Aquat Ecol (2007) 41:161–168
DOI 10.1007/s10452-006-9068-9

ORIGINAL PAPER

Microhabitat and morphometric variation in two species of *Prasiola* (Prasiolales, Chlorophyta) from streams in central Mexico

Rocío Ramírez Rodríguez ·
Javier Carmona Jiménez ·
Carlos Martorell Delgado

Received: 22 March 2006 / Accepted: 26 September 2006 / Published online: 24 November 2006

Abstract Prasiolales are characterized by high morphological plasticity. This problem in taxonomic delimitation of the species may be a result of environment heterogeneity. Habitat characteristics and morphological variation of *P. mexicana* and *P. nevadensis*, two species of freshwater leaf-like thallus green algae, were investigated in five sites in central México where the morphological traits of these species overlap. Comparisons were made between habitat characteristics among streams and transects with and without *Prasiola* samples. Although no consistent correlation was found between environmental variables and morphological traits, a significantly wider range of habitat characteristics of current velocity and irradiance and morphometric variation (lamina length, lamina diameter, cell length, surface of the thallus, thickness and number of layers) suggested that *P. mexicana* was more plastic than *P. nevadensis* in its physicochemical requirements. Our results suggest that small variation of microhabitat conditions in *P. nevadensis* (irradiance and current velocity) could explain its very restricted geographic range in streams in central Mexico.

Keywords Ecology · Freshwater algae · Microhabitat · Morphometric variation · Temperate streams · Mexico

Introduction

Prasiolales green algae have been cited as abundant and frequent components of stream macroalgal communities, and form massive green thalli in temperate, tropical, and boreal climates throughout the world (Starmach 1972; Sheath and Cole 1992; Hamilton and Edlund 1994; Naw and Hara 2002; McClintic et al. 2003; Ramírez and Carmona 2005). The genus *Prasiola* is characterized by a monostromatic laminar thallus and vegetative cells with stellate or lobed chloroplasts containing a single pyrenoid. In Mexico it has been reported in most taxonomic studies involving stream macroalgal communities (Agardh 1847; Knebel 1936; Ortega 1984; Ramírez et al. 2001; Ramírez and Cantoral 2003; Ramírez and Carmona 2005).

The systematic position of *Prasiola* has been controversial, because of morphological similarities with marine genera such as *Ulva, Monostroma, Enteromorpha*, and *Rosenvingiella* (Smith 1950; Bravo 1965) and overlapping morphometric

R. R. Rodríguez · J. C. Jiménez (✉) ·
C. M. Delgado
Departamento de Ecología y Recursos Naturales,
Universidad Nacional Autónoma de México, Facultad
de Ciencias, Circuito exterior s/n, Ciudad
Universitaria, Coyoacán, A.P. 70-620, C.P. 04510
México City, D.F., México
e-mail: jcj@hp.fciencias.unam.mx

variation and plasticity within *Prasiola* (Hamilton and Edlund 1994; Naw and Hara 2002; Rindi et al. 1999; Rindi and Guiry 2004). Ramírez and Carmona (2005) analyzed taxonomic characteristics of the two stream species of *Prasiola* in central Mexico. They argued that morphometric variations (plant length and cell length and diameter) and morphological characteristics (surface texture of the thallus) could be explained by environmental factors. Information about algal ecology and ecophysiology in Prasiolales can be found elsewhere (Friedmann 1959, 1960). Cole and Akintobi (1963) noted that gametes of *P. stipitata* Suhr and *P. meridionalis* Setchell & Gardner were released from the thallus when it was kept at low temperature and in short periods of light. In the same way, Anderson and Foster (1999) reported that high insolation affects the distribution and abundance of *P. meridionalis*. In terrestrial species such as *P. calophylla* (Carmichel) Meneghini and *P. crispa* (Lighfth) Meneghini it developed above firm substrata and its asexual reproduction was related to the warmest season of the year (Rindi et al. 1999).

Studies of *Prasiola* have been oriented toward supraspecific characteristics (Rindi et al. 1999; Sherwood et al. 2000; Naw and Hara 2002; Ramírez and Carmona 2005), demonstrating that ecological information is still lacking. The purpose of this study was to evaluate the effect of physical factors (current velocity, depth, irradiance, and substratum type) on small-scale (microhabitat) spatial distribution, morphometric adaptations, and percentage cover of *P. mexicana* and *P. nevadensis* populations in five streams in the central region of Mexico.

Material and methods

Fieldwork was performed at the end of the rainy season and winter (November–February), these being the most favorable growth periods for four populations of *Prasiola mexicana* and one of *P. nevadensis* in the region (Ramírez and Carmona 2005), in five sites located in the central region of Mexico (Table 1). Each sampling location consisted of randomly chosen stream segments, 10 m long. Observations were made of natural substratum (gravel, sand, pebbles, and boulders). Microhabitat analysis was conducted using the quadrant technique (Krebs 1989; Necchi et al. 1995), which evaluates the effect of microhabitat variables (current velocity, depth, subaquatic irradiance, and type of substratum) on scales of a few centimeters over the vegetative and reproductive characteristics of the lamina. Each sampling quadrant was a circle of 10 cm radius (area 314 cm^2). Type and size of sampling quadrants were defined from preliminary tests and previous research (Necchi et al. 1995; Ramírez and Carmona 2005). Sample size consisted of 10 quadrants, each separated by 1 m; their locations were determined by random numbers between 0 and 180°. In one of these positions we situated a sampling site with the algae (presence quadrants) and also looked for a point without the algae (absence quadrants) to evaluate differences between microhabitat characteristics. We sampled 20 sampling quadrants per segment (ten presence quadrants and ten absence quadrants).

Temperature, pH and specific conductance were measured for each river segment with a Conductronic PC-18 conductivity meter. Microhabitat variables were measured in situ at the center of each sampling quadrant. Current velocity and irradiance were measured as close as possible to the algal growth using a Swoffer 2100 current velocity meter and a Li-Cor LI-1000 quantum meter with a flat subaquatic sensor of photosynthetic active radiation (PAR), respectively. Algal cover (%) was recorded by the same person within each sampling quadrant by visual estimation with a 175 cm^2 viewfinder. Five laminas were collected per sampling quadrant and preserved in 3% formaldehyde for subsequent evaluation in the laboratory. Specimens collected within quadrants were measured to determine the morphometric characteristics previously believed to be of taxonomic importance—diameter, length and thickness of lamina, diameter and length of vegetative cells, number of layers, and surface of the lamina (Ramírez and Carmona 2005). Measurements of diameter and length of lamina were made in replicate for five plants and cells; number of layers and surface dimensions were made in replicates of 20. Means were calculated for each morphometric characteristic.

Table 1 Location and characteristics of the sampling sites of *Prasiola mexicana* and *P. nevadensis* populations. Substratum (B = boulder)

Location, date, and hour	Temperature, °C	Conductivity, µS cm^{-1}	Dissolved oxygen mg L^{-1}	pH	Substratum[a], %	Depth, cm	Irradiance[a], µmol photons, m^{-2} s^{-1}	Current velocity[a], m s^{-1}	Species
Site 1									
4° Dinamo 14.I.03 19°16′ N, 99°18′ O Hour: 11.10 am	7.2	60	10.0	7.2	B-100	0–6	45–387 227.3 ± 35.6	0–1.02 0.3 ± 0.1	*P. mexicana*
Site 2									
2° Dinamo 5.II.03 19°29′ N, 99°17′ O Hour: 10.10 am	7.6	66	12.0	7.8	B-100	0–11	130–1631 913.8 ± 157.6	0–0.69 0.1 ± 0.08	*P. mexicana*
Site 3									
Agua de Leones 1.III.03 19°17′ N, 99°19′ O Hour: 11.15 am	10.0	45	4.0	6.0	B-100	0–4	294–1342 762.4 ± 141.1	0 – 0.53 0.1 ± 0.06	*P. mexicana*
Site 4									
Molinos 12.II.05 19°40′ N, 99°26′ O Hour: 12:35 pm	14.0	60	–	7.8	B-100	0–15	60–1607 588.9 ± 143.2	0–0.50 0.2 ± 0.05	*P. mexicana*
Site 5									
Malila 4.XII. 03 20°45′ N, 98°45′ O Hour: 1:13 pm	16.0	262	8.8	7.5	B-100	0–7	18–35 27.6 ± 1.7	0–0.30 0.1 ± 0.1	*P. nevadensis*

[a] Values are minimum, maximum,and averages ± standard error, calculated from measurements taken with quadrants (presence). – denotes values were not recorded

We used a Mann–Whitney test to assess environmentally significant differences between presence and absence quadrants at each site and a Kruskal–Wallis test to assess morphometric and environmental differences among five populations of *Prasiola*. When these tests were significant, the Mann–Whitney test was conducted among all pairs of sites to detect which differed significantly. The substratum preference was evaluated by use of a log–linear regression model. Associations among percentage cover and morphometric data with microhabitat variables were tested using the Spearman correlation coefficient (Gotelli and Ellison 2004). Tests were performed with the software SPSS 12 and GLIM 4.

Results

Prasiola populations occurred under very diverse environmental conditions (Table 1, Fig. 1, 2). Similarities between the two species were boulder substratum and shallow depth; differences were conductivity, irradiance, and, possibly, current velocity and temperature. Environmental conditions had particular patterns for each species studied. The Mann–Whitney test applied between presence and absence quadrants revealed significant differences for depth ($U = 1.00$–17.50, $P < 0.05$) and substratum ($\chi^2 = 65.52$, $P < 0.001$) in the five sites and current velocity in sites 2 and 5 ($U = 16.00$–19.50, $P < 0.05$). The Kruskall–Wallis test revealed significant differences in all the morphometric characteristics and percentage cover among the five populations (Fig. 2): percentage cover ($H = 17.34$, $P < 0.05$), lamina length ($H = 54.4$; $P < 0.001$), cell length ($H = 59.5$; $P < 0.001$), cell diameter ($H = 53.7$; $P < 0.001$), thallus undulations ($H = 26.55$; $P < 0.001$), thickness ($H = 105.4$; $P < 0.001$), and cell layers ($H = 76.9$; $P < 0.001$).

Some significant correlations were found between environmental conditions and morphometric data (Table 2). These were found to be significant at one site only, however, or when the correlation was significant at two sites it had opposite signs. Thus, there was no consistent relationship among form and environment. Positive correlations were obtained for the morphometric variables lamina length and lamina diameter in four sites ($\rho = 0.69$–0.94; $P < 0.05$), lamina length and cell length in sites 2 and 4 ($\rho = 0.65$–0.83; $P < 0.05$), cell length and cell diameter in sites 1, 2, 4, and 5 ($\rho = 0.68$–0.93; $P < 0.05$), thickness and number of layers in sites 1, 2, and 5 ($\rho = 0.79$–0.89; $P < 0.01$), and lamina length and thickness ($\rho = 0.63$; $P < 0.05$) and number of layers ($\rho = 0.71$; $P < 0.05$) in site 5. The negative correlation was for lamina length and thallus undulations ($\rho = -0.68$; $P < 0.05$) in site 5.

Discussion

It has been reported that environmental heterogeneity is responsible for morphological variation in other Chlorophyta populations (Branco and Necchi 1998; Vieira and Necchi 2002). Our data show, however, that despite their large morphological variation these two *Prasiola* species have no plastic response to the conditions they experience. The same lack at pattern has been reported for Characea populations (Vieira and Necchi 2002). More studies are needed to establish whether it is common in other algae. *Prasiola mexicana* occurred under a wide range of microhabitat conditions, suggesting they tolerate substantial variations of current velocity and irradiance. In this study the current velocity was in the range 0.05–2 m s^{-1} reported by Hamilton and Edlund (1994) and McClintic et al. (2003) for *P. fluviatilis*, and similar to those for *Prasiola* sp. in low or zero current velocity (Naw and Hara, 2002). Rindi and Guiry (2004) proposed that emersed Prasiolales populations have evolved different mechanisms to exist in subaereal conditions. One such mechanism is a physiological adaptation for fixing bicarbonates in the photosynthetic process and Mycosporine-like amino acid (MAAs) production for ultraviolet filtration (Raven and Johnson 1991; Lud et al. 2001; Rozema et al. 2002; Gröniger and Häder 2002; Karsten et al. 2005). Another adaptation is the protection given by several lamina layers for humidity retention and assimilation of nutrients in *P. crispa* (Lud et al. 2001).

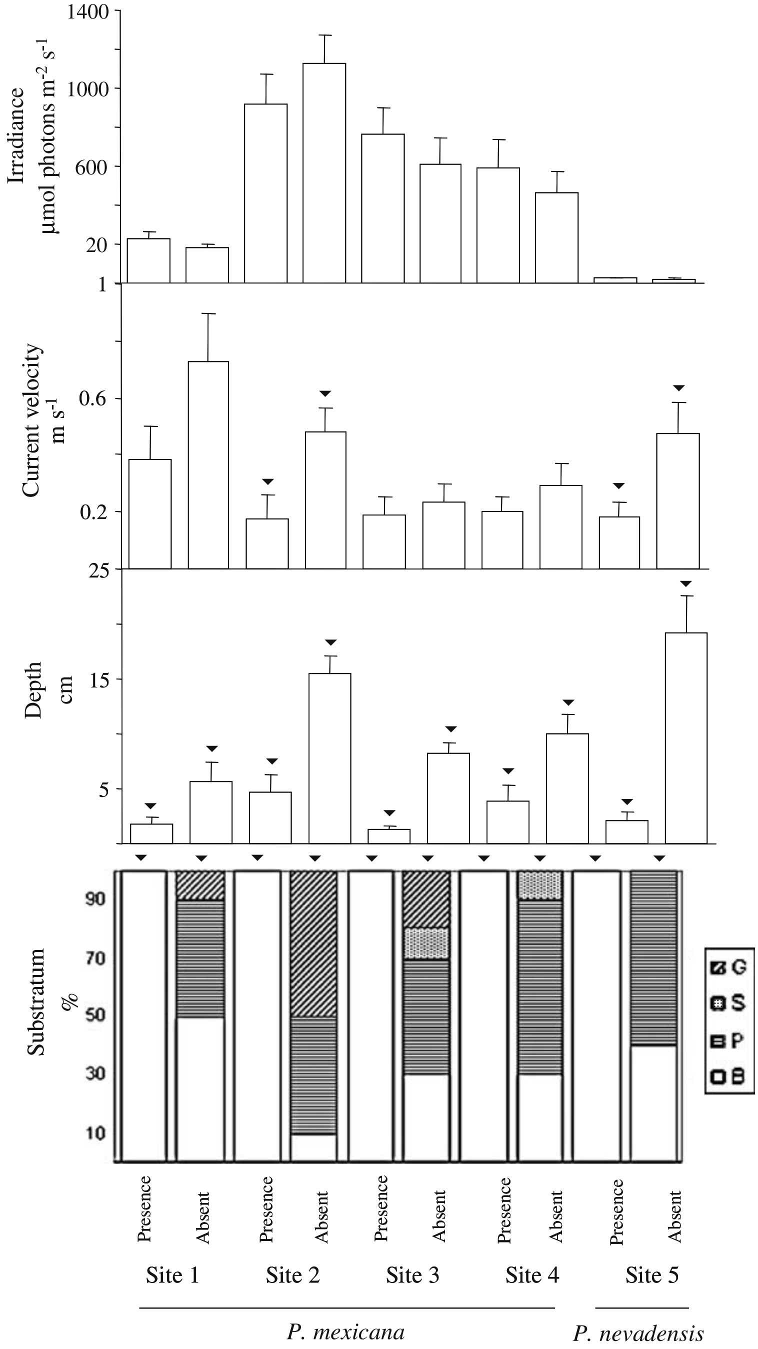

Fig. 1 Irradiance, current velocity, depth, and dominant substratum (*G* = gravel, *S* = sand, *P* = pebbles and *B* = boulders) (*n* = 10, mean ±1 SE) for *Prasiola mexicana* and *P. nevadensis* populations in presence and absence quadrants in the fives sites studied. *Arrow heads* show differences between presence and absence quadrants for the same population according to the Mann–Whitney test (α = 0.05). Sites are numbered in accordance with Table 1

Prasiola mexicana populations in central Mexico have a preference for boulder substrata that is shared by several freshwater species in the genus; for example, *P. japonica* Yatabe, *P. formosana* Okada, *P. sinica* Jao (Starmach 1972), *P. fluviatilis* (Sommerf.) Aresch (Hamilton and Edlund 1994; McClintic et al. 2003), and *Prasiola* sp. (Naw and Hara 2002). This characteristic is apparently related to the stable substrata and the time for rhizoidal holdfast development. Greater tolerance of microhabitat conditions and high percentage cover can explain the relatively large number of temperate streams in central Mexico with *P. mexicana*, including

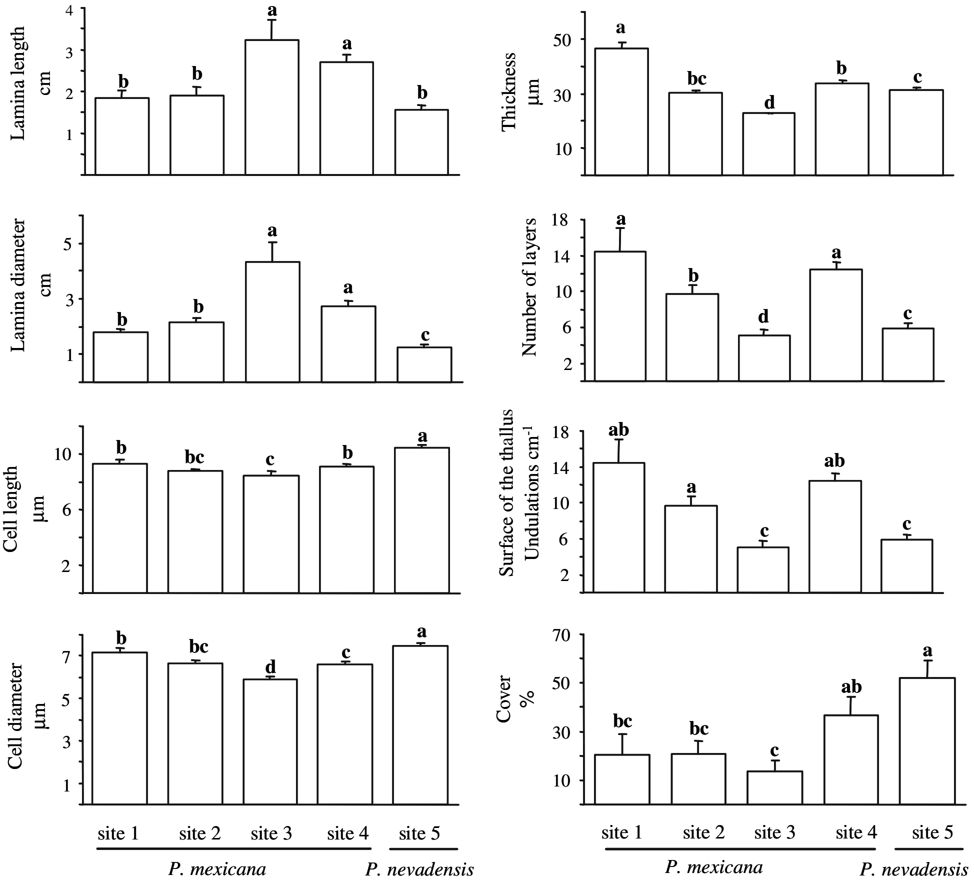

Fig. 2 Morphometric and percentage cover values (mean ±1 SE) for populations of *Prasiola mexicana* and *P. nevadensis* in the five sites studied. Sites with the same letter do not differ significantly (Mann–Whitney test, $\alpha = 0.05$). For morphometric variables $n = 200$

Table 2 Significant values of Spearman's ρ correlation coefficient among environmental variables and morphometric characteristics for *Prasiola* populations

Variables	Characters	Site 1	Site 2	Site 3	Site 4	Site 5
Current velocity	Thickness	0.76**	–	–	–	–
	Percentage cover	–	–	0.71*	–	–
	Lamina length	–	–	–	0.66*	–
	Number of layers	–	0.63*	–	–	–
Irradiance	Percentage cover	–	–	–0.68*	0.65**	–
	Lamina length	–	–	–0.65*	–	–
	Undulations	0.67*	–	0.67*	–	–
	Cell diameter	–	–	–	–	–0.63**
Depth	Lamina length	–0.65*	–	–	–	–
	Lamina diameter	–0.72*	–	–	–	–
	Thickness	–	0.63*	–0.65*	–	–
	Number of layers	–	0.64*	–	–	–
	Undulations	–	–	–0.65*	0.85**	–

Sites are numbered in accordance with Table 1 * $P < 0.05$, ** $P < 0.01$; – = non-significant values pristine (Ramírez and Cantoral 2003) to little-disturbed habitats (Ramirez et al. 2001). Thus, these results suggest that *P. mexicana* is a highly tolerant species similar to other Chlorophyceae populations, for example *Stigeoclonium helveticum* Vischer with tolerance of high irradiance (1010–1235 μmol photons m^{-2} s^{-1}), low depth (20.2–21.7 cm), and variable current velocity (0.47–0.97 m s^{-1}) (Branco and Necchi 1998).

Our results suggest that *P. nevadensis* grows in a narrow range of current velocity and irradiance and in higher-conductivity waters than *P. mexicana*. *P. nevadensis* also grows on boulder substrata. The significant negative correlation of irradiance with cell diameter and the narrow range of irradiance in this study indicate limited tolerance of high irradiation; this is dissimilar to the populations of *Prasiola mexicana* in this study. Similar trends of low irradiance have been observed for several stream populations of filamentous and parenchymatous Chlorophyta and Prasiolales species, for example *Rhizoclonium hieroglyphicum* (C. Ag) Kütz (105 μmol photons m^{-2} s^{-1}), *Chara* sp. (300 μmol photons m^{-2} s^{-1}, Necchi 2004), and *P. fluviatilis* (riparian cover 75%, McClintic et al. 2003). This suggests that *P. nevadensis* is a shade-tolerant species. This information about the ecological distribution and environmental requirements of *P. nevadensis* is consistent with its taxonomic designation (Ramírez and Carmona 2005), but the survey is not sufficient to complete our taxonomic knowledge. The significant cell length and diameter differences between *P. nevadensis* and the four populations of *P. mexicana* confirm the importance of these morphometric characteristics in distinguishing between these species, as proposed by Ramírez and Carmona (2005). The significant correlations found between morphometric and microhabitat variables also suggest that *P. nevadensis* has limited plasticity to cope with the typical spatial heterogeneity of lotic habitats (Whitton 1975). Narrow microhabitat range can explain the relatively low frequency of this species in the streams of Central Mexico (Montejano et al. 2004; Ramírez and Carmona 2005).

Acknowledgements This research was partial funded by the Program ‘‘Ecosystems management and human development: La Magdalena basin, Mexico City’’— UNAM. The authors are indebted to Professor G. Garduño, M. Ramírez, and O. Sandoval for help with fieldwork and to David Adams for the English language review.

References

Agardh JG (1847) Nya alger fran Mexico. Oversigt al kongl. Vetenskaps-Akademiens förhandlinglar 4:5–17

Anderson BA, Foster MS (1999) The effects of insolation and grazing on populations of the splash zone alga *Prasiola meridionalis* (Chlorophyta). Phycologia 38:349–355

Bravo LM (1965) Studies on the life history of *Prasiola meridionalis*. Phycologia 4:177–194

Branco C, Necchi O, Jr. (1998) Microhabitat and morphometric variation of two chaetophoracean (Chaetophorales, Chlorophyta) species in tropical streams of southeastern Brazil. Phycological Res 46:169–174

Cole K, Akintobi S (1963) The life cycle of *Prasiola meridionalis* Setchell & Gardner. Can J Bot 41:661–668

Friedmann I (1959) Structure, life-history and sex determination of *Prasiola stipitata* Suhr. Ann Bot 23:571–597

Friedmann I (1960) Gametes, fertilization and zygote development in *Prasiola stipitata* Suhr. Nova Hedwigia 1:333–350

Gotelli NJ, Ellison AM (2004) A primer of ecological statistics. Sinauer Associates, Inc, U.S.A

Gröniger A, Häder D-P (2002) Induction of the synthesis of an UV-absorbing substance in the green alga *Prasiola stipitata*. J Photochem Photobiol 66:54–59

Hamilton PB, Edlund SA (1994) Occurrence of *Prasiola fluviatilis* (Chlorophyta) on Ellesmere Island in the Canadian Arctic. J Phycol 30:217–221

Karsten U, Friedl T, Schumann R, Hoyer K, Lembcke S (2005) Mycosporine-like amino acids and phylogenies in green algae: *Prasiola* and its relatives from the Trebouxiophyceae (Chlorophyta). J Phycol 41:557–566

Knebel G (1936) Monographie der algenreihe der Prasiolales, insbersondere von *Prasiola crispa*. Hedwigia 75:1–120

Krebs C (1989) Ecological methodology. Harper and Row, Publishers, U.S.A

Lud D, Buma AG, Van De Poll W, Moerdijk TC, Huiskes A (2001) DNA damage and photosynthetic performance in the Antarctic terrestrial alga *Prasiola crispa* ssp. *antarctica* (Chlorophyta) under manipulated UV-B radiation. J Phycol 37:459–467

McClintic A, Casamatta DA, Vis ML (2003) A survey of algae from montane cloud forest and alpine streams in Bolivia: macroalgae and associated microalgae. Nova Hedwigia 76:363–379

Montejano G, Cantoral-Uriza E, Carmona J (2004) Algas de ambientes lóticos en la cuenca baja del río Pánuco. In: Luna I, Morrone J, Espinoza D, (eds) Biodiversidad de la Sierra Madre Oriental, Las prensas de Ciencias, México, D.F, pp 111–126

Naw MW, Hara Y (2002) Morphology and molecular phylogeny of *Prasiola* sp. (Prasiolales, Chlorophyta) from Myanmar. Phycological Res 50:175–182

Necchi O, Jr. (2004) Light-related photosynthetic characteristics of lotic macroalgae. Hydrobiologia 525:139–155

Necchi O, Jr., Branco L, Cesar C (1995) Comparison of three techniques for estimating periphyton abundance in bedrock streams. Archive für Hydrobiologie 134:393–402

Ortega MM (1984) Catálogo de algas continentales recientes de México. Universidad Nacional Autónoma de México. México

Ramírez RR, Carmona J (2005) Taxonomy and distribution of freshwater *Prasiola* (Prasiolales, Chlorophyta) in central Mexico. Cryptogamie Algologie 26:177–188

Ramírez V, Beltrán MY, Bojorge GM, Carmona J, Cantoral UE, Valadez F (2001) Flora algal del río La Magdalena, Distrito Federal, México. Boletín de la Sociedad Botánica de México 68:51–73

Ramírez V, Cantoral UE (2003) Flora algal de ríos templados en la zona occidental de la cuenca del Valle de México. Anales del Instituto de Biología 74:143–194

Raven JA, Johnson AM (1991) Photosynthetic inorganic carbon assimilation by *Prasiola stipitata* (Prasiolales, Chlorophyta) under emersed and submersed conditions: relationship to the taxonomy of *Prasiola*. Brit Phycol J 26:251–254

Rindi F, Guiry MD (2004) Composition and spatial variability of terrestrial algae assemblages occurring at the bases of urban walls in Europe. Phycologia 43:225–235

Rindi F, Guiry MD, Barbiero RP (1999) The marine and terrestrial Prasiolales (Chlorophyta) of Galway City, Ireland: a morphological and ecological study. J Phycol 35:469–482

Rozema J, Björn O, Bornman JF, Gaberščik A, Häder D-P, Trošt T, Germ M, Klisch M, Gröniger A, Sinha RP, Lebert M, He Y-Y, Buffoni-Hall R, Bakker NV, Van De Staatt J, Meijkamp BB (2002) The role of UV-B radiation in aquatic and terrestrial ecosystems—an experimental and functional analysis of the evolution of UV-absorbing compounds. J Photochem Photobiol 66:2–12

Sheath RG, Cole KM (1992) Biogeography of stream macroalgae in North America. J Phycol 28:448–460

Sherwood AR, Garbary DJ, Sheath RG (2000) Assessing the phylogenetic position of the Prasiolales (Chlorophyta) using *rbc*L and 18S rRNA gene sequence data. Phycologia 39:139–146

Smith G (1950) The fresh-water algae of United States. McGraw–Hill, USA

Starmach K (1972) Flora Słodwodna Polski. Tome 10: Chlorophyta III. Panstowe wydawnictwo Naukowe, Warszawa-Kraków

Vieira J, Necchi O, Jr. (2002) Microhabitat and plant structure of Characeae (Chlorophyta) populations in streams from São Paulo State, southeaster Brazil. Criptogamie Algologie 23:51–63

Whitton B (1975) River ecology. Studies in ecology, vol 2. Blackwell Scientific Publications, U.S.A