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Taxonomy and distribution of freshwater *Prasiola* (Prasiolales, Chlorophyta) in central Mexico

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Abstract – Seventeen samples and specimens of *Prasiola* from central Mexico, representing two species, have been studied in laboratory culture. The most common species, *P. mexicana*, is characterized by large leaf-like thalli (≥ 90 mm), bifurcating branches and a smooth margin. The lamina apex has numerous aplanospores and the margin is polystromatic (≥ 8 layers). Specimens with a corrugated surface and rhizoidal cells in the middle region were also noted. The second species, *P. nevadensis*, is characterized by small, leaf-like thalli (≥ 30 mm), crenate margins, and a monostromatic lamina. Two relevant morphological features were noted in culture: multilocular gametangia with possible female gametes, and the formation of small cellular packets fixed to the apical margin of the lamina. *Prasiola nevadensis* is here reported for the first time for Mexico, which also represents only the second report for the species. *Prasiola mexicana* was found in subaerial or submerged conditions in cold rivers (2-16.4ºC), neutral pH (6.4-7.6) and low specific conductivity (35.5-94.2 $\mu S.cm^{-1}$). *Prasiola nevadensis* was collected submerged in a river with cold water (14ºC), acidic pH (5.5), and moderate specific conductivity (192 $\mu S\ cm^{-1}$). Both species were collected in high mountain regions (≥ 1300 masl).

Chlorophyta / distribution / México / *Prasiola* / Prasiolales / streams / taxonomy

Résumé – Taxinomie et distribution de *Prasiola* (Prasiolales, Chlorophyta) dulçaquicoles au Mexique central. On a observé deux espèces parmi dix-sept récoltes et spécimens de *Prasiola* du Mexique central maintenus en culture. La plus commune, *P. mexicana*, est caractérisée par de larges thalles foliacés (≥ 90 mm), des ramifications bifurquées et une marge lisse ; le sommet des thalles porte de nombreux aplanospores et montre une marge polystromatique (≥ 8 couches). On remarque aussi des spécimens avec une surface plissée et des cellules rhizoïdales dans la région médiane. La seconde espèce, *P. nevadensis*, est caractérisée by de petits thalles foliacés (≥ 30 mm), une marge crénelée et une lame monostromatique. Deux caractères morphologiques pertinents ont été notés en culture : des gamétanges multiloculaires contenant d'éventuels gamètes femelles et la formation de petits paquets cellulaires fixés à la marge apicale du thalle. *Prasiola nevadensis*, connu uniquement de son lieu d'origine, est signalé ici pour la première fois du Mexique. *Prasiola mexicana* a été trouvé en conditions subaériennes ou submergées dans des rivières froides (2-16.4 ºC), à pH neutre (6.4-7.6) et à conductivité spécifique basse (35.5-94.2 $\mu S.cm^{-1}$). *Prasiola nevadensis* a été récolté submergé dans une rivière à eau fraîche (14 ºC), à pH acide (5.5) et à conductivité spécifique modérée (192 $\mu S.cm^{-1}$). Les deux espèces ont été récoltées dans des régions de haute montagne (altitude ≥ 1 300 m).

Chlorophyta / distribution / México / *Prasiola* / Prasiolales / rivières / taxonomie

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INTRODUCTION

The genus *Prasiola* is included in the class Trebouxiophyceae and is defined by the following characteristics: a monostromatic laminar thallus, vegetative cells with stellate or lobed chloroplasts containing a single pyrenoid, biflagellate male gametes with cross-type flagellar insertion and a basal body disposed in counterclockwise fashion, closed mitosis with a persistent spindle in telophase, and cytokinesis by transverse wall deposition (O'Kelly *et al*., 1989; Sherwood *et al.*, 2000). The systematic position of the Prasiolales has been controversial due to the similarity of the type of mitosis and cytokinesis to the class Ulvophyceae. However, Friedl & O'Kelly (2002) analyzed the 18S rDNA nucleotide sequence and confirmed its placement in the Trebouxiophyceae. The family Prasiolaceae includes two genera: *Rosenvingiella* P.C. Silva, characterized by its filamentous organization, and *Prasiola* G. Meneghini, which has a monostromatic laminar thallus and oogametic reproduction (Friedmann, 1959a, 1959b; Bravo, 1965; Rindi *et al*., 1999). The delimitation of these two genera has been debated since some species of *Prasiola* have filamentous stages similar to *Rosenvingiella* (Bravo, 1965). Recently, Sherwood *et al*. (2000) found molecular differences between the two genera based on analysis of *rbc*L and 18S rRNA nucleotide sequences.

Species of the genus *Prasiola* are common inhabitants of marine, freshwater and terrestrial environments in temperate and tropical boreal regions (Naw & Hara, 2002). There are nine currently recognized freshwater species of *Prasiola* (Starmach, 1972; Naw & Hara, 2002): five reported in Asia, one with two varieties (*P. japonica* Yatabe, *P. formosana* Okada var. *formosana*, *P. formosana* Okada *var. coreana*, *P. sinica* Jao, *P. subareolata* Skuja, *P. yunnanica* Jao) and four reported in North America and Europe (*P. fluviatilis* (Sommerfeld) Areschoug, *P. nevadensis* Setchell *et* Gardner, *P. velutina* (Lyngbye) Wille and *P. mexicana* J. Agardh). The characteristics used to differentiate species include the habit, size, and thickness of the lamina, and the disposition, length, and cell size of the foot used to attach to the substratum (Starmach, 1972). Even so, identification is often problematic due to the subjectivity of observers and variability of some characters.

One of the species of the order Prasiolales, *P. mexicana*, has been found in six waterways of the high mountains of central Mexico (Ortega, 1984; Ramírez *et al*., 2001). However, information about its anatomical characteristics (vegetative and reproductive) and distribution is lacking. In this work, the anatomical structure, habitat, and geographic distribution of populations of *Prasiola* are presented in detail in addition to previous reports from the central region of Mexico.

MATERIALS AND METHODS

A total of 17 samples of *Prasiola* were collected from 8 localities between 19°-20° N, 98°-99° W, and at altitudes of 1,530 to 3,220 m. (Fig. 1). Samples were preserved in 3% formalin and deposited in **FCME** (Holmgren *et al*., 1990). The physical and chemical characteristics of the water were measured with a Conductronic electro-chemical analyzer (pH ± 0.02; conductivity ± 0.5 %; temperature ± 0.5).

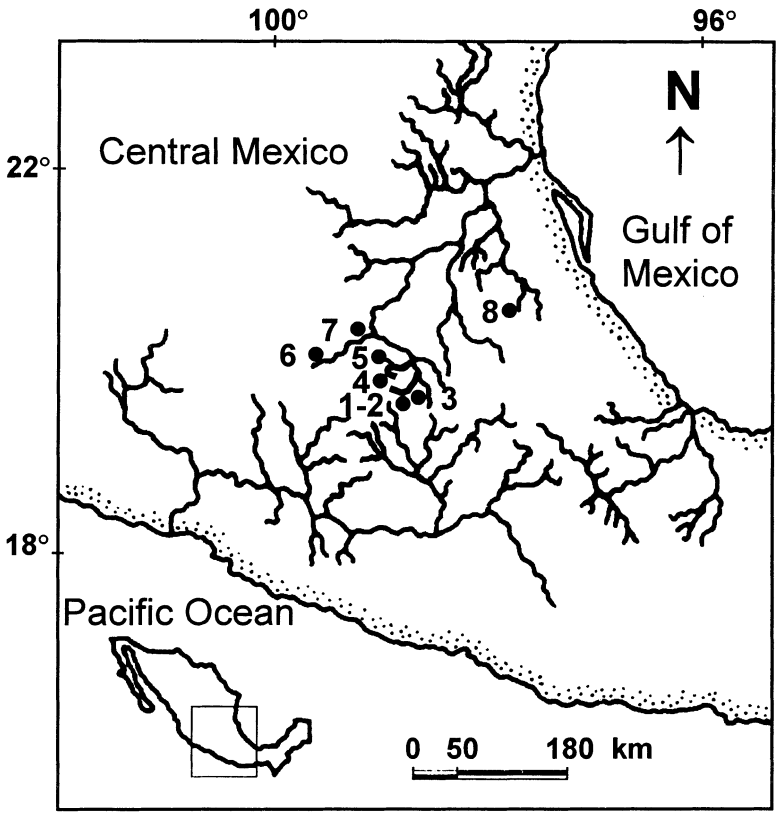


Fig. 1. Location of the study region in central Mexico with freshwater populations of *Prasiola* indicated (circles).

All of the characters considered to be of taxonomic importance in *Prasiola* were examined (Agardh, 1847; Collins 1909; Setchell & Gardner, 1920; Friedmann, 1959a, 1959b; Bravo, 1965; Starmach, 1972; Hamilton & Edlund, 1994; Van den Hoek *et al.*, 1995; Naw & Hara, 2002). Measurements were made in replicates of 20 and are represented as an interval containing a maximum, minimum, average, and standard deviation. The number of replicates was determined using the equation: n = (S/Ex), where S = standard deviation, x = average and E = a predetermined standard error (Southwood, 1978). Each specimen was observed and measured with a Olympus BX51 contrast interference microscope, with an SC35 system of microphotography. The thickness of the lamina was examined in sections made with an ultrafreeze microtome (Sartorius-Werke Mod. 27, Germany). A Student's t-test was used to compare morphological characters (gametes, aplanospores and vegetative cells).

Reproductive structures were observed in specimens from two populations maintained in culture: Xalancocotla, *coll. R. Ramírez*, 14.i.2003 and Malila, *coll. J. Carmona*, 01.iii.2003. Cultures were initiated from 10-15 mm segments of

thallus placed in sterilized tubes with distilled water and then vigorously agitated to remove epiphytes. The segments were later rinsed three times in distilled water in Petri dishes, and placed in culture tubes with 50 ml of culture media. Three replicates were transferred from each population. Each specimen was maintained in a 20:1 water-soil culture media for 60 days in climate-controlled chambers with fluorescent lights (Phillips 15 W) under the following conditions: temperature 14ºC; irradiance 98-150 μmol m^{-2} s^{-1} and a photoperiod of 12 h. A solution of Iodine (Johansen, 1940) was used to stain the pyrenoids.

RESULTS AND DISCUSSION

Morphological Analysis

Two groups of populations were recognized in central Mexico, both with characteristics referable to the genus *Prasiola* (Table 1, Figs 2-20): a laminar monostromatic thallus with a central chloroplast with lobed margin, a central pyrenoid surrounded by plates of starch, and oval to square vegetative cells generally arranged in packets of four (Figs 2-3, 6, 11-12, 15).

The first group (Table 1, Figs 2-10) included the 14 populations from the watershed of Mexico and corresponds to the descriptions of *P. mexicana* by Agardh (1847), Collins (1909) and Setchell & Gardner (1920) (Table 2). It was characterized by large (≥ 9.0 cm long), procumbent lamina with oval to irregular shape (Fig. 2) with one or many (≥ 4) fronds originating from the same base and a smooth lamina margin (Fig. 4). Bifurcating ramifications were sometimes present in the apical part of the lamina. It attaches to the substratum with a small mass of elongated, fibrous cells that form a rhizoidal holdfast (Fig. 5). The basal and intermediate sections of the lamina were monostromatic (Fig. 6), while the apical region had abundant aplanospores (≥ 8 layers) and a tendency to become polystromatic (≥ 130 μm thick) (Fig. 7). Vegetative cells were oval to square, 3.1-16.0 μm in diameter (mean 9.2 μm); length 6.1-19.8 μm (average 12.2 μm). Aplanospores were seen in both field and cultured specimen, were square or rounded, with a granulate cellular content, a diameter of 5.4-10.6 μm (average 7.7 μm), and a length of 4.5-10.8 μm (average 7.9 μm) (Fig. 7); these were easily distinguished superficially from the vegetative cells, and were found isolated or in groups (Figs 3, 5) in either the basal or apical region of the lamina. The aplanospores were liberated from the apical margin of the lamina upon rupture of the cell wall.

Two interesting morphological characteristics were observed in several specimens (populations 2-8). The surface of the lamina was frequently corrugated (Fig. 9) in specimens collected from terrestrial and high-velocity currents (Table 2), which seems to be related to the physical factors (current velocity or substratum), or biotic interactions (fungal or larval infections). This feature has not been described in any other freshwater species and further studies to characterize the microhabitat and morphological variation will be of interest. The second novel character was the presence of elongated and colorless cells in the folds of the corrugated specimen (Fig. 10). These cells are similar to those described by Fritsch (1961) and Naw & Hara (2002) as rhizoidal cells.

Table 1. Characteristics of freshwater populations of *Prasiola* in Mexico. Measurements are in µm and represent ranges, average and standard deviation.

Population and locality	*Lamina length (mm) LL*	*Lamina diameter (mm) LD*	*LL/LD*	*Thickness*	*Vegetative cell*		*Aplanosporangia*	
					Length	*Diameter*	*Length*	*Diameter*
1. MA87	7.0-10.0	5.0-9.0	1.1-1.8	24.6-32.9	8.3-16.0	6.3-16.0	4.5-8.8	5.4-10.6
Trout tank	8.8 ± 1.3	6.4 ± 1.6	1.4 ± 0.2	28.8 ± 2.9	11.2 ± 2.3	8.4 ± 1.8	6.9 ± 1.4	8.1 ± 2.2
2. MA1	5.0-26.0	3.0-14.0	0.7-2.6	22.3-27.5	6.1-18.5	3.1-13.5	–	–
Xalancocotla	14.4 ± 8.4	8.4 ± 4.7	1.8 ± 0.7	24.9 ± 1.8	10.7 ± 2.7	7.1 ± 2.4	–	–
3. MA9	2.0-14.0	6.0-19.0	0.7-1.5	–	9.7-15.5	7.2-11.7	–	–
Xalancocotla	8.3 ± 6.0	14.3 ± 7.2	1.1 ± 0.3	–	12.1 ± 1.8	8.9 ± 1.3	–	–
4. MA26	10.0-18.0	4.0-21.0	0.6-3.7	69.0-130.0	7.0-14.6	3.6-9.4	–	–
Xalancocotla	14.6 ± 3.2	10.0 ± 6.5	1.9 ± 1.1	88.3 ± 16.3	11.0 ± 2.1	7.6 ± 1.6	–	–
5. MA36	4.0-17.0	4.0-15.0	0.2-1.7	–	7.2-18.3	7.2-10.3	–	–
Xalancocotla	9.5 ± 5.5	9.0 ± 4.9	1.3 ± 0.7	–	12.4 ± 2.3	9.1 ± 0.9	–	–
6. MA102	5.0-6.0	4.0-5.0	1.0-1.5	–	9.4-19.8	7.6-12.8	–	–
Xalancocotla	5.5 ± 0.7	4.5 ± 0.7	1.2 ± 0.3	–	13.7 ± 3.5	10.3 ± 1.8	–	–
7. MA94	6.0-15.0	9.0-29.0	0.3-1.6	–	9.4-15.8	8.1-11.5	–	–
Purification plant	10.0 ± 3.7	15.0 ± 8.4	0.7 ± 0.5	–	11.9 ± 1.9	9.3 ± 0.9	–	–
8. MA 154	9.0-25.0	11.0-29.0	0.8-0.8	18.0-22.3	7.2-16.9	7.4-13.5	7.2-7.6	5.8-8.3
Purification plant	17.0 ± 11.3	20.0 ± 12.7	0.8 ± 0.02	20.4 ± 1.3	11.4 ± 3.2	9.9 ± 1.9	7.4 ± 0.2	7.4 ± 1.1
9. CB5	12.0-90.0	7.0-41.0	0.2-2.1	21.6-29.3	9.7-16.2	7.2-13.7	–	–
Agua de leones	29.3 ± 29.9	34.3 ± 29.6	1.0. ± 0.6	25.6 ± 2.0	12.7 ± 1.9	9.7 ± 1.5	–	–
10. CB32	13.0-18.0	14.0-19.0	0.7-1.0	–	9.9-16.7	7.4-13.5	–	–
Agua de leones	15.0 ± 2.6	17.0 ± 2.6	0.8 ± 0.1	–	12.7 ± 2.0	10.2 ± 1.9	–	–
11. DL18	16.0-47.0	25.0-45.0	0.6-1.0	30.2-49.9	9.2-15.1	7.2-13.3	8.8-10.8	6.7-9.9
San Borja	31.5 ± 21.9	35.0 ± 14.1	0.8 ± 0.2	41.4 ± 6.1	11.5 ± 2.3	9.3 ± 2.3	9.6 ± 0.7	7.8 ± 1.2
12. DL26	8.0-12.0	9.0-20.0	0.6-0.8	–	10.8-15.5	7.2-12.4	–	–
San Borja	10.0 ± 2.8	14.5 ± 7.7	0.7 ± 0.1	–	12.7 ± 1.7	9.5 ± 1.6	–	–
13. SL25	5.0-20.0	4.0-16.0	0.2-1.5	21.0-28.0	9.9-19.6	8.3-14.9	–	–
San Luis Ayucán	11.0 ± 6.4	8.5 ± 5.1	0.9 ± 0.6	23.0 ± 2.3	13.6 ± 2.8	10.0 ± 1.5	–	–
14. VC22	8.0-29.0	4.0-29.0	0.5-2.0	16.2-19.5	9.2-17.6	7.4-12.8	–	–
Villa del Carbón	15.2 ± 8.3	14.8 ± 10.3	1.2 ± 0.5	18.2 ± 1.0	13.7 ± 2.8	10.0 ± 1.6	–	–
15. PA3292	19.0-26.0	11.0-28.0	0.6-2.3	20.7-28.0	11.8-19.8	8.6-15.5	17.2-39.5	15.0-25.8
River Malila	21.4 ± 4.4	21.0 ± 7.1	0.7 ± 0.3	23.9 ± 1.9	14.5 ± 2.7	11.7 ± 1.8	22.9 ± 9.2	18.0 ± 4.4
16. PA3800	14.0-28.0	9.0-25.0	0.6-2.2	–	9.0-19.6	8.8-18.7	–	–
River Malila	20.2 ± 5.5	15.6 ± 5.8	1.4 ± 0.6	–	14.1 ± 2.8	12.8 ± 2.3	–	–
17. PA4097	17.0-31.0	9.0-19.0	1.0-3.0	–	10.8-22.3	8.3-14.6	–	–
River Malila	23.6 ± 5.5	14.6 ± 5.1	1.8 ± 0.8	–	13.9 ± 2.8	10.6 ± 1.6	–	–

The second group (Table 1, Figs 11-20) included the three populations from the Malila River, and were identified as *P. nevadensis* according to the characteristics described by Setchell & Gardner (1920), with some differences in cell size (Table 2). This group was characterized by its small lamina (≥ 3.0 cm long),

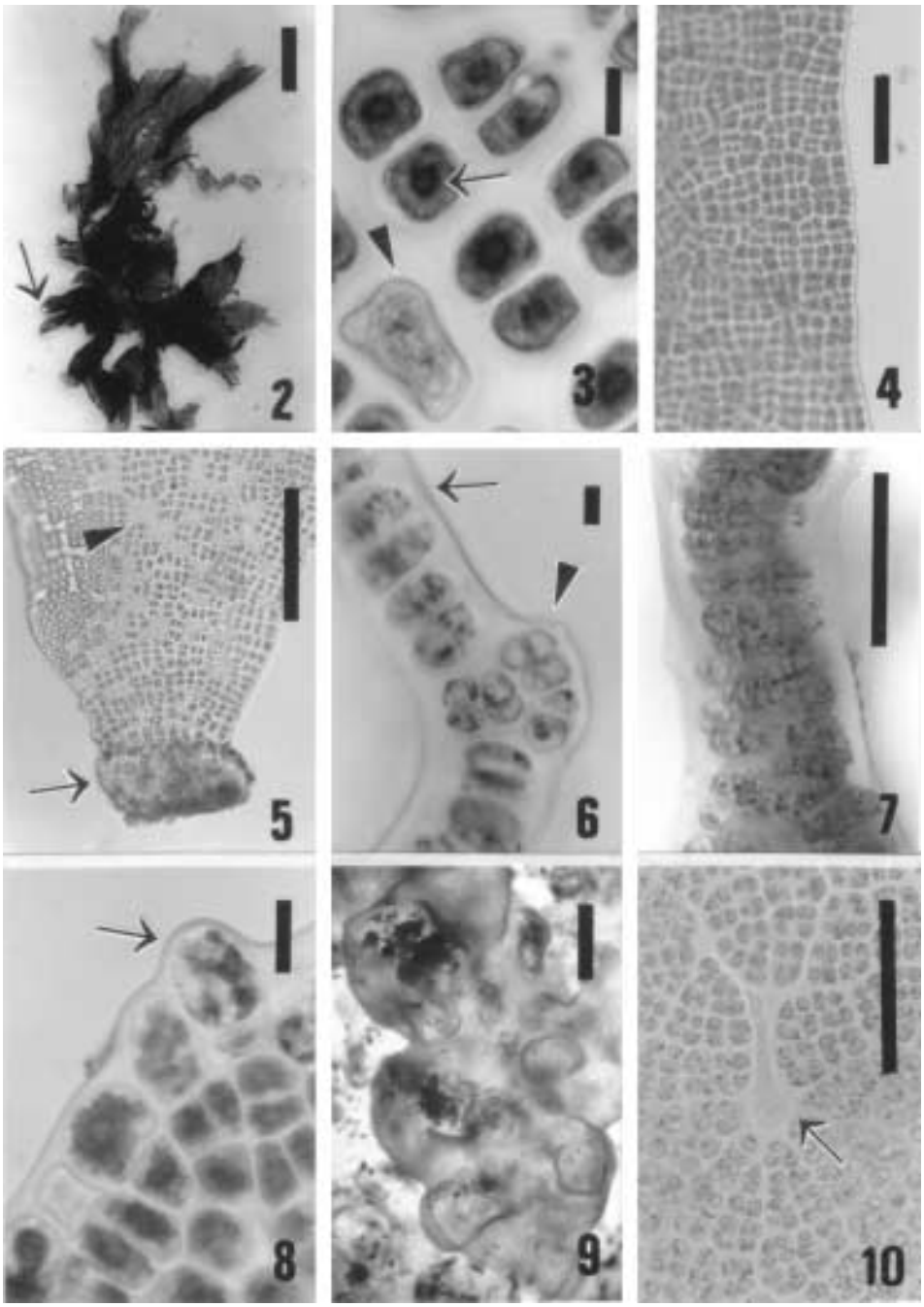


Table 2. Characteristics of freshwater populations of *Prasiola* in Mexico and species reported in the literature.

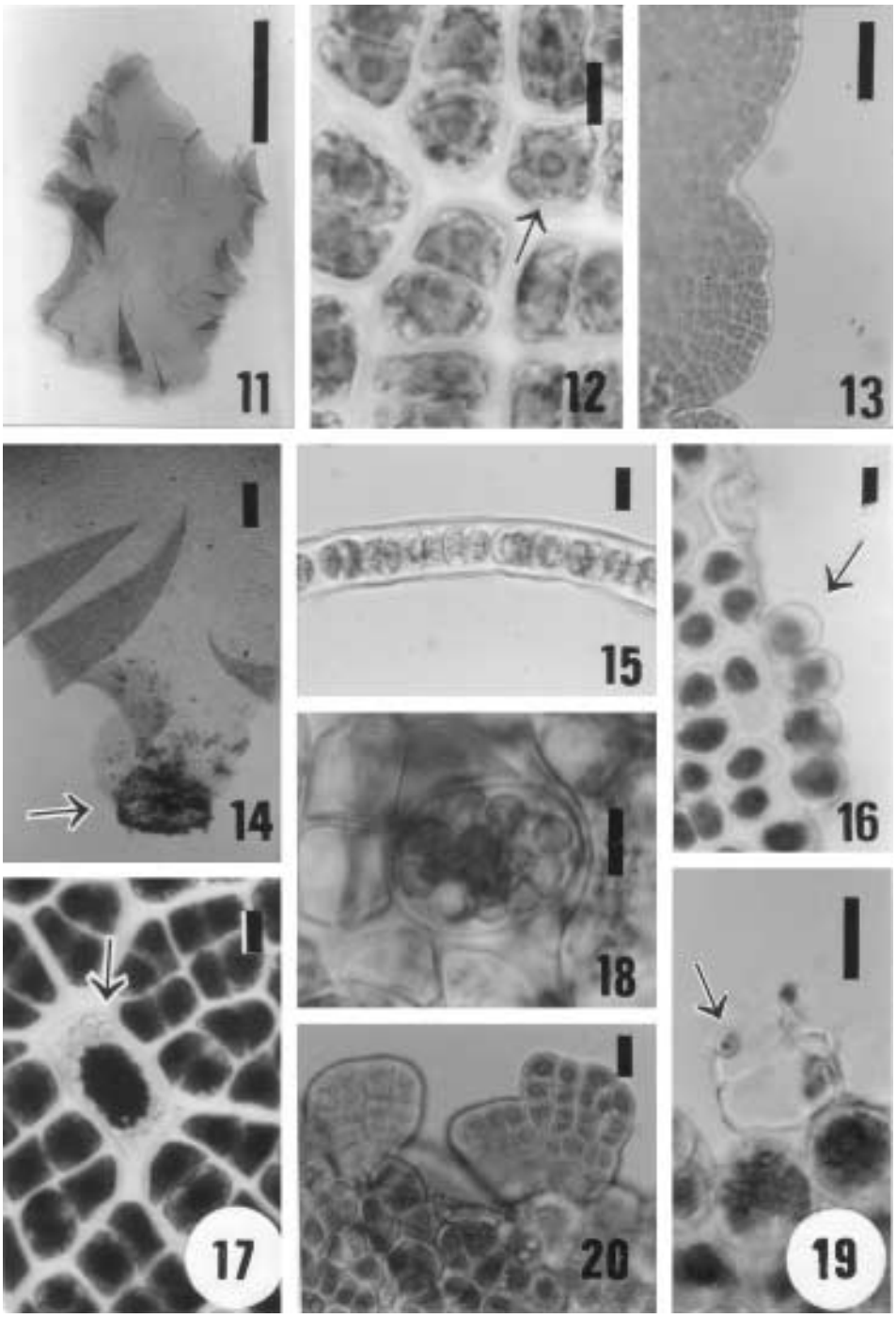
Taxon	*Size of frond Width × length (cm)*	*Thickness (μm)*	*Cell size Width × length (μm)*	*Reference*
P. mexicana	0.3-4.5 × 0.2-9	16.2-130	3.1-16 × 6.1-19.8	(this work)
P. nevadensis	1.4-2.1 × 2-2.3	20.7-28	8.3-18.7 × 9-22.3	(this work)
P. fluviatilis	0.02-0.3 × 4.5-95.0	2-4	3-5.7 × 3.2-6.1	Hamilton & Edlund (1994)
P. formosana var. *coreana*	0.4-2.3 × 1.0-7.5	20.0-28.0	3-5 × 10-17.5	Naw & Hara (2002)
P. formosana var. *formosana*	0.2-2.6 × 2.0-10.7	25.0-30.0	2-5 × 3-7	Naw & Hara (2002)
P. japonica	0.5-4.0 × 1.0-20	19.0-25.0	7.5-10 × 17.5-22.5	Starmach (1972) Naw & Hara (2002)
P. mexicana	– × 10.0	24.0-52.0	6.0-16 × –	Setchell & Gardner (1920) Starmach (1972)
P. nevadensis	0.5-1.0 × 2.0-3.0	14.0-25.0	2.7-4.1 × 4.1-8	Setchell & Gardner (1920)
P. sinica	3.0 × 9.0	30.0-33.0	5-8 × 9-13	Starmach (1972)
P. subareolata	0.5 × 6.0	45.0-54.0	6-8 × 28-38	Starmach (1972)
P. velutina	0.03-0.07 (0.11) × 1–2	–	– × 4-12	Starmach (1972)
P. yunnanica	4.0 × 20.0	51.0-87.0	5.0-7.0 × 7.0-12.0	Starmach (1972)
Prasiola sp.	0.5-5.0 × 2.0-12.0	20.0-35.0	4.0-13.0 × 12.0- 20.0	Naw & Hara (2002)

– = not reported.

smooth surface, oval form, and one or two fronds that originate from the same base (Fig. 11). It attaches to the substratum with a small mass of elongated, fibrous cells that form a rhizoidal holdfast (Fig. 14). All sections of the lamina tended to be monostromatic and were at least 28 μm thick (Fig. 15). Vegetative cells were oval to square, larger than in populations of *P. mexicana*, 8.3-18.7 μm in diameter (average 11.7 μm); length 9.0-22.3 μm (average 14.1 μm). In natural conditions round to irregular aplanospores were found with a granular cellular content and dimensions of 15.0-25.8 μm in diameter (average 18.0 μm) and 17.2-39.5 μm in length (average 22.9 μm); these were seen to be grouped or isolated at the apex and margin of the lamina surface (Figs 16-17).

Three previously unreported characteristics were observed in the three populations. The margin of the entire lamina was lobed and crenate (Fig. 13). This feature was consistent and allowed the differentiation of *P. nevandensis* from *P. mexicana*. Setchell & Gardner (1920) included figures of *P. nevadensis* where a

Figs 2-10. Morphological characteristics of *Prasiola mexicana.* **2.** General view of the lamina showing bifurcated branches (arrow)(**CB** 5). **3.** Detail of the lobed chloroplast, pyrenoid stained with iodine (arrow), and aplanospores (arrowhead) (specimens in culture). **4.** Detail of the lamina margin (specimens in culture). **5.** Detail of the rhizoidal holdfast (arrow) and a group of aplanospores (arrowhead) (**MA** 26). **6.** Transverse section of the monostromatic region (arrow) and formation of aplanospores (arrow) (**MA** 87). **7.** Transverse section of the polystromatic region (**MA** 87). **8.** Lamina margin with aplanospores (arrow) (specimens in culture). **9.** Surface view of the corrugated lamina (**MA** 87). **10.** Rhizoidal cells in the middle region of the lamina (arrow) (**MA** 87). Bar scale: 10 mm for Figs 2, 9; 100 μm for Figs 4-5, 7, 10; and 10 μm for 3, 6, 8.



lightly crenate margin can be seen, although it was not described in the text; this characteristic, however, is not mentioned for any other freshwater species (Starmach, 1972). The second novelty was the presence of multilocular, ovoid gametangia in the margins of the thallus; these were found to have hyaline gametes with a small pyrenoid, lacking flagella (Figs 18-19), and were from 3.2-5.4 µm in diameter (average 4.3). The gametes were significantly smaller than the aplanospores ($p < 0.0001$) and agree with the description of the female gametes described for marine populations of *P. stipitata* (3.2-5.0 µm in diameter; Friedmann, 1959a, 1959b) and *P. meridionalis* (3.0-6.0 µm in diameter; Bravo, 1965). The gametes were liberated upon rupture of the wall of the gametangium (Fig. 19). This is the first report of gametes for this species (Setchell & Gardner, 1920; Starmach, 1979). The third characteristic of the cultured specimens was the development of packets of cells fixed to the apical margin of the adult lamina (Fig. 20); these packets were probably produced by *in situ* germination of the aplanospores, as described in *P. stipitata* (Friedman, 1959). The cellular packets were found separately and showed a tendency to be filamentous. In order to identify the range of morphological plasticity in this alga, more detailed studies on its reproduction and seasonal morphological changes are needed.

Distribution

The populations studied were found in 8 waterways of first and second order, at altitudes above 1500 m and in humid to subhumid temperate climates. *Prasiola mexicana* was collected in high montane rivers (altitude > 2,200 m) in areas similar or close to previous records for central Mexico (Setchell & Gardner, 1920; Ramírez *et al*., 2001). Its distribution is reported to include the United States, Mexico and Costa Rica (Collins 1909; Sherwood *et al*., 2000; Ramírez *et al*., 2001). Collections of *P. nevandensis*, previously undescribed in Mexico, represent the second record of the species, and extend its range to the equator (40º N *vs*. 20º N). On the basis of this record, it is suggested that the distribution of this species might be much broader, as is the case for other temperate North American species found in central Mexico, *Sirodotia suecica* Kylin and *Paralemanea annulata* (Kützing) Vis *et* Sheath (Carmona, 1997; Carmona & Necchi, 2002). It will be necessary to explore waterways of similar characteristics (mountainous areas with cold water) in more tropical latitudes to confirm this hypothesis.

In terms of environmental characteristics (Table 3), populations of *P. mexicana* grew in seven shallow (0.1-20.0 cm) rivers with cold water (2.0°C a 16.4°C), a slightly acidic to basic pH (6.4-7.6), low conductivity (35.5-94.2 $\mu S.cm^{-1}$),

Figs 11-20. Morphological characteristics of *Prasiola nevadensis*. **11.** General view of the lamina (**PA** 4092). **12.** Detail of the lobed chloroplast and pyrenoid stained with iodine (arrow) (specimens in culture). **13.** Detail of the lamina margin (**PA** 4092). **14.** Detail of the rhizoidal holdfast (arrow) (**PA** 3800). **15.** Transverse section of the monostromatic region (**PA** 4092). **16.** Detail of the lamina margin with a group of aplanospores (arrow) (specimens in culture). **17.** Aplanospores isolated with granular cytoplasm (arrow) (specimens in culture). **18.** Detail of the gametangium in the lamina margin (specimens in culture). **19.** Liberation of gametes (arrow) via rupture of the gametangial wall (specimens in culture). **20.** Cellular packets fixed to the apical margin of the adult lamina (specimens in culture). Bar scale: 10 mm for Figs 11, 15; 100 µm for Figs 13-14; and 10 µm for 12, 16-20.

Table 3. Physical and chemical characteristics of streams containing freshwater populations of *Prasiola* in Central Mexico. Number of sampling sites are those represented in Figure 1.

Sampling site	*Populations*	*Altitude (masl)*	*Climate*[a]	*Temperature (°C)*	*pH*	*Specific conductance ($\mu S.cm^{-1}$)*	*Current velocity ($cm.s^{-1}$)*	*Shading*[b]	*Depth (cm)*	*Substratum*[c]	*Associated species*
1. Trout tank, river La Magdalena	1	3000	$C(w_2)(w)$	4	6.4	65	60	A-C	0-20	1	*Cocconeis placentula* var. *lineata* (Ehrenberg) Van Heurck, *Hydrococcus cesatii,* Rabenhorst, *Cymbella silesiaca* Bleisch.
2. Xalancocotla, river La Magdalena	2, 3, 4, 5, 6	3150	''	3-11	6.7-7.7	65-90	0-85	A-C	2	1-2	*Synedra ulna* (Nitzch) Ehrenberg, *Melosira varians* J. Agardh, *Cymbella mexicana* (Ehrenberg) Cleve, *Gomphonema* sp, *Diploneis elliptica* (Kützing) Cleve, *Placoma regulare* Broady *et* Ingerfeld.
3. Purification plant, river La Magdalena	7, 8	2800	''	2-11	7.3-7.6	90-94	0-85	C	0-11	1-2	*Ulothrix* sp.
4. Agua de Leones	9, 10	3220	''	7-12	7.0-7.6	35-36	90	A	0-5	1	*C. placentula* var. *lineata*, *Xenotholos* sp, *Fragilaria* sp, *P. regulare*
5. San Borja	11, 12	2800	''	10-16	6.9-7.2	60-60	35-85	A	0-10	"	*C. placentula* var. *lineata*, *Cymbella* sp, *Navicula* sp
6. San Luis Ayucán	13	2664	''	16	6.6	79	60	A	10	''	*Paralemanea mexicana* (Kützing) Vis *et* Sheath
7. Villa del Carbón	14	2227	''	14	7.0	76	35-85	A	0-8	''	–
8. River Malila	15, 16, 17	1300	C(fm)	14-17	5.5-7.2	192	60-85	C	0-20	''	*C. placentula* var. *euglypta* (Ehrenberg) Grunow, *Xenotholos huastecanus* Gold, Montejano *et* Komárek, *Chamaesiphon* sp.

[a] = García (1973): $C(w_2)(w)$ = Temperate, dry winter < 5%; C (fm) = Temperate, no dry season.
[b] = A, open; B, parttly shaded; C, shaded.
[c] = 1, predominantly bedrock; 2, predominantly boulder.

and a wide range of light intensity (low-high) and water current speed (10-85 cm.s^{-1}). In populations 2 and 10, specimens were collected in the splash zone from the water to 10 cm above the maximum level of the current. The temperature data are similar to those reported by Sheath & Cole (1992) and Ramírez *et al*. (2001). The most frequent substratum was andesitic-basaltic rock. Occasionally trunks or metal tubes served as a substratum as well. *Prasiola mexicana* commonly grew with other algae such as *Placoma regulare* and *Cocconeis placentula* var. *lineata*, and was occasionally seen with *Paralemanea mexicana*. Populations of *P. nevadensis* grew in a river of humid temperate climate with cold water (14.0°C) that was acidic (pH = 5.5), had medium specific conductivity (192 µS.cm^{-1}), was shallow (0.2-20.0 cm), and had low irradiance and high water current speed (60-85 cm.s^{-1}). The predominant substratum was lime-lutite and *P. nevadensis* grew in association with other algae such as *Xenotholos huastecanus*. This is the first report of the habitat of *P. nevadensis*.

Description of the Mexican material

Prasiola mexicana J. Agardh, 1847. (Figs 2-10)
Monostromatic thallus 2.0-9.0 cm long, 0.3-4.5 cm wide, length/width 0.2-3.7; with bifurcating ramifications at apex. Lamina surface smooth or corrugated and margin smooth to lobed; thickness of 16.2-130.0 µm; 1 to 4 laminae per rhizoidal holdfast. Cells oval to rectangular, 6.1-19.8 µm long, 3.1-16.0 µm in diameter. Aplanospores 4.5-10.8 µm long, 5.4-10.6 µm in diameter in the polystromatic layer (4-8 layers).

Populations examined: (Numbering corresponds to the list of Table 1). **1)** D.F., Magdalena Contreras, Trout tank, *coll. J. Carmona*, 19.xii.1998 (FCME MA87); **2)** Xalancocotla, *coll. J. Carmona*, 23.xi.1997 (FCME MA1); **3)** *coll. M. Ramírez*, 23.xi.1997 (FCME MA9); **4)** *coll. J. Carmona*, 8.ii.1998 (FCME MA26); **5)** (FCME MA36); **6)** *coll. M. Bojorge*, 4.xi.1999 (FCME MA102); **7)** Purification plant, *coll. J. Carmona*, 24.i.1999 (FCME MA94); **8)** *coll. M. Ramírez*, (FCME MA154); **9)** Cuajimalpa, Agua de Leones, *coll. J. Carmona*, 19.vi.1999 (FCME CB5); **10)** *coll. E. Cantoral*, 28.xi.1999 (FCME CB32); **11)** San Borja, *coll. J. Carmona*, 15.v.1999 (FCME DL18); **12)** *coll. G. Montejano*, 15.v.1999 (FCME DL26); **13)** Estado de México, Jilotzingo, San Luis Ayucán, *coll. M. Ramírez*, 18.vii.2001 (FCME SL25); **14)** Villa del Carbón, river Villa del Carbón, *coll. J. Carmona*, 18.vii.2001 (FCME VC22).

Prasiola nevadensis Setchell *et* Gardner, 1920. (Figs 11-20)
Monostromatic thallus, 2.2-2.3 cm long, 1.4-2.1 cm wide, length/width 0.5-2.3. Lamina surface smooth, margin lobed and crenate; lamina 20.7-28.0 µm thick. 1-2 laminae per rhizoidal holdfast. Cells oval to rectangular, 9.0-22.3 µm long, 8.3-18.7 µm diameter. Aplanospores 17.2-39.5 µm long, 15.0-25.8 µm in diameter, in the polystromatic layer (1-2 layers). Gametangia ovoid with elliptical or oval gametes, without flagella, 3.2-5.4 µm in diameter.

Population examined: (Numbering corresponds to the list of Table 1) **15)** Hidalgo, Molango, river Malila, *coll. J. Carmona*, 15.ii.1992 (FCME PA3292); **16)** *coll. G. Montejano*, 10.iii.1999 (FCME PA3800); **17)** *coll. J. Carmona*, 6.xi.2002 (FCME PA4097).

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