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OBSERVATIONS ON THE GENUS *PINNULARIA* SECTION DISTANTES (BACILLARIOPHYTA) FROM MACEDONIA; DIVERSITY AND DISTRIBUTION

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Pinnularia is one of the largest and widely distributed freshwater diatom genera. Nevertheless, dedicated taxonomic studies to the section Distantes of *Pinnularia* are rather few. A notable diversity of *Pinnularia* taxa from the section Distantes has been observed from a variety of aquatic habitats on several mountain areas in Macedonia. In total, 12 taxa have been observed, of which three are described as new species: *Pinnularia idsbensis*, *P. micevskii* and *P. subalpina*. The main morphological features are given for all of the observed taxa. The distinctive features for the new species, in comparison with allied taxa, are also provided. Additional notes are given for the distribution and habitats where every taxon was observed. The habitats vary in type and altitude including high mountain lakes of glacial and non-glacial origin, springs, streams, rivers, rivulets and peat bogs, as well as aerated old drinking water wells and permanent or temporary wet rocks.

Key words: diatoms; *Pinnularia*; section Distantes; diversity; Macedonia

INTRODUCTION

The section Distantes (Cleve) Patrick in Patrick & Reimer [1] of the genus *Pinnularia* Ehrenberg [2] was introduced by Cleve [3] as a group of taxa sharing linear-lanceolate to elliptical valves with distinctly broad and distantly spaced striae, as distinguishing features from the remaining *Pinnularia* taxa. Nevertheless, a valid taxonomic rank was given to this group with the description of the section Distantes within the genus *Pinnularia* by Patrick in Patrick & Reimer [1].

The diversity of taxa belonging to the genus *Pinnularia* was emphasized as early as the end of the 19th century by Cleve [3] and also on several occasions afterwards (Mayer [4], Hustedt in A. Schmidt et al. [5], Patrick & Reimer [1]). More recent revisions of diatom floras from different geographical regions such as Europe (Krammer [6, 7]), South America (Metzeltin & Lange-Bertalot [8, 9]), Asia (Metzeltin et al. [10]), and the Antarctic and Subantarctic (Van de Vijver et al. [11], Van de Vijver [12], Van de Vijver & Zidarova [13], Van de Vijver et al. [14]) confirmed the notable diversity and wide distribution of *Pinnularia* taxa.

The section Distantes of *Pinnularia* has seldom been a focus of a thorough treatment, with the contributions of Mayer [4], Bock [15], Krammer [7] and Van de Vijver & Zidarova [13] being the most comprehensive to date. Mayer [3] focused on many forms around *P. borealis* Ehrenberg [2] and *P. lata* (Brébisson) Rabenhorst [16], while Bock [15] compiled a complete set of the iconotype drawings known at that time of the taxa from this group. In his treatise of the European *Pinnularia* taxa Krammer [7] introduced ten new taxa in the section Distantes, three species and seven varieties. Most of his infraspecific additions are in *P. borealis* (four), two in *P. rabenhorstii* (Grunow) Krammer [7] and one in *P. dubitabilis* Hustedt [17]. A noteworthy diversity of the section Distantes has also been demonstrated for the Antarctic and Subantarctic region. Two infraspecific taxa in *P. rabenhorstii* and one species have been introduced by Van de Vijver et al. [11] from Ile de la Possession. Later on, Van de Vijver [12] described two species from King George

Island, Van de Vijver & Zidarova [13] contributed five taxa, four species and one variety in *P. borealis*, from Livingston Island, and Van de Vijver in Van de Vijver et al. [14] made an addition of one species from Ile Amsterdam.

Recently, Souffreau et al. [18] applied the principles of molecular phylogenetics and presented evidence on a distinct Antarctic lineage of *P. borealis*. An assessment of the molecular divergence revealed *P. borealis* as the oldest known diatom species complex, and the Antarctic lineage of this taxon is estimated to have diverged after the geographical and thermal isolation of the Antarctic continent. In addition, the temperature-dependent growth characteristics (lower optimal growth temperature and upper lethal temperature) indicate a niche differentiation in the Antarctic lineage of *P. borealis* (Souffreau et al. [18]).

An appealing diversity of taxa from the section Distantes of *Pinnularia* has been observed on different mountains in Macedonia (Levkov et al. [19]). Nevertheless, the diversity of taxa from this group was not restricted to mountain areas only, but also observed in the lowland regions in Macedonia (Petrovska & Stojanov [20, 21], Krstic & Stojanovski [22]). The aquatic habitats of importance for these taxa vary in type and altitude and include high mountain lakes of glacial and non glacial origin, mountain springs, streams, rivers and rivulets, and also peat bogs. In addition, somewhat extreme habitats as wet rocks, of permanent or temporal moisture, and old wells used as a drinking water source have also been found as an appropriate environment for these taxa (Krammer [7]). Hence, this study is an attempt for a proper documentation of the diversity of *Pinnularia* taxa from the section Distantes in Macedonia. Still, we would refrain from pointing to this observation as a final one. Many additional taxa, observed with a single or a few specimens only, need correct identification and therefore we believe that this systematic group is even more diverse in the area studied.

EXPERIMENTAL SECTION

The samples examined were collected on various sampling campaigns from 1995 to 2012. The mountains where samples were collected are: Šar Planina in the western part of Macedonia, Jablanica and Pelister in the south-western part, Kožuf and Nidže in the southern part, and Osogovo in the eastern part of Macedonia. In addition, samples were collected from Mountain Bistra and the region of Mariovo in the central part of Macedonia. The altitude range of the sampling sites varies between 200 and 2500 m a.s.l.

The aquatic habitats sampled include high-altitude lakes of glacial and non-glacial origin, springs, streams, rivers and rivulets, ponds and pools of various size and also peat bogs. Furthermore, aerated habitats such as wet walls and rock pools of permanent or temporary aqueous periods, and even old drinking water wells have been sampled. The substrate sampled was of organic sediment, sand, rock scrape, submerged plants, macrophyte algae and a variety of moss species of different moisture.

The organic content was removed from the samples by acid digestion, with the addition of 2 ml of K_2MnO_4 and 4 ml of HCl to a small (ca 2 ml) subsample. Permanent microscope slides were mounted in Naphrax®. Slide observations were performed with a Nikon E–80i light microscope, and photomicrographs were made with a Nikon Coolpix 600 digital camera. Photomicrographs were made to show most of the size diminution of each taxon. The sequence of the taxa in the plates is based on morphological similarity among the taxa, exceptions made only for better utilisation of the available space. The observations on the taxa in the text follow the sequence of the taxa in the plates. The proper identification of these taxa was based on the works of Mayer [4], Bock [15], Krammer [7], Van de Vijver [12], Van de Vijver et al. [11, 14] and Van de Vijver & Zidarova [13], whereas the terminology used for the descriptions of the taxa mainly follows Krammer [7], Patrick & Reimer [1], Van de Vijver et al. [11] and Van de Vijver & Zidarova [13]. An identification key is provided at the end as assistance in the identification of the taxa.

All samples and microscope slides are housed at the Macedonian National Diatom Collection (MKNDC) at the Institute of Biology in Skopje, Macedonia. The type slides are also housed at the MKNDC and isotype slides are deposited at the Friedrich Hustedt Diatom Study Centre (BRM).

RESULTS AND DISCUSSION

In total, 12 taxa have been observed, four species and eight named varieties (Table 1). Of these, three species are described as new: *Pinnularia idsbensis*, *P. micevskii* and *P. subalpina*.

Table 1. An overview of the main morphological features of the *Pinnularia* taxa, section Distantes, observed in Macedonia

Taxon	Valve outline/ Valve margins	Valve apices	Central area	Length/Width (μm)	Striae (in 10 μm)
P. borealis* var. *borealis	Linear-elliptic/ weakly convex	Unprotracted, rounded	Rhombic-lanceolate to rectangular	23.5–43.0/ 7.5–10.5	4–6
P. borealis* var. *islandica	Linear-elliptic/ weakly convex	Unprotracted, broadly rounded	Rhombic-lanceolate to lanceolate	25.0–47.5/ 9.5–13.0	4–6
P. borealis* var. *scalaris	Linear to linear-lanceolate/parallel	Weakly protracted, rounded	Rhombic-lanceolate to rectangular	34.5–51.0/ 8.0–10.0	4–7
P. borealis* var. *subislandica	Linear-lanceolate/ weakly convex	Not or weakly protracted, truncate	Rhombic-lanceolate to rounded	22.5–42.5/ 7.0–9.0	4–6
P. dubitabilis* var. *dubitabilis	Rectangular, linear/ Parallel	Unprotracted, rounded to truncate	Absent	28.0–40.0/ 7.0–8.5	4–5
P. idsbensis	Linear/consistently parallel	Unprotracted, broadly rounded	Rhombic-lanceolate	33.0–71.5/ 11.0–14.0	4–6
P. lata* var. *lata	Linear-elliptic/convex	Unprotracted, broadly rounded	Asymmetrical, lanceolate	128.5–148.0/ 36.0–39.5	2–3
P. lata* var. *minor	Linear-elliptic/slightly convex	Unprotracted/narrowly rounded	Rhombic-lanceolate, rarely rectangular	36.0–47.0/ 12.5–14.0	5–7
P. micevskii	Linear/parallel	Unprotracted, subrostrate	Asymmetrical, rhombic-lanceolate	81.0–112.0/ 22.0–26.0	3–4
P. rabenhorstii* var. *franconica	Linear/consistently parallel	Protracted, subrostrate	Rhombic-lanceolate to rounded	43.5–61.0/ 11.0–14.0	4–7
P. rabenhorstii* var. *rabenhorstii	Linear-lanceolate/ weakly convex	Unprotracted, broadly truncate	Rhombic-lanceolate to lanceolate or rounded	44.0–72.5/ 13.0–17.0	4–5
P. subalpina	Linear-elliptic to elliptic/parallel to strongly convex	Unprotracted, broadly rounded	Asymmetrical, rhombic-lanceolate	52.0–84.0/ 20.0–21.5	3–5

Pinnularia micevskii Levkov, Pavlov & Nakov sp. nov. (Figs 1: 1–3; 2: 1–4)

Description: Valves broadly linear, with parallel margins and unprotracted, broadly rounded, subrostrate apices. Valve length 81.0–112.0 μm, and valve width 22.0–26.0 μm (n = 15). Axial area broad, 1/5 to 1/6 of valve width, linear, expanded near central area. Central area asymmetrical, large rhombic lanceolate, 1/2 of valve width. Central area well defined, bordered on each side by 2–4 shortened striae. Irregular silica ornamentation present in central and axial areas, in some specimens also observable within interstriae. Raphe weakly to moderately lateral, outer raphe fissures slightly curved and inner fissures nearly linear. Proximal raphe endings deflected towards one valve side, terminated with large and distinct, tear-drop shaped central pores. Distal raphe fissures sickle-shaped, positioned in distinct terminal areas, clearly discernible in LM. Transapical striae broad, closely spaced, radiate in mid-valve and parallel near valve apices, 3–4 in 10 μm. Interstriae of equal or narrower width than striae.

Type (here designated): Mountain of Šar Planina, Lake Crno, sediment, depth 0.5 m, collection date: 7 August 2003, leg. Z. Levkov (Accession no. MKNDC 003048). Holotype! illustrated in Fig. 1: 1 (circled specimen on slide MKNDC 003048/A). Slide BRM Zu9/47 (isotype!).

Etymology: The specific epithet is given to honour late Acad. Kiril Micevski, from the Institute of Biology in Skopje, for his pioneering efforts and remarkable contribution to plant taxonomy in Macedonia.

Distribution: *Pinnularia micevskii* has been observed on Mt. Šar Planina, in the type locality, within the epipelon from glacial Lake Crno, at an altitude of 2165 m a.s.l. This species was also observed on Mt. Jablanica, as an epiphyte on mosses from a peat bog above glacial Lake Podgorechko, at an altitude of 1950 m a.s.l. *Pinnularia micevskii* was fairly abundant in both samples.

Observations: *Pinnularia micevskii* is most similar to *P. rabenhorstii* var. *rabenhorstii* (Grunow) Krammer [7] in respect to the valve outline, the shape of the valve apices and the central area. A