Phycologia (2009) Volume 48 (1), 70–74 Published 2 January 2009

Research Note

A proposed protocol for nomenclaturally effective DNA barcoding of microalgae

Katharine M. Evans and David G. Mann*

Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh, EH3 5LR, United Kingdom

K.M. Evans and D.G. Mann. 2009. A proposed protocol for nomenclaturally effective DNA barcoding of microalgae. *Phycologia* 48: 70–74. DOI: 10.2216/08-70.1.

A mechanism for giving DNA barcodes nomenclatural status in microalgae via culture-derived epitypes is demonstrated with reference to four species of the freshwater diatom *Sellaphora*. A fifth, *Sellaphora caput*, is described as a new species to illustrate application of barcoding via a holotype. Using *cox*1 barcodes, it is shown that *S. capitata* has a worldwide distribution, consistent with the ubiquitous dispersal hypothesis.

Key Words: Biogeography, *cox*1, Diatom, DNA barcoding, New species, Molecular typification, Nomenclature, *Sellaphora*, Ubiquitous dispersal hypothesis

INTRODUCTION

In this paper, we demonstrate how DNA barcodes can be made nomenclaturally effective for existing species of microalgae or for new species that cannot be preserved as living or metabolically inactive cultures. The basic problem, which has been identified previously (e.g. Hoef-Emden *et al.* 2007), is to reconcile barcoding with conventional nomenclature to provide a binding molecular typification. Our explanation and examples concern diatoms because barcoding is already feasible in this group and a nomenclatural mechanism is urgent, but the principles are general.

Those who use diatoms as bioindicators or for biological research need accurate taxonomy and therefore encounter three problems: (1) the diversity of diatoms is unknown but undoubtedly much greater than ever envisaged (Mann & Droop 1996), (2) identification becomes ever more difficult as existing taxonomic aids are rendered obsolete by new discoveries, and (3) contrary to the long-held belief that all taxa are cosmopolitan, species (Kooistra *et al.* 2008) and even genera (Vyverman *et al.* 1998, 2007) can possess biogeographies. The effects of inadequate taxonomy can be considerable (cf. Darling *et al.* 2004) but may not become obvious until after it has become impossible to rework the original material. Traditionally, diatom taxa have been characterized and identified by aspects of valve morphology, but exclusive reliance on these traits is an insufficient basis for future work because cryptic diversity is too great (Sarno *et al.* 2005; Amato *et al.* 2007; Vanormelingen *et al.* 2008), the extent of phenotypic plasticity is usually unknown, and describing and communicating slight morphological differences is very difficult, even among experienced taxonomists (cf. Kelly *et al.* 2002).

The freshwater morphospecies *Sellaphora pupula* (Kützing) Mereschkowsky *sensu lato* exemplifies current difficulties. Within the United Kingdom alone, there are at least 36 subtly different species within this single morphospecies (Mann *et al.* 2004, 2008), and molecular phylogenetic studies (18S rDNA, *rbc*L and *cox*1) have demonstrated the prevalence of morphological homoplasy and paraphyly or polyphyly of previous taxa (Evans *et al.* 2007, 2008). Even after 25 years of studying *Sellaphora*, one of us (DGM) is not confident that he can identify many *Sellaphora* species without extra, nonmorphological information, such as provenance (which presupposes an existing, accurate catalogue of diversity) or the results of mating experiments (which are obviously impractical for routine identification). Such cases are not uncommon and are prime candidates for the development of DNA barcoding (Hebert *et al.* 2003). We recently demonstrated the power of barcoding in *Sellaphora* (Evans *et al.* 2007). We trialed four candidate genes and, in line with animals (Hajibabaei *et al.* 2006) and red algae (Saunders 2005; Robba *et al.* 2006), proposed part of the mitochondrion-encoded cytochrome oxidase I (*cox*1) as the gene of choice because it is short, variable, easy to align, and also a useful phylogenetic marker in combination with other genes. We now routinely use *cox*1 barcoding to identify *Sellaphora* clones and discover new species and, in collaboration with Caroline Souffreau (University of Ghent) and Dr Rosa Trobajo (IRTA, Catalunya), have extended barcoding to *Pinnularia* and *Nitzschia*, with promising results (unpublished).

In a recent survey of British *Sellaphora*, Mann *et al.* (2008) specified GenBank *cox*1 sequences that might act as barcodes for five formally recognized *Sellaphora* species and eight informally named genodemes (putative species). The aim was to provide unambiguous reference data for identification. However, in order for barcodes to have real value, it is essential to find a way to give them formal nomenclatural status, as 'molecular types', and this was not achieved in our earlier paper (Mann *et al.* 2008). There are two obstacles: (1) there is no provision in the International Code of Botanical Nomenclature (McNeill *et al.* 2006) for designating gene sequences as types, and (2) existing

* Corresponding author (d.mann@rbge.org.uk).

diatom types are almost invariably cleaned preparations of the silica cell walls. By contrast, type specimens of macroalgae sometimes contain DNA that is sufficiently intact to allow use in phylogeny (e.g. Hughey *et al.* 2002) and hence potentially also for barcoding. Cultures of algae are acceptable as types if kept metabolically inactive, such as by cryopreservation (McNeill *et al.* 2006, Article 8.4), but this is not yet possible for most diatoms, including *Sellaphora* (Dr V.A. Chepurnov and O. Chepurnova, personal communication), and it may never be. Furthermore, because of the obligate link between size restitution and sexual reproduction in most diatoms, living cultures are particularly liable to genetic alteration with time, and the high frequency of heterothallism in the pennates (which are by far the most speciose group) means that many species, including those dealt with here, cannot be maintained as monoclonal cultures beyond a few months or years.

We will employ five species of *Sellaphora* as worked examples. The mechanism we use to give a barcode nomenclatural effect is via the holotype, if the species is described for the first time, or via an epitype, that is, 'a specimen or illustration selected to serve as an interpretative type when the holotype, lectotype, or previously designated neotype, or all original material associated with a validly published name, is demonstrably ambiguous and cannot be critically identified for purposes of the precise application of the name of a taxon' (McNeill *et al.* 2006, Article 9.7). Four *Sellaphora* clones from Blackford Pond, Edinburgh, and one from Loch Leven, Perthshire, were isolated, vouchered, analysed, and photographed as described in Evans *et al.* (2007, 2008) to provide types. All slide preparations are kept in the diatom herbarium of the Royal Botanic Garden Edinburgh (E).

For specimen records to gain formal BARCODE status in global sequence databases, including GenBank, seven stringent requirements are in place: (1) a species name (although it can be interim), (2) voucher data (catalogue number and institution at which the voucher is stored), (3) collection record (collector, collection date, and location with GPS coordinates), (4) identifier of the specimen, (5) *cox*1 sequence of at least 500 base pairs (bp), (6) PCR primers used to generate the amplicon, and (7) deposit of accompanying, overlapping electropherograms (Ratnasingham & Hebert 2007). To adhere to the seventh of these requirements, *cox*1 regions of the five type specimens were sequenced using superior chemistry to that employed previously (Evans *et al.* 2007). The 665-bp diatom *cox*1 region was amplified using primers GazF2 and KEdtmR as detailed in Evans *et al.* (2007), and PCR products were purified using ExoSAP-IT (USB Corporation). Sequencing was conducted in 10-µl volumes using 0.32 µM of PCR primer, 1 µl of BigDye v3.1, and 2 µl of sequencing reaction buffer (Applied Biosystems). Sequencing PCR conditions were 25 cycles of 95°C for 30 s, 50°C for 20 s, and 60°C for 4 min. Excess dye-labelled nucleotides were removed using the Performa DTR V3 cleanup system (EdgeBio), and sequence products were run on an ABI 3730 DNA sequencer (Applied Biosystems). Forward and reverse reads were edited and aligned using Sequencher 4.5 (GeneCodes Corporation). Full 665-bp reads were obtained for all type specimens; GenBank BARCODE accessions are listed with each species. To document the distributions of the five species as an example of barcode use, all available *cox*1 data for these five species were uploaded to GenBank as standard submissions.

Specimens from four clones are illustrated in Figs 1–9 and agree morphologically with previous descriptions of *S. pupula* (Mann 2001; Mann *et al.* 2004), *S. blackfordensis* D.G. Mann & S. Droop and *S. capitata* D.G. Mann & S.M. McDonald (Mann *et al.* 2004), and *S. caput* K.M. Evans & D.G. Mann *sp. nov.* (Mann *et al.* 2008, as the 'caput' deme). Accordingly, these clones are designated as epitypes or (for *S. caput*) the holotype, the type specimens taking the form of preserved cell walls and DNA, with associated information (*cox*1 sequences); the fifth species, *S. bacillum* (Ehrenberg) D.G. Mann, was illustrated by Jahn *et al.* (2008).

Sellaphora pupula

EPITYPE DESIGNATED HERE: Material of clone BLA21 (= SEL 721B), as preserved on slide E4215 (E), illustrated in Fig. 1, barcoded in GenBank accession FJ147204, and with preserved DNA at (E) as EDNA 08-01132. The epitype is selected to clarify the nature of the lectotype specimen at England Finder reference M45/2 on slide BM 17918 (Natural History Museum, London), which was designated and illustrated by Mann (2001, figs 2–6); this epitype supplements and does not replace the previous epitype illustrations and specimen designated by Mann *et al.* (2004) and available online at http://rbg-web2.rbge.org.uk/algae/research/types/Sellaphora_pupula_type.html.

Clone BLA21 (isolated 15 January 2008) and the previous epitypes were all derived from epipelon of soft mud at the SW end of Blackford Pond, Edinburgh (55°55′29″N, 3°11′49″W; UK National Grid Reference NT 253709).

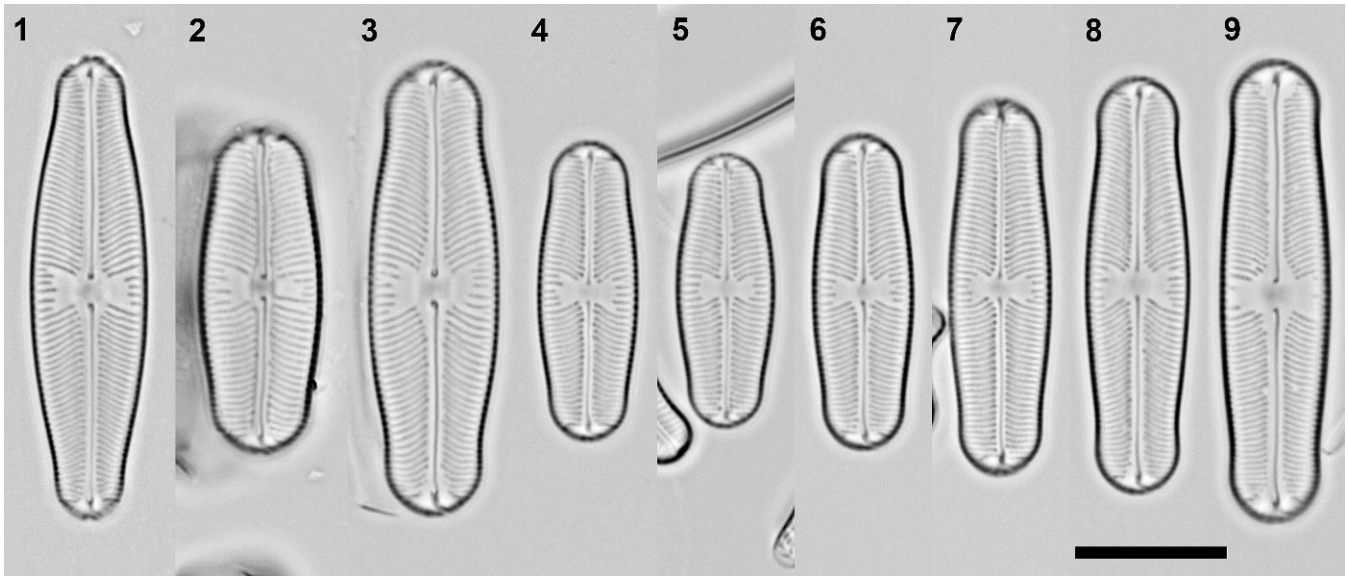
Sellaphora blackfordensis

EPITYPE DESIGNATED HERE: Material of clone BLA17 (= Bl 85), as preserved on slide E4701 (E), illustrated in Fig. 2, barcoded in GenBank accession EF164948, and with preserved DNA at (E) as EDNA 06-04760. The epitype is selected to clarify the nature of the holotype on slide E16/4 at England Finder reference S39/4, which was designated and illustrated by Mann *et al.* (2004, fig. 19) and is shown online at http://rbg-web2.rbge.org.uk/algae/research/types/Sellaphora_blackfordensis_type.html.

Clone BLA17 (isolated 5 October 2004) and the holotype were both derived from epipelon of soft mud at the SW end of Blackford Pond, Edinburgh (location as described previously).

Sellaphora capitata

EPITYPE DESIGNATED HERE: Material of clone BLA18 (= Bl 89), as preserved on slide E3608 (E), illustrated in Fig. 3, barcoded in GenBank accession EF164947, and with preserved DNA at (E) as EDNA 06-04765. The epitype is selected to clarify the nature of the holotype on slide E16/4 at England Finder reference R41/0, which was designated and illustrated by Mann *et al.* (2004, fig. 20) and is shown



Figs 1–9. Valves from type specimens of *Sellaphora* species. Scale bar = 10 μm.
Fig. 1. Supplementary epitype of *S. pupula*: isolate BLA21 on slide E4215.
Fig. 2. Epitype of *S. blackfordensis* isolate BLA17 on slide E4701.
Fig. 3. Epitype of *S. capitata* isolate BLA18 on slide E3608.
Fig. 4. Holotype of *S. caput* isolate LEV2 on slide E4391.
Figs 5–9. Epitype figures of *S. caput* from the LEV2 source population on slide E4241/4 from Loch Leven.

online at http://rbg-web2.rbge.org.uk/algae/research/types/Sellaphora_capitata_type.html.

Clone BLA18 (isolated 5 October 2004) and the holotype were both derived from epipelon of soft mud at the SW end of Blackford Pond, Edinburgh (location as described previously).

Sellaphora bacillum

In a previous paper (Jahn *et al.* 2008), we applied the same approach as adopted here to specify a 'type' *cox*1 sequence for *S. bacillum* (Ehrenberg) D.G. Mann, but we did not fulfil the requirements for it to be designated an official BARCODE. We have now upgraded the sequence and record (EF164941) to official quality and status.

Sellaphora caput* K.M. Evans & D.G. Mann, *sp. nov.

(Figs 4–9)

Valvae lineari-ellipticae, polis latis capitatis ad subcapitatis rostratisve in specimenibus parvis, 18−38 × 6.25−7.3 μm (in culturis vetustioribus, magnitudo descrescens ultra 18 ad c. 11 μm). Striae radiatae, paulum curvatae, brevioribus intercalatis ad centrum, 22.5−26 (plerumque 23−25) in 10 μm. Areolae invisibiles (LM). Area axialis angustissima. Area centralis nec ornamenta nec porcata, transverse rectangularis ad ± subfasci angulis acutis dictu Angliae 'bow-tie' similis. Transtra polaria parallela ad paulum radiantia.

Valves linear-elliptical with broad capitate poles, becoming subcapitate or rostrate in small specimens, 18−38 × 6.25−7.3 μm (decreasing beyond 18 to *c.* 11 μm in old cultures). Striae radiate, slightly curved, with shorter ones intercalated at the centre, 22.5–26 (usually 23–25) in 10 μm. Areolae invisible in LM. Axial area very narrow. Central area neither ornamented nor ridged, transversely rectangular to ± bow-tie-shaped. Polar bars parallel to slightly radiate.

HOLOTYPE: Material of clone LEV2 (= SEL 832L), as preserved on slide E4391 (E), illustrated in Fig. 4, barcoded in GenBank accession FJ151419, and with preserved DNA at (E) as EDNA 08-01116. Clone LEV2 (collected 12 March 2008 and isolated 15 March) was derived from epipelon at the SW end of Loch Leven, Perthshire (56°10′46″N, 3°20′33″W; UK National Grid Reference NT 167993).

EPITYPES DESIGNATED HERE: Because the holotype represents a small section of the size reduction cycle, it is supported by epitype illustrations in Figs 5–9 from a slide (E4241/4) of the cleaned epipelon from which clone LEV2 was isolated.

ETYMOLOGY OF THE EPITHET: Though originally inspired by the Latin word for 'head', the epithet *caput* is to be regarded as an indeclinable noun in apposition, to maintain maximum compatibility with the catalogue of *Sellaphora* diversity by Mann *et al.* (2008), who provide further illustrations and information about the species.

DISTRIBUTIONS: Using the barcode sequences as references, we have confirmed the presence of *S. pupula* in Blackford Pond and Dunsapie Loch (55°56′43.2″N, 3°09′12.5″W; GenBank accession FJ042923), Scotland; *S. blackfordensis* in Blackford Pond, Dunsapie Loch (EF164949), the Royal Botanic Garden Edinburgh pond (55°57′55.1″N, 3°12′20.8″W; FJ042934), Inverleith Pond (55°57′40.9″N, 3°13′4.6″W; FJ042936), Figgate Loch (55°57′02.3″N, 3°7′29.9″W; FJ042935), Balerno Millgate Pond (55°52′33.4″N, 3°19′58.0″W; FJ042937), and Balgavies Loch (56°38′49.4″N, 2°45′53.6″W; EF164935), Scotland, and Malham Tarn (54°5′53.9″N, 2°9′28.6″W; FJ042927), England; *S. capitata* in Blackford Pond, Dunsapie Loch (FJ042902), and Loch Leven (FJ042904), Scotland; Malham Tarn (FJ042905), England; Merelbeke

Pond (51°0′45.6″N, 3°44′49.8″W; FJ042907), Belgium; and Lake Purrumbete (38°17′1″S, 143°12′55″E; FJ042906), Australia; *S. bacillum* in Blackford Pond and St Margaret's Loch (55°57′10.6″N, 3°09′37.0″W; EF164930), Scotland, and Malham Tarn (FJ042924) and a pond near Ashford-in-the-Water (53°13′50.5″N, 1°42′7.7″W; FJ042925), England; and *S. caput* in Loch Tulla (56°33′12.9″N, 4°45′2.6″W; FJ042926) and Loch Leven, Scotland. In no case was *cox*1 divergence greater than 1% between isolates regarded as the same species; in most cases it was much less. All these records are backed by voucher slides in E.

Deposit of vouchered sequences in GenBank provides a secure basis for biogeographical and ecological analysis. Thus, clear demonstration of *S. caput* in Loch Leven and Loch Tulla goes against the morphology-based survey of Mann *et al.* (2008) by showing that the species can occur in eutrophic as well as dystrophic habitats. The *S. capitata* records represent clear evidence, independent of morphology-based identification, that a freshwater diatom species can have a worldwide distribution, as required for the ubiquitous dispersal hypothesis (e.g. Finlay 2002); although, it remains to be established if this is natural or the result of recent accidental introduction by humans.

DISCUSSION

These examples illustrate how barcodes can be used to stabilize taxonomy by giving them nomenclatural status via holotypes or epitypes. An alternative would have been simply to mention a gene sequence in a new or emended description. We rejected this because the function of a description is to *circumscribe* a taxon, that is, to specify the *range* of variation among individuals that a particular taxonomist decides should be included within a particular species, variety, and so on. Not surprisingly, the circumscription, and hence the description, usually changes as knowledge increases. Like any fast-evolving part of the genome, the *cox*1 barcode region can also be expected to vary within species, and indeed, it *does* vary within several of the species we treat here. A description of these species would therefore have to list several *cox*1 sequences in order to circumscribe them, and the list would grow or shrink according to subsequent taxonomic research. Typification, on the other hand, has little to say about circumscription, serving instead to determine what name should be applied to each group of individuals; the barcode's value is to improve typification, as an unambiguous molecular reference point.

Specification of a barcode as part of the holotype (as for *S. caput*) seems straightforward. Our proposal to add a barcode to an existing taxon via an epitype clone and its preserved remnants, and associated information requires extra steps beyond those required for a new species but no more than would be needed if the epitype were a photograph. For example, it must be true that the epitype and the holotype or lectotype it supports are the same taxonomically; if not, the types must be redefined (McNeill *et al.* 2006, Article 9.18). Hence, morphological agreement between the epitype material and the lectotype or holotype must be as exact as the lectotype or holotype will allow. In the case of two or more species that differ scarcely or not at all in their morphology and that have previously been confused with each other, the choice of barcoded epitype will often be arbitrary. The provenance of the original material may provide guidance, but we consider that it would be inadvisable to insist on geographical or ecological congruence between epitype and lecto- or holotype in every case because pre-barcode identifications will be inherently ambiguous. For extinct species, of course, our proposals offer no aid.

ACKNOWLEDGEMENTS

This work was supported by a Natural Environment Research Council Fellowship (NE/C518373/1) to KME.

REFERENCES

Amato A., Kooistra W.H.C.F., Levialdi Ghiron J.H., Mann D.G., Pröschold T. & Montresor M. 2007. Reproductive isolation among sympatric cryptic species in marine diatoms. *Protist* 158: 193–207.

Darling K.F., Kucera M., Pudsey C.J. & Wade C.M. 2004. Molecular evidence links cryptic diversification in polar planktonic protests to Quaternary climate dynamics. *Proceedings of the National Academy of Sciences of the United States of America* 101: 7657–7662.

Evans K.M., Wortley A.H. & Mann D.G. 2007. An assessment of potential diatom "barcode" genes (*cox*1, *rbc*L, 18S and ITS rDNA) and their effectiveness in determining relationships in *Sellaphora* (Bacillariophyta). *Protist* 158: 349–364.

Evans K.M., Wortley A.H., Simpson G.E., Chepurnov V.A. & Mann D.G. 2008. A molecular systematic approach to explore diversity within the *Sellaphora pupula* species complex (Bacillariophyta). *Journal of Phycology* 44: 215–231.

Finlay B.J. 2002. Global dispersal of free-living microbial eukaryote species. *Science* 296: 1061–1063.

Hajibabaei M., Janzen D.H., Burns J.M., Hallwachs W. & Hebert P.D.N. 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America* 103: 968–971.

Hebert P.D.N., Cywinska A., Ball S.L. & Dewaard J.R. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B* 270: 313–321.

Hoef-Emden K., Küpper F.C. & Andersen R.A. 2007. Meeting report: Sloan Foundation workshop to resolve problems relating to the taxonomy of microorganisms and to culture collections, arising from barcoding initiatives; Portland ME, November 6–7, 2006. *Protist* 158: 135–137.

Hughey J.R., Silva P.C. & Hommersand M.H. 2002. Solving taxonomic and nomenclatural problems in Pacific Gigartinaceae (Rhodophyta) using DNA from type material. *Journal of Phycology* 37: 1091–1109.

Jahn R., Mann D.G., Evans K.M. & Poulíčková A. 2008. The identity of *Sellaphora bacillum* (Ehrenberg) D.G. Mann. *Fottea* 8: 121–124.

Kelly M.G., Bayer M.M., Hürlimann J. & Telford R.J. 2002. Human error and quality assurance in diatom analysis. In: *Automatic diatom identification* (Ed. by J.M.H. Du Buf & M.M. Bayer), pp. 75–91. Series in Machine Perception and Artificial Intelligence, vol. 51. World Scientific Publishing Co., Singapore.

Kooistra W.H.C.F., Sarno D., Balzano S., Gu H., Andersen R.A. & Zingone A. 2008. Global diversity and biogeography of *Skeletonema* species (Bacillariophyta). *Protist* 159: 177–193.

MANN D.G. 2001. The systematics of the *Sellaphora pupula* complex: typification of *S. pupula.* In: *Lange-Bertalot-Festchrift. Studies on diatoms, dedicated to Prof. Dr. Dr. h. c. Horst Lange-Bertalot on the occasion of his 65th birthday* (Ed. by R. Jahn, J.P. Kociolek, A. Witkowski & P. Compère), pp. 225–241. A.R.G. Gantner, Ruggell, Liechtenstein.

MANN D.G. & DROOP S.J.M. 1996. Biodiversity, biogeography and conservation of diatoms. *Hydrobiologia* 336: 19–32.

MANN D.G., MCDONALD S.M., BAYER M.M., DROOP S.J.M., CHEPURNOV V.A., LOKE R.E., CIOBANU A. & DU BUF J.M.H. 2004. Morphometric analysis, ultrastructure and mating data provide evidence for five new species of *Sellaphora* (Bacillariophyceae). *Phycologia* 43: 459–482.

MANN D.G., THOMAS S.J. & EVANS K.M. 2008. Revision of the diatom genus *Sellaphora*: a first account of the larger species in the British Isles. *Fottea* 8: 15–78.

MCNEILL J., BARRIE F.R., BURDET H.M., DEMOULIN V., HAWKSWORTH D.L., MARHOLD K., NICOLSON D.H., PRADO J., SILVA P.C., SKOG J.E., WIERSEMA J.H. & TURLAND N.J. 2006. *International Code of Botanical Nomenclature (Vienna Code)*. Regnum Vegetabile 146. A.R.G. Gantner, Ruggell, Liechtenstein.

RATNASINGHAM S. & HEBERT P.D.N. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* 7: 355–364.

ROBBA L., RUSSELL S.J., BARKER G.L. & BRODIE J. 2006. Assessing the use of the mitochondrial *cox*1 marker for use in DNA barcoding of red algae (Rhodophyta). *American Journal of Botany* 93: 1101–1108.

SARNO D., KOOISTRA W.H.C.F., MEDLIN L.K., PERCOPO I. & ZINGONE A. 2005. Diversity in the genus *Skeletonema* (Bacillariophyceae). II. An assessment of the taxonomy of *S. costatum*-like species with the description of four new species. *Journal of Phycology* 41: 151–176.

SAUNDERS G.W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical Transactions of the Royal Society of London B* 360: 1879–1888.

VANORMELINGEN P., CHEPURNOV V.A., MANN D.G. & VYVERMAN W. 2008. Genetic divergence and reproductive barriers between sympatric but morphologically heterogeneous clones of *Eunotia bilunaris* sensu lato (Bacillariophyta). *Protist* 159: 73–90.

VYVERMAN W., SABBE K., MANN D.G., KILROY C., VYVERMAN R., VANHOUTTE K. & HODGSON D. 1998. *Eunophora* gen. nov. (Bacillariophyta) from Tasmania and New Zealand: description and comparison with *Eunotia* and amphoroid diatoms. *European Journal of Phycology* 33: 95–111.

VYVERMAN W., VERLEYEN E., SABBE K., VANHOUTTE K., STERKEN M., HODGSON D.A., MANN D.G., JUGGINS S., VAN DE VIJVER B., JONES V., FLOWER R., ROBERTS D., CHEPURNOV V.A., KILROY C., VANORMELINGEN P. & DE WEVER A. 2007. Historical processes constrain patterns in global diatom diversity. *Ecology* 88: 1924–1931.

Received 25 August 2008; accepted 23 September 2008
Associate editor: Alan Millar