermum* were observed in variable quantities in some isolates: moderately abundant (25-50) in one isolate of *A. pygmaea* (94, Figs 3-4) and "Chantransia" (1) and sparse (few) in another isolate of "Chantransia" (13).

ANOVA (one way) revealed significant differences ($F = 13.3$-211.8, $p < 0.001$) for all morphometric characters among isolates (Fig. 6). No clear groupings for branch angle (Fig. 6) were evidenced by ANOVA and Newman-Keuls test, with a gradient from narrow (< 20 °) to open angles (> 40 °, Fig. 1). For cell length three groupings were detected (Fig. 6): one with long cells formed by three isolates of "Chantransia" (1, 13 and 96), an intermediate with the two isolates of *A. hermannii* and one with short cells represented by all remaining algae (*A. pygmaea* and "Chantransia"). For cell diameter, two groupings were evidenced (Fig. 6): one with large cells formed by four isolates of "Chantransia" (1, 13, 70 and

96) and another with small cells including all isolates of *A. pygmaea* and other "Chantransia" stages. Two groupings were detected for monosporangial length (Fig. 6): one with long monosporangia formed by two isolates of "Chantransia" (1 and 13) and one of *A. pygmaea* (90) and another with short monosporangia including all remaining isolates (*A. hermannii*, *A. pygmaea* and "Chantransia"). For monosporangial diameter, ANOVA and Newman-Keuls test revealed three groupings (Fig. 6): one with large monosporangia formed by two isolates of "Chantransia" (1 and 13), an intermediate including one isolate of *A. pygmaea* (90) and the third containing all remaining isolates (*A. hermannii*, *A. pygmaea* and "Chantransia"). The first has the typical morphology of *A. macrospora*.

Morphology under different combinations of temperature, irradiance and photoperiod

Vegetative and reproductive characters of the acrochaetioid algae generally responded to most conditions of temperature, irradiance and photoperiod tested (Tab. 2-3, Figs 7-8).

Tab. 3. Results of F (ANOVA - two way) for vegetative and reproductive characteristics of the freshwater acrochaetioid algae tested under distinct treatments of temperature, irradiance and photoperiod. Significant values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Alga/variable*	*Treatments*			*Treatments*		
	Temperature	*Irradiance*	*Interaction*	*Photoperiod*	*Irradiance*	*Interaction*
'Chantransia' (1)						
Cell length	5.61**	5.85**	0.72	6.86*	1.97	6.28*
Cell diameter	1.77	15.43***	2.88*	0.96	0.49	1.76
Branch angle	1.11	1.57	4.30**	1.61	1.33	0
Monosporangial length	–	–	–	2.33	0.53	5.40*
Monosporangial diameter	–	–	–	56.42***	1.57	5.48*
'Chantransia' (13)						
Cell length	16.67***	1.68	11.54***	1.29	1.79	0.47
Cell diameter	3.89*	15.35***	3.57*	3.16	0.08	3.46
Branch angle	0.48	0.57	2.73*	1.04	13.29***	0.06
Monosporangial length	0.88	2.65	0	–	–	–
Monosporangial diameter	10.46**	0.10	2.79	–	–	–
A. hermannii (38)						
Cell length	21,87***	0.08	10,29***	6.10*	0.01	2,16
Cell diameter	6.86***	0.20	0.27	28.00***	7.18**	6.47*
Branch angle	0.74	2.49	3.66*	0.16	0.02	0.12
Monosporangial length	0.97	0.11	2.40	–	–	–
Monosporangial diameter	10.85**	3.58	3.07–	–-	–	
A. pygmaea (55)						
Cell length	56.19***	57.61***	17.94***	0.98	5.65*	0.40
Cell diameter	10.84***	0.09	22.21***	4.93*	0	0.06
Branch angle	2.42	3.15	1.43	0.15	1.40	0.68
Monosporangial length	16.01***	22.16***	15.16***	0.80	0.28	2.18
Monosporangial diameter	31.87***	1.04	27.35***	7.68**	10.81**	0.23

* according to Table 1.

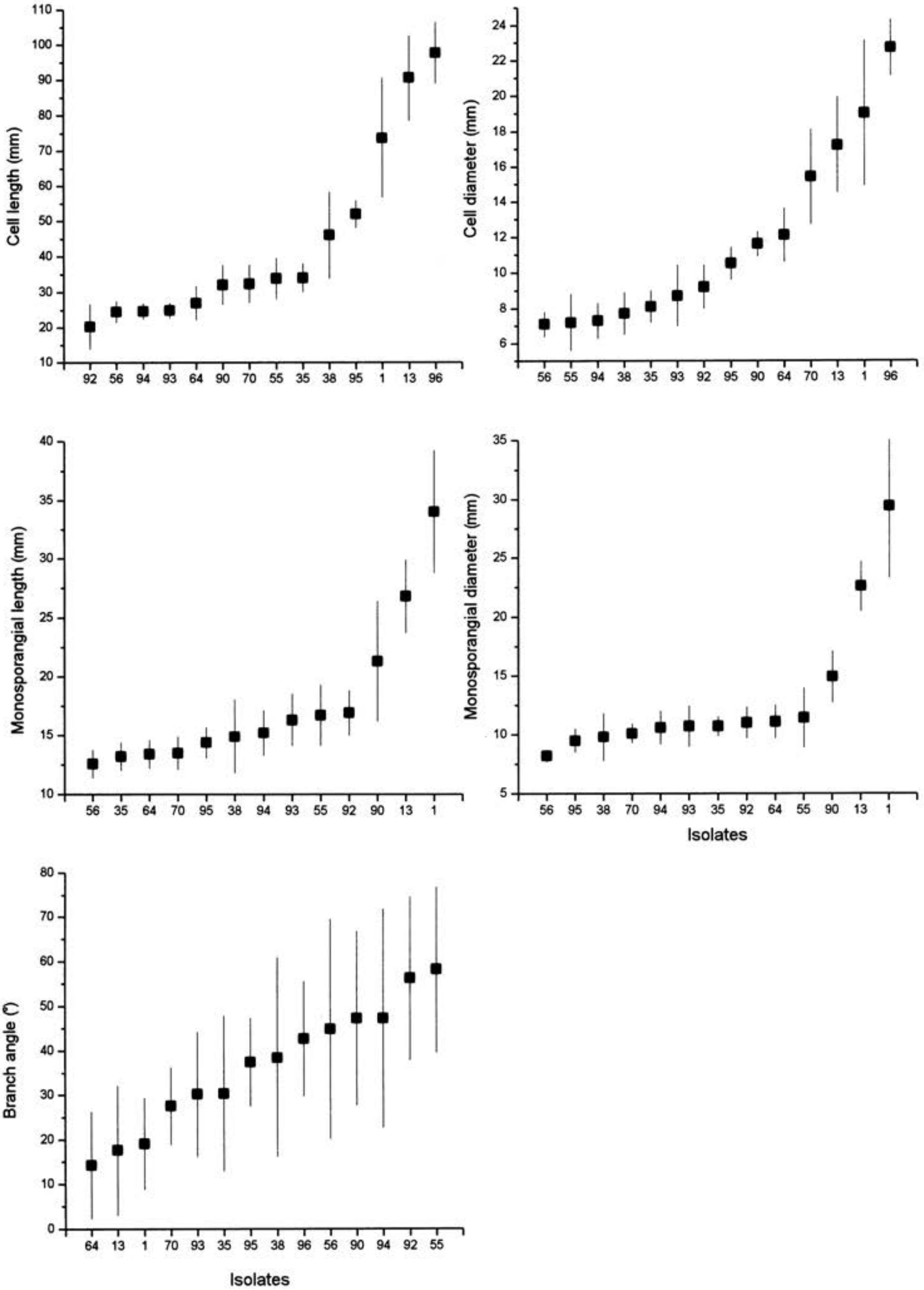


Fig. 6. Values (mean ± 1 standard-deviation, n = 20) of morphometric variables for the freshwater acrochaetioid algae analysed under optimal culture conditions. Values refer to tetrasporangia, instead of monosporangia, for *A. hermannii* (95); monosporangia were not observed for "Chantransia" (96). Isolate numbers according to Tab. 1.

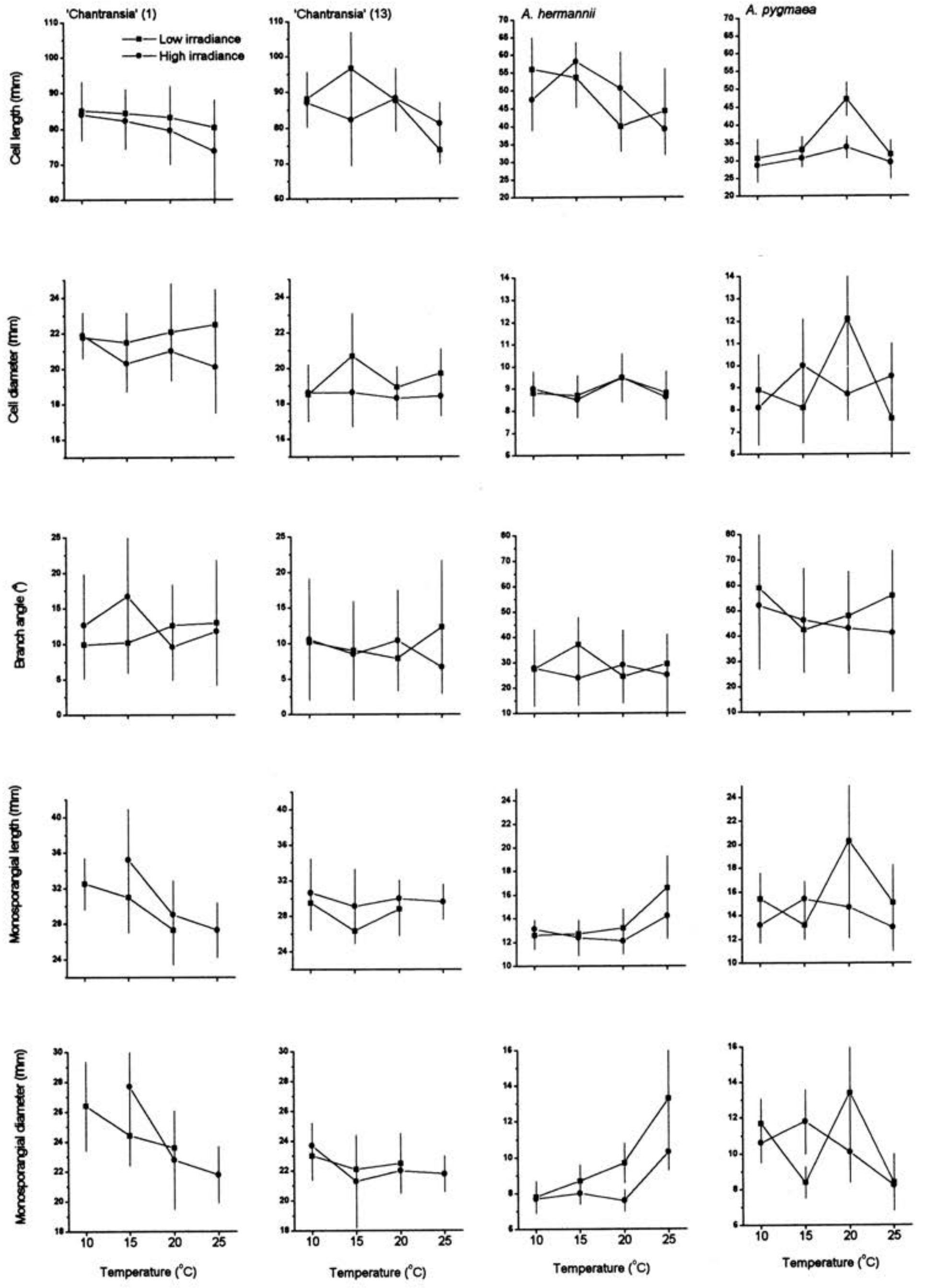


Fig. 7. Values (mean ± 1 standard-deviation, n = 20) of morphometric variables for the four freshwater acrochaetioid algae tested under distinct treatments of temperature and irradiance. Low and high irradiances correspond to 65 and 300 μmol photons m^{-2} s^{-1}, respectively.

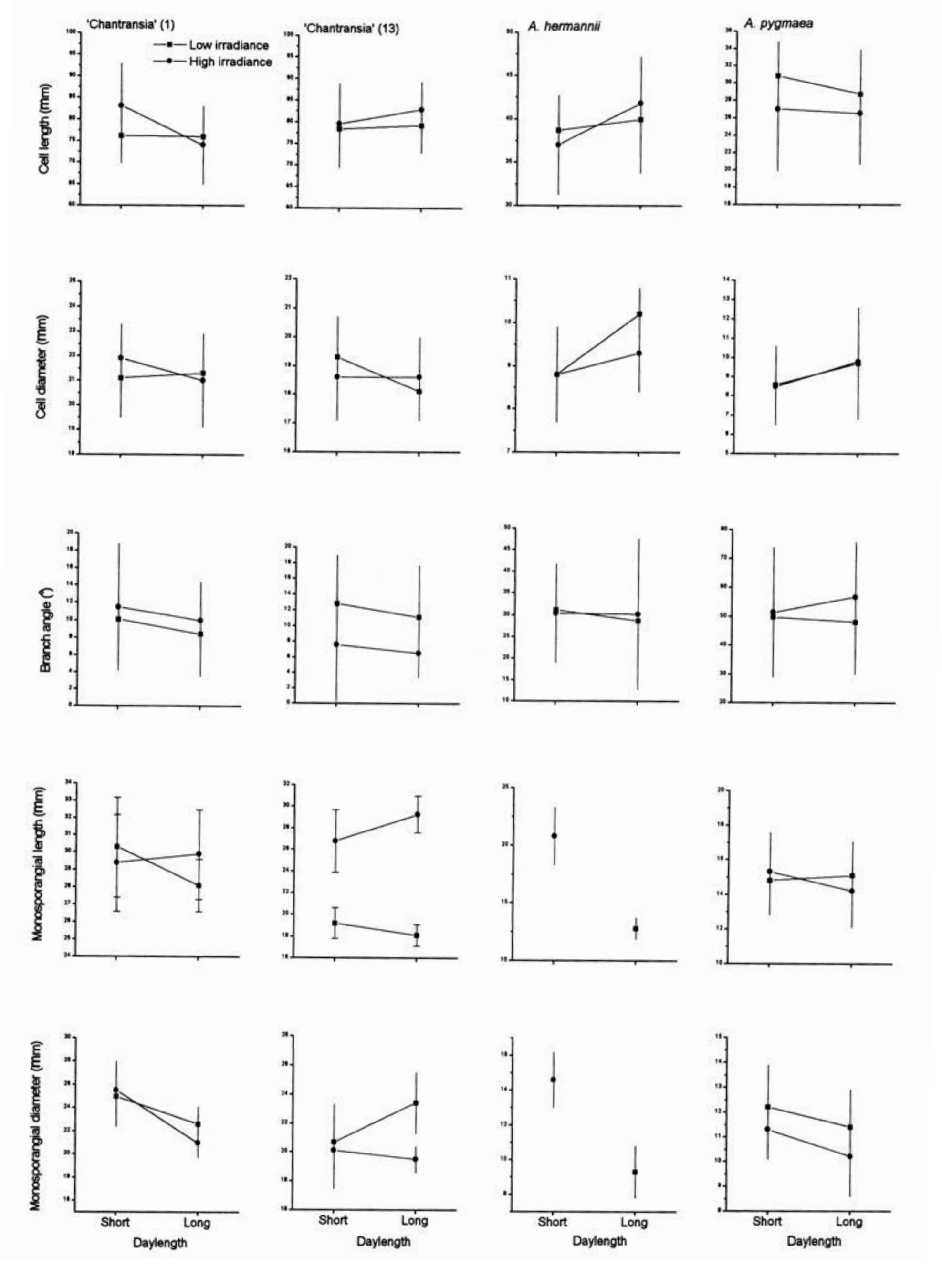


Fig. 8. Values (mean ± 1 standard-deviation, n = 20) of morphometric variables for the four freshwater acrochaetioid algae tested under distinct treatments of photoperiod and irradiance, under constant temperature (20 °C). Long day – 16:8 light/dark; short day – 8:16 light/dark. Low and high irradiances correspond to 65 and 300 µmol photons m^{-2} s^{-1}, respectively.

Plant colour varied within two discrete colour ranges for the algae tested (Tab. 2): blue-green for *A. pygmaea* (codes 21-22, rarely 23) and the “Chantransia” stages (codes 22-24) and red for *A. hermannii* (codes 4-5, rarely 3). As a rule, plants had darker colours at low irradiances and short days. The “Chantransia” stages were pale at high irradiances and low temperatures (10-15 °C) or yellow-greenish (code 1E) at high irradiance and temperature (25 °C) in isolate 1.

No overall trend was observed for vegetative characters in response to temperature, irradiance and photoperiod for all algae tested (Figs 7-8). A clear decreasing trend for cell length with temperature (both at low and high irradiances) was observed in one “Chantransia” isolate (1) but no clear trend was found in *A. hermannii, A. pygmaea* and the other “Chantransia” (Fig. 7). ANOVA (two way) revealed significant differences for temperature in all algae tested, whereas only in two for irradiance (Tab. 3). Significant interactions of temperature and irradiance were detected for all algae, except “Chantransia” (1). No evident trend was observed for cell diameter in relation to temperature (Fig. 7), but the algae responded to temperature and irradiance, singly or interactively (Tab. 3), except *A. hermannii*, which had no significant interaction. Few significant differences were generally found for cell length and diameter in response to photoperiod and irradiance, as well as weak interactions between them, except for cell diameter in *A. hermannii* (Tab. 3, Fig. 8). No significant differences were found singly for branch angle in response to temperature and irradiance but interactions were detected for all algae, except *A. pygmaea* (Tab. 3, Fig. 7). In addition, no significant values were found in response to photoperiod and irradiance (except irradiance in one “Chantransia”), with no interaction between them (Tab. 3, Fig. 8).

No major trend was observed in terms of quantity of monosporangia (Tab. 2), with moderate to abundant production under variable conditions of temperature, irradiance and photoperiod for all algae tested. Monosporangia were not produced by “Chantransia” stages at extreme temperatures (10 or 25 °C) both at low and high irradiances, nor by *A. hermannii* at short day and low irradiance or long day and high irradiance.

Monosporangial length showed a decreasing trend with temperature (both at low and high irradiances) in one “Chantransia” isolate (1), whereas an increasing trend was observed in *A. hermannii*, which was more evident at low irradiance (Fig. 7). No clear trend was detected in *A. pygmaea* and the other “Chantransia”. Few significant differences and weak interactions of temperature and irradiance were found for all algae, except *A. pygmaea* (Tab. 3). The same trends were observed for monosporangial diameter, except for significant temperature differences in three algae (Tab. 3, Fig. 7). Few significant differences were found in response to photoperiod and irradiance (except for monosporangial diameter in *A. pygmaea*), as well as weak interactions between them (except in one “Chantransia” isolate) (Tab. 3, Fig. 8).

Gametangia, carposporangia and tetrasporangia were not observed under the experimental conditions tested in this study, although, juvenile gametophytes of *Batrachospermum* were produced by one isolate of “Chantransia” (1) under all conditions and also by *A. pygmaea* at 10 °C and high irradiance (Tab. 2).

DISCUSSION

Some isolates originally identified as *A. pygmaea* were proven to be unequivocally “Chantransia” stages owing either to production of juvenile gametophytes or to derivation from carpospores. On the basis of similarity with respect to the diagnostic characters (blue-greenish colour, monosporangia $\cong$ 15 µm in diameter, Necchi *et al*., 1993b; Necchi & Zucchi, 1995) among all isolates examined, all are interpreted as "Chantransia" stages. On the basis of results of the present study and previous suggestions (Israelson, 1942; Necchi & Zucchi, 1997; Pueschel *et al*., 2000) there is no reason to retain *A. pygmaea* as a member of the Acrochaetiaceae. We found no clear association of the morphology of *A. pygmaea* with any particular species of *Batrachospermum*, *Paralemanea* or *Thorea*, thus it should be regarded as a complex involving many species of the Batrachospermales *sensu lato*, as was also the case with *A. macrospora* (Necchi & Zucchi, 1997). Pueschel *et al*. (2000) suggested that some limited value could be gained by retaining these two names in informal use to refer to the different morphological forms of "Chantransia", i.e. *pygmaea* "Chantransia" and *macrospora* "Chantransia". We agree with their conclusion and propose that we refer to one of the morphologies of the "Chantransia" stage when referring to this life history phase. On the other hand, there is no sound basis for associating a particular species with one "Chantransia" morphology, since each one (*macrospora* and *pygmaea*) can be aligned with several species (Necchi & Zucchi, 1997; this study). Even a same species can produce "Chantransia" stages with the two morphologies, for instance *B. ambiguum* (this study, isolate 35 $\cong \pm$ *pygmaea*) and in a previous investigation (Necchi & Zucchi 1997, population 7 $\cong$ *macrospora*). The same is expected to occur in other batrachospermalean species.

Most criteria proposed by Skuja (1934) for distinguishing *Audouinella* from "Chantransia" are not easily applicable or reliable (Necchi & Zucchi, 1997). In this study, we found no value in the branching pattern and monosporangia abundance, confirming the conclusions of that previous investigation. In contrast, the distinction based on plant colour (reddish vs. blue-greenish) has proven to be useful, the former being true *Audouinella* species and the latter representing "Chantransia" stages. No exception to this pattern has ever been reported for freshwater acrochaetioid algae (Necchi & Zucchi, 1997) and it was shown to be conservative at distinct temperatures, irradiances and photoperiods. Thus, plant colour is here proposed as the only vegetative character that can be unequivocally applied to distinguish *Audouinella* from "Chantransia", providing that an accurate and objective evaluation is made. The reddish colour of *Audouinella* species results from a higher concentration of phycoerythrin in comparison to phycocyanin (Kaczmarczyk & Sheath, 1992; Zucchi & Necchi, 2001). In terms of reproductive features, the presence of sexual reproductive structures (carpogonia and spermatangia), carposporangia or tetrasporangia, are also reliable characters to indicate that an acrochaetioid alga is an independent species. Such structures have never been observed in blue-greenish forms, but reported in reddish species in natural (Drew, 1935; Korch & Sheath, 1989; Necchi *et al*., 1993a; Carmona & Necchi, 2001) or culture conditions (tetrasporangia, in this study). On the other hand, unequivocal evidence that an acrochaetioid alga is a "Chantransia" stage is the production of juvenile gametophytes or, alternatively, the development of the acrochaetioid morphology from carpospores germinated from a batrachospermalean species. Both are relatively difficult to observe in field populations (Necchi & Zucchi, 1997) but are possible in laboratory culture, as demonstrated in this study.

The production of juvenile *Batrachospermum* gametophytes was observed in four isolates of "Chantransia" stage, one (isolate 1) under all experimental conditions tested, two (isolates 13 and 94) at optimal culture conditions (temperature 20 °C, photoperiod 12:12 h and irradiance 140-160 µmol photons m^{-2}

s^{-1}) and one (isolate 55) only at specific experimental conditions (temperature 10 °C, photoperiod 12:12 h and high irradiance, 300 µmol photons m^{-2} s^{-1}). The conditions of the first isolate (temperatures 10-25 °C, photoperiods – light/dark – 8:16, 12:12 and 16: 8 h and irradiances 65 and 300 µmol photons m^{-2} s^{-1}) were much wider than usually described for the production of juvenile gametophytes from a "Chantransia" stage of any *Batrachospermum* species. This isolate was previously reported to produce gametophytes by Necchi & Zucchi (1997), Glazer *et al*. (1997) and Pueschel *et al*. (2000); their induction is relatively easy and occurs under quite variable laboratory conditions. On the other hand, conditions favouring gametophyte development in other isolates were much narrower, as was also shown by other authors (Huth, 1979; Chesnick & O'Flaherty, 1986). The production of juvenile gametophytes by these "Chantransia" isolates occurred at different conditions than those reported by Huth (1979) for *B. gelatinosum* (Linnaeus) De Candolle (as *B. moniliforme*): temperature (15 °C), photoperiod (14:10 h) and irradiance (9-18 µmol photons m^{-2} s^{-1}) Chesnick & O'Flaherty (1986) also described more abundant development of gametophytes in *Batrachospermum* sp. under distinct environmental parameters: temperature 15 °C, irradiance 46-93 µmol photons m^{-2} s^{-1} and variable photoperiod (8:16, 12:12 and 16:8 h). Thus, previous information from culture studies suggests that temperatures around 15 °C and low irradiances were generally favourable to induce gametophyte production in the Batrachospermaceae, whereas photoperiod did not seem to be critical. Our results demonstrate that induction of gametophytes occurs under much wider conditions, reinforcing the suggestion that the requirements are probably species-specific (Necchi & Zucchi, 1997).

The ranges found for morphometric characters in the acrochaetioid algae tested were within or close to those reported for natural populations, including the "Chantransia" stages, with *macrospora* and *pygmaea* morphologies (Necchi & Zucchi, 1995; Necchi *et al*., 1993b) and *A. hermannii* (Drew, 1935; Israelson, 1942; Necchi & Zucchi, 1995; Necchi *et al*., 1993a). These results allow us to draw a similar conclusion to that of Garbary (1979), who studied marine species of *Audouinella*. He found that, while phenotypic plasticity was present in the genus, with temperature, irradiance and photoperiod affecting diverse morphological aspects, no species showed variation outside the limits based on traditional taxonomic studies. In our study no overall trend was observed for vegetative or reproductive characters in response to temperature, irradiance and photoperiod for the algae tested, but some trends (increasing or decreasing) were found for specific algae (e.g. "Chantransia" stages and *A. hermannii*) or characters (cell length). Effects of temperature and irradiance on morphological characters were more evident, as well as the strong interactions between these variables, whereas few differences were generally found in response to photoperiod and irradiance, with weak interactions between them for most features and algae. A similar trend was observed for growth of freshwater red algae under the same experimental conditions (Zucchi & Necchi, 2001).

Acknowledgements. This research was supported by a FAPESP grant-in-aid (98/07537-8) and a CNPq research grant (520551/96-6) to ONJ and a CAPES fellowship to MRZ. We are grateful to Maria Helena Carabolante for laboratory assistance; to Franklyn D. Ott, Kansas, USA, and John A. West, Melbourne University, Australia for kindly providing culture isolates.

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