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A new species of *Punctastriata* (Bacillariophyta, Fragilariophyceae) from temporary streams in southern Portugal

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The genus *Punctastriata* D.M.Williams & Round (1988: 278) contains species that are difficult to identify under light microscopy (LM) (Wetzel & Ector 2021). Their striae are composed of several rows of minute areolae that open interiorly into a single depression. It is the shadow created by this depression under LM that closely resembles the striae in species of *Staurosirella* D.M.Williams & Round (1988: 274); striae that are often said to have a punch hole appearance (Morales & Manoylov 2006). Thus, scanning electron microscopy (SEM) is often required to confirm the presence of a species of *Punctastriata* in a sample.

Besides the typical multiseriate striae, produced by the formation of multiple viminules in the entire striae (both of these are the defining feature of the genus), the species share all the remaining features with other small araphid genera lacking a rimoportula, such as the already mentioned *Staurosirella*, but also *Nanofrustulum* Round, Hallstainsen & Paasche (1999: 345), *Opephora* P.Petit (1888: 130–131), *Pseudostaurosira* D.M.Williams & Round (1988: 276), *Pseudostaurosiropsis* E.Morales (2001: 116), *Sarcophagodes* E.Morales (2002: 111), *Stauroforma* R.J.Flower, Jones & Round (1996: 53), and *Staurosira* Ehrenberg (1843: 45) (see table 1 in Morales *et al*. 2019).

To date, nine species have been included in *Punctastriata* (see table 1 in Wetzel & Ector 2021). These have been reported from around the globe, but by our own finding, it appears that new species can still be found, even in areas that have previously received attention. This is the case of temporary streams in southern Portugal, ecosystems with dynamic benthic communities, even in the dry period, when diatoms persist in the stream beds in reduced, shallow pools or as dry biofilms (Novais *et al*. 2020).

New species description

Punctastriata obstinata **E.Morales, M.L.García & Novais *sp. nov*. (Figs 1–20 LM, 21–26 SEM)**

Frustules rectangular in girdle view (Figs 19, 20, 23), joined together by interlocking spines (Figs 19, 20; chains of up to 4 cells were seen in treated samples). Valves broadly elliptical to faintly ovoid, slightly heteropolar (Figs 1–18). Broadly rounded head pole (e.g. Fig. 2), slightly pointier foot pole (e.g. Fig. 6). Length 4.5–6.6 µm, width 3.7–4.8 µm, striae 11–13 in 10 µm. Axial area narrow, zig-zag shape (Figs 21, 22), externally depressed with respect to virgae (Figs 23, 24). Internally, axial area at the same level as raised costae (Fig. 22). Striae with 3–6 rows of elliptical to round areolae (Figs 21–25). Striae with stretched ovate shape, the longest and narrower extreme on valve face (Fig. 21). All areolae open into single internal depression running from valve face to mantle (Figs 22, 25). Virga flared on both extremes of striae, narrower than striae (Figs 21–24). Vimines slender and short (Figs 21, 23, 24). Viminules present in entire stria (Figs 21, 23, 24). Well-developed volae, arising from the areolar inner periphery and projecting inwards, with mineral depositions (Figs 23, 24, 25). Spines originating from virgae at the valve face/mantle junction; solid, with elliptical base of about the same width of the vimines they sit on. Spine body flattened and retorted (concave) on its back (if frontal part facing the valve face) and having a flared, skirt-like projection, sometimes surrounding the entire base of the spine (black arrows in Figs 23, 24). Spine tip bi- or

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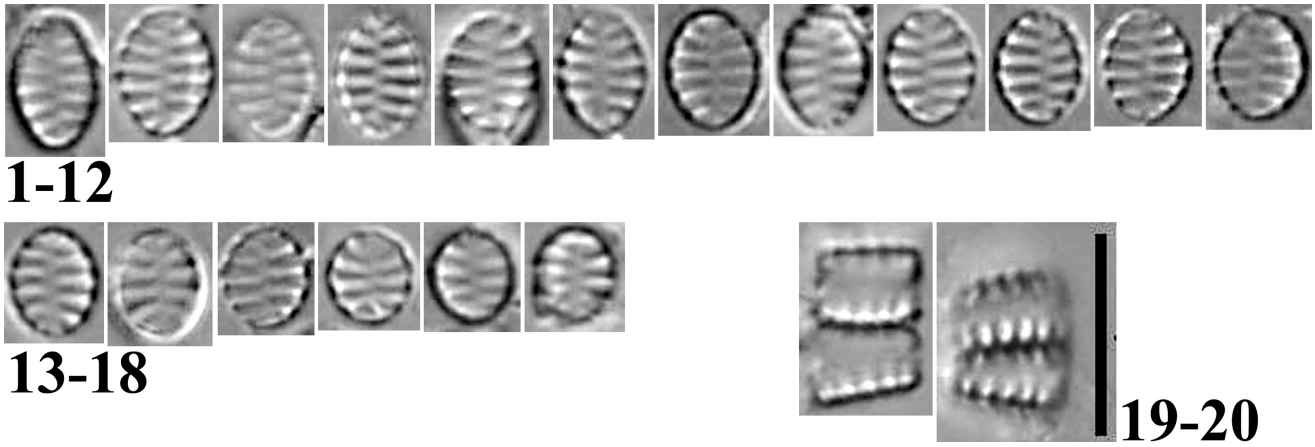
trifurcate (Figs 21, 23, 24). Second row of spines often present on valve mantle and of similar characteristics to the first row, but tend to have a conical body and originate from virgae or from vimines (white arrows in Figs 21, 23). Stipules absent. Apical pore fields present on both extremes of valves, but smaller at head pole (dashed arrows in Figs 22, 26), composed of several pores that open into a single elliptical to rounded internal depression (Fig. 26). Girdle elements variable in number, open (white arrow heads in Figs 21, 24), lacking pores, with larger valvocopula (Figs 21, 23, 24).



FIGURES 1–20. LM images of *Punctastriata obstinata sp. nov*. from type material BR-4654, Oeiras Stream, Portugal. 1–18. Valve views arranged in a size diminution series, notice the overall elliptic to slightly ovate shape and the slightly pointy foot pole seen in some valves; Fig. 2 is the holotype. 19–20. Girdle view of short chains. Scale bar: 10 µm (Fig. 20).

Type:—PORTUGAL. Alentejo region, Almodôvar Council: Oeiras Stream, sampling point in the stream, 37º 32' 51.45" N, 7º 59' 45.01" W, *M.H.Novais, 19th September 2017* (holotype BR-4654= Fig. 2).

Etymology:—The epithet "*obstinata*" refers to the harsh conditions that this and other taxa are able to withstand in temporary streams. These resilient communities thrive even in the face of completely dry conditions.

Ecology and distribution:—The new species was found in a epilithon sample scrubbed from 5 hand-sized rocks, from a pool with no stream flow during summer, the dry season in southern Portugal (for collection methods see Novais *et al.* 2020). The stony stream bed (boulders 70 %, cobbles 30 %) was 90 % dry, and rocks had filamentous algal growths. The pool size was 9×16 m and its depth 0.24 m, turbidity 18.25 NTU, and it was less than 20 % shaded. Water temperature was 18.3 ºC (air temperature was 27.2 ºC at 1:00 PM), pH 9.5, electrical conductivity 5059.5 $\mu S \cdot cm^{-1}$, dissolved oxygen 36.8% sat., phosphates 0.23 $mg \cdot L^{-1}$, and nitrates 27.20 $mg \cdot L^{-1}$.

Major impacts on the stream (besides the seasonality of precipitation being higher during winter and causing a high degree of erosion and sediment transport, filling the ca. 20 m-wide stream bed from side to side and ca. 2 m above the bottom) are cattle raising and agriculture in the contiguous area. Also the stream is visited by Iberian black pigs. The land use within 5 m of the bank tops is characterized by the presence of broad leaf/mixed woodland (semi-natural), scrub and shrubs and rough unimproved grassland/pasture.

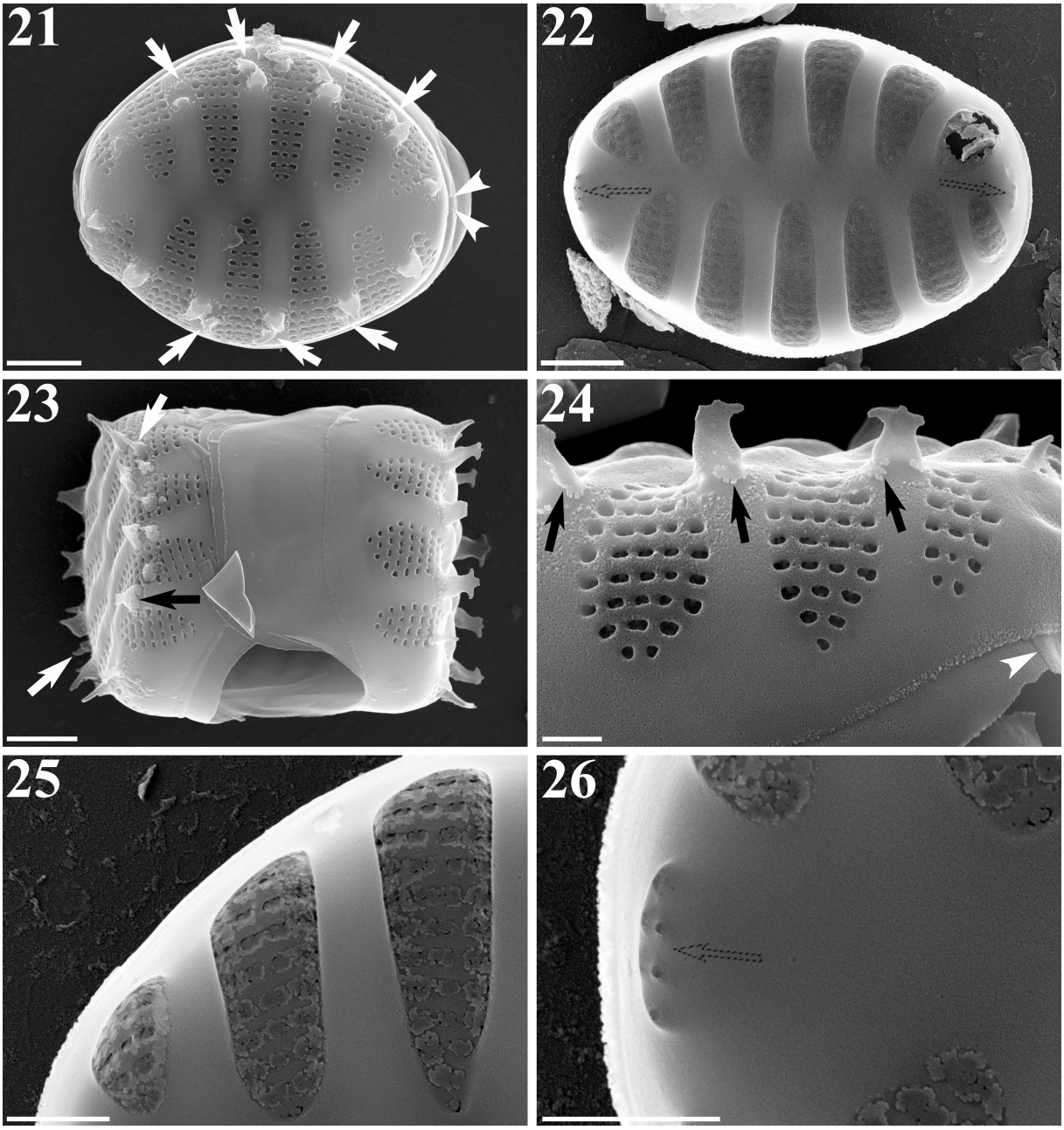
The new species was also found, but in low abundance, in the Limas Stream (Serpa Council, 37º 49' 19.91" N, 7º 37' 21.18" W, collected 20th September, 2017. Pool 0.3 m deep, temperature 33.8 ºC (ca. 12:00 am), pH 8.8, electrical conductivity 752.5 $\mu S \cdot cm^{-1}$, dissolved oxygen 79.8 % sat., phosphates 0.16 $mg \cdot L^{-1}$, and nitrates 1.92 $mg \cdot L^{-1}$).

Accompanying flora:—*Punctastriata obstinata sp. nov.* reached a relative abundance of 12% in the Oeiras Stream (0.8% in the Limas Stream sample) and was originally identified as *Staurosirella pinnata* (Ehrenberg) D.M.Williams & Round (1988: 274). It was accompanied by *Pseudostaurosira* sp. 1 (40.6% of relative abundance), *P. elliptica* (Schumann) Edlund, E.Morales & Spaulding (17.7%, 2006: 58), *Navicula cryptotenella* Lange-Bertalot (3.7%, In Krammer & Lange-Bertalot 1985: 62), *Pseudostaurosira* sp. 2 (3.06%), *Epithemia adnata* (Kützing) Brébisson (2.4%, 1838: 16), *Epithemia sorex* Kützing (2.2%, 1844: 33), and other species with abundances <1.5%. The undetermined species are currently under description.

Comments:—Following Table 1 in Morales *et al.* (2019), which contains the salient features of small araphid genera lacking rimoportulae, the new species fits within *Punctastriata* appropriately. Namely, the profuse production of vimines on each entire stria and the resulting multiseriate rows of areolae, are features present in *P. obstinata sp. nov*.

At the species level and comparing the features of the new taxon to those contained in table 1 in Wetzel & Ector (2021), the following characters are unique to the species. First, the new taxon often has two rows of spines, one of them located on the valve mantle. The population found in the Oeiras Stream has many representatives that lack this double row of spines or have

them weakly developed. Thus, even if the character of a double row of spines is unique to this taxon, it is inconstant. In the case of Figs 23 (valve on the right side of the image) and 24, only a single row of spines can be seen.



FIGURES 21–26. SEM images of *Punctastriata obstinata sp. nov*. from type material BR-4654, Oeiras Stream, Portugal. 21. Valve view showing second row of spines on valve mantle (white arrows) and open copula (white arrowheads). 22. Valve internal view showing apical pore fields (dashed arrows), the volae that are well-developed, and the raised axial area-virgae together with the recessed striae. 23. Girdle, tilted view of frustule showing second row of spines on mantle (white arrows), and skirt-like, flared projection of the spines (black arrow). 24. Close up on valve mantle showing spines with skirt-like, flared projections in formation (black arrows) and open copula (white arrowhead). 25. Internal view of striae showing multiseriate striae with very small areolae and volae extending inwards, accumulating mineral material. Notice the striae lies in a single internal depression running from valve face to mantle. 26. Close up on internal elliptic opening of the apical pore field (dashed arrow). Notice small round poroids. Scale bars: 0.5 µm (Figs 25, 26), 1 µm (Figs 21–24).

Incipient spines growing on the valve mantle can also be seen in *P. linearis* D.M.Williams & Round (1988: 278) (see study of material collected from the type locality in Wetzel & Ector 2021), but these seem to be elements along a disorganized, single row of spines, rather than the two distinct rows in *P. obstinata sp. nov*.

Second, the apical pore fields developing on both apices in *P. obstinata sp. nov*. are only present in *P. mimetica* E.Morales (2005: 128), but the valve shape in the latter is cruciform to rhomboid with acuminate to subrostrate apices. Therefore, for species with an elliptical to ovoid shape within *Punctastriata*, *P. obstinata sp. nov.* is the only one having well-developed

apical pore fields on both valve apices (Fig. 22). The slight heteropolarity of *P. obstinata sp. nov*. is clearly shown in the degree of development of the apical pore fields. Fig. 22 shows a smaller apical pore field on the right side of the image.

The flaring on the spines in a skirt-like fashion is a third notorious characteristic in the new species. We have refrained from using the term "stipule" for this structure since the stipule originates at the base of the spine, from its posterior side (the one facing away from the valve). In the case of *P. obstinata sp. nov.*, the skirt-like projections originate from the sides of the spine and then they envelope even part of the spine body, as can be seen in Fig. 23.

The skirt-like projections have also been observed in *P. glubokoensis* D.M.Williams, Chudaev & Gololobova (2009: 480), which are interpreted by the authors as "two small flat projections" at the base, parallel to the sternum. As far as what has been shown in the literature, the skirt-like projections are only present in the spines of *P. obstinata sp. nov*. and *P. glubokoensis*. Regarding the production of spines, the latter species infrequently produces paired spines, but they grow side by side on the virga (Williams *et al*. 2009).

Based on the observations above, it is inferred that the distinguishing features of the new species are those of apical pore fields and the double row of spines. In table 1 in Wetzel & Ector (2021), it is evident that spines are variable structures in *Punctastriata*, and that this variability includes, among others, position (on virgae, vimines, both, or on the mantle, as shown here), presence/absence of projections, structure of the body (hollow or solid, flat or cylindrical), features of the tips (spatulate, conical, bi- or trifurcate), etc. Spines, therefore, are structures worth exploring in species distinction.

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