

The distribution of *Oxyrrhis marina*: a global disperser or poorly characterized endemic?

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It is often argued that microscopic organisms typically have cosmopolitan distributions. This argument has been criticized as it fails to acknowledge the many protists that have limited geographic distributions. *Oxyrrhis marina*, a common heterotrophic dinoflagellate in many intertidal and coastal habitats, is regarded as globally distributed, yet despite its prominent role as an experimental planktonic organism there are few explicit studies of this species' geographic range. An analysis of 846 water samples globally confirmed the wide geographic distribution of *O. marina* and extends this morphospecies' range into the southern hemisphere. We did not find *O. marina* beyond 63°N latitude, implying that it is rare, or even absent, in Polar seas. Despite being rare in open water, *O. marina* inhabits the coastal waters of remote islands, such as Hawaii and the Azores. Beneath the apparent broad spatial range are some emerging patterns on the distribution of distinct genetic clades that form two divergent lineages that may represent different species. Two of the clades have discrete, non-overlapping distributions (one in the European Atlantic and western Mediterranean, and the second in the eastern Mediterranean Sea): one has a broad distribution (both coasts of North America and also in the eastern Mediterranean Sea), and one is limited to culture collections from the Baltic Sea and Red Sea. Thus, the Mediterranean Basin appears to be a diversity hotspot for *Oxyrrhis*, and there is a mixture of wide distributions and endemism. These issues are discussed with respect to protist biogeography.

KEYWORDS: dispersal; speciation; cosmopolitan species; biogeography

INTRODUCTION

Oxyrrhis marina, a common heterotrophic dinoflagellate in many intertidal and coastal habitats, is typically regarded as cosmopolitan or globally distributed (e.g. see AlgaeBase—<http://www.algaebase.org/>—date accessed 15 October 2009). It is surprising, therefore, that despite extensive research using *O. marina* as a model marine protist (Montagnes *et al.*, 2011a), there

are few explicit studies of this species' natural distribution. Thus, the apparent global occurrence of *O. marina* is poorly characterized. As a consequence, we lack the basic data that underpin an understanding of the ecological and evolutionary processes that generate present patterns of diversity, and this inevitably creates problems when studies attempt to interpret patterns of divergence (e.g. functional or genetic) between isolates of *O. marina*.

Below, we first provide an indication of why it is important to assess model-species distributions in marine environments. We then indicate the scope of knowledge regarding the distribution of *O. marina*, indicate why this species can act as a model, in this respect, and finally suggest which data are required to improve our use of *O. marina* as a model.

DISTRIBUTIONS OF MARINE SPECIES

Most macroscopic species (defined here as organisms >10 mm in size) have relatively restricted geographic distributions that presumably reflect a combination of their ecological requirements, evolution and historical contingency (see [Finlay and Fenchel, 2004](#)). The relative influences of these processes depend upon the taxon, but there remains some debate about the general importance of such processes in generating observed biogeographic patterns. For example, a contrast may be made between (i) taxa that evolve at a point and subsequently disperse outwards to realize their geographic distribution and (ii) species whose evolution is driven by vicariance or allopatry are characterized by no obvious centre of origin ([Heads, 2005](#)). An issue that remains central to any debate about biogeographic patterns is a species' ability to disperse across heterogeneous landscapes and, therefore, its potential to colonize and exploit new habitats, and to maintain gene flow over wide areas.

As an extreme biogeographic pattern, cosmopolitanism (organisms that occur globally or quasi-globally) nonetheless has been described for a substantial number of large species, particularly marine taxa which may be distributed by currents or allowed to disperse due to the connectivity of the oceans (e.g. [Graves, 1998](#); [Watts et al., 1998](#)). On the one hand, the pelagic marine environment is perceived to be relatively more stable and typically lacking in barriers to dispersal, when compared with terrestrial landscapes or even benthic marine systems ([Palumbi, 1994](#); [Graves, 1998](#); [Sexton and Norris, 2008](#)). For species with life-history traits suitable for wide dispersal, particularly free-living pelagic species, such uniformity potentially permits broad geographic distributions. On the other hand, however, this picture is an overly simplistic, and somewhat outdated, view of a marine environment that is now recognized to be heterogeneous. Indeed, numerous studies document effective barriers to dispersal (e.g. oceanic fronts), and concomitant genetic divergence, in a broad range of marine taxa ([Darling et al., 2004](#); [Gallindo et al., 2006](#); [Hansen and Hemmer-Hansen,](#)

[2007](#); [Patarnello et al., 2007](#)). Nevertheless, there is also contemporary evidence for extensive and cosmopolitan distributions for a broad range of marine organisms. With this comparison in mind, it is somewhat surprising that the underlying biology of “cosmopolitan” species, such as *O. marina*, is often overlooked, as they should possess interesting life-histories.

One possible reason for a relative lack of recent interest in the biology of cosmopolitan species appears to be a degree of scepticism towards this phenomenon. An extensive geographic range, for example, may be an artefact of inadequate taxonomic resolution, which combined with undersampling in the marine environment, could lead to unrecognized cryptic species being amalgamated into a morphospecies with artificially large distributions ([Knowlton, 1993](#); [Klautau et al., 1999](#)). With the increasing number of molecular-genetic studies that have identified cryptic species in apparently widely distributed taxa ([Todaro et al., 1996](#); [Klautau et al., 1999](#); [Westheide and Schmidt, 2003](#); [Fukami et al., 2004](#); [Derycke et al., 2008](#); [Lowe et al., 2011a](#), but cf. [Aitken et al. 2007](#)), reports of cosmopolitanism are typically regarded as more apparent than actual and the debate appears to have reduced.

MICROBIAL DISTRIBUTIONS

For microbial species, in contrast, controversy has developed with conflicting theories and predictions about the extent and distribution of (free-living) microbial diversity, and ultimately whether microbial biogeography is fundamentally different to that of macroorganisms. On the one hand it has been argued that microscopic or “small” organisms (<1 mm) typically have a cosmopolitan distribution in terrestrial, freshwater and marine environments ([Finlay and Clarke, 1999](#); [Finlay, 2002](#); [Fenchel and Finlay, 2003, 2004](#); [Finlay and Fenchel, 2004](#)). These authors argue that wide geographic ranges are a consequence of huge absolute population sizes (rather than inherent properties of certain taxa), which drive wide dispersal potential and maintain a low probability of local population extinction. Since endemism largely generates patterns of species diversity of large organisms, there is an apparent effect of scale, with the diversity of small species exceeding that of larger organisms at local scales, whereas the feature is reversed at larger scales (e.g. global at the extreme). The corollary is that the distribution of small organisms is less dependent on historical contingency and that free-living microbial species are less likely to have biogeographies. Clearly, the above arguments are relative, being based on the size of the organism, its dispersal rate, and the

size of the spatial niche that it occupies, but some support for the notion of very wide or cosmopolitan distributions follows from a number of studies on eukaryotic microbial taxa, such as diatoms, foraminiferans and diplomonads (Hasle, 2002; Casteleyn *et al.*, 2008; Kooistra *et al.*, 2008; Lara *et al.*, 2009); in some cases these imply that certain protist species are capable of global gene flow (Darling *et al.*, 2000; Pawlowski *et al.*, 2007).

Several flaws in the “ubiquity model” have been highlighted, including poor taxonomic resolution and under-sampling (Heger *et al.*, 2009; reviewed by Dolan, 2005; Foissner, 2006, 2008), with perhaps the most serious being an apparent failure to acknowledge the many studies that demonstrate many protists have limited geographic distributions (Wilkinson, 2001; Chao *et al.*, 2006; Smith and Wilkinson, 2007; reviewed by Foissner, 2006, 2008; Vanormelingen *et al.*, 2008). Although it is reasonable to assume that biogeographic and evolutionary concepts used in macroorganismal biology are applicable to free-living microbes, it is important recognize that the majority of free-living microbes tend to have larger geographic ranges than do macroorganisms. Accordingly, a “moderate endemism” counter argument has been proposed (Foissner, 2006, 2008), whereby protists, compared with prokaryotic microbes, generally have low abundances, and low rates of dispersal that result in moderate extinction rates and moderate (~30%) levels of endemism.

Anecdotally, the literature indicates *O. marina* to be cosmopolitan, isolates of which have been collected from throughout the northern Atlantic and Pacific Oceans, as well as the Mediterranean, Baltic and Red Seas. The older literature (1841–1917), reviewed by Kofoed and Swezy (1921), indicates a wide distribution of *O. marina*, including: Kiel (Germany), Plymouth (England), Genoa harbour (Italy), Jersey, Marseilles (France), Palingbrug (Belgium), Tengerick (New Guinea), more broadly in the Mediterranean and White Seas and, most unusually, in a salt lake in Kharkoff (Russia). Kofoed and Swezy (1921) also noted that *O. marina* is a regular inhabitant of marine aquaria around Europe: Frankfurt, Helgoland and Heidelberg (Germany), Naples (Italy), Roscoff and Villefranche (France). Thus, even as early as the turn of last century, *O. marina* was recognized to have a wide distribution.

One problem with these data are the increasingly numerous examples of morphologically simple protist ‘species’ that have been shown, using molecular-genetic techniques, to harbour extensive cryptic diversity (de Vargas *et al.*, 1999; Darling *et al.*, 2004; Schekenbach *et al.*, 2006; Slapeta *et al.*, 2006). *Oxyrrhis marina* is no exception, as it possesses few easily observable

cytological structures (see Lowe *et al.*, 2011a, for taxonomic assessment), yet recent work (Lowe *et al.*, 2005a, 2010) has identified several divergent lineages within what is described as “*Oxyrrhis marina*”, culminating in support for the notion of distinct species (Lowe *et al.*, 2010, 2011a). The apparent wide distribution of “*O. marina*” may be a simple consequence of over-conservative taxonomy. We, therefore, argue that it is difficult to interpret a range of experimental studies on protists that have uncovered extensive ecophysiological variation between isolates (Lowe *et al.*, 2005a; Weiss and Rammer, 2006), as it is not known whether this variation reflects adaptation within a single eurytopic species (i.e. one that is able to tolerate a wide range of environments) or has simply described a number of species-specific responses.

In the following sections, we highlight the current extent of our understanding of *O. marina* spatial and temporal distributions. In particular, we stress that explicit studies of *O. marina* distributions are scarce and that our current knowledge of distributions is almost entirely derived from a synthesis of methodological observations from the literature. Further, in light of recent studies describing high levels of functional and genetic variation within the *O. marina* morphospecies (Lowe *et al.*, 2005a, 2010, 2011a), we indicate that the majority of studies on *O. marina* are confined to few isolates that likely belong to disparate phylogenetic groups. We emphasize that far from assuming cosmopolitanism on the part of *O. marina*, future studies must address the distributions of potentially multiple, cryptic *Oxyrrhis* species. Finally, it is apparent, that in a biogeographic context, *O. marina* has the potential to be an important model organism to assess demographic parameters determining the processes that drive species distributions and geographic patterns of functional and genetic diversity in free-living protists.

“*OXYRRHIS MARINA*” IS BROADLY DISTRIBUTED AND LOCALLY ABUNDANT

Oxyrrhis marina was described over 150 years ago from a salt marsh habitat in Belgium (Fig. 1), and early work mentioned above characterized its putative wide distribution; typically it is now widely regarded as a single, broadly distributed species. A citation search using the keyword “*Oxyrrhis*” in *Web of Knowledge* (<http://wok.mimas.ac.uk/>) returned 114 papers published between 1938 and 2009 (date of search September 2009) that reported 38 isolates/sampling locations (in 64 cases no



Fig. 1. Summary of the distribution of 38 defined isolates of *O. marina* synthesized from a review of 157 manuscripts. The diameter of the symbols is proportional to the frequency that an isolated is cited in the literature. Note that, despite a wide geographic distribution, work is biased on two isolates: (i) CCAP1133/5 isolated from Finland ($n = 17$ manuscripts) and (ii) an isolate from Villefranche-Sur-Mer ($n = 11$ manuscripts). The majority of strains ($n = 24$) were reported in the literature just once.

details of the strains were provided, or a reference only was provided, and 19 papers could not be accessed by the authors). On the basis of these published data, the *O. marina* morphospecies is best described as broadly distributed, inhabiting areas of the Atlantic and Pacific coasts of the USA, the Gulf of Mexico, the Atlantic coasts of Europe, the Mediterranean and Baltic Seas, Persian Gulf, the Indian Ocean and the western Pacific (Fig. 1). Throughout this distribution, however, *O. marina* has been isolated only from coastal areas, and predominantly from intertidal pools. This is potentially a sampling bias as a result of the particular ease with which littoral environments can be accessed (compared with open waters). *Oxyrrhis marina* is unlikely to be an exclusively intertidal species, since it has been identified from plankton surveys of neritic environments, estuaries and coastal embayments (Quevedo and Anadón, 2000; Johnson *et al.*, 2003; Begun *et al.*, 2004; Jeong *et al.*, 2007; Orlova *et al.*, 2009). Despite these observations, *O. marina* does not appear to be common in neritic habitats as other, direct comparisons of lagoon and coastal sites have not reported its presence (Lopez-Flores *et al.*, 2006). Indeed, in a summary of the distribution of dinoflagellates in UK waters, Dodge (1982) classifies *O. marina* as a eurytopic species that inhabits pools, estuaries and marshes, rather than open water. Given its distinctive morphology (see Lowe *et al.*, 2011a), it seems unlikely that *O. marina* has been overlooked during plankton surveys. Thus, it is unlikely that it is a common component of the coastal and oceanic plankton community, although periodically it may form red tides in shallow embayments (Begun *et al.*, 2004).

Clearly, the observations made above are more anecdotal than systematic; crucially, we lack data about locations where *O. marina* appears to be absent, or at

least rare. As part of our research into levels of genetic and ecophysiological variation of *O. marina* throughout its range, we have acquired 846 samples from 40 countries. The distribution of samples that yielded *O. marina* reinforces the observations made above and also extends this morphospecies' range into the southern hemisphere, into Australia, South Africa and Brazil (Fig. 2). We did not find *O. marina* beyond 63°N latitude (e.g. northern Norway, Iceland), implying that it is rare, or even absent, in Polar seas. However, it has to be stressed that relatively few (<20) samples were collected from these areas; thus we may simply lack power to detect *O. marina* in these locations (see Lowe *et al.*, 2011b). Indeed, some reports of *O. marina* at northern latitudes (e.g. White Sea, and Tromsø, Norway; Hansson, 1997), suggest that *O. marina* may occur in Polar seas. A second feature of this species' apparent distribution is that, despite being rare in open water, it inhabits the coastal waters of remote islands, such as Hawaii and the Azores (Fig. 2). Given an apparent rarity in open water, speculation about dispersal mechanisms highlights our ignorance of this basic process; for example, clonal reproduction could allow even just a few cells of *O. marina* to persist for relatively long periods (and therefore travel large distances, e.g. by ocean currents), with colonization success simply an inevitable outcome of continued, but low frequency, dispersal from neritic areas over evolutionary time. Alternatively, *O. marina* may be associated with, and thus dispersed by, macroalgal rafts. Finally, it is possible that anthropogenic dispersal (such as transport in ballast water) has extended the natural distribution of this species. Indeed, genetic data provide evidence for trans-Atlantic transport of *O. marina*, possibly associated with shipping

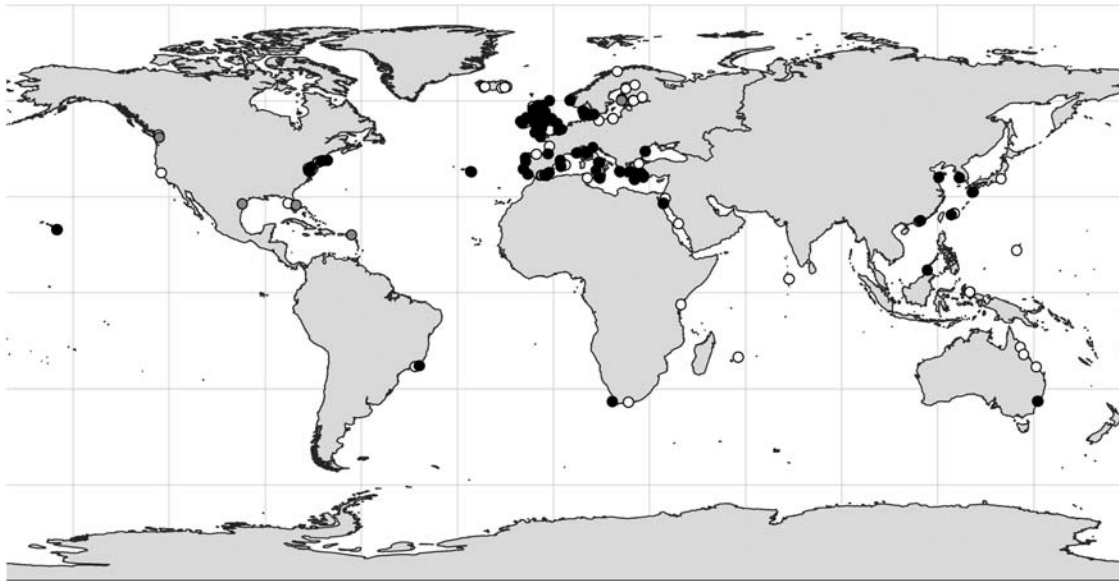


Fig. 2. Summary distribution of global sampling effort for *O. marina*, indicating areas that were positive (filled circles) and negative (open circles); grey circles indicate sample location of *O. marina* isolates that are maintained in culture collections.

routes (Lowe *et al.*, 2010), and *O. marina* is present in ballast water (Burkholder *et al.*, 2007). Given the long history of human global movements using ships, exploring this possibility raises a broader issue of disentangling natural patterns (i.e. currents) and processes from anthropogenically enhanced distributions.

Despite the preceding discussion, this assessment of the literature and our own sampling support the notion that “*O. marina*” is broadly distributed and common in coastal environments and thus occupies a range of contrasting tropical and temperate environments (Fig. 2), which is perhaps not surprising given its tolerance to estuarine/intertidal pool habitats (for details of physiological tolerances see Lowe *et al.*, 2011b; Montagnes *et al.*, 2011b). One intriguing feature of our recent sampling, however, is that we have not yet isolated *O. marina* from samples taken from the eastern Baltic Sea (the positive sample in Figs. 2 and 3 is a strain maintained in a culture collection [CCAP1133/5], which was isolated from coastal waters in Finland in 1951). Droop (1953) reported *O. marina* to be a common inhabitant of intertidal pools from the area surrounding Tvärminne, Finland, and Hansson (1997) reported *O. marina* from the Gulf of Finland and the south and west Baltic. Interpreting negative results is problematic, however, as our failure to isolate *O. marina* from the Baltic Sea could occur for a number of reasons. For example, our samples ($n = 27$) from the Baltic Sea were collected between April and July, and the population growth of *O. marina* may occur during early spring or in the late summer. Counter to this argument is the onset of the

spring phytoplankton bloom in the southern Baltic Sea during April (Wasmund *et al.*, 1998) and studies in the UK (Fig. 4, Kimmance, 2001, Unpublished PhD Thesis) that demonstrate *O. marina* is more abundant during the summer.

Given that misidentification of *O. marina* by Droop and others is extremely unlikely, if future, rigorous sampling continues to fail to yield *O. marina*, it has become rare or even gone extinct. Possibly, as the Baltic Sea has experienced a range of environmental changes associated with pollution during the past decades, *O. marina* may have been eliminated. If supported, this would represent an important addition to the loss of the sturgeon *Acipenser sturio* from the Baltic. More widely, the present records of redlisted protists (<http://www.iucnredlist.org/> date accessed 18 October 2009) are limited to 15 species of phaeophyceae. A confirmed decline or loss of *O. marina* from such a broad geographic area would be the first example of a marine protist neoextinction from a relatively wide area. Crucially, this work emphasizes basic gaps in our knowledge about protist distributions and that this deficiency will continue to hamper any serious efforts to conserve protist biodiversity (see also discussion by Cotterill *et al.*, 2008). As more investigations expand our catalogue of protist species (e.g. Gross, 2007; Foissner, 2008), the question remains: what have we lost?

Beneath the relatively broad geographic patterns mentioned above, a small number of specific studies have quantified the spatial and temporal distribution of *O. marina* within a shore, although they are restricted in



Fig. 3. Detailed distribution of sampling effort for *O. marina* in European coastal waters, indicating areas that were positive (filled circles) and negative (open circles); grey circles indicate sample location of *O. marina* isolates that are maintained in culture collections.

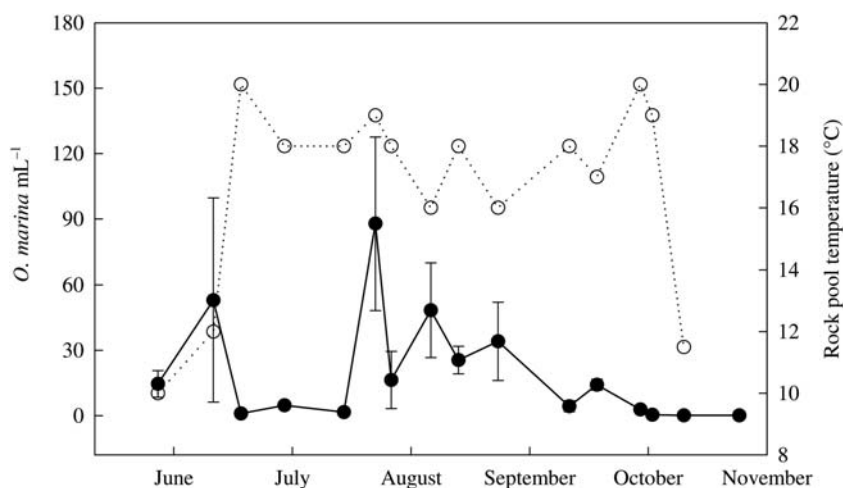


Fig. 4. Seasonal variation in temperature (open circles) and abundance of *O. marina* (filled circles) from intertidal pools at Port St. Mary, Isle of Man (2000). Data points are average (\pm SD) cell densities from three replicate samples (data from Kimmance, 2001, Unpublished PhD Thesis).

scope. For example, Jonsson (1994) associated *O. marina* abundance (described as *Oxyrrhis* sp.) with tidal cycle and flushing. Subsequently, Johnson (2000) identified a positive correlation between tidal height and abundance of *O. marina* on the Isle of Man, with greater numbers in the summer than in winter. Also relevant to our failure to find *O. marina* in some samples, particularly from the Baltic Sea, is work by Kimmance (2001, Unpublished PhD Thesis) who recorded seasonal

variation in the abundance of *O. marina* in intertidal pools (also on the Isle of Man), with peaks in abundance during late June and also between late July and September (Fig. 4).

Prominent in our analysis of the literature is the large number of *O. marina* isolates that are reported in only a single study. Almost certainly this reflects the relative ease with which isolates can be newly derived from the environment (see Lowe *et al.*, 2011b, for discussion on

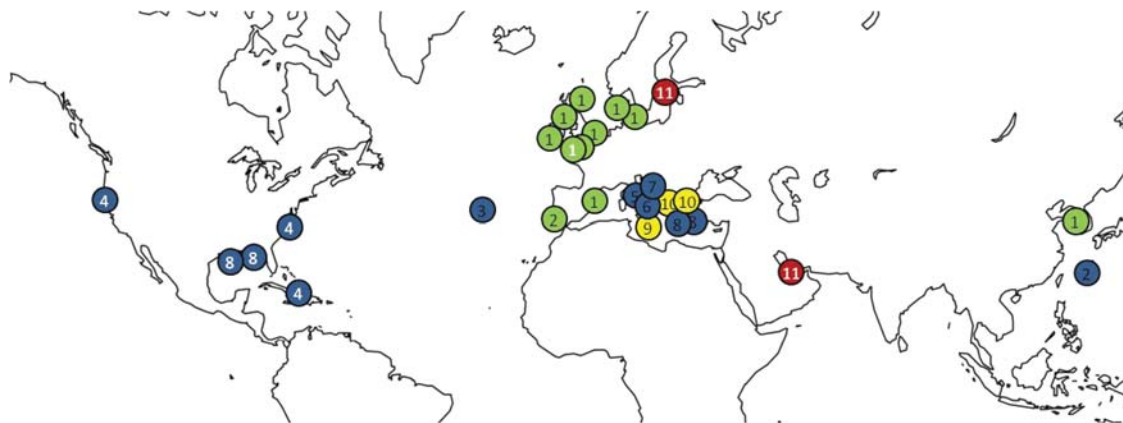


Fig. 5. Distribution of the four *O. marina* phylogenetic clades that have been identified to date. Numbers with circles refer to 11 distinct 5.8S ITS rDNA phylotypes within the four clades: clade 1 (green), clade 2 (blue), clade 3 (yellow) and clade 4 (red). White text refers to strains that were obtained from a culture collection; black text indicates that the strain was isolated from the environment (redrawn from [Lowe et al., 2010](#)).

this issue). In addition, there is also a bias for experimental work on just one or few strains that are kept in culture. The two most commonly studied isolates are CCAP1133/5 isolated from Finland (reported in 17 studies) and an isolate from Villefranche-sur-Mer (reported in 11 studies). Given the discussion on the apparent rarity (or even absence) of *O. marina* from the Baltic Sea, this raises the issues of: (i) “what” is the scientific community actually working on? and (ii) can experimental data on one strain be extrapolated to understand behavioural, demographic and evolutionary responses of *O. marina* elsewhere? In another paper in this issue [Lowe et al. \(2011a\)](#) provide guidance on this problem.

CRYPTIC DIVERSITY AND THE OCCURRENCE OF RESTRICTED DISTRIBUTION

Underlying the apparent broad spatial range there are some emerging patterns on the distribution of distinct genetic clades that have implications for experimental studies on a “model organism”. Most genetic characterization of *Oxyrrhis* has been directed towards uncovering its phylogenetic position relative to the dinoflagellates and apicomplexans (e.g. [Saldarriaga et al., 2004](#) who sequenced SSU rRNA, actin, α -tubulin and β -tubulin); however, the pattern of sequence variation at part of the SSU rDNA did uncover two lineages of “*O. marina*”. Unfortunately, the level of genetic diversity within *Oxyrrhis* at this genetic marker is relatively low which limits power to detect additional clades. More importantly, since only a small number of isolates were studied ($n = 2, 3, 11$ for [Saldarriaga et al., 2003](#);

[Cavalier-Smith and Chao 2004](#); [Lowe et al., 2005a,b](#), respectively) the geographic distributions of divergent lineages could not be determined. Thus, the most extensive spatial study completed to date ([Lowe et al., 2010](#)) used sequence data from cytochrome oxidase I (COX1) and a more polymorphic marker, the 5.8S internal transcribed spacer 1 and 2 rDNA (5.8S ITS rDNA), to determine the genetic relationships of 58 *O. marina* isolates; this study identified four distinct clades that formed two highly divergent lineages. Two of the *O. marina* clades had discrete, non-overlapping distributions, one with a broad distribution and one with few samples (summarized in Fig. 5): Clade 1 is European Atlantic and western Mediterranean in distribution, while Clade 2 occurs off both coasts of North America and was also found in samples from the eastern Mediterranean Sea. Clade 3 only occurs in the eastern Mediterranean Sea. Clade 4 was taken from culture collections and occurs in the Baltic Sea and Red Sea. The Mediterranean Basin appears to be a diversity hotspot for *Oxyrrhis* (and also the clades/species defined by [Lowe et al., 2010, 2011a](#)), as it is for many taxa ([Myers et al., 2000](#)). Thus, there is a mixture of wide distributions and endemism, albeit at a regional scale (e.g. Clade 3 is endemic to the Mediterranean), a pattern reported in some other recent research into protist biogeography ([Kooistra et al., 2008](#)).

Despite the broad scale of the study by [Lowe et al. \(2010\)](#), it remains unclear how broadly distributed (or local) the *O. marina* clades are, as there are some conspicuous gaps and areas without replicates in sampling. For example, both Clades 1 and 2 were represented by individual isolates from Korea and Japan, respectively. Whether these potentially broad distributions are driven by wide tolerance to varying conditions, high natural

dispersal potential by prevailing currents and/or anthropogenically enhanced transport (likely in ballast water, as mentioned above) remains to be determined. Nonetheless, within the *O. marina* morphospecies, at least one of the genetic lineages appears to be restricted to quite limited regions suggesting that allopatric divergence may occur in eukaryotic microbial species, and ubiquity is not a logical corollary of small size: a point previously emphasized in arguments (Foissner, 2006, 2008; Vanormelingen *et al.*, 2008) against the ubiquity model of microbial biogeography.

CURRENT AND FUTURE BIOGEOGRAPHIC RESEARCH

It is clear that our knowledge of the distribution of *O. marina* has improved substantially over the past few years but remains limited. Unlike other protists, such as diatoms and foraminiferans, *O. marina* has limited morphological characters that may be used to delineate species (see Lowe *et al.*, 2011a), and thus detailed biogeographic studies on species (will) rely heavily on molecular techniques, potentially limiting the range of laboratories that will expand the known distribution of *O. marina* clades. Indeed, the majority of genetic-based studies that have identified cryptic speciation in protists (e.g. Moreira and Lopez-Garcia, 2002; Lundholm *et al.*, 2003; Darling *et al.*, 2004; Lowe *et al.*, 2005a, 2010; Schekenbach *et al.*, 2006; Slapeta *et al.*, 2006; Amato *et al.*, 2007) have not extended this work to quantify levels of endemism or cosmopolitanism in these new clades/species, likely because this would require a huge sampling effort (but see e.g. Kooistra *et al.*, 2008). Indeed, under-sampling (at both local and global scales), combined with taxonomic uncertainties, continue to hamper our understanding microbial biogeography (Heger *et al.*, 2009). Thus, despite the impact of the discussion recently stimulated by Finlay and co-workers (Finlay and Clarke, 1999; Fenchel and Finlay, 2003, 2004; Finlay and Fenchel, 2004), there are still too few studies to make broad generalizations on the processes that drive adaptive divergence and speciation in free-living aquatic protists (see also Dolan, 2005 for review). For example, combined breeding experiments and genetic data support the idea that some species are globally distributed (Casteleyn *et al.*, 2008), while a combination of ecological processes and allopatric distributions have been implicated in driving divergence of planktonic foraminifera (Darling *et al.*, 2004); yet, other studies have argued for isolating mechanisms linked to behaviour rather than allopatry (de Vargas *et al.*, 1999;

Sexton and Norris, 2008). We have uncovered a mixture of widely distributed and endemic clades of the *Oxyrrhis* morphospecies, and support their separation into (at least) two distinct species (Lowe *et al.*, 2010; 2011a). At a basic level we argue that researchers must know which species of *Oxyrrhis* they are working on if their experimental work is to be placed in an evolutionary context; ideally, all future studies should genetically characterize their strain to provide this context. Beyond this, recognition of cryptic species raises questions about the mechanisms that permit or prevent their coexistence (Ortells *et al.*, 2003). Accumulation of such data on a global scale is an essential way forward to resolve the demographic (e.g. migration, population size fluctuations) and evolutionary drivers that are responsible for the present patterns of diversity contained within the genus *Oxyrrhis*; understanding the distribution of this species complex will be important to guide future *Oxyrrhis* research and the implications of experimental and genome studies (Slamovits and Keeling, 2011).

One key issue is the relative role of sexual processes for the creation of adaptive divergence—and the scale over which adaptive differences are evident. As an inhabitant of intertidal pools and estuaries, certain strains (or species) of *O. marina* are clearly eurytopic. Indeed, ecophysiological data indicate that *O. marina* inhabits waters that vary in a number of ways, including 5 and 60 PSU and 10 and 30°C (see Lowe *et al.*, 2011b; Montagnes *et al.*, 2011b). Indeed, a mixture of early and recent work has highlighted extensive ecophysiological variation within and among clones of *Oxyrrhis* (Droop, 1959; Lowe *et al.*, 2005a), and this appears typical of aquatic protists (Weisse, 2002, 2003; Weisse and Rammer, 2006). We know virtually nothing about the sexual ability of *O. marina* (see Montagnes *et al.*, 2011b). Interestingly, analysis of the genetic diversity of *O. marina* on a small spatial scale (i.e. <2 km) failed to uncover extensive clonal reproduction, with just six pairs (out of 81 isolates) of identical multilocus genotypes (Lowe *et al.*, in press) which raises questions about the extent of clonal reproduction at this scale. More generally, eurytopy, combined with high dispersal ability and large population sizes, is a key trait for acquiring a wide geographic range and geologic duration (cf. Jackson, 1974). Another key issue is to determine the relative impacts of putative ecological and physiological processes that determine absence (or likely rarity, since, as discussed above, earlier work reported *O. marina* from Tromsø and the White Sea) of *O. marina* from Polar regions. An obvious explanation at present is that our Arctic samples have been collected outside the spring bloom, and *O. marina* abundance was too low. Alternatively, our sampling methods (see Lowe *et al.*,

2011b) may be inappropriate for establishing cold-adapted strains. Clearly, patterns of presence and absence, and also diversity, of *O. marina* need to be investigated more thoroughly for other regions, particularly the Baltic Sea and tropical areas that are biodiverse (e.g. Philippines, Wallacea, Myers *et al.*, 2000). Finally, there is some indication that *O. marina* may form cysts, although it is not clear if these are resting cysts (Montagnes *et al.*, 2011b); appreciating the factors that stimulate cyst production may aid in our understanding of dispersal. Not only will such data highlight centres of origin, biodiversity hotspots, and opportunity for ecological specialization, but they will also provide the crucial baseline to assess the impact of climate change and altered distributions. Studies that couple patterns of functional genetic diversity with ecophysiological data will provide an interesting model to test which clades make the most significant changes in their distribution; for instance it might be appropriate to focus on genes associated with osmoregulation when examining salinity responses (e.g. Lowe *et al.*, 2005b).

Finally, human-mediated introductions have been commonly reported for relatively large marine species that have obvious ecosystem impacts, such as the ctenophore *Mnemiopsis leidyi* (Oguz *et al.*, 2008; Javidpour *et al.*, 2009). Numerous microscopic taxa have certainly had their ranges enhanced by anthropogenic transport (e.g. diatoms, Vanormelingen *et al.*, 2008; nematodes, Deryke *et al.*, 2008), with recent studies of ballast water highlighting the diversity of protists and potential for introduction of non-native species by shipping in particular (Burkholder *et al.*, 2007). Better descriptions of clade distributions of protist species will highlight allopatric populations that may, for example, be linked with shipping routes; with this in mind, studies of the distribution of clades and species within *Oxyrrhis* would seem ideally placed to examine the affect of shipping on organism distributions in the marine environment.

Beyond this consideration the broad distribution, high diversity and wide ecophysiological tolerance of the *O. marina* “complex” makes it a useful model to examine demographic processes amongst free-living protists. This genus, *Oxyrrhis*, is widespread, but there are differences between clades that need to be clarified by future sampling, particularly away from European seas. Such work will form the foundation to identify relative roles of, for example, Quaternary climate fluctuations and allopatric processes, as opposed to putative sympatric mechanisms, that drive adaptive divergence and speciation in aquatic free-living protists (e.g. see de Vargas *et al.*, 1999; Darling *et al.*, 2004; Sexton and Norris, 2008). Studies that use *O. marina* appear ideally placed to provide these data, and thus a wider

appreciation of the roles that protist biodiversity play in ecosystem function.

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