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The morphological variability of *Kobayasiella parasubtilissima* and *K. micropunctat*a in the Carpathian basin

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INTRODUCTION

Correct identification of diatoms has been and continues to be the foremost problem in diatomology. It is the key for obtaining accurate interpretation of water quality, paleo-limnological reconstruction as well as reliable molecular data. However the continuity or discontinuity between species – regarding their different features – is not always clear (Mann 1997). Theoretically, features have normal distribution; meaning the extreme values often overlap. That is why the interest in publications dealing with the morphological variability of biological entities – including diatoms – is permanent. More knowledge about the morphological variability of diatoms can also contribute to better understand the size-reduction process as well as their sexual behavior (auxosporulation).

The morphological variability of the most common species is reported frequently (e.g. Fukushima et al. 1985, 1986, Metzeltin 2007 and so on), but there are only few data on rare or infrequent species. This paper presents some observation about a finely striated group of diatoms that are mainly living in acidic environments.

The genus *Kobayasiella* Lange-Bert. (formerly *Navicula subtilissima* group) can be characterized by predominantly linear-lanceolate, narrow valves, with rostrate or capitate ends. The striae are usually not visible in light microscope (more than 30 in 10 µm). The striae pattern is characteristic: radiate in the centre, turning abruptly convergent near the apices. The unique raphe feature, noted so far only amongst *Kobayasiella* species, is the presence of an “umbilicus” or kink on the raphe. The correct identification of *Kobayasiella* taxa is difficult because the main features of the genus become visible only by SEM (Lange-Bertalot 1996, 1999, Vanhoutte et al. 2004).

Kobayasiella micropunctata (H.Germ.) Lange-Bert. and *Kobayasiella parasubtilissima* (H.Kobayasi & Nagumo) Lange-Bert. are very similar species, their dimensions overlap (*K. micropunctata* 19–22.5 x 3.0–4.0 µm and 36-40 striae/10 µm; *K. parasubtilissima* 22–34 x 3.5–4.5 µm and 40–44 striae/10 µm) and their distinctive features are only visible in the SEM (Kobayasi & Nagumo 1988). Different populations of these species were studied in the Carpathian Basin.

MATERIAL & METHODS

Algal samples were collected from different habitats (mosses, sediments, epiphytic samples, filamentous algae) in several mires between 1999 and 2007 in the Carpathian basin (Fig. 1, Table 1) and a crater lake.

In the lab, the samples were treated with hot hydrogen peroxide, washed several times and the cleaned material was mounted in Zrax (refractive index is 1.7). For light microscope analysis a LEICA DM LB2 was used (100 X HCX PLAN APO, and Fujifilm Digital Camera FinePix S2 Pro). Scanning electron microscopy was performed with a Hitachi S-2600N (Fig. 3, 34) and a Philips 510 at B (Fig. 12, 26–28.)

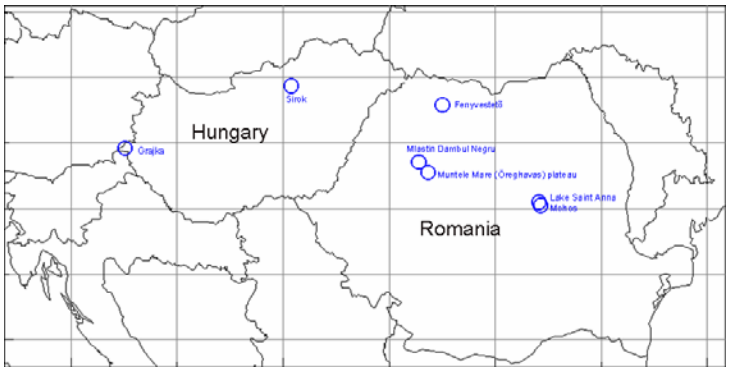

Fig. 1. The known *Kobayasiella* habitats in the Carpathian basin.

Table 1. The studied sites.

Site name	Latitude	Longitude	Elevation (m)	Peatland type	Mean pH
Grajka	N 46° 53'26"	E 16°14'19"	275	basin/floodplain fen	6
Sirok (Nyíres bog)	N 47°56'	E 20°11'	280	Raised bog	4.2
Lake Saint Anna	N 46°13'	E 25°89'	950	crater lake	4.2–6.5
Mohos	N 46°08'	E 25° 54'	1050	raised bog	3.6
Mlastin Dambul Negru	N 46°41'28"	E 23° 01' 53"	1093	raised bog	no data
Fenyves-tető	N 47°40'	E 24° 02'	1340	raised bog	3,9
Muntele Mare plateau	N 46°29' 36.4"	E 23°12'50.2"	1720	transition spring bog	no data

RESULTS

Kobayasiella micropunctata and/or *K. parasubtilissima* were found in seven peat bogs and/or lake. Their abundances varied on a wide scale: from the floristic detection level to dominancy. In four localities such rich populations could be found that allow us to perform a statistical analysis (at least 10 valves could be measured from the populations). The results are listed in the order of the elevation of the localities: from the deepest (275 m) to the highest (1720 m) (see Table 1).

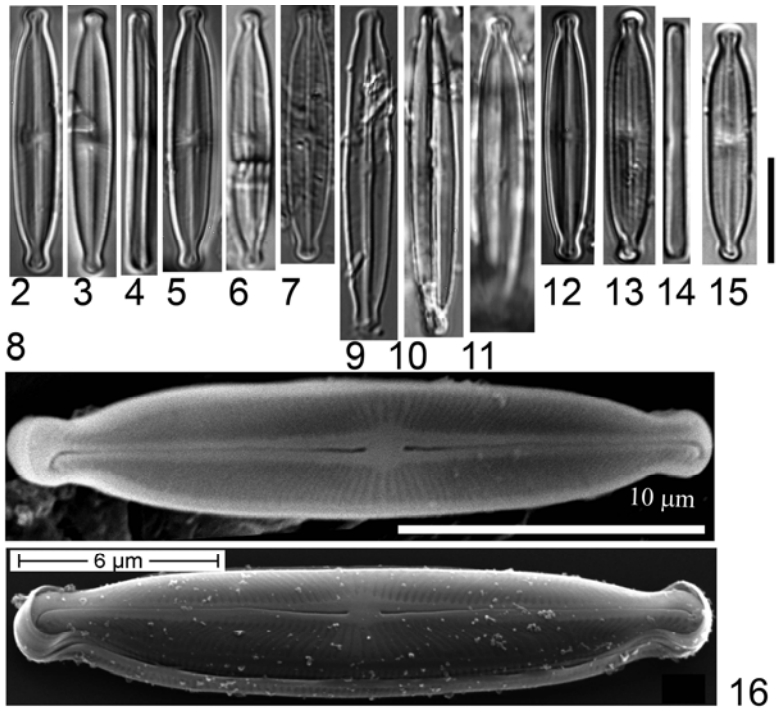

Fig. 2-16. Kobayasiella *micropunctata and K. parasubtilissima.* – 2-8. *K. micropunctata,* Grajka brook, LM (2-7), SEM, external view (8). – Fig. 9-11. *K. parasubtilissima*, 9. Sirok,10. Lake Saint Anna, 11. Mohos mire, LM. – Fig.12–16. *K. micropunctata*, Mlastin Dambul Negru, LM (12-15), SEM, external view (16). – Scale bar (LM) = 10 µm.

1. Grajka brook

Valves of this population are linear-lanceolate with capitate ends (Fig. 2-8). The lengths are 24–25 µm. (24.14 ± 0.38). Every measured valves' width was 4 µm (n=12). The raphe is straight, the terminal fissures are bent to the same side, they are characteristically fish-hook shaped (Fig. 8). Striae are radiate, alternately longer and shorter at the centre. The number of striae is about 40 per 10 µm, Each stria consists of a single line of areola. The length of valves fits to *K. parasubtilissima*, the shape of the terminal fissure to *K. micropunctata*. The number of striae fits both mentioned species. In spite of the dimensions but on the basis of raphe terminal fissure, this species is identified as *K. micropunctata*.

2.–4. Sirok, Lake Saint Anna and Mohos

In these three localities, *K. micropunctata* and *K. parasubtilissima* were rare, their occurrences were only at floristic detection level. Only one *Kobayasiella* specimen (Fig. 9) belonging to the studied group (*K. parasubtilissima/micropunctata)* was found at Nyíres-bog. Its length is 31 µm and width is 4.5 µm. The subcapitate end of the valva is noteworthy, because it differs from most studied populations in the Carpathian Basin. The rarity would not allow for SEM investigation. Otherwise Nyíres bog at Sirok provides suitable habitat for different *Kobayasiella* species (*K. madumensis, K. subtilissima)*. According to the dimensions this species is identified as *K. parasubtilissima*.

In Lake Saint Anna, where rich and abundant *Kobayasiella* assemblages could be found recently (Buczkó, Wojtal & Jahn subm.) and during the Holocene with five species (*K. elongata, K. madumensis, K. subtilissima, K.* sp.), representatives of the *K. micropunctata/parasubtilissima* group are sporadic. Four valves were measured, theirs lengths are 31 µm and widths 4 µm. This population can also be characterised by its subcapitate ends (Fig. 10). It is identified as *K. parasubtilissima.*

Also only one valve was detected in Mohos bog (Fig. 11). Its length is 32 µm and width 4.5 µm. According to the dimensions it is also identified as *K. parasubtilissima*.

5. Mlastin Dambul Negru

Valves are linear-lanceolate with capitate ends (Fig. 12-16). The lengths are 21–23 µm. (22.5 ± 0.84). Every measured valves' width was 4 µm (n=10). The raphe is straight, the terminal fissures are bent to the same side, they are characteristically fishhook shaped (Fig. 16). Striae are radiate, alternately longer and shorter at the centre. The number of striae is about 40 per 10 µm, Each stria consists of a single line of areola. The number of pores on the mantle is more than the number of transapical striae on the central part of valves (Fig. 16.) This species exactly corresponds to the description of *K. micropunctata*.

6. Fenyvestető

In this peat-bog the *Kobayasiella* population was abundant among the filamentous green algae and on a *Drepanocladus* moss. Here, its abundance reached 40 %. Otherwise it was absent from the lag zone of mire and among the *Sphagnum* species. Valves are linear-lanceolate with capitate ends (Fig. 17-28). The lengths are 23–31 µm (27.18 µm ± 2.08; n=30). The widths are 4–5 µm. The raphe is straight, the terminal fissures are bent to the same side, they end in an obtuse angle (Fig. 28). Striae are radiate, but their angles are variable (Fig. 26-27). The number of striae is 37–41 per 10 µm. The number of pores on the mantle is equal the number of transapical striae on the central part of valves (Fig. 28). Except for the number of striae (it must be 40–44 per 10 µm in the case of *K. parasubtilissima),* these features fit to *K. parasubtilissima*.

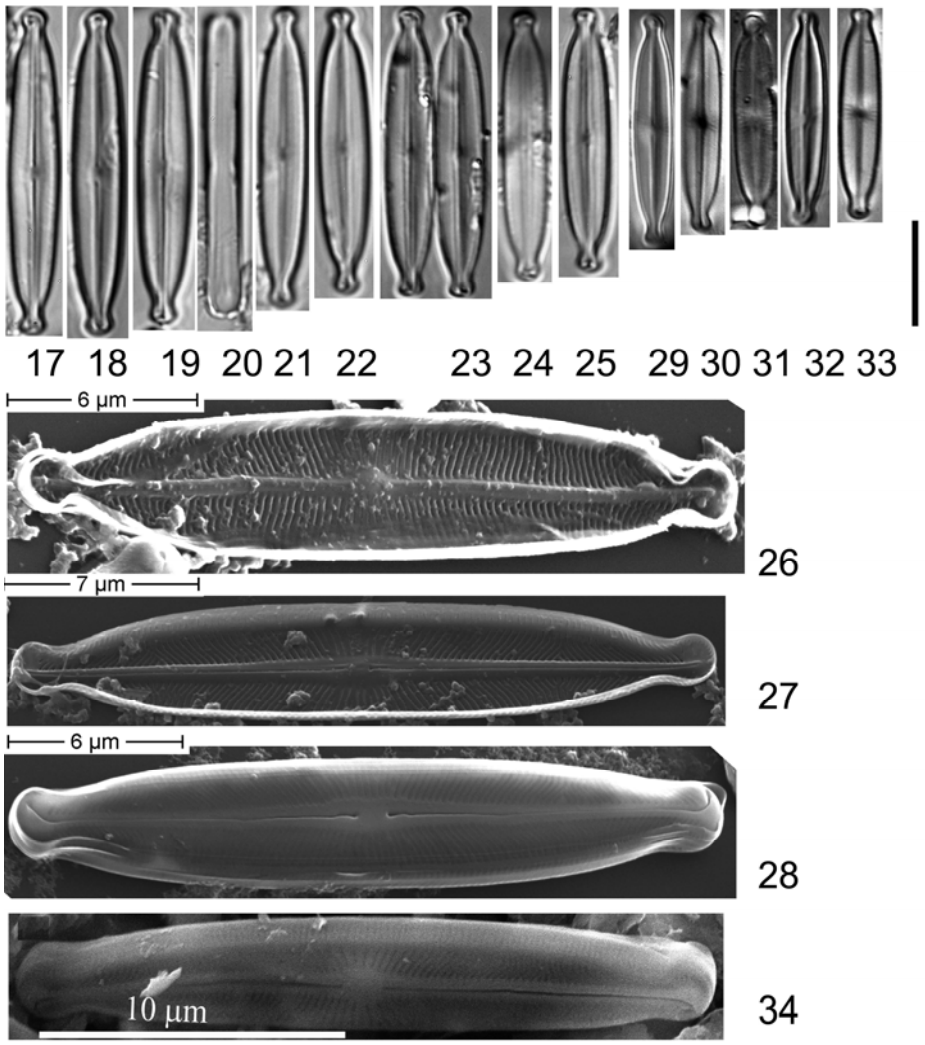

Fig. 17-34. *Kobayasiella parasubtilissima* and *K. micropunctata.* – 17-28. *K. parasubtilissima*, Fenyvestető, LM (17-25), SEM, internal view (26-27), external view (28). – 29-34. *K. micropunctata,* Muntele Mare plateau, LM (29-33), SEM (34). – Scale bar (LM) = 10 µm.

7. Muntele Mare plateau

Valves are linear-lanceolate with capitate ends (Fig. 29-34). The lengths are 19–21 µm. (20.39 ± 0.86). The widths of valves are 3–3.5 µm (3.17 ± 0.25) (n=13). The raphe is straight, the terminal fissures are bent to the same side, they are characteristically fish-hook shaped (Fig. 34). Striae are radiate, alternately longer and shorter at the centre. The number of striae is 40 per 10 µm. Each striae consists of a single line of areola. The number of pores on the mantle is more than the number of transapical striae on the central part (Fig. 34). This population clearly belongs to *K. micropunctata*.

DISCUSSION

Though the taxonomy of acidobiontic/acidophilous taxa is full of difficulties, to identify *Kobayasiella* species is important. Even the presence or absence of the genus *Kobayasiella* is uncertain; SEM or TEM investigations usually are necessary for proving their occurrences. The most similar two species in the genus are *K. micropuncata* and *K. parasubtilissima*.

Apart from the differences in dimensions and other numerical parameters (number of striae in 10 µm) there are three features that distinguish *K. micropunctata* from *K. parasubtilissima* (Kobayasi & Nagumo 1988). *K. micropunctata* has fish-hook shaped terminal fissures of the raphe, while the raphe of *K. parasubtilissima* ends in an obtuse angle. Both species have pore rows on the mantle, but in the case of *K. micropunctata* the number

of the mantle pores are higher than the number of transapical striae, while in the case of *K. parasubtilissima* they are equal. These two features are more or less well visible in the SEM. The third criterion “continuous striae closed externally by a hymenate pore occlusion with perforations arranged in a hexagonal array” can be observed only partially. The continuous striae are closed. The perforations can only be studied by TEM, which was not available during this study.

The studied populations in the Carpathian basin show differences from the original description. The valves of *K. micropuncata* are longer (up to 25 µm instead of 22 µm) as is published (Kobayasi & Nagumo 1988). Moreover, it seems that the length of valve decreases from lower to higher elevation. The average length of valves is 24.1 µm in the population that lives at 275 m a.s.l.; 22.5 µm in the population that was found at 1093 m a.s.l.; while at 1720 m a.s.l. this length is 20.4 µm. The differences could be due to different phases of the size reduction of the population, but maybe these can be different ecotypes as well. More data are necessary to test this phenomenon.

Some differences for *K. parasubtilissima* were found in the population at Fenyvestető; here the valves are wider than in the original description (5 µm), and the striation is scarcer.

On the basis of these results, we can summarize, that it is impossible to distinguish *K. micropunctata* and *K. parasubtlissima* by LM, and their dimensions in the Carpathian basin differ from earlier published data.

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