Morphological investigation of *Eunotia bidentula* W. Smith (*Eunotiaceae, Bacillariophyceae*) including its type material*

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Eunotia bidentula W.Smith (1856: 83) was first formally described without illustration from various localities in Britain and Ireland: Braemar and Arran (Scotland), Fell-end near Lancaster (England) and Barley Lough near Glengariff, Co. Cork (Ireland). In the catalogue of Smith's slides held in BM, there is an entry for *E. bidentula*, unfortunately lacking information about its source (Smith 1859: 4). Greville had previously examined specimens from Braemar (collected by John Hutton Balfour 1808–1884), probably identical to the material Smith commented upon in his description, and gave them the name "*Eunotia camelus* Ehr. ?" adding a single drawing showing two valves, one with high and one with low undulations dorsally and a straight ventral margin (Greville 1855: 254, pl. XI: fig. 1). Greville was doubtful about his identification, but went on to say: "This diatom, in the opinion of the Rev. Professor Smith, is certainly the *E. camelus* of Ehrenberg and Kützing, notwithstanding the inaccurate figure given by the latter, in which the base of the frustule is represented as concave" (Greville 1855: 254). Smith clearly had second thoughts noting that "The figure in the 'Microgeologie,' xxxix. 15, of *E. camelus*, Ehr., shows that the present cannot be that species" (Smith 1856: 83) and thus went on to consider the specimens as a new species (type specimens of *E. camelus* are illustrated in Reichardt 1995: pl. 1: figs 1–11). The localities listed by Smith in the protologue for *E. bidentula* should formally be considered syntypes. As he specifically refers to the Braemar specimens collected by Balfour and examined by Greville in his account, this is the most appropriate choice of lectotype.

Specimens from Braemar [Walker Arnott Collection 138, part of the Van Heurck Collection, Meise Botanic Garden (**BR**), Belgium] are here illustrated in LM (Figs 1–8) and SEM (Fig. 57), and specimens from the Nant Lliwdy, a stream in Wales, from a pool in the Kintail area, Scotland, and from a rockface in the ravine of the River Devlin, Glenveagh National Park, Co. Donegal, Ireland, with samples held at the diatom collection of Amgueddfa Cymru – National Museum Wales (**NMW**), are illustrated in LM (Figs 9–35) and SEM (Figs 36–56).

* In memory of our friend and colleague Luc Ector (1962–2022).

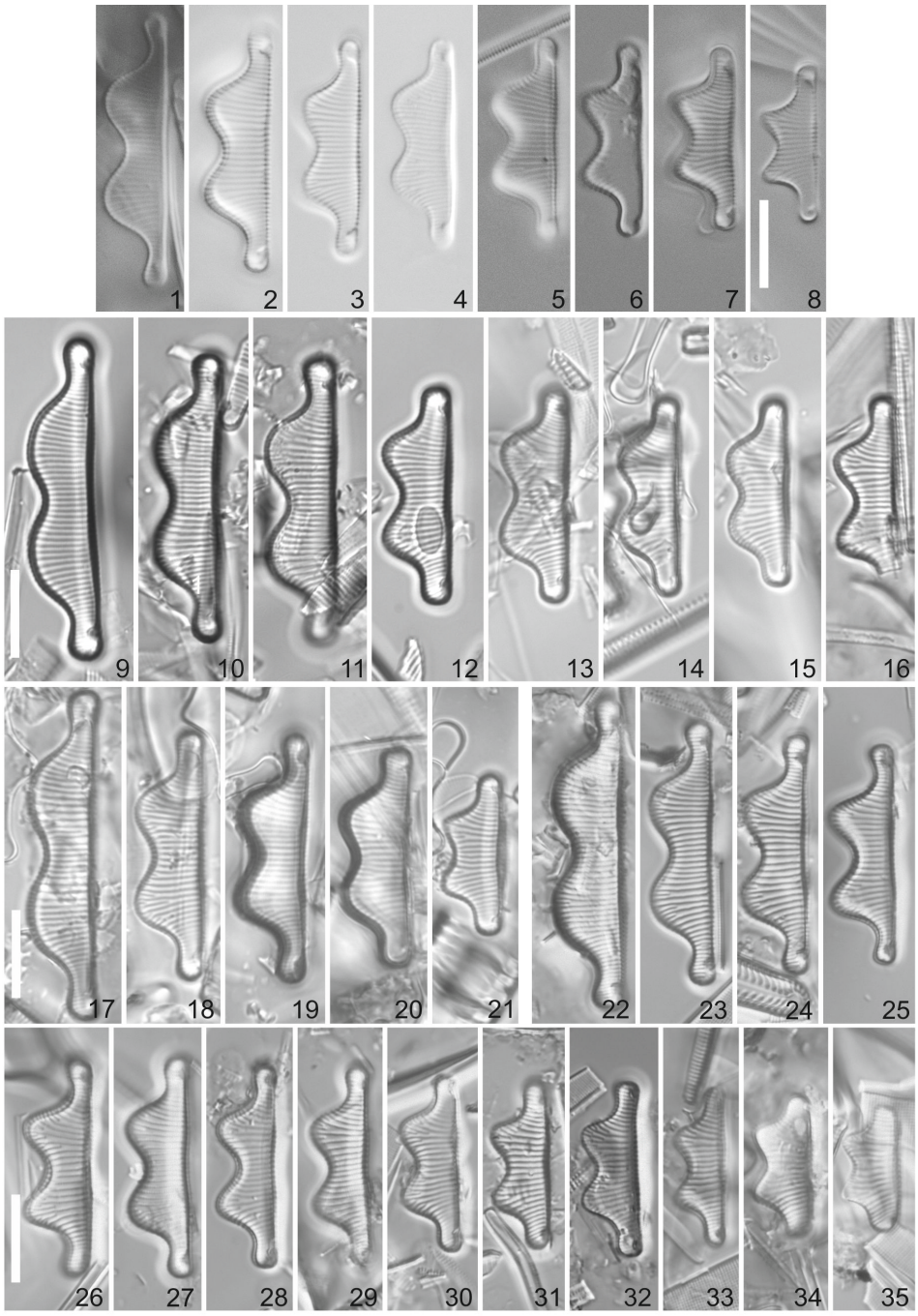
Eunotia bidentula W.Smith 1856: 83
Heterotypic synonym: *Eunotia bidentuloides* Foged, 53, pl. 8: fig. 13, 1977 (Bog near Healy Pass, Co. Kerry, Ireland).
Collection data: "Freshwater. Braemar, August 1854, *Dr. Balfour*. Fell-end near Lancaster, Aug. and Oct. 1854, *Mr. Johnson*. Arran, July 1854, *Dr. Arnott*. Barley Lough near Glengariffe [sic], Co. Cork, June 1855, *W. Sm*." (Smith 1856: 83), all syntypes; **lectotype, designated here**: "Braemer Dr Balfour", Walker Arnott 138 (**BR-4720**), Meise Botanic Garden, Belgium = Figs 1–8).

At **BM**, slides made from Smith's material, in all probability from the type locality in Scotland, are "Braemer, W.S. A 1", BM 19443 (Roper collection); "Braemer, W.S. A 1", BM 23082; Braemer, 142, BM 57262 (Wynne Baxter collection, 2633), "Braemer, Balfour 8 54", BM 523 (Greville collection); and from Lancashire, Fell End, 146, Walker-Arnott, BM 11761-2 (collection J. Deby); Fell End, Lancashire 137, Walker-Arnott, BM 11782-3 (collection J. Deby); Fell End, Lancashire, BM 57264 (Wynne Baxter collection, 2634); Fell End, Lancashire 54, Arnott, BM 526 (collection Greville). Greville's slides are labelled '*Eunotia camelus*'.

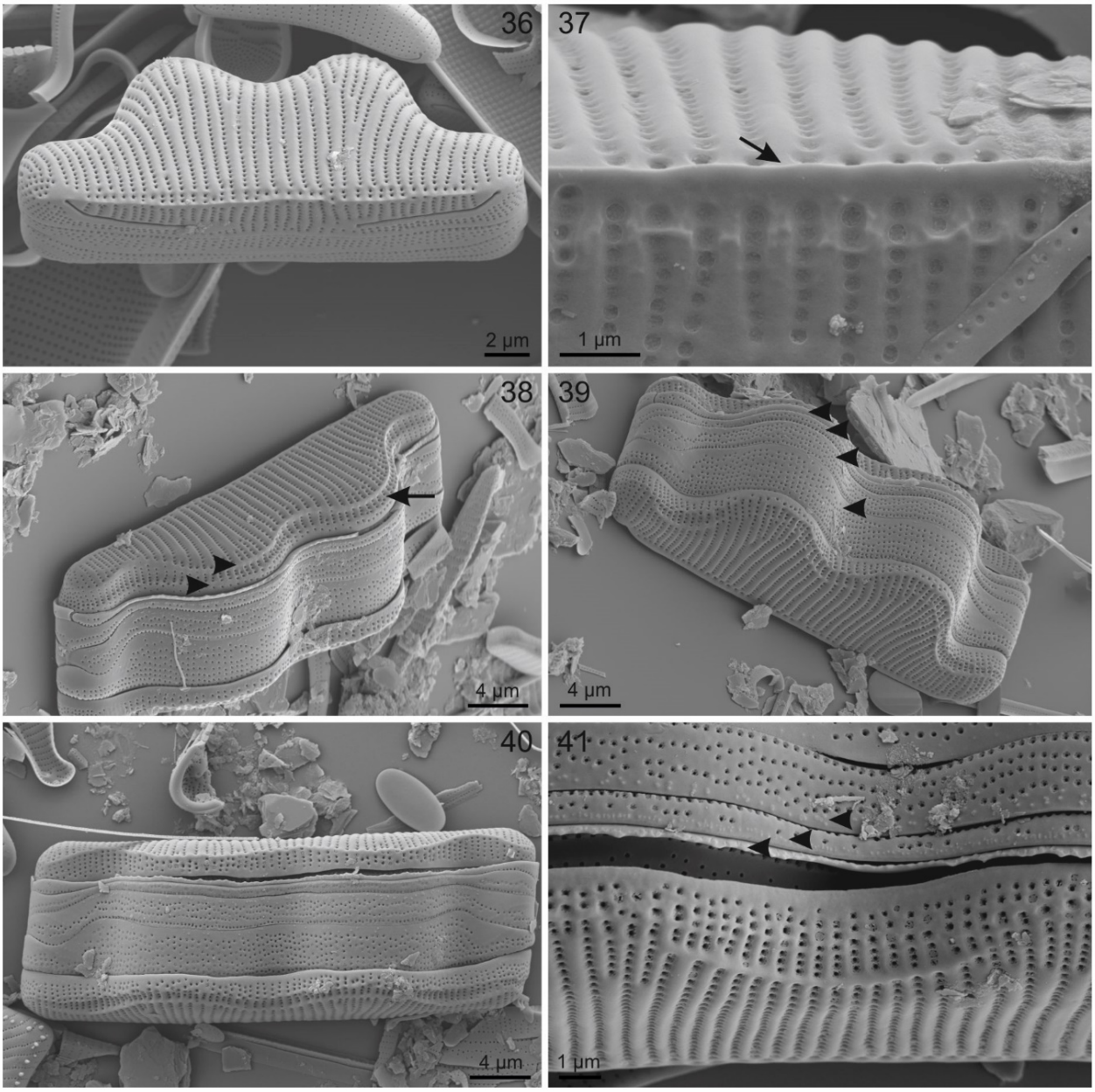
The *E. bidentula* valves have a dorsal margin with two prominent undulations and a straight ventral margin (Figs 1–35). The undulations vary in shape, broadly rounded in large valves, becoming narrower with decreasing valve size, and acute in the smallest valves (Figs 8, 21, 34, 35). Poles are protracted, broadly rounded, subcapitate in the largest valves and subcapitate to rostrate in medium-sized and smaller valves. Valve dimensions: type population [n=7]: length 18.0–31.0 µm, width 6.5–7.5 µm; populations from Wales, Scotland, and Ireland [n=50]: length 14.0–43.5 µm, width 5.5–8.5 µm. In LM, short, deflected terminal raphe ends can be seen near the ventral margin at the poles. Striae are equidistant, parallel in the central part of the valve, slightly radiate and curved in the distal part of the undulations, and parallel or slightly radiate at the poles (type population [n=7]: 15–17[18] in 10 µm; populations from Wales, Scotland, and Ireland [n=24]: 15–17 [18] in 10 µm, [measured in the central part of the valve]).

In SEM, a small ridge and hyaline area is visible at the valve face/mantle junction except at the distal part of the poles (Figs 37, 40–43). Striae are uniseriate and composed of circular areolae which are externally occluded by hymenes. Shorter striae of varying length can be intercalated dorsally (Figs 36, 38, 39). Striae continue onto the mantle, first interrupted by the marginal hyaline area at the valve face/mantle junction, then by a smaller, sometimes inconspicuous second hyaline area. The mantle on the dorsal side consists of a concave step, separated by a narrow hyaline area from the vertical, abvalvar section of the mantle (Figs 38–41). Areolae form irregular rows on the dorsal part of the mantle and on the bands (40, 41, 50–56). Ventrally, vertical rows on the mantle consist mostly of 9–11 areolae between the raphe slits, 1–2 areolae below the raphe adjacent to the mantle margin, and 4–6 areolae above the raphe (Figs 36, 42, 43). The cingulum consists of 4 open bands: a broad valvocopula with mostly 7–8 irregular rows of pores on the pars exterior, and 1–4 on the fimbriate pars interior (Figs 50, 51), a copula with several, often 4, irregular rows on the pars exterior, up to 8 rows of pores on the pars interior separated by a hyaline area from 3–4 rows on the fimbriate part of the pars interior (Fig. 52), and two 2 narrow pleurae with 1–2 rows visible (Figs 54, 55). Additionally, small nodules form irregular rows on the abvalvar parts of the bands (Figs 53–56). The raphe is located on the mantle, moderately sigmoid, bent more strongly towards the poles, terminating at the ventral margin or near the margin on the valve face (Figs 36, 42–44, 49). The sternum is very narrow and close to the margin or indistinct (Figs 36, 44). External raphe ends are small round or tear drop-shaped, internally the distal raphe ends terminate in helictoglossae. Hyaline areas are adjacent to the raphe slit and are moderately large at the raphe ends (Figs 36, 42–

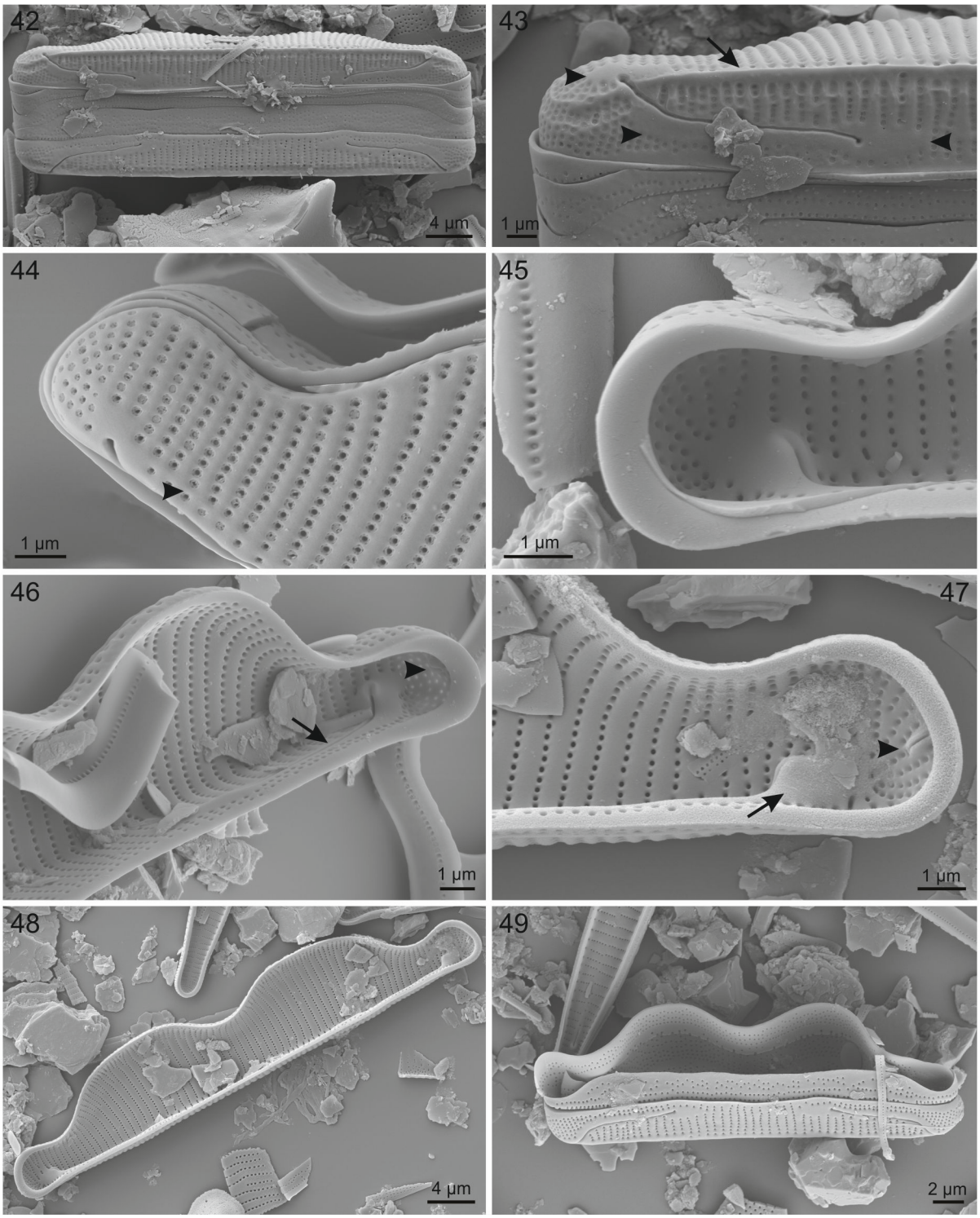
44). A rimoportula is present at one pole, located centrally at the apex, visible externally by an enlarged round pore (Figs 55, 56).



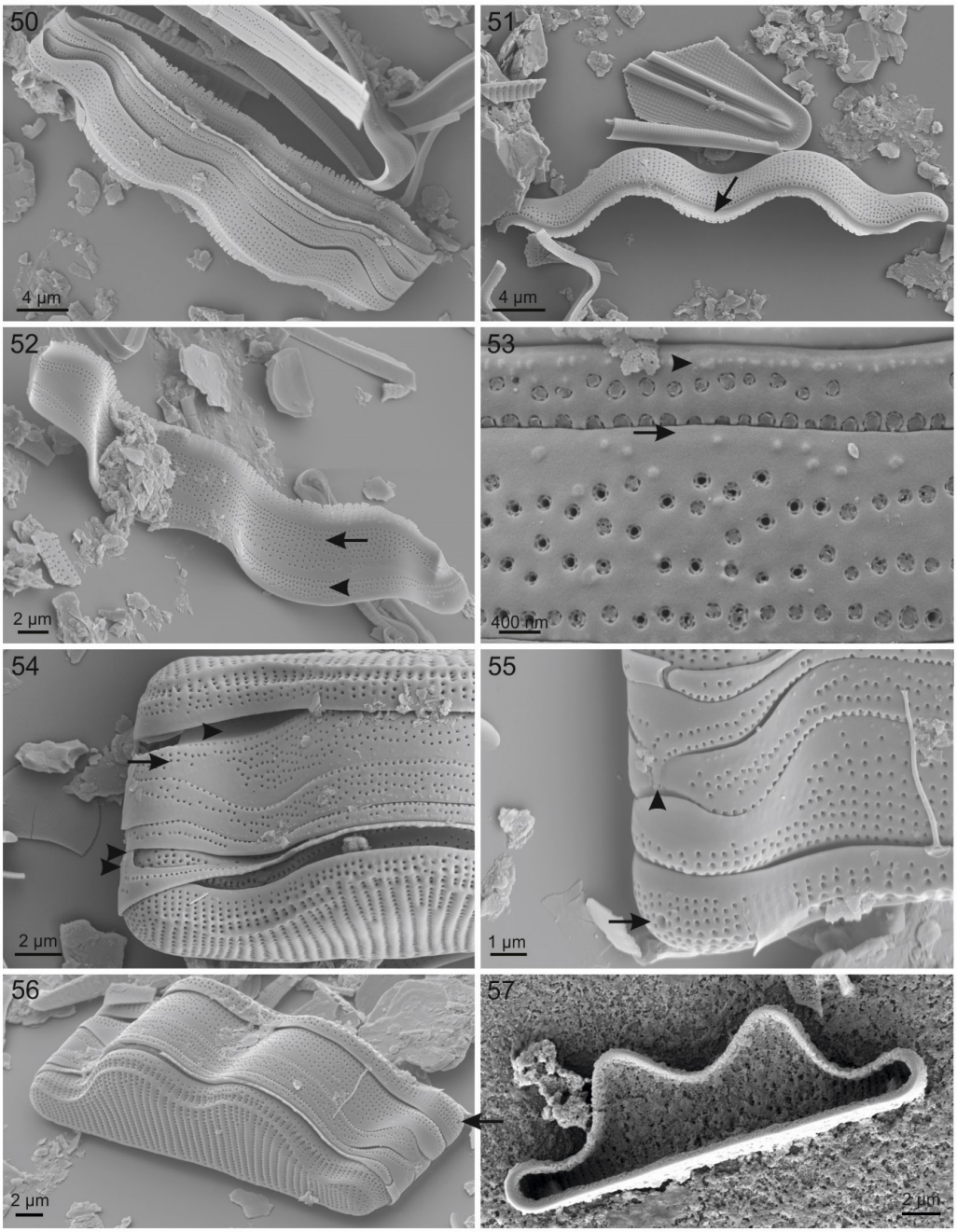
Figs 1–35. *Eunotia bidentula* W.Smith, LM. Cell diminution series of four populations: **Figs 1–8.** Lectotype: Walker Arnott 138, Meise Botanic Garden (**BR-4720**). **Figs 9–16.** Nant Lliwdy, Esgair Forest, Pantperthog, Machynlleth, Gwynedd, Wales, 52.63113°N, 3.89190°W, 392 m a.s.l., leg. Ingrid Jüttner, 02/11/2016 (**NMW**.C.2022.02.Esgair.Forest. Lliwdy.1.11/2016); **Figs 17–21.** pool, Coire na Criche, Kintail, Highland, Scotland, 57.211476°N, 5.361891°W, 730 m a.s.l., shallow pool with *Sphagnum* in upland area with grassland, leg. Alan Orange, 31/05/2005 (**NMW**.C.2011.034.2005. Scotland.5). **Figs 22–35.** rockface, ravine of River Devlin, Glenveagh National Park, Co. Donegal, Ireland, 55.00935°N, 8.116367°W, 170 m a.s.l., rock face with dripping water, leg. Ingrid Jüttner, 01/03/2013 (**NMW**.C.2013.006.02/13.Ireland.21.sto.aero).



Figs 36–41. *Eunotia bidentula* W.Smith, SEM. **Fig. 36.** Valve view and oblique view of the ventral mantle and cingulum of a frustule. **Fig. 37.** View of the small ridge at the valve face/mantle junction. **Fig. 38.** View of the valve face, stepped, dorsal mantle (arrow) and hyaline areas (arrowheads), and cingulum. **Fig. 39.** View of the valve face, valvocopula, copula and two pleurae of the epivalve (arrowheads). **Fig. 40.** Girdle view of the dorsal side of a frustule. **Fig. 41.** Girdle view of the central part of the dorsal mantle, pleurae and copula with marginal nodules (arrowheads). **Fig. 36.** Coire na Criche, Kintail, Highland, Scotland. **Figs 37–41.** Nant Lliwdy, Gwynedd, Wales.



Figs 42–49. *Eunotia bidentula* W.Smith, SEM. **Fig. 42.** Girdle view of a frustule showing the ventral mantle and external raphe slits. **Fig. 43.** Girdle view of the distal part of the mantle showing the external raphe slit and adjoining hyaline areas (arrowheads), occluded areolae and the marginal ridge (arrow). **Fig. 44.** External view of the pole with the distal raphe end and narrow sternum (arrowhead). **Fig. 45.** Internal view of the pole with the helictoglossa. **Fig. 46.** Internal view of part of the valve showing the internal raphe slit (arrow), helictoglossa and rimoportula (arrowhead). **Fig. 47.** Internal view of the pole with helictoglossa (arrow) and rimoportula (arrowhead). **Fig. 48.** Internal view of the whole valve. **Fig. 49.** Oblique view of the ventral valve mantle and valvocopula. **Figs 42–49.** Nant Lliwdy, Gwynedd, Wales.



Figs 50–57. *Eunotia bidentula* W.Smith, SEM. **Fig. 50.** View of the cingulum consisting of valvocopula, copula and two pleurae. **Fig. 51.** Valvocopula with fimbriate pars interior (arrow). **Fig. 52.** Copula with fimbriate pars interior (arrow) and pars exterior (arrowhead). **Fig. 53.** Copula with irregular row of nodules (arrow) and advalvar pleura with row of densely spaced nodules (arrowhead), pore occlusions are present or partly eroded. **Fig. 54.** View of the epicingulum with valvocopula (pars interior [arrowhead] and pars exterior (arrow), copula, and two pleurae, the ligula is visible on the abvalvar pleura (two arrowheads). **Fig. 55.** View of the valve at the pole showing the external opening of the rimoportula (arrow), the cingulum showing two open bands and the ligula on the advalvar pleura (arrowhead). **Fig. 56.** Oblique view of a frustule showing the dorsal undulations of the valves and bands, and the external opening of the single rimoportula (arrow). **Figs 50–56.** Nant Lliwdy, Gwynedd, Wales. **Fig. 57.** Internal view of one valve found in the lectotype material, Walker Arnott 138.

The size range reported in the literature is larger than the range found in our populations (e.g. Patrick & Reimer [max. length 50 µm, width 14 µm], Lange-Bertalot & al. 2011 [max. length 55 µm, width 10 µm]). The size diminution leads to a change in valve outline with smaller valves having acute undulations. The smallest valve of the type population (Fig. 8) and the two smallest specimens from Ireland (Figs 34, 35) are most similar in shape to the valve shown of *Eunotia bidentuloides* Foged. Foged (1977: 53) described this species from a bog near Healy Pass, Co. Kerry, Ireland, showing one photo (pl. 8: fig. 13). Foged gives a size range of 12–16 µm (length) and 5–6 µm (width). Later, Lange-Bertalot & al. (2011: 64, pl. 56: figs 15, 16) adjusted the range to be 12–18 µm (length) and 5–7 µm (width). These overlap with our size range but not with Foged's length of *E. bidentula* (14.0–43.5 µm [length], 5.5–8.5 µm [width] and 19–44 µm, 5–8 µm, respectively). Lange-Bertalot & al. (2011: 376) also mentioned that their specimen in fig. 16 conforms to both *E. bidentuloides* but also to smaller specimens on the type slide of *E. bidentula*. The stria density of *E. bidentula* of 15–18 in 10 µm, this study, and 14–20 in 10 µm in Lange-Bertalot & al. (2011) includes the range given by Foged (1977) for *E. bidentuloides* (16–18 in 10 µm). The only difference in Foged's description of *E. bidentuloides* is the "faintly convex ventral margin". We did not observe this in our smallest valves of *E. bidentula* but the "sharp swellings" of *E. bidentuloides*, the only other character given by Foged to distinguish the species from *E. bidentula* are like those in the smallest valves of the latter. We therefore conclude that *E. bidentuloides* most likely represents the smaller valves of *E. bidentula* and should thus be regarded as a synonym, as already stated in Krammer & Lange-Bertalot (1991: 226).

There are several similar species from tropical and subtropical areas. *Eunotia elucens* Metzeltin & Lange-Bertalot (2007: 93, pl. 78: figs 1–9) from Florida is a new name for *Eunotia bidentula* var. *elongata* Hustedt ex Simonsen (Simonsen 1987: 40, pl. 41: figs 4–5). It is larger (length 42–75 µm, width 7.5–9.5 µm) than *E. bidentula* and has a flatter profile with slightly flattened undulations. *Eunotia schneideri* Metzeltin & Lange-Bertalot (1998: 77, pl. 15: figs 1, 2) found in Venezuela and Brazil also has a flatter profile and a slightly lower stria density (12–15 in 10 µm) (Costa & al. 2017: pl. 67: fig. 12); the same applies to *Eunotia floweri* Metzeltin & Lange-Bertalot (2007: 96, 97; pl. 89: figs 15–18, pl. 90: figs 14–16) from Brazil (stria density 13–15 in 10 µm). *Eunotia herzogii* Krasske (1948: 426, pl. I: fig. 20), from Brazil, illustrated in Lange-Bertalot (1996: 70, pl. 71: figs 5–19) and in Costa & al. 2017 (pl. 67: figs 9–11) has some resemblance with larger valves of *E. bidentula*, but the two main undulations are wavy with two small humps, giving the valve outline a more irregular appearance, and the valves are narrower (width 4 µm). *Eunotia diodon* Ehrenberg (1837: 45; 1838: 192, pl. 21: fig. 23, originally described as a fossil from Lapland) from northern latitudes in the Holarctic (see Lange-Bertalot & al. 2011) is a larger species (length 35–65 µm, width 9.5–14 µm) with spines on the dorsal margin.

Smith (1856) reported *E. bidentula* from several localities in the Fell End area near Lancaster, England, Braemar and Arran, Scotland, and from Barley Lough, a glacial corrie lake, near Glengariff, Co. Cork, Ireland. *Eunotia bidentula* is more widely distributed in Ireland with Foged (1977: 53, pl. 8: figs 14–16, 20) reporting it from 30 localities in nine counties, most of them streams, lakes and bogs, and pH in most of them was between 4.0–6.5. Our specimens (Figs 9–35) from a small pool, a small stream (pH 4.2–5.3) and subaerial on a wet rock face were found in upland grassland areas. *Eunotia bidentula* was absent from other studied sites in the area of the stream which were in conifer plantations. *Eunotia bidentula* was reported from many acidic habitats in the Holarctic (Bishop & al., 2016 and included references). Often the species occurred at low numbers (e.g., Siver & al., 2005, the present study). Lange-Bertalot & al. (2011: 63, 64, pl. 56: figs 1–14, figs 6, 7, 15 = BM 23082, [15 as *E. bidentuloides*], "Braemer, W.S. A 1") showed specimens from Braemar, Scotland, the US and France, fossil and extant, and mentioned fens and

minerotrophic peat bogs as preferred habitats. Fallu & al. (2000: 27) regarded it as a potential indicator species for higher DOC. Habitats were usually nutrient poor and pH ranged from slightly to strongly acidic (Siver & Hamilton, 2011). In a study on lakes in the Adirondack Park, New York, USA, *E. bidentula* occurred in low pH and low alkalinity lakes and compared to most other *Eunotia* species at lower aluminium and DOC and higher phosphorus concentrations (Camburn & Charles, 2000). In North America it was reported from lakes and ponds from Northern Québec, New England to the south-eastern States (Patrick & Reimer, 1966; Fallu & al., 2000; Siver & al., 2005; Siver & Hamilton, 2011). *Eunotia bidentula* was found in lake surface sediments of cores indicating low pH conditions, and was abundant in Catala Lake, Vancouver Island, Canada, and in Lake Vera, Tasmania (Bradbury, 1986; Davis & al., 1994; Clague & al., 1999).

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