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Recent taxonomic research on the bladed Bangiales (Rhodophyta) has revealed that the diversity of this group along the Atlantic coast of the Iberian Peninsula is much greater than previously understood. Species in this group of red algae are a common element in the intertidal marine flora along the shores off the Iberian Peninsula. Between 2009-2011, intensive sampling throughout the Atlantic coast of the Iberian Peninsula has resulted in the collection of many specimens that cannot be placed in currently recognized taxa, although traditionally, all of them have been included in the genus *Porphyra* sensu lato. In the present study we used a combination of molecular analysis and detailed morphological observations to investigate diversity within this group. Molecular analyses were based on three genetic markers: the plastid gene *rbcL*, the mitochondrial gene *COI*, and the nuclear gene *nSSU*. The study of these genes revealed unexpected species richness and highlighted cryptic and misidentified taxa. The results showed that in this area, the genus *Pyropia* is currently the most speciose with up to five different species, the genus *Porphyra* is so far represented by three traditionally reported species, and finally, phylogenetic reconstruction allowed us to discover two new taxa of bladed Bangiales placed in separate clades that need to be described. These results alter the floristic composition of this group considerably and confirm the need to undertake taxonomic revisions, including the application of genetic tools and morphological studies to determine the extent of the diversity of this group in this area, and thus to be able to contribute to knowledge of the global diversity of the Bangiales. Contribution to the project PORPHIBER (Ref. CGL2008-00932/BOS).

1PO.13

INFLUENCE OF SUBSTRATE AND PH ON MICROALGAL DIVERSITY: A POTENTIALLY IMPORTANT FACTOR FOR SYMPATRIC SPECIATION

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Few investigations of protist speciation have been conducted, and therefore the drivers that influence

the species diversity and richness of these microorganisms remain to be fully elucidated. Huge protist diversity could be caused by limited distribution and/or by different preference of habitats, but in the case of aeroterrestrial green alga *Klebsormidium* the diversity is not mainly influenced by limited distribution, but mostly by habitat preferences. In the present study, we investigated ecological speciation in the genus *Klebsormidium*. We collected 100 strains from sandstone, basalt, granite, and limestone, sampling 2-3 localities per substrate. The sequences yielded a total of 27 genotypes having strict habitat preferences on substrate. To investigate the ecophysiological differences among the strains isolated from sandstone and limestone, we examined 12 strains representing four different genotypes. The strains were cultivated at five different pH levels ranging from pH 4 to pH 8. We determined the responses of the strains to the experimental pH conditions by (1) measuring the effective quantum yield of photosystem II, and (2) determining the growth rates after cultivation at different pH levels. Our results clearly showed a strong ecological differentiation of *Klebsormidium* clades. Strains isolated from two limestone clades showed the highest growth rates at pH 7 and pH 8 and had significantly lower growth rates at pH 4 and pH 5. Strains isolated from sandstone exhibited two different growth responses. Strains from clade E13 showed the highest growth rate at pH 6, whereas strains belonging to clade E1 showed almost identical growth rates at all of the investigated pH levels. In addition, we determined distinct ecophysiological differentiation among distantly and closely related lineages, thereby corroborating our hypothesis that the sympatric speciation of terrestrial algae is driven by ecological divergence. We clearly showed that pH is a critical ecological factor that influences the diversity of autotrophic protists in terrestrial habitats.

1PO.14

REPRODUCTIVE BARRIERS IN THE *SEMINAVIS ROBUSTA* SPECIES COMPLEX AND THEIR ROLE IN SPECIES DIVERSIFICATION

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Despite their enormous diversity and ecological importance, the understanding of diatom speciation is largely uncharted territory. Several studies showed a large cryptic species diversity in microalgae, with a large variety of reproductive systems in some species complexes. Since sexual reproduction is an obligate stage in the

life cycle of most diatoms, their evolutionary success may be related to this widespread variation in reproductive systems and rapid evolution of their highly sophisticated signalling systems involved in mating. In this study, we collected a set of *Seminavis robusta* strains from the Veerse Meer and the Grevelingenmeer (the Netherlands) and the spuikom (Belgium). Phylogenetic analysis based on *rbcL* and LSU rDNA sequences shows that these strains form three distinct cryptic lineages. Sexual reproduction can be induced with high efficiency in intra-group crosses, while inter-group mating successes drop as much as 70%. Interestingly, phylogenetic distance correlates with interbreeding capacity between mating group. This represents an interesting case in which we can dissect the contribution of different possible pre- and post-zygotic reproductive barriers between these recently diverged diatom species. We here focus on the identification of possible pre-zygotic isolating mechanisms, exploiting recent insights into the signalling mechanisms prior to mating in *S. robusta*. These include the production of a conditioning factor by both mating types which induce cell cycle arrest in the opposing mating type and the subsequent production of the pheromone L-dipropylamine by mating type minus cells that attract mating type plus cells. Combinatorial bio-assay experiments were set up with strains of both mating types from the three mating groups. The receptivity to conditioning factor was monitored by following cell cycle progression, while additionally for mating type minus strains the subsequent production of attraction pheromone L-dipropylamine was measured. The role of these pre-zygotic barriers to interspecies crossing in maintaining species boundaries will be discussed.

1PO.15

THE FRITSCH COLLECTION OF FRESHWATER, BRACKISH, AND TERRESTRIAL ALGAE ILLUSTRATIONS: CATALOGUING CHANGES IN ALGAL TAXONOMY OVER TIME

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The Fritsch Collection contains several million published figures and taxonomic entries on terrestrial, fresh- and brackish-water algae from worldwide distributions. With comprehensive citations, this unique reference collection is held by the Freshwater Biological Association at Windermere. Extensively used by researchers to assist in identification and for the resolution of taxonomic problems, the Fritsch Collection comprises species sheets organised by genus within the major

groups. Constantly updated, the Fritsch Collection illustrates the diverse and changing views that have been or are still held about the identification and taxonomy of many algae. Part of the author index is a searchable online bibliography at www.fritschalgae.info. Planning for an online version of the Collection is underway.

1PO.16

APPLICATION OF THE HIGHLY VARIABLE ITS2 MOLECULAR MARKER TO EVALUATE SPECIES AND GENERIC BOUNDARIES WITHIN THE CLASS EUSTIGMATOPHYCEAE (STRAMENOPILES)

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The Eustigmatophyceae is a class of coccoid stramenopile algae, which has been separated from the Xanthophyceae on the base of ultrastructure. Published analyses of nuclear 18S rDNA sequence data show the Eustigmatophyceae by far more diverse than previously recognized, with many species previously described within other classes still pending to be transferred to the Eustigmatophyceae. Little morphological diversity within the class has restricted the description of new species and hampered efforts to estimate the biodiversity of the group. Therefore, application of highly variable molecular markers is essential to study taxonomy, diversity and distribution of the group. In this study the ITS2 rRNA sequences were newly determined for 20 strains from the SAG and ACOI culture collections, representing 18 species from 9 described genera, most of them not sequenced so far, and compared to the 25 corresponding sequences currently available for the class. The ITS2 sequences are analysed for inter- and intra-specific variations including secondary structure models. The presence of extensive ITS2 intragenomic variation required cloning before the sequence analyses. The ITS2 sequence study demonstrated the need of re-evaluating genera and families boundaries within the Eustigmatophyceae, whereas no conflicts with current species delimitations were found. For example, the