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Scientific Editors

Aquatic Ecology of the Mondego River Basin
Global Importance of Local Experience

Coimbra • Imprensa da Universidade

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DIVERSITY OF FRESHWATER ALGAE

Abstract

In this paper we present an historical review of the published information on freshwater algae collected within the Mondego Basin and report on the algae identified in a survey covering the Basin area. The reviewed publications concern predominantly taxonomy and go back to the beginnings of the 19th century. In the last thirty years, the possibility of combining taxonomic and ultrastructural studies led to the establishment of a culture collection of microalgae at the Department of Botany, University of Coimbra (ACOI). Only a few ecological studies have been conducted in the area, quite recently, concerning the structure and dynamics of the phytoplankton.

A survey of the phytoplankton composition in 21 sites located from the highland areas to the coast revealed the presence of 388 taxa, the great majority being diatoms (232 taxa). Green algae, euglenophytes and silica-scaled chrysophytes were other well represented groups, with 60, 48 and 35 taxa, respectively. The remaining algal groups were poorly expressed by this survey. Higher algal variety was found in the eutrofic waters of the lowland rice-field areas of the Basin when compared with the oligotrophic waters of Serra da Estrela. However, a remarkable flora develops in the small ponds of this area in spring, due to the occurrence of rare specimens of chrysophytes and desmids.

Introduction

Biodiversity has recently become the focus of international debates, but algae have been largely omitted and neglected even by institutions such as the United Nations (Norton et al. 1996). Among the reasons for this neglect are certainly their microscopic size, the time consuming methods for identification of some species (such as electron microscopy or molecular biology), a reduction in the number of algal taxonomists and a reduction in funds to support systematic research. These reasons act synergistically to hamper the research on algal biodiversity (Andersen 1992).

However, the algae include the dominant primary producers of our planet and the main producers of oxygen. Finally, algae include at least seven distinct phylogenetic lineages

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of organisms that arose independently during geological time. Therefore, the biodiversity of algae in terms of species and phylogenetic groups seems to be unusually high.

In Portugal several valuable contributions for the knowledge of the algal flora have been made since the 19th century, but the algal diversity is still very far from being properly estimated. A Portuguese algal flora simply does not exist, has not been published so far, and experienced taxonomists are a very few. Also, in order to develop ecological algal studies, a strong taxonomic basis is required. As a consequence, only a small number of studies have been carried out.

Despite the Mondego River importance as one of the major rivers in central Portugal and the largest entirely Portuguese River, little published information is available on the diversity and ecology of microalgae present within the drainage area. To investigate the information available on algae for this Basin is equivalent to investigate the evolution of the phycological studies at the Department of Botany, University of Coimbra. Most of that information has been produced by academics working at this Institution and is scattered throughout publications made over almost two centuries. Contributions written by academics or naturalists of other main Botanical Institutions (in Lisboa or Porto) were most often based on material provided by collectors or researchers from Coimbra.

The purposes of this paper are (1) to compile this scattered information on freshwater microalgae of the Mondego Basin; (2) to present data from collections made in the area recently.

Historical review

The 19th century contributions

To our knowledge, the first references to algae in Portugal go back to Brotero's work Flora Lusitana (Brotero 1804), where a small list of marine and freshwater species was published. Specifically for the Basin area it was reported *Chara vulgaris* (in Quinta das Lágrimas, Coimbra and surroundings) and two brown macroalgae (in Buarcos, Figueira). However, under the generic designations of *Byssus* and *Conferva*, Brotero reported three other "algae verae", collected from the surroundings of Coimbra and from the Mondego (Mundae) margins.

Brotero was one of the first directors of the Botanical Garden of Coimbra. A botanical society in his honour, Sociedade Broteriana, was founded at the end of that century (in 1880) by initiative of Júlio Henriques, director of the Institution at the time. The society still exists and produces three different publications, the most important in terms of scientific contributions being the Boletim da Sociedade Broteriana (Bol. Soc. Brot.), which first number goes back to 1880.

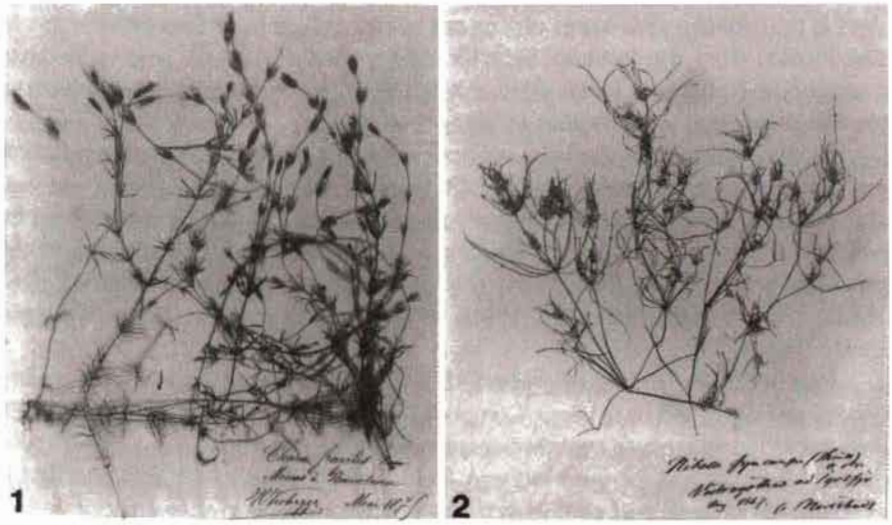
Within the scope of the society, an increase of phycological studies occurred at the turn of the century. The collection and interchange of botanical specimens (including algae) were promoted, with posterior identification by international experts such as Hauck, Kützing, Nordstedt (Santos and Mesquita 1981), several new taxa for

science being described. Much of this material became part of the valuable Herbarium of Coimbra and is still available, although losses have occurred. Lists summarising some of these studies were published or translated by Júlio Henriques (Henriques 1881, 1889) and report a total of 69 freshwater taxa, mainly chlorophytes (about 37), but also diatoms, cyanophytes, xanthophytes and rhodophytes. Most of these algae were collected from tanks or moist soil in Coimbra or around by the renowned A. F. Moller, supervisor of the Garden at that time.

The first half 20th century

Several important contributions for the taxonomical knowledge of the Portuguese algal flora, mentioning material from the Mondego Basin, were made during this period. Diatoms were studied by Carvalho and Zimmermann, the first reporting about 118 taxa for Serra da Estrela (Carvalho 1913) and the second about 28, mostly from Coimbra (tanks, the Botanical Garden, the Mondego River and some of the irrigation channels) (Zimmermann 1906, 1909, 1910, 1914).

About twenty years later, new references appear in the literature. A survey of green flagellates (order Volvocales) was done in 18 sampling points in Coimbra and 16 species were found, 3 being described as new for science (Moewus 1935). A study of charophytes, based mainly on material from the Herbarium of Coimbra (Figs 1 and 2), but also on some collections made by the author (working at the University of Lisbon) lists a total of 15 taxa, including 5 varieties of *Chara vulgaris* for the central region of the Mondego Basin (Cunha 1934, 1935, 1942, 1943).



Figs 1, 2. Two specimens of the genera *Chara* (Fig. 1) and *Nitella* (Fig. 2) from the Coimbra Herbarium, with at least 130 years.

Desmids and cyanophytes were collected and studied in the North of Portugal for more than twenty years by Sampaio, a naturalist at the Instituto de Botânica (Porto). In his synopsis that includes about 200 known desmids for Portugal, he mentions only two species of *Closterium* and one of *Cosmarium* for the Mondego Basin, collected in the Botanical Garden of Coimbra (Sampaio 1943, 1944). These species had been recorded by the author for the garden (Sampaio 1923). His studies of cyanophytes mention a total of 24 taxa, the majority for Coimbra and a few for Serra da Estrela (Sampaio 1933, 1934, 1947a,b).

Other studies of green algae include a revision of literature on Zygnemataceae (Alte 1945) and a first survey of desmids in Serra da Estrela (Rozeira 1947). The first mentions 8 taxa of *Spirogyra*, all collected by A. F. Moller from Coimbra and surroundings; the second reports 15 taxa from ponds near Lagoa Comprida.

After an interruption of about forty years, a renewal of phycological enthusiasm from academics at the Department of Botany, Coimbra, started in the late forties and afterwards, with no further interruption till the present. A major contribution for the knowledge of the green algae, in particular Oedogoniaceae, was made by Lacerda (Lacerda 1946, 1949). The author reported 32 taxa belonging to that family (28 to the genus *Oedogonium* and 5 to the genus *Bulbochaete*) based on specimens collected mainly in Coimbra and near Figueira da Foz. He also reported a few taxa belonging to other algal groups (Lacerda 1945, 1948).

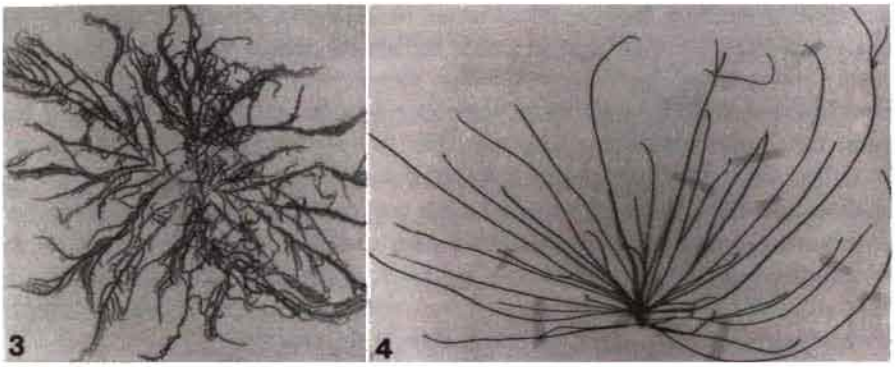
The second half 20th century

Taxonomical and ultrastructural studies

In this half century there was an increase in taxonomical studies of freshwater algae in the Mondego Basin area carried out by researchers from Coimbra and also by Vasconcellos from the Instituto Superior de Agronomia (Lisboa), who reported 6 macroscopic filamentous green algae and one rhodophyte infesting the rice fields of the Mondego Basin (Vasconcellos 1956).

In the 1950s and during a period of about thirty years, the freshwater rhodophytes were studied by the naturalist priest M. P. Reis, first at Coimbra and later at Aveiro. He described about 20 taxa new for science. For the Mondego Basin he reported a total of 26 taxa (not counting forms and varieties), 15 belonging to the genus *Batrachospermum* (Reis 1974) (Fig. 3) and 11 to the genera *Lemanea* (Reis 1961a) (Fig. 4), *Audouinella* (Reis 1961b, 1966), *Pseudochantransia* (Reis 1962) and *Thorea* (Reis 1957).

In the 1960s, Rodrigues reported on algae collected mainly in Coimbra (Rodrigues 1961) and near Lagoa Comprida (Serra da Estrela) (Rodrigues 1963) and Rino published four major contributions describing and illustrating more than 70 taxa new for Portugal and for the Mondego Basin (Rino 1967a,b, 1969; Rino and Santos 1968). Posterior published contributions from this author for the region are punctual (Rino and Pereira 1989), since he moved first to Mozambique, later to Aveiro and became engaged in the study of the algal flora of those regions. However his published



Figs 3, 4. Representatives of the genera *Batrachospermum* (Fig. 1) and *Lemanea* (Fig. 2), studied by M. P. Reis and kept at the Coimbra Herbarium.

and unpublished data and his knowledge on freshwater algae would certainly be enough for publication of a first Portuguese Flora of Freshwater Algae, a need that he mentioned in his first publication as an incentive to study algae.

Since the seventies, a similar taxonomic work on microalgae from the region has been continued at Coimbra by M. F. Santos. About 94 taxa were recorded as new for Portugal and the Basin in her six contributions (Santos 1970, 1971, 1973a,b, 1976; Santos and Morgadinho 1987). In order to have algal material to perform cytological studies, conditions were created at the Department for the establishment of algal cultures. The result was a collection of more than 900 (see below). Aspects of the cytology and ultrastructure of a few taxa collected from sites within the Basin have been studied and published (Mesquita and Santos 1976a,b, 1981) or presented at conferences (e.g. Santos et al. 1997; Osório et al. 1999). A survey of the algal flora in the Natural Park of Serra da Estrela was also prepared and waits publication. It lists a total of 574 taxa (125 new for the park), with illustrations of most.

Taxonomical studies of groups requiring the use of electron microscopy, such as the silica-scaled chrysophytes, were done more recently. The first published survey of these organisms in Portugal (and the Iberian Peninsula) reported 23 species from lakes, reservoirs and ponds of Central Portugal, 16 for the Basin area (Santos and Leedale 1993). Posterior studies (Calado and Craveiro 1995; Santos et al. 1996) added 11 new species for the region and included information on their ecology.

Phytoplankton

The first major attempted survey of phytoplankton in Portuguese waters is Nauwerck 's work (Nauwerck 1962). It refers to samples collected from lakes, rivers and reservoirs throughout the country; it provides lists and drawings of the algae found in those sites and information on the water physical and chemical parameters measured as well as biomass. Within the Mondego Basin, he sampled Lagoa Comprida, Covão do Vidoal, springs and moist stones near Lagoa Escura and the Mondego River at Coimbra (since we are revising the information on freshwater algae found within the Basin, we

include here the algae found on moist stones by the author, although "moist stones do not give phytoplankton"). The same author had already done a previous shorter report on the phytoplankton found in spring at two sites near Coimbra (Nauwerck 1959).

To our knowledge, only thirty years later another study was published, dealing with the dynamics of cyanophyte blooms in the Aguieira reservoir (Oliveira and Monteiro 1992). The blooms were followed during four months, in summer 1992, to evaluate their effects on water treatment processes, drinking water quality and zooplankton community. It was found that, under such cyanophyte biomass, used water treatment processes could not remove algae from drinking water, including some toxic species. It was also found that changes in dominant zooplanktonic species were related to the type of cyanophyte species responsible for the bloom.

More information on diversity, density and toxicity of cyanophytes in the Mondego reservoirs, Aguieira in particular, is given by Vasconcelos in his contribution for this book.

A study of silica-scaled chrysophytes from three shallow water bodies rich in organic matter, in the lowland region of the Basin, reported on the eutrophic taxa found and suggested the use of the saprobity system to a better delimitation of the ecological characteristics of eutrophic taxa (Santos et al. 1996).

The most comprehensive available study on composition and dynamics of the phytoplankton in the Mondego Basin has been done in the Coimbra reservoir (Craveiro and Santos 1997, 1998). The phytoplankton assemblages were studied during one year and compared to those present in the Mondego and Ceira rivers, before their confluence. Several physical and chemical parameters of the water were also measured. It was found that the phytoplankton community in this small reservoir was mainly conditioned by the flow. Only in late spring and summer, when the retention times increased to two or tree days and the mean values of flow became lower than 20 $m^3.s^{-1}$, a phytoplanktonic community different from the one entering with the tributaries could develop in the reservoir. During most of the year, the diatoms were the dominant group, but diversity increased in summer, a maximum value of biomass being due to *Aulacoseira ambigua, Peridinium umbonatum* and some Cryptophyceae.

The ACOI culture collection

The Culture Collection of Algae at the Department of Botany, University of Coimbra (ACOI) (http://www.uc.pt/botanica/ACOI.htm) was built in 1972 for institutional research purposes. The first list of strains included 167 taxa, mostly isolated from central Portugal (Santos and Mesquita 1986). Two posterior supplements added 88 taxa (Santos 1988) and 194 (Santos et al. 1993) to the collection. The present list is available on the web and includes more than nine hundred cultures, although the total number of strains in culture is about 4 thousand. They are mostly freshwater microalgae isolated from field samples collected across the country by many different persons. The ACOI collection provides experimental organisms for research and didactic purposes within the Department and other national and international Institutions.

A rather tiring and time-consuming process is needed to establish these cultures. Aquatic, free-living organisms are collected with a 20-30 µm mesh phytoplankton net

whereas algae attached to aquatic plants, stones or other substrates are removed with the help of a knife or a spoon. The samples must be kept alive, at low temperatures, until they reach the laboratory.

Isolation is performed by pipetting the cells directly from the field sample to a tube, a flask or a Petri dish with culture medium, under an inverted microscope. Some taxa, due to the small dimension or the low number of cells present in the field sample, may require to develop first on a Petri dish with solidified culture medium (streak cultures) (Venkataraman 1969). The isolation procedure usually has to be repeated several times, before a unialgal culture is established. Identification of the culture to species level (or lower) always requires the expertise of an experienced algal taxonomist.

Since the establishment of the ACOI, many types of culture media recipes, previously developed and published by authorities on this subject, were tried, modified or mixed in order to suit the algal growth requirements. Also, physical space and staff limitations to maintain the increasing number of cultures made it necessary to reduce the culture media to the fewer possible number. A total of only six different media (see the web site for recipes), are used to grow the nine hundred identified species and the other thousands waiting for identification to species level. Therefore, the cultures are maintained on media that are not necessarily the best for their growth.

Qualitative phytoplankton survey

Since the first reports on phytoplankton of Portuguese waters (Frade 1951; Nauwerck 1962), several studies were made throughout the country (e.g. Oliveira 1982, 1984, 1987; Coutinho 1990; Vasconcelos 1990), but only a very few included reservoirs or sites in the Mondego River or Basin, as mentioned.

Here we report on a recent qualitative survey aimed to assess the diversity of the freshwater microalgae in the Mondego Basin. We also provide taxonomical lists and illustrations of some taxa.

Methods

Phytoplankton samples were collected with a 20-30 µm mesh net, in a total of 21 sites within the Mondego Basin. Eighteen were sampled once in April or June 1996, a few of these were sampled again every two months from March 1997 to March 1998 and the remaining three were sampled once or twice during that period. The sampling sites include ponds and reservoirs in Serra da Estrela Natural Park, main river and tributaries in the central area of the Basin and ponds, marshes and irrigation channels in the lowland region (Fig. 5).

In the field, each sample was divided in two replicates: one was kept in ice for posterior observation and isolation purposes, the other was immediately fixed with 5% formalin. Some physical and chemical water parameters (such as pH, conductivity, dissolved oxygen, ammonia, nitrates, nitrites, orthophosphates, silica and calcium) were determined, either in the field using WTW portable meters or in the laboratory by

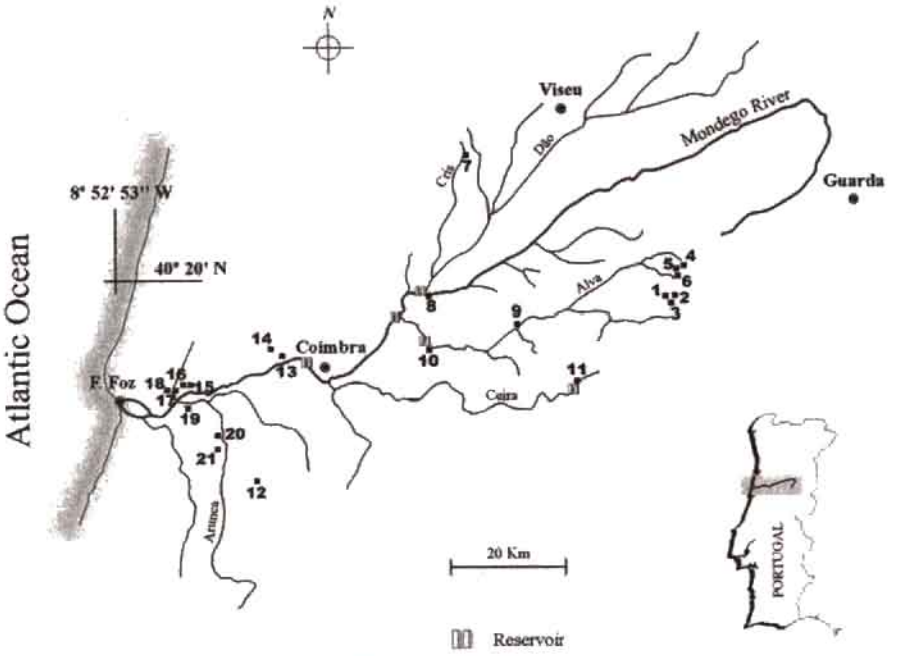


Fig. 5. Location of the sampling sites within the Mondego Basin. Serra da Estrela Natural Park: 1, Covão do Curral; 2, Pond near Lagoa Comprida; 3, Lagoa Comprida; 4, Vale do Rossim Reservoir; 5, Erva da Fome Reservoir; 6, Lagoacho Reservoir. Mondego River and tributaries: 7, Cris River, near Tondela; 8, Aguieira Reservoir; 9, Alva River; 10, Fronhas Reservoir; 11, Alto Ceira Reservoir. Lowland areas: 12, Pond near Srª da Estrela; 13, Poço dos Albinos; 14, Ribeira de Ançã; 15, Marsh near Montemor; 16, Irrigation channel near Montemor; 17, Pond near S.ta Olaia; 18, Irrigation channel near S.ta Olaia; 19, Pond near Marujal; 20, Irrigation channel near Soure; 21, Ribeira near Casal da Rola, Soure.

standard methods, but this information will be presented elsewhere, since it is not within the scope of this work.

The samples were generally examined with a Leitz – Biomed or Leica DMRB light microscope. Isolation and culture were performed as previously described (see the ACOI section), from samples collected in 1996 and 1997/1998. The whole phytoplankton was studied in eleven samples out of the eighteen collected in 1996 (sites 1– 4, 6, 7, 10, 11, 15, 16 and 20). To study the diatoms, subsamples were oxidised with concentrated nitric acid for one hour, then washed in distilled water and mounted with Naphrax resin for preparation of permanent slides.

To identify the silica-scaled chrysophytes (or particular details of other algae), the samples collected in 1996 were examined by transmission or scanning electron microscopy. For transmission (TEM), one small drop of material was placed on formvar and carbon coated grids, air-dried and directly examined with a Siemens Elmiskop 101. For scanning electron microscopy (SEM), a larger drop was dried onto aluminium foil glued to a stub, sputter – coated with gold for five minutes and observed with a Jeol JSM 5400.

Results and discussion

The qualitative examination of the samples allowed the identification of 387 taxa (see Tables 1 and 2, Appendix 1), 231 being diatoms (class Bacillariophyceae) (Table 1).

Forty diatom genera were found, *Navicula* and *Nitzschia* being the most represented, followed by *Eunotia, Fragilaria* and *Cymbella*. The majority of the species are considered of broad geographical distribution and occurrence, either in Portugal or in central Europe, such as *Achnanthes minutissima, Eunotia minor, Frustulia rhomboides, Navicula angusta*. They usually occur in oxygenated waters, with a pH slightly acidic to moderate and relatively poor in nutrients (nitrates, nitrites and orthophosphates). Taxa that markedly prefer less oxygenated waters but higher nutrient concentrations, such as *Cyclotella meneghiniana, Melosira varians, Navicula phyllepta* and *Nitzschia acula*, occurred and were dominant in lentic waters of the irrigation channels, marshes and ponds. Rich and diverse communities were found in lowland water systems such as sites 15 and 16 when compared with sites at higher altitude, such as 3 and 4 (Serra da Estrela Natural Park) (see Table 3, Appendix 1). Low silica concentrations in these sites (less than 1.5 $mg.L^{-1}$) may explain the reduced number of taxa registered. We should bear in mind, however, that many of the diatom taxa listed in Table 1 are not phytoplanktonic organisms, only temporarily being found in suspension.

A hundred and fifty-six taxa, belonging to other algal groups, were identified during this general phytoplankton survey in the Mondego Basin (Table 2, Appendix 1). The list is particularly rich in Euglenophyta of the genera *Trachelomonas* and *Phacus* and in Chlorophyta, especially Chlorococcales and Desmidiaceae. The presence of these euglenophytes is worth mentioning; they usually appear in lentic waters during spring, summer and autumn, in particular when there is an enrichment in organic matter, the conductivity is medium to high (from 250 to 1900 $\mu S.cm^{-1}$), the pH around 6.0 to 7.5, and several inorganic ions are present (Pereira 1999). These conditions therefore existed at several of the sampled water bodies, mainly in the lowland areas. The occurrence of *Trachelomonas* further indicates significant levels of iron, because this element is often found in the mineralised lorica of such organisms (Rino and Pereira 1991, Pereira 1999).

Fifty-two species were isolated into culture, mainly from the samples collected in Serra da Estrela, and are now part of the ACOI collection (Table 2, marked with an asterisk). The great majority belongs to the green algal genera *Staurastrum, Staurodesmus, Euastrum* and *Closterium*. Representatives of the other groups, although frequent and sometimes abundant in the sampled areas, are not well represented in the culture collection, either because they are quite common and therefore not selected for isolation or because they do not survive in culture or need special conditions and constant surveillance to keep alive.

A total of 35 species of silica-scaled chrysophytes was registered in this study (Table 2). The most frequent were *Paraphysomonas vestita, Mallomonas acaroides, M. punctifera, M. tonsurata, Synura curtispina* and *S. petersenii*, all quite common in our freshwaters and other parts of the world. Organisms rare from other surveys, such as *Mallomonas calceolus* (Calado and Craveiro 1995), *M. retifera* (Santos and Vicente

1999) and *Spiniferomonas breakneckii* (Santos and Leedale 1993), were found in Serra da Estrela. Reported here for the first time for Portugal is *Paraphysomonas capreolata*; it was found in site 18, an irrigation channel of eutrophic water, with a conductivity of about 500 $\mu S.cm^{-1}$ and a pH of 6.5. Four other taxa are new for the Basin area. The present survey and others conducted in the region (published or unpublished) show the presence of a rich flora of silica-scaled organisms, particularly in the marshes, irrigation channels and small ponds of the rice-field area. Forty-two species, mainly belonging to the genus *Mallomonas*, are now known for the Mondego Basin from a total of 53 reported for Portugal.

Final remarks

We may say, in conclusion, that a very rich, diverse and interesting flora of freshwater algae is already known for the Mondego Basin but that it still waits to be revealed to its full splendour. Illustrations of some taxa, that may give an idea of this richness, are compiled on eight plates (see Appendix 2). Higher variety can be found in the marshes, irrigation channels, small ponds and lagoons of the lowland rice-field areas. On the other end, in highland water bodies, an attractive flora usually develops in spring and some ponds are worth to preserve, due to the occurrence of quite rare algal species.

A few groups, such as the rhodophytes, some greens and the silica-scaled chrysophytes have deserved particular attention from phycologists and have been reasonably searched for and taxonomically investigated in the surroundings of Coimbra, the rice-field area and easily accessible water bodies in Serra da Estrela. The present survey gives a good insight on diatoms (more than 200 taxa) and euglenophytes (about 50). Further estimation of the diversity of these groups is still required and less explored areas should be investigated.

Other groups in particular the cyanophytes and the yellow-green representatives, need special attention in future surveys. It is of great importance and public health service to improve our taxonomic knowledge on cyanophytes, since this is indispensable to quickly determine the presence of potentially toxic species in water supply systems and to take adequate measures to prevent the formation of toxic blooms.

Further progresses in the study of freshwater algae require the urgent publication of a Portuguese Algal Flora that integrates existing disperse information on taxonomy (descriptions and iconography), distribution, ecology and other relevant data. It is essential that easily reading and consulting guides become available to teachers and students, as well as to common people, simply interested in the preservation of Life and Nature; these guides would help them to program field trips, to collect and identify the most frequent species and to apprehend the otherwise inconceivable biodiversity of these freshwater microscopic organisms.

It is recognised that algae have the capacity to integrate and reflect the environmental conditions they are submitted to. This is particularly true for diatoms, since more studies with this group have been performed. The evaluation of water

quality in rivers, lakes and other water systems, based on the profound knowledge of the species auto-ecology is today a current practice, world-wide applied (see Descy and Coste 1991). This survey allowed us to recognise exclusively from a taxonomical point of view the algal flora dominant in the Basin; however, the extension and patrimonial value of this region would certainly deserve and require a more detailed investigation in the future. It is essential to characterise the auto-ecology of the dominant species, to recognise the diversity of their environmental requirements, in order to be able to use that information for water quality evaluation and monitoring purposes. That knowledge is essential to apply and improve the existing biological indices to Portuguese waters, namely the diatomological indices (Descy and Coste 1991; Coste 1992) and to compare results with other studied areas (see Gil et al. 1989; Almeida 1998; Pereira 1999).

The isolation of algal strains into culture is one way of preserving their genetic potential for the future, now that the aquatic habitats are massively destroyed and their biodiversity lost. Furthermore, in culture they are more easily accessible for use and exchange as experimental organisms, either by academics or for commercial purposes. These reasons justify future work aiming the conservation and expansion of the ACOI collection and the improvement of its available database.

Many similar remarks and topics of needed research on freshwater algae in the Mondego Basin area or the whole country could be given. As a final remark, we may say that collaboration among institutions and people dealing with algae is fundamental for further phycological progresses.

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