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COMPARATIVE STUDY OF *NAVICULA HASTA* AND *N. RAKOWSKAE*: THEIR MORPHOLOGY AND DISTRIBUTION

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Despite numerous reports of *Navicula hasta* in the literature its distribution still remains unknown. More detailed studies of *Navicula hasta* and related taxa resulted recently in the separation of several new species. One of these, *Navicula rakowskae* Lange-Bertalot, was described from a karstic spring in Poland. The holotype of *N. hasta*, a portion of the type material from the Natural History Museum in Budapest and the material collected from Poland (Krakowsko-Częstochowska Upland) were examined with LM and SEM. Based on the protologue and the material examined the morphological characters of both species are described. Their distribution is briefly discussed on the basis of the material collected and the reliable records in the literature.

Key words: Bacillariophyceae, diatoms, distribution, morphology, *Navicula*, taxonomy

INTRODUCTION

Diatoms are being used increasingly in a wide range of studies as powerful bioindicators of both short and long-term environmental climatic changes. On the other hand problems with identification (ROUND 2001) could result in uncertain reports of the taxa and in some cases the range of their requirements/preferences and distribution appears to have been overestimated.

Josef PANTOCSEK described *Navicula hasta* in 1892. In the protologue he stated that material containing *N. hasta* originates from Tertiary, freshwater sediments from three localities (Bibarczfalva, Köpecz and Bodos) in Transsylvania.

However, line drawings of this species published in 1903, referred to the protologue of *Navicula hasta* were based only on material originating from Köpecz. According to Krenner's work in the diatom collection of Pantocsek in the Natural History Museum, Budapest, the slide number 122 was assigned as the holotype of *N. hasta*. This slide was made by Kinker and was made of Bodos material (KRENNER 1980).

A survey of the literature reveals that *Navicula hasta* has been reported from various parts of the world (*e.g.* MEISTER 1913, SILVA 1946, CLEVE-EULER 1953, KENICHIRO and AYAKO 1991, MPAWENAYO 1996, HODGSON *et al.* 1997, COCQUYT 1998, STOERMER *et al.* 1999). On the other hand, according to LANGE-BERTALOT

(2001) the only certain recent locality is the geologically very old Lake Aokiko in Japan, whereas specimens from other localities appear to be similar, but also show some differences and "certainly or probably do not belong to this taxon". The uncertainty of the status of *Navicula hasta* was taken into account by LANGE-BERTALOT (2001).

The problem of *Navicula hasta* identity extends back to the early 20th century. This has been caused by some LM observations in which *N. hasta* was identified in Central Europe (*e.g.* HUSTEDT 1930). Recognising taxonomic problems with *N. hasta*, recently LANGE-BERTALOT (2001) was able to differentiate a number of morphological entities from Europe including description of a few novel taxa. One of the newly described species, *Navicula rakowskae* LANGE-BERTALOT (2001) and the long established *N. hasta* show a certain extent of morphological similarity under the light microscope. The length, width and arrangement of transapical striae are similar for both species and partially overlap. The separating feature for both taxa was the density of the lineolae (LANGE-BERTALOT 2001).

The aim of our study was a comparison of other ultrastructural features of both species *i.e. Navicula hasta* and *N. rakowskae*.

MATERIALS AND METHODS

The type material of *N. hasta* deposited in the Pantocsek collection was re-investigated. The small portion of the type material from Bodos was treated with distilled water and mounted in Naphrax. The recent material containing *N. rakowskae* from the Krakowsko-Częstochowska Upland (Poland) was collected from springs and their outlets (Kobylanka stream, Krztynia river and Wiercica river). Water temperature was 6.2–9.0 °C, conductivity was moderate, generally 240–480 μScm^{-1}, and the pH values usually varied from 6.1 to 8.0.

The recent samples were treated with HCl, washed several times with distilled water, and boiled in concentrated H_2O_2 with small amount of $KClO_3$ in order to remove organic matter. After washing several times with distilled water, the material was air-dried on coverglasses and mounted in Naphrax. The LM observations were made with a Nikon Optiphot microscope equipped with Nomarski interference optics and Nikon FX-35 photomicroscopy unit. SEM observations (Philips ESEM-35) were made on cleaned Au-sputtered material.

Data on the distribution of the species were obtained from various published sources including: monographs, papers and *Iconotheca of Algae* of the Department of Phycology, W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

The material studied is deposited in the collection of the Department of Phycology, W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków and the Department of Botany, Natural History Museum, Budapest.

RESULTS

Based on examination of a portion of the type material from Bodos and material collected in karstic springs of the Krakowsko-Częstochowska highland a morphological comparison of *Navicula hasta* and *N. rakowskae* was made.

The specimens observed from Bodos type material (*e.g.* Figs 1–3) conform to *N. hasta* as illustrated by LANGE-BERTALOT (2001), but they differ from the holotype material (Fig. 4).

Navicula hasta Pantocsek (Bodos material) (Figs 1–12)

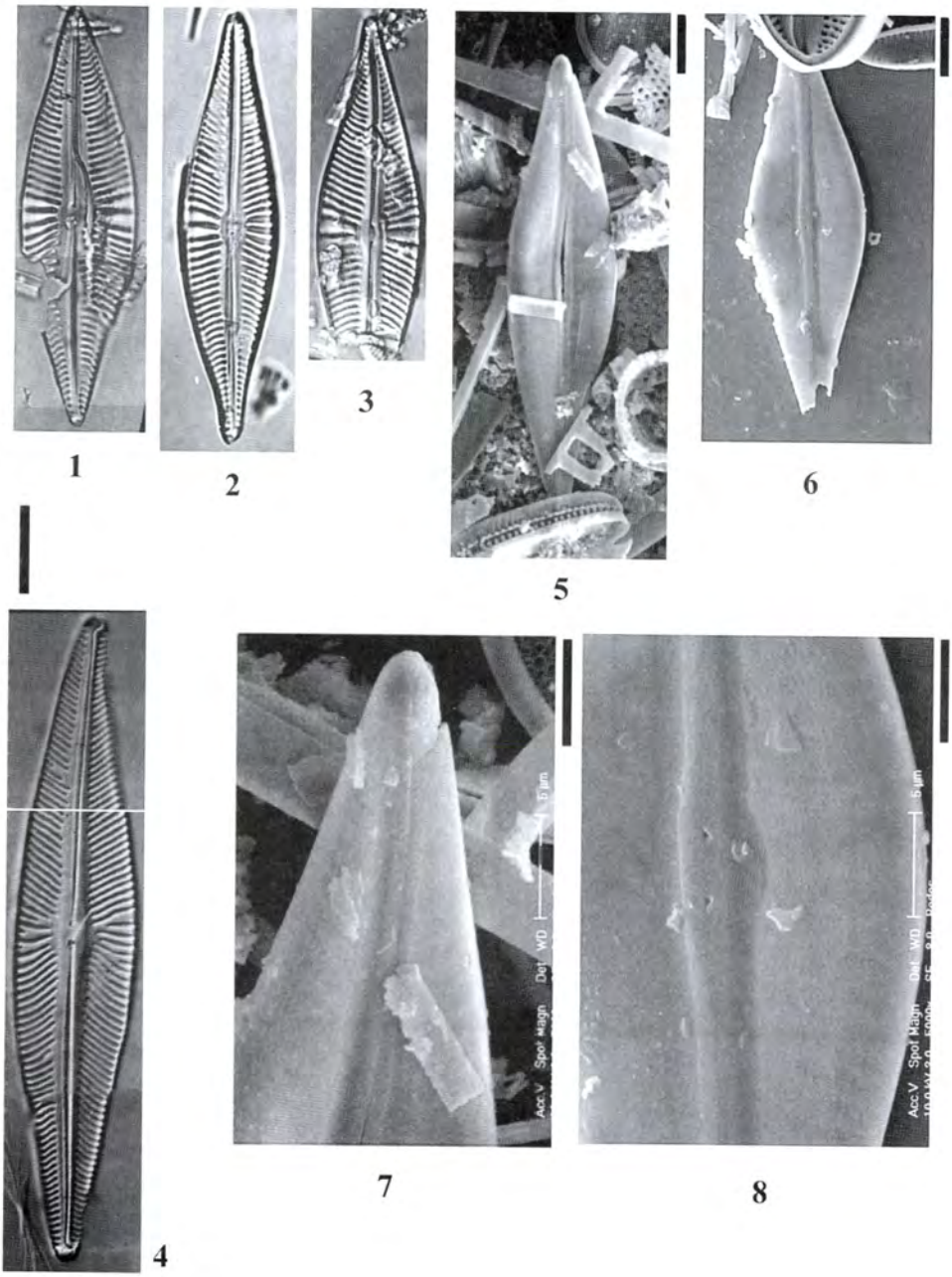
Light microscopic observations. Valves rhombic-lanceolate with cuneate apices, 76.8–98 μm long, 16.8–21.6 μm wide. Raphe undulate, thread-like with sickle-like apical external endings. The central external endings slightly expanded and deflected towards secondary side. Axial area moderately broad, parallel, slightly expanding towards central area. Central area small, elliptical, due to gradually shortened middle transapical striae (Figs 1–4). Striae radiate throughout, lineate, 6.2–7.5 in 10 μm. The lineolae parallel to valve margin *ca* 24 in 10 μm.

Electron microscopic observations. External valve view (Figs 5–8): valve face bilaterally curving into raphe sternum and mantle. Raphe sternum raised above valve surface due to shallow depressions running at its both sides (Fig. 8). External central raphe endings expanded, deflected to the secondary valve side. External apical raphe endings slightly hooked. Internal valve view (Figs 9–12): raphe-sternum thickened, internal fissures open laterally, except at centre and poles. Raphe terminates at the apices in a simple helictoglossa (Figs 11–22).

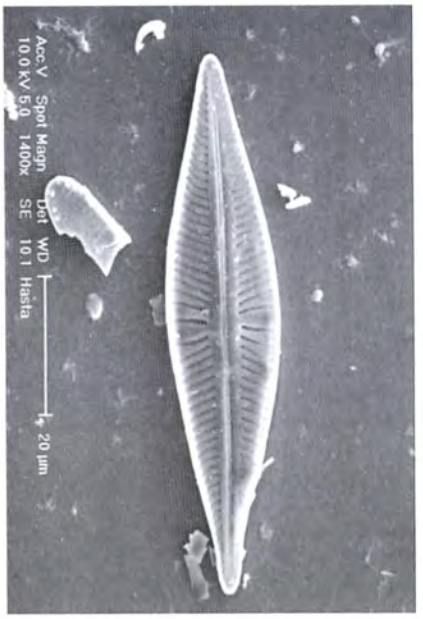
Distribution. A survey of the published sources revealed that *Navicula hasta* (conforming to Bodos population) has been reported mainly from fossil material from Europe (SCHULZ 1926, HAJÓS 1976, GLESER *et al.* 1974, KHURSEVICH and GORJUNOVA 1986, WITKOWSKI *et al.* 2001), Asia (SKVORTZOW 1937, OKUNO 1952, HIRANO 1979), and from Africa (GASSE 1980).

Navicula rakowskae Lange-Bertalot (Figs 13–21)

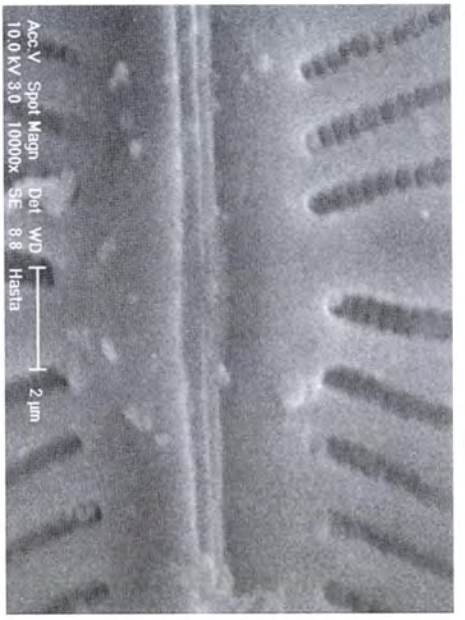
Light microscopic observations. Valves narrow lanceolate with cuneate apices, 69–94.3 μm long, 11.5–14.0 μm wide. Raphe undulate, thread-like with



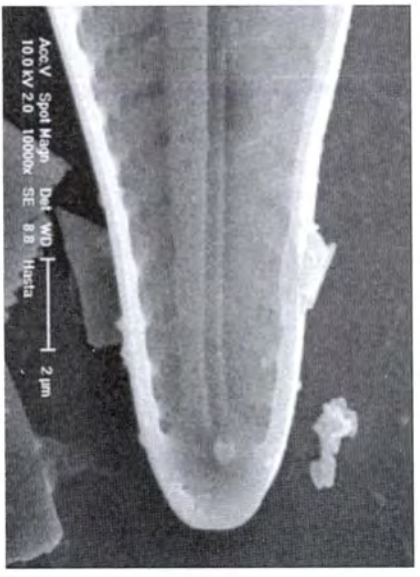
Figs 1–8. *Navicula hasta* Pantocsek. 1–3 = LM, Bodos material; 4 = LM, holotype; 5–8 = SEM, external view of the valve.



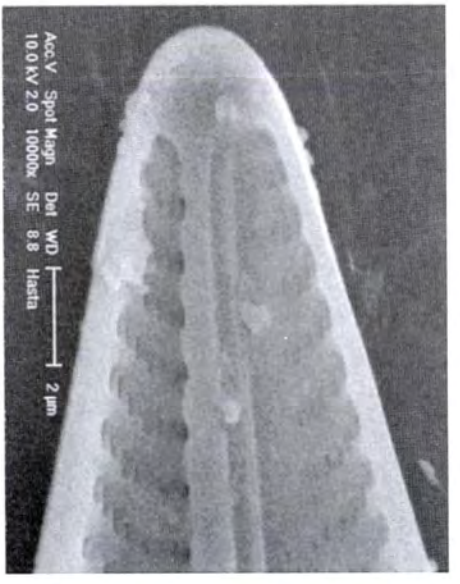
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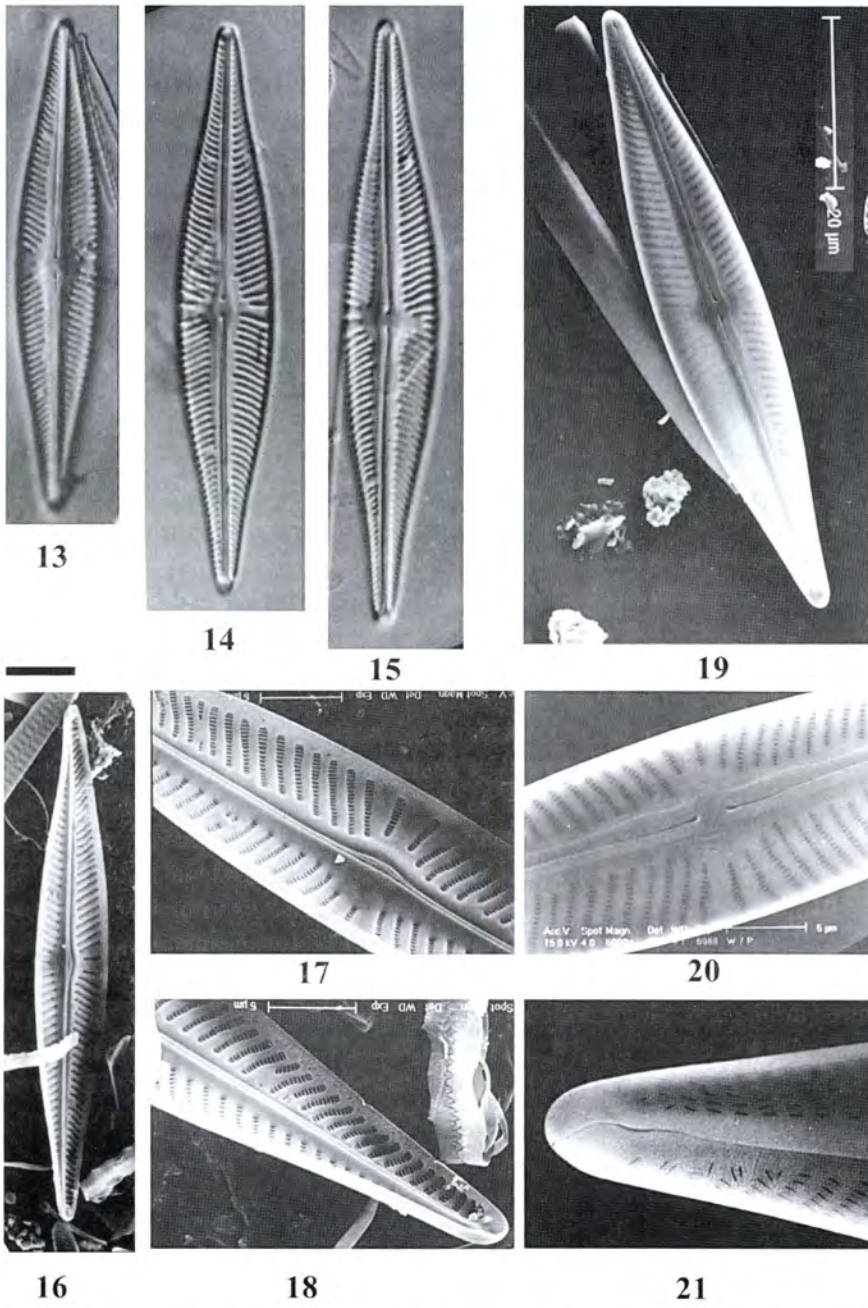


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12

Figs 9–12. *Navicula hasta* Pantocsek. 9–12 = SEM, internal view of the valve.



Figs 13–21. *Navicula rakowskae* Lange-Bertalot. 13–15 = LM; 16–18 = SEM, internal view of the valve; 19–21 = SEM, external view of valve. (Scale bars: Figs 1–6, 13–16 = 10 µm; Figs 7–8, 17–18, 20 = 5 µm; Figs 9, 19 = 20 µm; Figs 10–12, 21 = 2 µm).

sickle-shaped external apical endings. External central endings pore-like, deflected to the secondary valve side. Axial area moderately broad, lanceolate, widening towards central area. Central area small, elliptical, due to the gradually shortened middle transapical striae. Some striae near the apices are interrupted by hyaline markings (Figs 13, 15). Transapical striae radiate throughout, 7.5–12 in 10 μm. Lineolae *ca* 28–30 in 10 μm.

Electron microscopic observations. External valve view (Figs 19–21): valve face almost flat. Raphe sternum raised above the valve surface, bordered by shallow depressions occurring at both sides. The surface not broader around the central nodules. External central raphe endings expanded into slightly crooked pores, deflected to the secondary valve side. Terminal raphe endings hooked (Fig. 21). Lineolae (30–36 in 10 mm) parallel to valve margin throughout the valve, except apices, where are parallel to the transapical axe (Fig. 20). Internal valve view (Figs 16–18): raphe sternum thickened, internal fissures open laterally, except at centre and apices. Internal central raphe endings simple at apices terminating in a simple helictoglossa.

Distribution. This species is not rare in oligo-mesotrophic, oligo-mesosaprobic calcareous waters of northern part of Kraków-Częstochowska Upland, especially on the surface of sand in slowly flowing water. In the southern part of the Upland (waters of higher trophy and saprobity) it occurs rarely.

DISCUSSION

Detailed investigations by LANGE-BERTALOT (2001) have shown that *N. hasta* is a complex of species, differing in their valve morphology. According to LANGE-BERTALOT (2001) the population of *Navicula hasta* in the fossil type material presumably represents a "transient stage" between diatoms belonging to a very small subsection *Navisantiqua* (*N. perturbata* Jurlij, *N. haueri* Grunow, and *N. lacusbaicali* Skvortzow et Meyer) on the one hand, and the taxa in the section of *Navicula* on the other. At present, some of the forms related to *Navicula hasta* are known only from fossil, Tertiary material (e.g. *Navicula haueri* Grunow, *N. gurovii* Pantocsek, and *N. turris* Hustedt). Other taxa allied to *N. hasta*, such as *N. hastatula* Lange-Bertalot et Miho, *N. lacusbaicali* Skvortzow et Meyer, *N. parahasta* Lange-Bertalot et Miho, *N. perturbata* Jurilj, with the sole exception of *N. rakowskae*, occur in lacustrine habitats of geologically old lakes (such as Lake Baikal and Lake Ochrid).

The primary aim of our work was the comparison of two similar, probably related taxa. It appeared very quickly that the concept of *Navicula hasta* is uncertain,

Table 1. Morphological features of frustules of *Navicula hasta* and *N. rakowskae*.

	Navicula hasta				*Navicula rakowskae*	
	PANTOCSEK (1903) protologue	holotype (designated by KRENNER)	LANGE-BERTALOT (2001) (Köpecz)	this study (Bodos)	LANGE-BERTALOT (2001) protologue	this study (Kraków-Częstochowska Upland)
valve shape	lanceolate with obtusely rounded apices	lanceolate	strictly lanceolate, apices somewhat obtusely to almost acutely rounded	rhombic-lanceolate with cuneate apices	narrow lanceolate to almost linear lanceolate, tapering to the finally somewhat indistinctly protracted rather acutely than obtusely rounded apices	narrow lanceolate with cuneate ends
length	63–76 µm	123 µm	60–90 (or more than 100?) µm	76.8–98 µm	80–100 µm	69–94.3 µm
breadth	14–18 µm	22 µm	15–19 (or up to 25) µm	16.8–21.6 µm	12.5–16 µm	11.5–14.0 µm
pattern of striation	radiate	radiate	radiate throughout or the most distal pair parallel to convergent	striae radiate throughout	striae rather strongly radiate throughout except the last pair, or only a single one in subpolar position becoming parallel to slightly convergent	striae radiate throughout the valve except only in subpolar region becoming parallel
striae density	8–10 in 10 µm	7.5 in 10 µm	8–10 in 10 µm	6.2–7.5 in 10 µm	8–10 in 10 µm	7.5–12 in 10 µm
lineolae density		27 in 10 µm	constantly comparatively coarse, *ca* 26 in 10 µm	*ca* 24 in 10 µm	30–31 in 10 µm	*ca* 28–36 in 10 µm

Table 1 (continued)

	Navicula hasta			N. rakowskae	
central area	small, elliptic	irregularly widened more or less transapically	small, elliptic due to the shortened striae	variable outline expanded more or less transapically	distinct, irregular, formed by gradually or irregulary shortened middle striae
arrangement of striae in the middle part of the valve	"ad centrum longiores et reviores" the central area formed by longer and shorter striae	central area formed by alternately longer and shorter striae more distantly spaced	formed by shortened alternately striae irregularly	striae more distantly spaced	striae more distantly spaced

and should be re-investigated. In this case all documented reports (or materials deposited in herbaria) should be compared with lectotypified material. The reliability of other reports is very limited.

Comparing the material studied with data from the literature we observed a morphological similarity of the population *of N. hasta* from Bodos and the population from Köpecz, which has been illustrated by LANGE-BERTALOT (2001). These populations are similar in respect of valve outline, arrangement of striae, and other ultrastructural features, and partially overlap the original diagnosis by Pantocsek (Table 1). However, some of the specimens observed by us were of larger size and had a lower number of striae in 10 μm.

It is also uncertain if the known holotype was originally designated by Pantocsek (KRENNER 1980). The specimen known as the holotype differs from our material, Pantocsek's description, and data in the literature (Table 1, Figs 1–4), and its value as the holotype is questionable. Despite an extensive search of Bodos material we have not found any specimen that more closely matches the holotype. Because the present concept of *N. hasta* is unclear (Pantocsek cited in his protologue illustrations of two specimens (syntypes), which do not conform with the holotype specimen. Therefore in our

opinion, designation of a neotype or at least lectotypification of *N. hasta* should be made.

The detailed LM and SEM studies of valve morphology of *N. hasta* (from Bodos) and *N. rakowskae* under the light microscope and scanning electron microscope showed that the similarities between these two species are mainly in length, width and raphe structure. Other features *e.g.* valve outline, striae density, and their density, shape of axial area, structure of the external valve surface are different. The study confirms also differences in density of lineolae between *N. hasta* and *N. rakowskae*, stated by Lange-Bertalot as separating criteria. On the other hand *N. rakowskae* should be compared with the recently lectotypified *N. hasta* var. *gracilis* Skvortzow (OHTSUKA and TUJI 2002), known from recent material from Japan.

In the course of investigating the distribution of *N. hasta* we have found that numerous documented reports actually relate to other taxa of the genus *Navicula*. In Poland a description of *Navicula hasta* was published in Polish by SIEMIŃSKA in 1963. Since then, the taxon has been reported from a few scattered localities as recent species (*e.g.* CHUDYBA 1979, KADŁUBOWSKA 1985, KALINOWSKA-KUCHARSKA and LIGOWSKI 1976, OLEJNICZAK and SZELĄG 1979, KALINOWSKA-KUCHARSKA 1984), but these reports were not supported and validated by any iconographic documentation. Of eight reports from Poland (from the Tatra Mountains, KAWECKA 1965, 1966) only two presumably concern *Navicula hasta* sensu lato. The observed specimens were similar to *N. hasta* documented by CLEVE-EULER in 1953 (pp. 135–136, fig. 774c), which according to LANGE-BERTALOT (2001) conforms to *N. hasta* covered by the protologue. The population of *N. hasta* reported by RAKOWSKA (1996) is at present separated as *N. rakowskae*.

The results confirm significant morphological differences between *N. hasta* (sensu LANGE-BERTALOT 2001) and *N. rakowskae*.

A revision of *Navicula hasta* should be made in the future. At that time additional information may become available for determining the validity of records in the literature. The authors are interested in recovering additional reports and comments for future use.

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