JOURNAL OF PLANKTON RESEARCH | VOLUME 33 | NUMBER 4 | PAGES 555–567 | 2011

# Who is *Oxyrrhis marina*? Morphological and phylogenetic studies on an unusual dinoflagellate

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Received May 13, 2010; accepted in principle July 28, 2010; accepted for publication July 30, 2010

Corresponding editor: John Dolan

*Oxyrrhis marina* is an extensively studied morphospecies and a common protist model used to examine a range of ecological processes. Further, as a result of a number of unusual cytological and genetic features, *Oxyrrhis* is increasingly a target for the study of evolutionary patterns and genome organization within the Alveolata. However, a small number of early morphological studies and recent phylogenetic data suggest that *O. marina* represents more than one species. As different research groups employ different *O. marina* isolates (which are potentially highly divergent strains or different species), the context in which comparisons between isolates can be made is difficult to assess. In this paper, we explore the literature that has contributed to the definition of *O. marina*, highlighting the unusual characteristics possessed by *O. marina* that have motivated much of the study on this organism and informed its key phylogenetic position. In addition, we assess historical and contemporary evidence for multiple *Oxyrrhis* species. Based on this assessment, in particular recent molecular genetic data, we assert that *O. marina* represents two species: *O. marina* and *O. maritima*. Based on historical observations, we also indicate that a third species (*O. tenticulifera*) may occur, although there are no contemporary data to support or refute this designation. Extensive cryptic diversity has important implications for researchers studying *Oxyrrhis*: caution must be exercised in characterizing *Oxyrrhis* isolates for experimental study (i.e. it is inappropriate to report assessments concerning poorly characterized isolates), and comparative studies of multiple isolates are required to assess individual, population and species level variation in the genus. Finally, in a broader context, the ecological and evolutionary processes driving diversity in free-living protists remains poorly understood. Model protists such as *O. marina* and *O. maritima* for which we are beginning to recognize and characterize an extensive pool of variation present ideal opportunities to unravel these fundamental processes.

KEYWORDS: protist diversity; phylogenetic species; intraspecific variation



doi:10.1093/plankt/fbq110, available online at www.plankt.oxfordjournals.org. Advance Access publication August 25, 2010

## INTRODUCTION

*Oxyrrhis marina* is an extensively studied morphospecies (Montagnes *et al.*, 2011a), exhibiting a wide geographic distribution (Watts *et al.*, 2011). Much study of *O. marina* has been motivated by the recognition that it possesses unusual cytological and genetic features (e.g. Leander and Keeling, 2004; Slamovits *et al.*, 2007); accordingly, *O. marina* has become a significant target for the study of evolutionary patterns and genome organization within the Alveolata (Slamovits *et al.*, 2007; Zhang *et al.*, 2007; Slamovits and Keeling, 2011). Despite this, the study of variability within the *O. marina* morphospecies has been ignored.

Recent work indicates that levels of genetic divergence within this taxon may be extensive, which coupled with substantial physiological variation is potentially sufficient to infer that *O. marina* represents more than one species (e.g. Lowe *et al.*, 2010). Such diversity is alarming, as researchers around the world continue to isolate strains and conduct experiments on this "species" (Fig. 1). Here, we provide a brief historical guide to the morphological and phylogenetic literature that has defined *Oxyrrhis*—we highlight why *O. marina* is an important model organism, but also indicate that this taxon harbours extensive cryptic diversity, which has remained poorly described.

Superficially, *O. marina* is easily recognized (e.g. Dodge, 1982) and easy to isolate from the natural environment (Lowe *et al.*, 2011); while such characteristics make *O. marina* simple and practical to study, they also present significant problems. Approximately 40 *O. marina* isolates are reported in the literature. However, most of these are poorly characterized beyond their gross morphology. Consequently, the bulk of studies are not interpretable in a comparative context, and despite recent evidence of substantial genetic variation (Lowe *et al.*, 2010), there are limited molecular, physiological, morphological or ultrastructural data to corroborate such diversity or aid the delineation of potentially multiple species in the genus. This presents a dilemma: *O. marina* is commonly employed as a "model" to examine a broad range of ecological, physiological and behavioural responses (see other papers in this special issue). However, different research groups employing different isolates of *O. marina* are potentially working on highly divergent strains or even different species. Thus, the context in which comparisons between isolates can be made is difficult to assess.

Indeed, a review of the literature since 1950 (Fig. 1a and b; Table I) indicates that ~160 studies examined various aspects of *O. marina* biology and reveals that: (i) most studies examine a single strain (74 examined one strain, 64 provided no isolate information, 14 were not traceable by the authors); (ii) many isolates are reported only once in the literature (38 isolates are reported, 30 of which are reported once or twice); and (iii) most laboratories (research groups) work on only a single strain. As a result, there are few comparative studies, and our ability to resolve potential strain and species differences are limited. The articles in this special issue expand on various aspects of *O. marina* biology. To place these, and future work, in a taxonomic context, it is essential that we first explore our current understanding of what *O. marina* is and the extent of diversity in this taxon.

In this paper, two major issues are examined. First, we explore the morphological, cytological and molecular literature that has contributed to the definition of

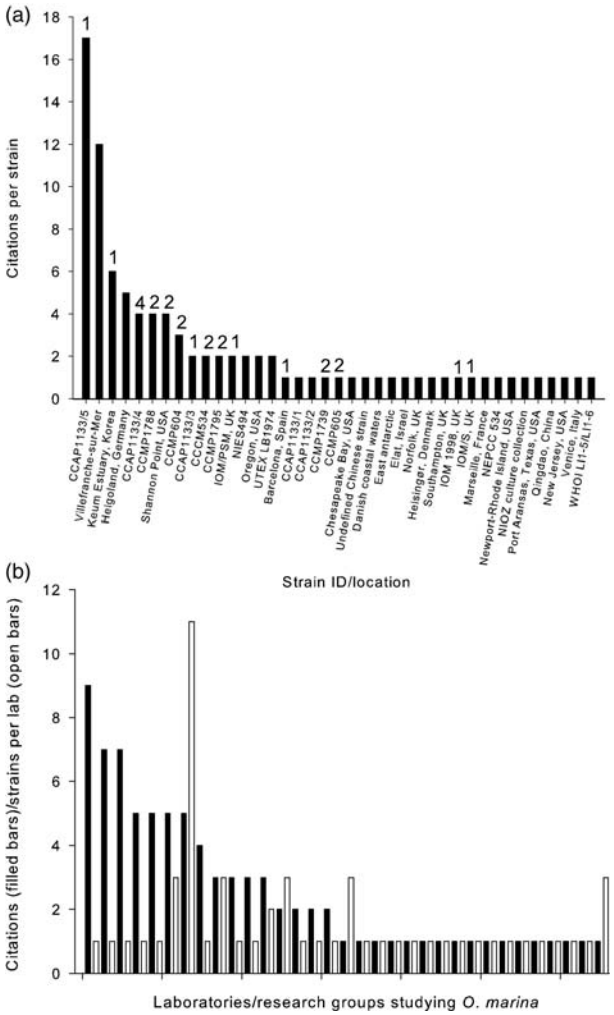


**Fig. 1.** A **s**ummary of *Oxyrrhis marina* strains reported in the literature between 1950 and 2009: (**a**) number of citations for the 38 named strains occurring in the literature, numbers above bars indicate, where know, to which clade a strain belongs (see Lowe *et al*, 2010, and Fig. 6); (**b**) "*Oxyrrhis* related output" (i.e. papers reporting observations/experiments concerning *O. marina*) and strain usage of the 33 laboratories/research groups reporting studies of *O. marina*.

*Table I: A summary of studies (post 1950) providing morphological, cytological, ultrastructural or molecular phylogenetic data concerning Oxyrrhis marina*

| | Study | Strain/isolate | Number of strains |
|---|---|---|---|
| Phylogeny/genetic | | | |
| Lenaers *et al.* (1991) | Dinoflagellate phylogeny | Villefranche-sur-Mer (France) | 1 |
| Saldarriaga *et al.* (2003) | *O. marina* and *Perkinsus marinus* are early branches of the dinoflagellates | CCCM534, NIES494 | 2 |
| Cavalier-Smith and Chao (2004) | Protalveolate phylogeny and systematics | WHOI LI1-5/LI1-6, NIES494, Chinese strain | 3 |
| Leander and Keeling (2004) | Early evolutionary history of dinoflagellates and apicomplexans | CCCM534 | 1 |
| Saldarriaga *et al.* (2004) | Evolutionary history of dinoflagellates | N/A (review article) | – |
| Lowe *et al.* (2005) | Intraspecific diversity of *O. marina* | IOM/PSM, S, P; CCMP1795, 604, 1739, 1788, 605; CCAP1133/3, 4, 5 | 11 |
| Slamovits *et al.* (2007) | Characterization of the mitochondrial genome of *O. marina* | CCMP1788 | 1 |
| Slamovits and Keeling (2011) | Plastid-derived genes in *O. marina* | CCMP1788 | 1 |
| Zhang and Lin (2008) | mRNA editing and spliced-leader RNA *trans*-splicing groups *Oxyrrhis*, N*octiluca*, *Heterocapsa* and *Amphidinium* as basal lineages of dinoflagellates | CCMP1795 | 1 |
| Morphology/cytology | | | |
| Hausmann (1973) | Structure and mode of function of trichocysts | Not stated | – |
| Clarke and Pennick (1972) | Occurrence of flagellar scales | CCAP1133/1, 2 | 2 |
| Clarke and Pennick (1976) | Occurrence of body scales | CCAP1133/2, Gorleston-on-Sea (UK), CCAP1133/4 | 3 |
| Brown *et al.* (1988) | Cytoskeletal microtubular system | Not stated | – |
| Roberts *et al.* (1993) | Cortical microtubular cytoskeleton | CCCM 534 | 1 |
| Hohfeld and Melkonian (1998) | The microtubular cytoskeleton | UTEX LB 1974 | 1 |
| Hohfeld *et al.* (1994) | Immunolocalization of centrin | UTEX LB 1974 | 1 |
| Kato *et al.* (2000) | Microtubule organization during division | Villefranche-sur-Mer (France) | 1 |
| Nuclear structure | | | |
| Cachon *et al.* (1979) | Nuclear division | Villefranche-sur-Mer (France) | 1 |
| Triemer (1982) | A unique mitotic variation in *O. marina* | Tuckerton, New Jersey (USA) | 1 |
| Gao and Li (1986) | Nuclear division in *O. marina* | Qingdao (China) | 1 |
| Kato *et al.* (1997) | Major basic nuclear protein and its localization on chromosomes of *O. marina* | Villefranche-sur-Mer (France) | 1 |
| Flagellar structure | | | |
| Roberts (1985) | Flagellar apparatus | Newport, Rhode Island (USA); UTEX LB1974 | 2 |
| Cachon *et al.* (1988) | Ultrastructure of the flagellar apparatus | Villefranche-sur-Mer (France) | 1 |
| Cosson *et al.* (1988a) | Swimming behaviour of *O. marina* | Villefranche-sur-Mer (France) | 1 |
| Cosson *et al.* (1988b) | Structure and function of the flagella | Villefranche-sur-Mer (France) | 1 |
| Godart and Huitorel (1992) | Effects of calcium on the longitudinal flagellum | Villefranche-sur-Mer (France) | 1 |
| Godart *et al.* (1992) | Composition/properties of the nanofilaments in the paraflagellar rod of *O. marina* | Villefranche-sur-Mer (France) | 1 |
| Cachon *et al.* (1994) | Nanofilament-dependent motility in dinoflagellates | Not stated | – |

The *O. marina* strain/isolate identity and the number of strains/isolates examined in each study are also noted.

*O. marina*—highlighting in particular the historical and contemporary evidence for multiple *Oxyrrhis* species. In parallel, we review the unusual characteristics possessed by *O. marina* that have motivated much of the study on this organism and informed its key phylogenetic position within the alveolates. We do not purport to have conducted an exhaustive review of the literature (which is extensive and covers >160 years); instead, we highlight key studies and provide sufficient guidance to allow researchers to further explore the topic. Ultimately, we indicate that while the phylogenetic position of *O. marina* is now reasonably well established, in other regards it remains poorly characterized. Most critically, we assert that *O. marina*, *sensu lato*, actually represents more than one species, for which we provide new diagnoses and a justification for this reclassification. Finally, we indicate that the recognition of extensive diversity within the *Oxyrrhis* genus provides productive new avenues of research based on this important model organism.

## MORPHOLOGICAL STUDIES OF *OXYRRHIS MARINA*

### Gross morphology

*Oxyrrhis marina* Dujardin (Dujardin, 1841; Fig. 2a, Table II) was originally described as oblong, oval bodied, with pointed anterior, obliquely notched anteriorly, possessing "several" flagella protruding sideways from the notch centre. Diagnostic features were: colourless, sub-cylindrical, rough bodied cell, with rounded posterior, 0.05 long (no units, but remarks on magnification of the original figure indicate 44 μm long). The type location was the Mediterranean (likely on the French coast), but, as was typical of protistan studies of the time, no type material was deposited.

The first main revision by Saville-Kent (Saville-Kent, 1880) provided further details (Fig. 2b, Table II) based on the literature and observations of isolates from Jersey (UK). The revision provided information on: two flagella, one extending and the other coiled within the oral aperture; swimming and feeding behaviour (e.g. the longitudinal flagellum being responsible for trapping prey, while the transverse flagellum pushes it into the oral cavity); division by transverse fission; an anterior contractile vacuole; and, in illustrations, a posterior ventral bulge (or tentacular lobe) within the posterior ventral depression.

Several other older *O. marina* reviews exist. Senn (Senn, 1911) extensively reviewed the literature and

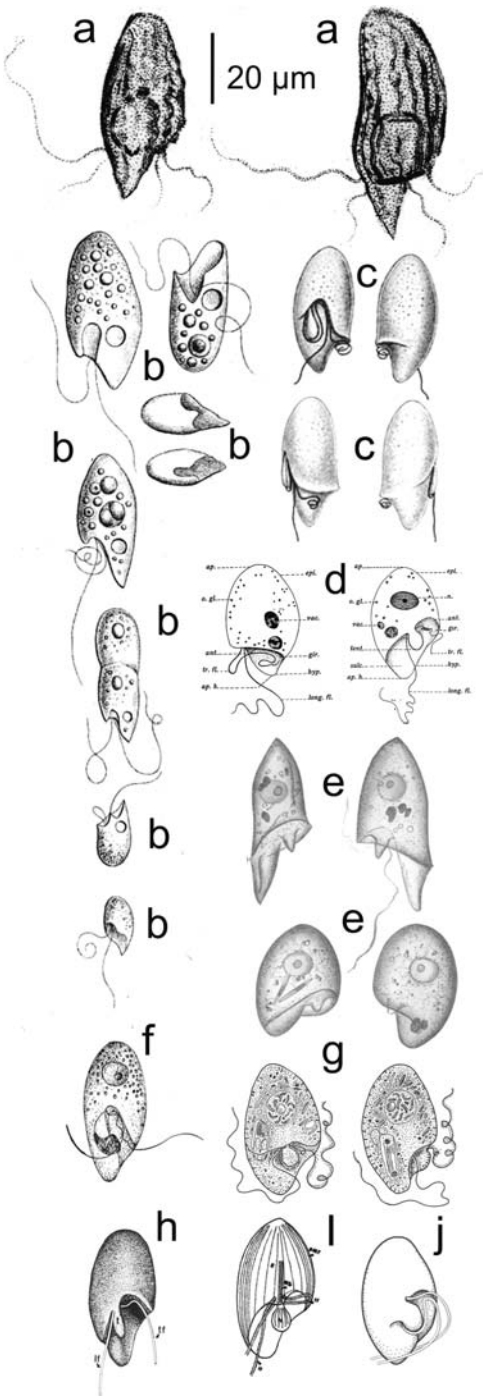


**Fig. 2.** Illustrations of *Oxyrrhis marina* over the last 160 years: (a) the original description (Dujardin, 1841); (b) eight drawings by Saville Kent, including variation in size and division (1880); (c) four of many illustrations by Senn (1911); (d) two illustrations from many provided by Hall (Hall, 1924); (e) four illustrations, indicating osmotic influence on cell size, by Diskus (Diskus, 1956); (f) an illustration from a guide to protozoa of Woods Hole (Calkins, 1902); (g) the two general illustrations presented in Dodge and Crawford (Dodge and Crawford, 1971a); (h) a simple schematic presented in Roberts (Roberts, 1985); (i) a schematic, indicating ultrastructure and microtubules (Brown *et al.*, 1988); (j) our own general illustration. All illustrations presented to be associated with the scale bar.

*Table II: The designations, and naming authorities, for species in the genus Oxyrrhis*

| *Oxyrrhis* | Date | Length (μm) | Flagella | Shape | Location |
|---|---|---|---|---|---|
| *marina* Dujardin | 1841 | 44[a] | Several | Oblong, oval bodied, rounded posteriorly | Mediterranean |
| *marina* Kent | 1880 | 28–51 | 2 | Body conical, subcylindrical, rounded posteriorly | St Helier, Jersey |
| *phaeocysticola* | 1900 | 20 | 2 | Rounded posterior, pointed anterior, excavated oral region with trunk-like projection | Helgoland, Germany |
| *tentaculifera* Conrad | 1939 | 38 | 2 | More voluminous than *O. marina* | Belgium |
| *maritima* Van Meel | 1969 | 16–24 | 2 | Twice as long as wide; has a tentacle | Belgium |

[a]No units were provided in the original description—value is inferred by the authors.

provided new details (Fig. 2c), indicating: no observable contractile vacuole; the flagella insert on either side of the ventral bulge; *O. marina* was a dinoflagellate, possibly related to *Gymnodinium*; and there was only one *Oxyrrhis* species. Hall (Hall, 1924) provided a later review, which included new observations of binary fission (Fig. 2d). However, of the older literature, we suggest that Kofoid and Swezy (Kofoid and Swezy, 1921) provide the best synthesis and most rigorous diagnosis of the genus and species (for veracity, reported in full below); they also supported the notion that there is only one species of *Oxyrrhis*, placed within the Gymnodinioidae.

### *Diagnosis of* Oxyrrhis

> Body subovoidal, asymmetrically contracted on the left posteriorly; girdle postmedian, incomplete distally, lacking postmargin; sulcus spreading posteroventrally, divided anteriorly by pendant tentacular lobe; transverse flagellum originating to the left and the longitudinal to the right of the lobe; nucleus with beaded chromatin; marine. (Kofoid and Swezy, 1921).

### *Diagnosis of* O. marina

> Body elongate oval, asymmetrical posteriorly; girdle imperfect on right side, without a postmargin; flagella midventral; stout tentacle-like lobe pendant between the two flagella, dividing the broad undeveloped ventral sulcus; colourless; length, 10−37 μm; marine. (Kofoid and Swezy, 1921).

Three other free-living *Oxyrrhis* species have been described: *O. phaeocysticola* Scherffel, 1900; *O. tentaculifera* Conrad, 1939 and *O. maritima* van Meel, 1969 (Fig. 3, Table II). *Oxyrrhis phaeocysticola* (Fig. 3a) was distinguished as *Oxyrrhis*-shaped, including possessing a ventral bulge, but its swimming pattern was flagella first, in contrast to *O. marina*, which swims with the flagella in the posterior (e.g. Scherffel, 1900). *Oxyrrhis maritima* (Fig. 3b) and *O. tentaculifera* (Fig. 3c) were both isolated from Belgian coastal waters. *Oxyrrhis maritima* was ambiguously distinguished as larger and rounder than *O. marina*, while *O. tentaculifera* was defined as possessing a long tentacle (probably a longer version of the ventral bulge indicated above), extending from the notch, but otherwise, it was superficially similar to *O. marina*. *Oxyrrhis phaeocysticola* was moved to the genus *Hemistasia* (Elbrächter *et al.*, 1996), thus creating the new combination *Hemistasia phaeocysticola*. *Oxyrrhis tentaculifera* and *O. maritima* were synonymized with *O. marina* by Dodge (Dodge, 1982), whose reasoning was that as *O. marina* exhibits considerable morphological variation, these two species were insufficiently different from *O. marina*. We suggest that the description by Conrad (Conrad, 1939) of *O. tenticulifera* is sufficiently distinct (particularly the presence of a conspicuous, long tentacle) to stand as a distinct species, although the lack of corroborating observations of this morphotype limits further

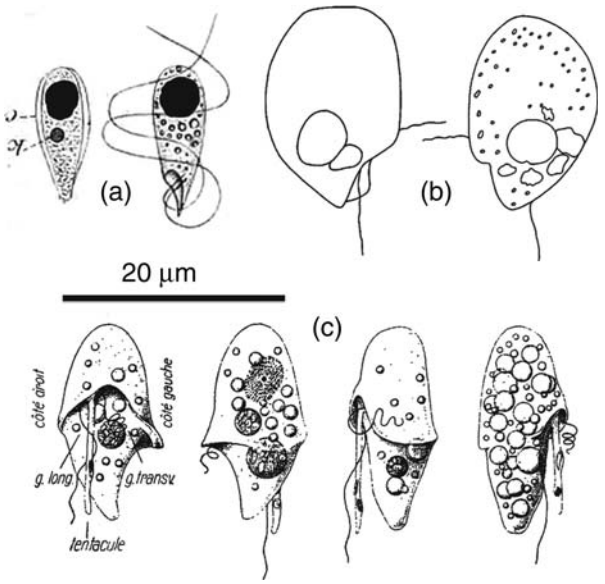


**Fig. 3.** Illustrations of the other three species described in the genus *Oxyrrhis*: (**a**) *O. phaeocysticola* Scherffel, 1900, moved to *Hemistasia phaeocystidicola* (Scherffel) comb. nov. (Elbrächter *et al.*, 1996); (**b**) *O. maritima* van Meel, 1969; (**c**) *O. tentaculifera* Conrad, 1939. Scale bar applies to all illustrations.

consideration. Regardless, the question of multiple *Oxyrrhis* species has not been revisited until relatively recently (see below); thus the current genus *Oxyrrhis* contains only the original species, *O. marina*.

Other observations of gross morphology are distributed throughout the literature (Fig. 2). For example, isolates of *O. marina* collected from Venice were illustrated with a ventral bulge and were noted to vary extensively in cell size and shape in response to a range of osmotic conditions (Diskus, 1956; Fig. 2e). Cell shape and size appear to be highly variable in *O. marina*: Triemer (1982) noted changes in shape following food ingestion; concurrently, in our own experience of culturing large numbers of *O. marina* isolates, variation in size occurs depending on food concentration and culture status (e.g. Kimmance *et al.*, 2006). Furthermore, our own recent observations on clonal isolates collected across Europe suggest clone-specific variation in cell size (C. Lowe, unpublished results), although whether these differences are systematic and correlated with phylogenetic identity is not yet clear.

## *Oxyrrhis*: an unusual dinoflagellate

As noted above, early studies recognized *O. marina* to be a dinoflagellate, though a somewhat unusual one. Virtually all of the subsequent morphological and ultrastructural work has focused on providing data to characterize *O. marina* and to assess its affinity within the alveolates. Below, we briefly review the morphology of *O. marina*, moving from larger to smaller structures, and highlight significant findings.

### *Cysts*

There are two independent descriptions of cyst formation in *Oxyrrhis*. Hall (Hall, 1924) noted thin-membrane covered cysts induced by both excess and lack of food—to our knowledge this is the only report of cyst formation in *O. marina*. A more recent study indicated that *Oxyrrhis* sp. formed robust adherent cysts (Jonsson, 1994). In this work *Oxyrrhis* sp., from intertidal rock pools of Brittany, France, formed 10 μm spherical cysts that adhered to the substrate ~0.5 h prior to pools being covered by the incoming tide; excystment occurred 18 h later as the tide receded a second time, and *Oxyrrhis* sp. were then free swimming for 6 h. Though gross morphology, based on scanning electron microscopy, suggested that the cells observed in this study were identical to *O. marina*, Jonsson (Jonsson, 1994) conservatively referred to "*Oxyrrhis* sp." as circatidal adherent cysts have not been reported in *O. marina*.

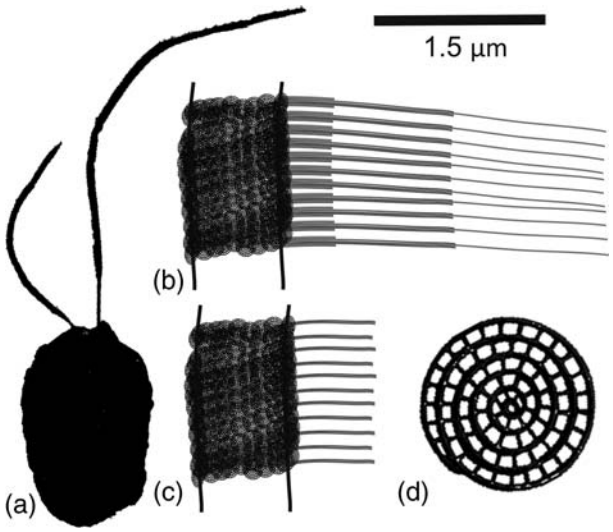


**Fig. 4.** Illustrations to indicate the extent and position of scales and mastigonemes on *O. marina*: (**a**) a silhouette of a cell, indicating a widening of the flagella caused by scales (redrawn from Clarke and Pennick, 1972); (**b** and **c**) Indications of the position and size of scales and mastigonemes on the transverse (b) and longitudinal (c) flagella (modified from illustrations, micrographs, and descriptions in Clarke and Pennick (Clarke and Pennick, 1972, 1976); (**d**) a scale (modified from Clarke and Pennick, 1976). The scale bar is associated with (b) and (c), only. For the size of cells and scales see the text.

### *The ventral bulge or tentacle*

This medial, ventral structure is well documented in the earlier literature, and as indicated above, its length was used to diagnose *O. tentaculifera*. In *O. marina*, it is relatively small (~5 μm), is constricted proximally and is located below the horizontal ridge (Dodge and Crawford, 1971a,b). See Cortical microtubular arrangement section for more details of this structure.

### *Flagellar structure*

The structure and function of the *O. marina* flagellar apparatus is well documented (Dodge and Crawford, 1971a,b; Roberts, 1985; Cachon *et al.*, 1988, 1994; Roberts and Roberts, 1991; Godart *et al.*, 1992) and differs from those of other dinoflagellates, in that the transverse flagellum lacks a broad striated strand (Cachon *et al.*, 1988). Unique features are that the transverse flagellum of *O. marina* possesses a row of complex mastigonemes, while the longitudinal flagellum possesses simple mastigonemes; both flagella are covered with scales, except at the proximal ends (see *Scales* below, Clarke and Pennick, 1972; Fig. 4a).

The flagellar root system has been studied in detail in two strains (from Newport, Rhode Island, and Texas Culture Collection strain LB1974; Roberts, 1985); this and other studies indicate that *O. marina* differs from

other dinoflagellates in several respects, including: the breadth of the posteriorly directed microtubular root; the orientation of connective structures and electron dense core of the ventral microtubular root; and the occurrence of fibres that parallel the flagella (Roberts, 1985; Roberts and Roberts, 1991).

The two flagella of *O. marina* differ in function, as recognized by early researchers, e.g. Saville-Kent (Saville-Kent, 1880) and Senn (Senn, 1911). The structure and function of the two flagella were rigorously investigated using an isolate from Villefranche-sur-Mer (Cachon *et al.*, 1988); this study indicated that the waves produced by the longitudinal flagellum are planar and symmetrical, and the transverse flagellum, which is coiled, produces helical waves and is responsible for the cork-screw-like swimming motion, propelling the cell forward (see Boakes *et al.*, 2011).

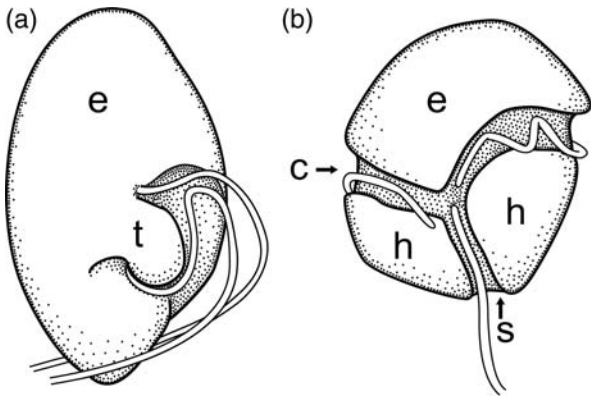


**Fig. 5.** Schematic illustrations of (**a**) *Oxyrrhis marina* and (**b**) a generalized athecate dinoflagellates indicating: the epicone (e), the hypocone (h), the tentacle (t)/ventral bulge, the cinglum (c) and the sulcus (s).

*Scales*

*Oxyrrhis marina* is distinguished from other dinoflagellates by possessing flagellar and body scales (Clarke and Pennick, 1972, 1976). Scales cover both flagella of two isolates, LB1133/1 (isolated from Långskar, Finland—now culture CCAP1133/5, maintained by the Culture Centre of Algae and Protozoa, Oban) and LB1133/2 (isolated from Essex, UK). Scales also occur on the whole cell surface of three isolates: LB1133/2, LB1133/4 (isolated from Bahrain—now CCAP1133/4, maintained by CCAP, Oban) and a sample from Norfolk (Clarke and Pennick, 1976).

Both flagellar and body scales are ellipsoidal or circular spiral plates 0.15–0.175 × 0.2 μm (Fig. 4d), each having a tight spiral of two to five turns (Fig. 4b and c, Clarke and Pennick, 1972, 1976). The scales cover the flagella, although they may be absent close to the cell; they are arranged lengthways around the flagella, forming a helix, with rows overlapping. On the transverse flagellum, the row of mastigonemes runs parallel to a row of scales (Fig. 4b). Imbrications of body scales appear in some (but not all) areas of the cell surface (Clarke and Pennick, 1976).

*Trichocysts*

As noted in some of the earliest descriptions, the cell surface is rough, with staggered double rows of projections running along the cell length (Clarke and Pennick, 1976); these are concentrated at the anterior end and appear to be associated with the region where trichocysts abut the cell surface. Dodge and Crawford (Dodge and Crawford, 1971a,b) suggest that the trichocysts of *O. marina* are similar in structure to those of other dinoflagellates, although possibly more rigorous study will reveal differences, as more recent detailed study of extrusomes of other alveolates show unique structures (e.g. Cavalier-Smith and Chao, 2004).

*Cortical microtubular arrangement*

The cortical structure of *O. marina* is similar to that of other alveolates (see Dodge and Crawford, 1971a); however, its microtubular cytoskeleton differs from that of dinoflagellates (see Roberts *et al.*, 1993 Fig. 17 for a comparison of cortical microtubular arrangements in *O. marina* and dinoflagellates). The longitudinal microtubules of *O. marina* are intact from pole to pole, and do not abut the transverse microtubules; this is in contrast to typical dinoflagellates in which the transverse microtubules abut the longitudinal at both the anterior and posterior ridges of the cingulum. Notably, *O. marina* does have a distinct transverse band associated with the ventral ridge, which may be homologous to the structures underlying the anterior ridge of the cingulum in other dinoflagellates. In contrast, there appears to be no transverse microtubules, homologous to those of the lower region of the cingulum (Roberts *et al.*, 1993). The ventral bulge (see above) has been suggested to be a reduced hyposome (i.e. the posterior half of the cell below the cingulum; Fig. 5; Brown *et al.*, 1988), but the lack of associated transverse microtubules prevents comparison to the same structures in other dinoflagellates.

*Mitotic apparatus, division and chromatin structure*

Dinoflagellates exhibit unusual nuclei, with a range of structural and molecular modifications that distinguish them from the "typical" eukaryote model of nuclear and chromosomal organization (Hausmann *et al.*, 2003). For some time, it has been recognized that the nuclear structure of *O. marina* differs from that of the typical dinoflagellate (e.g. Hall, 1924, but see Slamovits

and Keeling, 2011). *Oxyrrhis marina* generates an intranuclear mitotic spindle during mitotic cell division, in contrast to the extranuclear spindle of most dinoflagellates (Cachon *et al.*, 1979; Triemer, 1982; Gao and Li, 1986). As in other dinoflagellates, the nuclear envelope of *O. marina* persists throughout mitosis. However, unlike dinoflagellates, plaques (from which the mitotic spindle is generated) appear on the nuclear envelope during prophase (Triemer, 1982; Gao and Li, 1986). Chromosomal and chromatin structures in dinoflagellates are also atypical of eukaryotes and exhibit birefringent periodic banded or arched structures (Cachon *et al.*, 1979; Triemer, 1982; Gao and Li, 1986); these features have not been reported in *O. marina*, although in other respects its condensed chromosomal structure is dinoflagellate-like. Additionally, the typical eukaryote complement of DNA-associated histones is absent in dinoflagellates, and again *O. marina* differs from dinoflagellates by possessing a single 23 kDa histone-like DNA-associated protein (Kato *et al.*, 1997).

Of final note, to our knowledge there are no data to indicate whether *O. marina* is haploid, diploid or polyploid. Most dinoflagellates are haploid (Hausmann *et al.*, 2003), although diploidy occurs in some genera (e.g. *Noctiluca*, Zingmark, 1970; Pfiester and Anderson, 1987; Montagnes *et al.*, 2011b).

## *Oxyrrhis* taxonomy and phylogeny

The above morphological, ultrastructural and cytological studies provide extensive data to infer the taxonomic position of *O. marina* relative to other alveolates; indeed, much of the ultrastructural study of *O. marina* has been stimulated by the recognition that it is a somewhat aberrant dinoflagellate. Two conflicting taxonomic positions have been proposed for *O. marina*: either basal to (or an early branch of) the dinoflagellate lineage, suggesting an ancestral state; or derived, occurring within the Gonyaulaceae. Several authors (e.g. Cachon *et al.*, 1979; Taylor, 1980; Loeblich, 1984; Kato *et al.*, 2000) support the basal position for *O. marina* based on the argument that the flagellar apparatus, reduced sulcus and girdle, cortical microtubular structure and the apparent intermediate nuclear and chromosomal organization are all primitive. Contrastingly, other authors (see Cavalier-Smith and Chao, 2004) infer a highly derived position for *O. marina*, based on rDNA phylogenies and the subsequent argument that the presence of histone-like proteins, an intranuclear mitotic spindle and the reduction of sulcus and cingulum grooves support this derived position (see Cavalier-Smith and Chao, 2004 for their reasoning).

Two striking conclusions arise from a summary of the morphological literature: (i) *O. marina* is a dinoflagellate, but its position within the group is uncertain; and (ii) despite early descriptions of multiple *Oxyrrhis* species, most studies accept the opinions of Kofoid and Swezy (Kofoid and Swezy, 1921) and Dodge (Dodge, 1982) that only the single species *O. marina* exists. In the next section, we examine molecular phylogenetic data to further consider these two issues.

## Molecular phylogenetic studies of *Oxyrrhis marina*

Genetic data have inevitably been applied to examine the taxonomic and phylogenetic affiliation of *O. marina*. Molecular genetic studies provide support for both proposed positions of *O. marina*, although the majority of recent studies support that it is an early branching dinoflagellate, or a close ancestral lineage, branching after perkinsids. The first phylogenetic study to include *O. marina* (Lenaers *et al.*, 1991) assessed phylogenetic relatedness within the dinoflagellates based on sequence data for two divergent domains of the 24S rRNA gene in 12 species. This study supported a basal position, placing *O. marina* as an early emerging dinoflagellate, preceding the Peridiniales. A subsequent study (Saldarriaga *et al.*, 2003) based on SSU rDNA and sequences for actin, alpha-tubulin and beta-tubulin highlighted the two opposing phylogenetic positions. Phylogenies based on SSU rDNA sequences indicated a derived branching position within the Gonyaulacales, but noted that the affiliation should be interpreted cautiously as a result of the highly divergent *O. marina* rDNA sequence (Saldarriaga *et al.*, 2003). Conversely, in the same study actin, alpha-tubulin and beta-tubulin genes of *O. marina* were not noticeably divergent, and in phylogenetic trees based on all three proteins individually and in combination *O. marina* branched at the base of the dinoflagellate lineage. A study using these genes plus HSP90 achieved similar results (Leander and Keeling, 2004). Furthermore, a recent analysis including an extensive data set with 30 protein-coding genes strongly supported the basal position of *Oxyrrhis* (Slamovits *et al.*, 2007). Finally, studies of mitochondrial genome structure and RNA editing mechanisms also lend support to an early branching position for *O. marina* relative to the dinoflagellates, in particular, since *Oxyrrhis* was found to completely lack mitochondrial RNA editing, which is found in all other dinoflagellates (Slamovits *et al.*, 2007; Zhang and Lin, 2008). Although some authors (e.g. Cavalier-Smith and Chao, 2004) have maintained a derived position for *O. marina*, this hypothesis has not received further support and in

the light of the current wealth of data (i.e. multiple protein phylogenies, mitochondrial genome structure and RNA editing mechanisms), we assume that the basal position of *Oxyrrhis* relative to dinoflagellates is the correct interpretation.

## Contemporary evidence for cryptic *Oxyrrhis* species

The above review highlights that the taxonomic and phylogenetic affiliations of *O. marina* are well described, if not entirely agreed upon. In contrast, our understanding of genetic, physiological and morphological variability within *O. marina* is limited. Indeed, while early morphological studies argue for multiple *Oxyrrhis* species, assessments of variability between different *O. marina* strains and isolates are rare. Given the increasing number of examples of cryptic diversity in a broad range of free-living protist taxa (e.g. Darling *et al.*, 2004; Slapeta *et al.*, 2005), this lack of study represents an important oversight. In fact, recent studies of *O. marina* suggest that high levels of genetic diversity occur within the current *O. marina* morphospecies (Cavalier-Smith and Chao, 2004; Lowe *et al.*, 2005, 2010). In the following section, we examine assessments of variability within *O. marina*, highlight that current observations of morphological and cytological variation are scarce and indicate that genetic studies reveal extensive diversity. Based on the strength of the molecular phylogenetic data, we propose that there are two *Oxyrrhis* species—*O. marina* and *O. maritima*—for which we provide new diagnoses (the existence of a third species, *O. tenticulifera*, is also discussed below). Ultimately, this re-designation reflects the extent of diversity within the genus and provides an important framework to direct future comparative morphological, physiological and genetic studies.

### *Combining morphological and molecular data*

Six studies have examined variation between *O. marina* isolates (Table I). Of the morphological and cytological studies, only Clarke and Pennick (Clarke and Pennick, 1972, 1976) and Roberts (Roberts, 1985) compared *O. marina* isolates, based on scales and flagellar structure, respectively, and neither noted variation. The most extensive assessments of diversity within *O. marina* are phylogenetic, although these too are limited. Three studies have quantified the level of genetic variation between *O. marina* isolates based on a single gene (rDNA) and a small number of isolates ($n = 2$, 3 and 11 for Saldarriaga *et al.*, 2003; Cavalier-Smith and Chao, 2004; Lowe *et al.*, 2005, respectively). These studies indicate: (i) an exceptionally high level of divergence in the basal *O. marina* branch (Saldarriaga *et al.*, 2003) and (ii) two divergent lineages that have been proposed as separate species (Cavalier-Smith and Chao, 2004; Lowe *et al.*, 2005). Following this, a recent assessment of diversity within *O. marina* examined 5.8S ITS rDNA and mitochondrial cytochrome c oxidase I (COI) in 58 *O. marina* isolates; this work supported two highly divergent lineages, each composed of two distinct clades (Fig. 6; Lowe *et al.*, 2010). Based on the COI gene, sequence divergence between lineages was 10.5% (within lineage divergence was <1% in both cases). Mitochondrial COI sequences in particular are now commonly used to aid species delineations across a broad range of organisms (Hebert *et al.*, 2003; Sites and Marshall, 2003; Frezal and Leblois, 2008), with for example, 3–11% divergence (at COI) used to delineate species across a range of protist taxa (e.g. Evans *et al.*, 2005; Chantangsi *et al.*, 2007; Gentekaki and Lynn, 2009; Lin *et al.*, 2009). Comparisons of these divergence estimates strongly support the occurrence of two *Oxyrrhis* species.

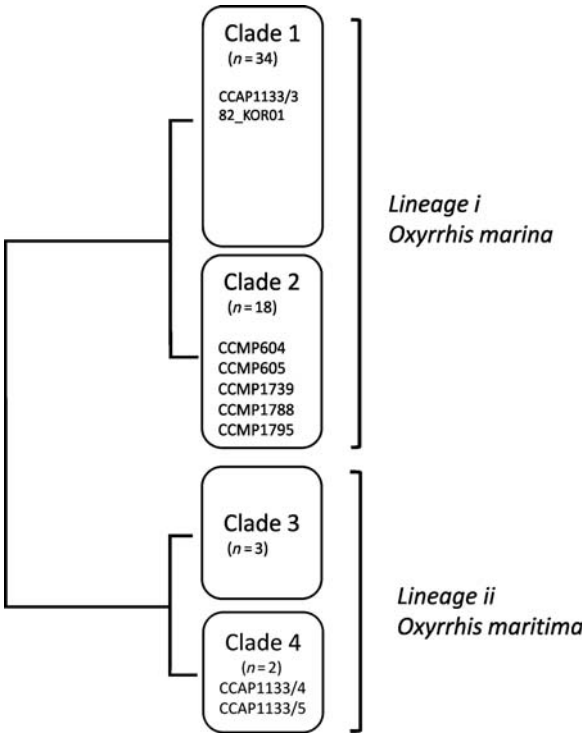


**Fig. 6.** Cladogram (redrawn from Lowe *et al.*, 2010) of the four *Oxyrrhis* clades defined based on 5.8S ITS rDNA and mitochondrial COI sequence data. Representations of the four clades are scaled according to the number of isolates know to belong to each clade. Indicated are the proposed species names for the two *Oxyrrhis* lineages and the most commonly used *Oxyrrhis* strains for which affiliations are known (CCAP and CCMP indicate the source culture collection: CCAP—Culture Collection of Algae and Protozoa, Dunstaffnage, UK; CCMP—Provasoli—Guillard National Center for Culture of Marine Phytoplankton,West Boothbay Harbour, ME, USA).

*Table III: Criteria for the re-designation of species names in the genus Oxyrrhis*

| Criteria for species assignment to *Oxyrrhis* lineages | Lineage *i* | Lineage *ii* | Citation |
|---|---|---|---|
| Environmental prevalence (i.e. occurrence in the ~150 samples that have been collected by us, to date) | 83 | 17 | Unpublished data |
| Global breadth of distribution | Broad | Narrow | Watts *et al.* (2011) |
| History of study (years), based on isolation date of commercial cultures | <20 | >50 | This study |
| Citations for the single most studied strain in each lineage | 6 | 17 | This study (Fig. 1a) |
| Citations for all stains within each lineage | 25 | 21 | This study (Fig. 1a) |
| Number of confirmed isolates within each lineage | 52 | 5 | This study, Lowe *et al.* (2010) |
| Species designation | *marina* | *maritima* | |

Oxyrrhis marina *is more than one species*

Based on the molecular evidence detailed above, we propose two *Oxyrrhis* species: *O. marina* and *O. maritima* (see diagnoses below). Following recommendations by Foissner *et al.* (Foissner *et al.*, 2002), we have adopted the use of previously employed species names; thus we resurrect the synonymized specific epithet *O. maritima* to denote the second *Oxyrrhis* species.

A third species, *O. tentaculifera*, may also occur. As noted, in our opinion, the description of *O. tentaculifera* (Conrad, 1939) is sufficiently distinct to stand as a separate species—although contemporary observations and DNA sequence data for this species are clearly required to support its existence and assess its precise relationship to the two other *Oxyrrhis* species. For completeness and to highlight this species as a subject for future study, a diagnosis of *O. tenticulifera* is included below.

## Amended diagnoses

*Diagnosis of* Oxyrrhis

Cell subovoidal, asymmetrical posteriorly; girdle postmedial, not extending to dorsal surface; sulcus spreading posteroventrally; flagella midventral; tentacular lobe occurs between two flagella, dividing the broad undeveloped ventral sulcus; brackish to marine; generally intertidal but occasionally open water.

*Diagnosis of* Oxyrrhis marina *Dujardin, 1841*

Length, 20–30 μm, but occasionally twice this size; appears colourless but with pink pigmentation that is apparent in concentrated cultures; tentacular lobe never extends beyond cell posterior; for mitochondrial cytochrome c oxidase I > 97% identity to accession number FJ853710 (Strain CCMP604).

*Diagnosis of* Oxyrrhis maritima *Van Meel, 1969*

Length, 20–30 μm, but occasionally twice this size; appears colourless but with pink pigmentation that is apparent in concentrated cultures; tentacular lobe never extends beyond cell posterior; brackish to marine; for mitochondrial cytochrome c oxidase I > 97% identity to accession number FJ853706 (strain CCAP1133/5).

*Diagnosis of* Oxyrrhis tentaculifera *Conrad, 1939*

Length, 16–24 μm; cell approximately two times as long as wide; compressed dorso-ventrally; tentacular lobe extends beyond cell posterior, used for prey capture and at times adhesion to surfaces; cell colourless. Type location: brackish marsh, Belgium (51°17′N, 3°12′E).

## Reasoning for diagnosis of three species of *Oxyrrhis*

High levels of cryptic genetic diversity are now documented for many free-living protist taxa. Such variety raises important questions—is extensive genetic variation paralleled by functional diversity, and does this need to be accounted for in evaluations of physiological responses and ecological interactions? Clearly, the use of experimentally tractable model organisms, such as *O. marina*, is an important strategy to address these questions. However, failure to recognize the sources and extent of cryptic variation in these organisms is problematic.

For *Oxyrrhis*, the designation of two species highlights for future studies that: (i) a more cautious approach must to be taken in selecting and characterizing *Oxyrrhis* isolates for experimental study (i.e. it is inappropriate to report assessments concerning poorly characterized isolates) and (ii) comparative studies of multiple isolates are required to assess individual, population and species level variation in the *Oxyrrhis* genus. Such recommendations are clearly relevant to all protist species and it should now be exceptionally clear that new species designations should include morphological and genetic data, and where possible examination of multiple isolates to assess variability.

Our reasoning for the designation of lineage *i* and lineage *ii* (Fig. 6, Table III; Lowe *et al.*, 2010) as

*O. marina*, and *O. maritima*, respectively, follows (Table III). As indicated above, there are no morphological data to tie the proposed molecular-based species to the original description of *O. marina*, nor does type location (i.e. coastal Mediterranean, France) provide a criterion to assign species names (as representatives from all clades occur in the French Mediterranean; Lowe *et al.*, 2010; Lowe, unpublished results). The original description of *O. maritima* as larger and rounder than *O. marina* offers a potential distinguishing morphological characteristic; however, our observations to date (unpublished results) do not suggest a difference in cell size between *Oxyrrhis* lineages. Furthermore, the small amount of work that recognizes ecophysiological differences between isolates (Lowe *et al.*, 2005) offers no guidance on defining "ecotypes". Therefore, we have chosen to designate species based on the least disruptive classification, using occupied names of junior synonyms. In this respect, there are a range of criteria that suggest that *O. marina* should be represented by lineage *i*—it is the most prevalent, has the widest distribution and has the highest number of confirmed isolates, and therefore changing its name would be most disruptive (Table III)—the overriding reason, however, is simple: there are only two well-studied (Lowe *et al.*, 2010) isolates of *Oxyrrhis* that are available from commercial culture collections in lineage *ii*, while there are six, well-studied, commercially available isolates in lineage *i*. Thus, by assigning the specific epithet *maritima* to lineage *ii*, we minimize the need to reassign names to past work and minimize future confusion.

### Recommendations for future studies

Our recommendations for species designation are based on phylogenetic data only. Clearly then, there is scope to re-visit, in a comparative context, many morphological studies conducted on single *O. marina* isolates to better define the extent of diversification within the genus. We suggest that studies of flagellar scales, tentacular structure and size, cyst formation and potentially flagellar rootlet structure will be fruitful directions for such work. In addition, the recognition of several species in the genus provides further avenues of research for which these model organisms may be applied. For example, as a novel intermediate taxon at the base of the dinoflagellate lineage, *Oxyrrhis* is increasingly a target for the study of evolutionary patterns and genome organization within the alveolates. The occurrence of distinct species within the *Oxyrrhis* genus represents a useful pool of variation to study processes that occurred during the evolution of the dinoflagellates and the development of derived "*Oxyrrhis*" characteristics. Finally, in a broader context, our understanding of the ecological and evolutionary processes that drive patterns of diversity and speciation in free-living protists as a whole remains poor. Model protists such as *O. marina* and *O. maritima* for which we are beginning to recognize and characterize an extensive pool of variation present ideal opportunities to unravel these fundamental processes.

## ACKNOWLEDGEMENTS

The Authors would like to thank Emily Roberts, Michael Steinke and Naoji Yubuki for constructive comments on the manuscript. We would also like to thank two anonymous reviewers for their suggestions.

## FUNDING

This work was, in part, supported by a UK NERC grant (NE/F005237/1) awarded to P.C.W., C.D.L. and D.J.S.M.

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