

Taxonomy and systematics of Mediterranean seaweeds: historical landmarks and new perspectives

Dear Colleagues,

The tree of phycology in the Mediterranean is an old olive. The roots are many, deep, and embrace the whole Europe. The fruits these days are rare but still precious. And yet, the flora that we still have problems to describe, has probably been different through past geological times. Wegener's theory of continental drift proposed early this century has changed our views not only in the geological context but also as regards the evolution of life on the planet as a whole.

THE ORIGINS OF THE MEDITERRANEAN FLORA (see *Flora Mediterranea* 12: 98-100. 2003)

...Thus, the major geological events that have marked the species composition of the Mediterranean Sea started at the Palaeozoic, and what can be postulated in connection to these events is that speciation took place either as a result of **Dispersal** or **Vicariance**. To realise that we need to look at the work of one man, the entomologist Willi Hennig, who is universally recognized as the founder of phylogenetic systematics. Hennig expressed the belief that speciation largely occurred in a dichotomous pattern, and that sister-taxa could be traced and connected to their hypothetical ancestor by finding their common derived characters (synapomorphies). This view reflected the dogma that phylogeny can be inferred if the genetically fixed characters could be traced in pairs of taxa. Because only such characters are inherited from a common ancestor to its descendants, relic characters (plesiomorphies) or characters formed by epigenetic or environmental factors should be avoided as they create homoplasious structures. Thus, the most important issue in Hennig's theory was the epistemological justification of phylogeny in light of genealogical traits. The sister-taxon relationship involves in itself another axiome, visually a constant dichotomous speciation pattern which gives rise to sister-taxa of equal rank. As a result, series of speciation events through time are nested and create the hierarchy of life. Hennig illustrated speciation as the tokogenetic relationships between individuals that are forced to divide because of the appearance of a barrier, and this classical view of speciation made the basis of recognizing two distinct patterns of the

phenomenon:

- 1) when a barrier splits the original population and the isolated subpopulations differentiate independently (vicariance), and
- 2) when a species succeeds to cross a barrier by natural or artificial way, showing originally a disjunct distribution (dispersal).

Hennig also suggested certain criteria, such as ontogeny and knowledge from the fossil record to identify and polarise the synapomorphies, but today the most commonly used method is the outgroup comparison. Along with parsimony that is justified to minimise the assumptions and increase the explanatory power of the data, these phylogenetic methods, and others that calculate similarity/dissimilarity, are now computerized and extremely powerful to infer relationships among taxa. Finally, resampling methods such as the parsimony jackknifing guarantee the rapid search of large data sets to find well-supported clades (Cladistics 1998, vol. 14, pp. 303 to 338).

A drawback with these methods is that reticulation is not detectable, and therefore a good knowledge of the study group is required to find and exclude the hybrids from the phylogenetic analyses. Examples of Hennigian systematics can be viewed in the subfamily Lithophylloideae. The relationships between the genera *Pseudolithophyllum* Lemoine, *Lithophyllum* Philippi, and *Titanoderma* Naegeli have been in debate many years. Today most workers agree that *Lithophyllum* and *Titanoderma* are distinct genera, while *Pseudolithophyllum* is recognized by few. However, of the generic characters that circumscribe *Pseudolithophyllum*, most are plesiomorphies (because they occur also in all other lithophylloid genera), while the presence vs absence of a single character (i.e., coaxial vs non-coaxial perithallium) is simultaneously used to distinguish *Pseudolithophyllum* from *Lithophyllum*. Such a complement relation is usually met with in taxonomic keys, and has been criticized since Aristotle (see Parts of animals, 642b 21), while in modern cladistics defines the notion of paraphyly (i.e., because *Pseudolithophyllum* lacks autapomorphies, we are forced to define it by characters present in the common ancestor it has with *Lithophyllum* excluding species of the sister-genus by a negative occurrence of its characters). In other words, what is common praxis in taxonomy, i.e. to dichotomise by the presence/absence criterion, in phylogenetic systematics cannot define sister-taxa.

Molecular data have even questioned the monophyly of the Lithophylloideae. In a recent paper (see Phycologia 1999, vol. 38 nr 3), *Titanoderma* has been recognized as the sister-taxon of the subfamily Amphiroideae, and *Lithophyllum* as the sister-taxon of this group, rendering Lithophylloideae paraphyletic. From the biogeographic point of view it is interesting to note that if the other lithophylloid genera *Goniolithon* Foslie and *Tenarea* Bory, which are endemic in the

Mediterranean, are distinct from *Titanoderma* (a widely distributed genus with fossils dated since Eocene; i.e., 38 MYA), it would indicate that they are Tethyan or Palaeotethyan relics. The alternative hypothesis is that *Titanoderma* is paraphyletic and that *Tenarea* along with *Goniolithon* represent advanced species of this complex.

A revision of the former Antithamnieae has been recently published (see Opera Botanica 1996, vol. 128) in light of phylogenetic systematics. Monophyly has been postulated in many genera, on the basis of morphological data and after having examined all taxa in this complex. In the case of *Antithamnion* Naegeli, this name has accommodated more than 150 taxa in the past, while the revision ended up with 30 species only. Many of them remain poorly known, one more species has been described, but it is certain now that the systematics of *Antithamnion* are standing on a firmer basis. The phylogenetic reconstruction has shown the presence of three distinct subgroups within the genus. Their existence as natural groups is confirmed by their biogeography that in many cases is congruent with the systematic results. In other words, sister-taxa partly overlap in distribution or occupy nearby areas, due to vicariance, while dispersal is easily recognized because it is limited to few taxa, which are known to be introductions or whose disjunct distributions deviate notably from the rest of the group. One group is apparently native to the Indo-Pacific, the remaining two include Mediterranean taxa which appear as advanced members within each group.

The fact that dispersal is so limited in *Antithamnion* (and even less in *Pterothamnion* Naegeli) suggests that phylogenetic reconstructions of other groups of algae having more limited spreading mechanisms (e.g., the coralline red algae) may support concrete biogeographic scenarios.

Pterothamnion shows a similar degree of speciation with *Antithamnion* having about 30 species within three major groups. There are also similarities in the biogeography of the two genera. For example, there is also an entirely Pacific group in *Pterothamnion* (although limited to the eastern Pacific), while Mediterranean taxa also appear as advanced members within the other two groups. In both genera, there is a particularly rich in species section occurring in southern Australia. The warm-temperate character of *Antithamnion* is mirrored by the larger number of Mediterranean endemics, while in *Pterothamnion* there is a clear North Pacific-North Atlantic group indicating the cold-temperate character of this genus. It can be hypothesized that these two genera had a parallel evolution, and that their Mediterranean members are neoendemics, but there are no fossils or molecular evidence that allow us to estimate taxon ages at present.

The phylogeny of *Mesophyllum* Lemoine is a project to be compared with the systematics and biogeography of the antithamnioids. In contrast to *Antithamnion* and *Pterothamnion*,

Mesophyllum [sensu lato] is widespread and has a fossil record of at least 100 MY. About 50 species are described. It is closely related to *Synarthrophyton* Townsend, a genus of about seven species, that is only known from the Southern Hemisphere. Three new species of *Mesophyllum* have recently been recognized in Europe, two from the Arctic Ocean where the genus was previously unknown. The group has also been studied in the NE Pacific and therefore it is likely that a monograph will be achieved soon. Preliminary results based on data of 16 species indicate that vegetative characters are congruent with certain reproductive features, and that the Arctic species may be related to deep-water Mediterranean endemics.

MOLECULES vs MORPHOLOGY

The advent of molecular methods in biology has revolutionized the entire study of life. Even if the greatest discoveries have been achieved in other groups of organisms than algae (e.g., Archaeobacteria), it is widely recognized that the new methodologies have provided extremely powerful tools to investigate relationships between taxa at almost all levels of classification and also between individuals within populations. The vast majority of molecular research has focused on the early evolution of algae, studying the origins of plastids and trying to clarify the relationships between procaryotes and eucaryotes in light of gene phylogenies that show either a single or multiple endosymbiotic events (see Pl. Syst. Evol. Suppl. 1997, vol. 11). A second body of researchers investigates relationships within groups such as the Dasycladales, the brown algae, and the red algae. We need to emphasize the limited work at species and genus level, which actually encompasses the interests of the taxonomists that at present are vanishing with alarming rhythm.

Attention should also be drawn to the fact that the new methodologies have not solved all of the problems, particularly as some old ones appeared again mutated. For example the problem of distinguishing between homologous and analogous genes has been documented in the globin group of vertebrates, after the discovery of paralogous genes (caused by gene-duplication, and which may underestimate the age of taxon divergence) or xenologous genes (caused by lateral transfer and which may overestimate the age of taxon divergence), and this phenomenon is also suspected to occur in algae. This revealed at least one superiority of the morphological data. For instance, in comparing the carpogonial branch in red algae, we can be sure that we are studying a homologous character throughout the group. How can we know studying parts of a gene in different species? A second problem is that molecular data often include large numbers of homoplasies. Consistency indices of molecular trees can be so low that dropping your data set on the floor and reassembling randomly you may even get a better phylogenetic signal (!) To give an

example, it has been shown that inclusion or exclusion of certain taxa in molecular trees render Ceramiales either monophyletic or paraphyletic, which demonstrates that taxon-relationships are based on homoplasies (instead of unique synapomorphies) that under other circumstances (i.e., exclusion of certain taxa) show different topologies.

Incongruence between gene trees is also known which shows the need to investigate and compare more gene phylogenies before molecular data should be considered trustworthy in the phylogeny of taxa. On the other hand, there are also workers who are convinced that in the red algae the SSU trees are in good agreement with data provided by anatomy and ultrastructure.

Another problem remains to explain why monophyly is denied in cases of rather apparent generic affiliation. Such cases of non-monophyletic genera are for example *Jania* Lamouroux and *Bossiella* Silva or *Hildenbrandia* Nardo. Finally, it should be remembered that the number of taxa analyzed remains small, viewed under the eyes of a taxonomist. Monophyly is not postulated in most molecular studies, so far, because taxon selection is usually based on 'accessibility' and this makes the application of molecular results in classification limited.

For instance, within the Bangiophycidae we still lack a phylogeny using sequences of the Rhodochaetales, Phragmonemataceae, Compsopogonaceae, and most genera of the Porphyridiaceae. Similarly within the former Gigartinales *sensu lato*, the absence of several families, such as the Nemastomataceae, Acrosymphytaceae, Schmitzielloideae, Choreocolacaceae, Gloisiphoniaceae, Peyssonneliaceae, Calosiphoniaceae, Caulacanthaceae, Cruoriaceae, Cystocloniaceae, Hypneaceae, Polyidaceae, Risoellaceae, Sarcodiaceae, Wurdemanniaceae (?), Naccariaceae (?) is questioning any classification attempt before **several** representatives of these groups are examined. Interestingly, many of these families have Mediterranean taxa as types.

In the order Gelidiales, several molecular studies have tried to solve relationships between taxa, that in neither case represent taxonomically monophyletic groups. For example, in the genus *Gelidiella* J. Feldmann et Hamel only the type has been examined, while at least four other species are known in the Mediterranean alone. In the genus *Ptilophora* Kuetzing only two species were analyzed of the 12 recognized. Similarly, of the 11 species of *Gelidium* Lamouroux known in Europe, only five are included in these studies.

In the Ceramiales, we still lack information of the most primitive tribes Warrenieae and Delesseriopseae, while the situation within the former Antithamieae is hopelessly limited to a single species (out of some 150 placed in 18 genera and 8 tribes). This very species, after being advertised in several papers as *Antithamnion* sp. has finally (?) got a more indicative name as

Antithamnionella spirographidis (Schiffner) Wollaston (see Eur. J. Phycol. 1998, vol. 33, p. 189). We can now hope that its molecular sister-taxon *Callithamnion*/*Aglaothamnion* sp. will become *Callithamnionella* Feldmann-Mazoyer in which case it will match the morphological results (see Opera Botanica 1996, vol. 128).

It is common knowledge that classification starts from taxa of lower rank and proceeds hierarchically, gathering all published information, but the molecular work so far seems to deny that trying to get answers the other way around. But in the latter case, inevitably, we have to abandon many species and genera, families and orders of unknown (in the best case speculative) affinities...in order to get THE molecular tree that shows the major lineages of life. In a recent paper on eucaryote phylogeny (see Cladistics 1998, vol. 14), based on SSU data, even this approach has failed, as the major lineages of life collapsed in a polytomy. This despite the fact that groups of wider molecular divergence were underrepresented (e.g., the red algae were represented by eight taxa only, while more than 70 taxa of green plants were included). In this analysis, interestingly, *Porphyra* C. Agardh becomes a Florideophycidae and *Porphyridium* Naegeli just qualifies to be a rhodophyte (57 % support) which suggests that many other rhodophytes would probably cluster basally in the polytomy.

While to extract DNA from the remaining types of genera and families is now a matter of time, to attempt explaining molecular data with morphological classifications is more difficult, particularly as molecular trees are not well-supported and modern morphological revisions of the majority of algal groups are lacking. Yet, it is our taxonomic understanding of the nature of taxa that enable us to interpret and confirm molecular phylogenies. Nobody supports the clustering of *Griffithsia* C. Agardh with *Gelidium* Lamouroux, while all have accepted that the recently discovered Rhodogorgonales is more likely to be the sister-group of the Corallinales or that *Hildenbrandia* and *Apophlea* Harvey are closely related.

Many new species and significant morphological data remain to be discovered but, despite the advent of new optic systems, electronic microscopes and culture techniques, to extract and interpret morphological data remains laborious and time-consuming as compares to DNA-sequencing. The coding of morphological characters for phylogenetic analyses is also more dubious than the alignment of nucleotide sequences. In doing the former, it is essential to interpret characters after decomposing structures into their individual characteristics. This also solves the problem of character weighting, because individual characteristics are generally controlled by single copy genes and thus they can be given equal weight in the analysis. It is also significant to avoid distinguishing between characters and character states, when the various individual states

cannot be considered as homologous and mutually exclusive (as it is apparently the case with nucleotide bases). With morphological data, such information is not known *a priori*, and therefore it is essential to treat all structures as independent characters, in a binary (absence/presence) way, whose homology can be tested without loss of information.

The major benefit with morphology is that you can continuously evaluate your data as new collections become available and complex structures are re-assessed. On the contrary, molecular sequences are fixed, for the genes concerned, and 'curious' topologies remain a puzzle leaving possible different interpretations (non-homologous genes, large number of homoplasies, taxonomic misidentifications, long branch attractions).

Molecular studies have also created several new taxa, such as the new orders Plocamiales and Halymeniales, while the reinstatement of Sphaerococcales and the new order Dumontiales is under discussion. Yet, this is not because nucleotides have provided unmistakable synapomorphies, or new insights in the evolution of red algae, but because the treatment of a large number of characters (i.e., nucleotide bases) and taxa in a hierarchic dichotomous pattern leads inevitably to many clades (particularly when homoplasies are frequent). On the other hand, the Linnean classification is inadequate to provide ranking for all recognizable clades, so that a final classification must necessarily compromise between the limited number of ranks and the information that we think merits ranking. Thus, it is likely that a revised Kylinian system, with few orders, will finally get acceptance to maintain the Linnean hierarchy in a proportional way. Another solution to this problem is to adopt the taxonomic phylogeny, as it is advocated by some workers.

As I said above molecular studies focus on different interests and so far have failed to provide conclusive evidence of monophyletic groups within classes, orders, families, or genera. Molecular sequences, although bear the name of taxa, are not representative of them, in the way taxonomists are used to accept names, because single genes tell us nothing about the variation in reproduction, morphology, anatomy or ecology that species, genera or tribes are established upon. This has been the territory of taxonomists who after having accumulated all that basic information are seeing often their data to be neglected or misinterpreted.

Molecular data so far point to phylogenies of particular genes and any assumption that this might reflect taxon-relationships is an overestimation.

The resampling techniques that are used as a statistical measurement to support conclusions tell us nothing about how much the data reflect reality, but only if there is a phylogenetic signal in the actual data set or not.

Despite all these problems, new phylogenies are published every month, as new sequences are analyzed and compared. Molecular methods are considerably easier to apply, and fast, in a way that taxonomists could not even dream about. It is believed that a consensus of molecular evidence, pointing to a single phylogenetic tree of life, will slowly emerge, but until now the progress in my opinion is limited.

It is also hoped that molecular data will throw some light in the existing taxonomic chaos within certain genera, such as *Ceramium* Roth, and in the Corallinales, but it is also essential that morphological research will continue to provide independent data sets for comparison and also to clarify the uncertain status of hundreds of taxa. To do the latter, taxonomists need to adopt modern methods of systematics and gather our efforts towards the following goals:

- 1) the alpha-taxonomic work must continue using the type method as a rule, to describe the species composition in the Mediterranean Sea and elsewhere,
- 2) species concepts need to be clarified in light of biosystematic and/or molecular evidence, and
- 3) monographic research must be based on phylogenetic systematics, using morphological and/or molecular data. Such studies can present scientific hypotheses of taxon relationships which enable us to infer biogeographic scenarios and, in the best case, the latter can be used to support the systematic results.

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