

**On the synonymy of *Nitzschia frustulum* var.  
*subsalina*, *N. inconspicua* and *N. boliviana* sensu  
Trobaño et al. 2013**

***Sobre la sinonimia de Nitzschia frustulum* var. *subsalina*, *N.*  
*inconspicua* and *N. boliviana* sensu *Trobaño et al. 2013***

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In 2013, Trobaño and collaborators published an important contribution to the clarification of the taxonomy of some taxa of *Nitzschia* Hassall belonging to the Section Lanceolatae. This constitutes a group of relatively small species and infraspecific taxa that are common in freshwater samples and that are also commonly misidentified in routine ecological and floristic works. The importance of the Trobaño *et al.* paper is exacerbated by the inclusion of light (LM) and electron microscopy (SEM) data on type material of *Nitzschia frustulum* var. *subsalina* Hustedt, *N. inconspicua* Grunow and *N. boliviana* E.Morales & Vis (among others). Revision of type material is a necessary step in the clear definition of taxonomic and ecological boundaries, information that can be later used to define, for example, biogeographical distributions and usefulness of taxa in bioindication [11][14][15].

After comparison of LM and SEM images of *N. frustulum* var. *subsalina*, *N. inconspicua* and *N. boliviana*, Trobaño *et al.* [23] concluded that the three were conspecific and that they should be put in synonymy under *N. inconspicua* as the oldest validly published name. There are, however, some differences in the type population of *N. boliviana*, originally described from the Yungas of La Paz, Bolivia [13], which were not taken into account by Trobaño and collaborators. In the present manuscript, I discuss these differences in support of a separation of *N. boliviana* from other species in the Section Lanceolatae.

Table 1 shows a comparison among the taxa treated here. Data in this table were gathered from, and are circumscribed to, type material presented in Trobaño *et al.* [23] and Morales & Vis [13]. Before comparisons are made, it is convenient to first delimit well the range of variability of *N. boliviana* in type material. In plate 2 of

the supplementary information, Trobajo *et al.* [23] present a size diminution series for *N. boliviana* from type material that is incorrect. The first and largest valve on the right of the upper row clearly does not belong to *N. boliviana* and does not appear to have been considered during calculations of size ranges (see their table 1). The more acutely-ended apices and the features of the raphe canal and fibulae in this specimen are different from the rest of the members in the mentioned series.

All characters considered in Table 1 are relevant in taxonomic treatments of *Nitzschia* spp. as shown by Mann [10]. The three taxa cannot be entirely separated based on valve measurements since there are overlaps in ranges for length, width, and density of stria and fibulae. Following Geissler [3][4], Wendker & Geissler [25], Trobajo *et al.* [20] and Trobajo & Cox [21], these features are unreliable for defining affinities or taxonomy within *Nitzschia* since such features vary with environmental conditions such as salinity, turbulence and nutrient concentrations.

Looking at other features in Table 1, however, it is possible to find differences in *N. boliviana* when compared with the other two taxa. First, shape of valve is never linear and its apices are characteristically concave on the secondary side of the valve, a feature that is maintained even in small valves (figs 260-264 in Morales & Vis [13]; figs 15-17, and plate 2, figs 2-20 in Trobajo *et al.* [23]). At the central area of the valve there is no constriction on the primary side, the proximal terminations of the raphe are both deflected towards the abvalvar side of the mantle and there is no external depression (figs 271-276 in Morales & Vis [13]; figs 80-83 and plate 2, figs 21-22 in Trobajo *et al.* [23]). From the same figures it can be seen that the external surface of the valves is flat, while in internal view the raised costae are clearly observable. Also, it is observed that the velum is placed in a very shallow position with respect to the valve external surface. The fibulae are biconcave with extensions onto valve face and mantle that can be bi or tripartite. The interfibular spaces are square and the central area is obvious only in some valves (figs 260-264 in Morales & Vis [13]; figs 15-17 and plate 2, figs 2-20 in Trobajo *et al.* [23]). Finally, the second girdle band does not possess a ligula (fig. 274 in Morales & Vis [13]; fig. 82 in Trobajo *et al.* [23]).

Some of the above features are different from those of *N. inconspicua* or *N. frustulum* var. *subsalina*, and some others are different from those in both taxa. From Table 1, it is also clear that *N. frustulum* var. *subsalina* and *N. inconspicua* have differences between them, raising questions about their conspecificity.

**Table 1:** Comparison of selected features of the three species of *Nitzschia* discussed in this manuscript. Morphometric values are kept as they appeared in Morales & Vis [24] and Trobajo *et al.* [24].

Character	<i>N. frustulum</i> var. <i>subsalina</i>	<i>N. inconspicua</i>	<i>N. boliviana</i>
<b>Shape of valve</b>	Linear-lanceolate with cuneate ends becoming elliptic in small valves. Small central constriction on side with fibular canal	Linear-lanceolate with cuneate ends becoming elliptic with sharp ends in small valves. Central constriction on primary side in larger valves	Lanceolate with subrostrate ends, concave on secondary side, even in small valves. No central constriction
<b>Valve measurements</b>	Length: 8-16.8 Width: 2.4-2.9 Stria density: 25.1-27.8/10µm Fibulae: 10.1-15/10µm	Length: 6-11.5 Width: 2.6-3.1 Stria density: 23.7-28.7/10µm Fibulae: 10.6-17/10µm	Length: 6-9.6 Width: 2.4-2.8 Stria density: 27.5-30/10µm Fibulae: 12.5-15/10µm
<b>Valve face surface</b>	External: sometimes with raised costae Internal: Conspicuously raised costae	External: sometimes with raised costae Internal: slightly raised costae	External: completely flat Internal: Conspicuously raised costae
<b>Areolae/Velum</b>	Shallow/Central	Deep/Internal	Shallow/Central
<b>Proximal raphe ends</b>	Straight, not depressed	Straight or slightly deviated in same direction toward valve face and slightly depressed	Deviated in same direction toward abvalvar side of mantle, not depressed
<b>Fibulae</b>	X-shaped with bipartite extensions onto valve face and mantle; square to round interfibular spaces; central area conspicuous	Biconcave with extremes fused onto valve face and mantle so extensions are difficult to see; round interfibular spaces; central area conspicuous	Biconcave with bi to tripartite extensions onto valve face and mantle; square interfibular spaces; central area obvious only in some valves
<b>Girdle bands</b>	Not shown	Second band ligulated	Second band non-ligulated

The features included in Table 1 and used to differentiate the three taxa included in this discussion are rather stable as shown by micrographs of the type populations. This stability allows the conclusion that the three populations can indeed be separated into three discriminate sets. In the absence of culturing and molecular data, something can still be said regarding the reliability of these distinguishing features. Some features of the raphe, central nodule, girdle bands, position of velum, shape of fibulae and distance between fibulae at the central area have been used to delimit species boundaries. Shape of valves and constrictions at the central area could be variable, but are often used as support characters to define species.

Though the distal ends of the raphe in nitzschioids has been shown to vary from valve to valve during formation of the frustule (e.g., Pickett-Heaps [16]), the central raphe endings have been little explored as a contributing feature in species delimitation. Mann [10] argued that presence or absence of a central raphe termination is questionable as a character defining species (see also discussion in Trobajo & Cox, [21]), but the deflection of such terminations (when they exist) and the presence of depressions at the valve central nodule have not been clearly studied in culture or from natural populations. The type materials of *N. frustulum* var. *subsalina*, *N. inconspicua* and *Nitzschia boliviana* show no variation regarding deflection or lack thereof and presence/absence of depressions. Therefore, until shown otherwise, they should be regarded as differentiating features at the species level. Other authors have made similar observations, though not on type material [5][9][6][25], while Trobajo & Cox [21] suggested that further studies are needed to show variability of these features.

The position of velum is one of the least discussed features in relation to genetic or environmentally-related variability. However, a review of the type material of several species of the Section Lanceolatae shows that this feature does not vary at the population level (e.g., Trobajo & Cox [21], Tudesque *et al.* [24] and Trobajo *et al.* [23]). Alakananda *et al.* [1] used this feature in combination with others and argued that they are useful to separate species within the Section, which is also the case for the three taxa analyzed here. At the moment, it is unclear if the stability in the position of the velum shown in type materials should be extrapolated to all species within the Section. This is because some morphological variants such as “type 2” found in type material of *N. abbreviata* Hustedt ex Simonsen and *N. imisitata* Hustedt exhibit a high variability in pore shape, and probably also in velum features and position, though this has not been shown [22] [23].

The features of girdle bands, except for their closed/open nature, and the occurrence and disposition of perforations, have also been little explored in the literature. Girdle bands of two of the three type populations discussed here were illustrated, and one (*N. inconspicua*) is shown to have ligulated copulae (fig. 79 in

Trobajo *et al.* [23]), while *N. boliviana* lacks this structure. Obviously, observations of girdle elements of one or two specimens are insufficient to derive hard conclusions, yet the difference illustrated by Trobajo *et al.* [23] is worth exploring further.

The shape of fibulae and interfibular spaces have been long used as a feature to distinguish species even under LM. As stated by Denys & Lange-Bertalot [2], extensions of fibulae associated to costae and striae can be useful not only for identification, but also for circumscription of morphologically closely related taxa (see also Alakananda *et al.* [1]). Especially at the LM level, the shape of the fibulae can be a helpful distinguishing character when used in association with other features such as those discussed here [12] [19] and has even been suggested to indicate evolutionary patterns in certain nitzschioid groups [7]. The distance between median fibulae (the ones flanking the central nodule) have been shown not to vary significantly with environmental conditions and are considered stable characters at the population level [20]. In the type materials presented by Trobajo *et al.* [23], variability in the shape and distance of the fibulae can be seen at the individual level, but at the population level the variability is different for each of the three taxa in question.

Examination of type material of several infrageneric taxa in *Nitzschia*, including those in the Section Lanceolatae, showed that valve shape is a reliable character to distinguish taxa at the species level (e.g., Trobajo & Cox [21], Tudesque *et al.* [24], Hlúbikova [8] and Trobajo *et al.* [23]). Some variation exists from one individual valve to another, but this is the expected variability found in most diatom populations (Pickett-Heaps *et al.* [17]). Valve shape in populations of *N. frustulum* var. *subsalina*, *N. inconspicua* and *N. boliviana* found in type material are rather stable as shown in LM and SEM illustrations presented in Morales & Vis [13] and Trobajo *et al.* [23], therefore, the comparison shown in Table 1 is reliable and can be used in combination with the other features discussed here to differentiate each of the three discussed taxa.

Culture experiments have shown that valve shape is variable under different conditions in *N. frustulum* (Kützing) Grunow, and thus, it is an unreliable feature for identification or species boundary delimitation. A more recent molecular study showed that valve outline could not give a clear differentiation of clades or genotypes in the *N. inconspicua* complex (Rovira *et al.* [18]; where –as per the analysis implemented here–, an even wider morphological variability than that presented in Trobajo *et al.* [23] is shown). Here, I show that valve outline can be used in combination with other features to establish discrete morphological variants, which up to this date, and in the absence of clear genetic information, can be considered as different morphological taxa.

Mann [10] warned about the cons of a “sparingly-divided classification”. Therefore, until we do not have a clear picture of the limits among taxa at the ecological and molecular level, it is convenient to adopt a splitter approach, especially taking into account that differing morphologies could be indicative of given environmental conditions (i.e., the result of natural selection) and that responses to those conditions might be taxa or even clone-specific [21][18]. Moreover, Rovira *et al.* [18] argue based *rbcL* and LSU genetic distances that the *N. inconspicua* complex could contain several cryptic species. The splitting of apparently cryptic morphologies based on a joint analysis of several qualitative characters, such as the analysis done here, provides a strong hypothesis to be tested in the molecular arena; a hypothesis drawn on morphological characters that go beyond the simple reductionist morphometry.

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