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Phycological Herbaria as a Useful Tool to Monitor Long-Term Changes of Macroalgae Diversity: Some Case Studies from the Mediterranean Sea

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Received: 30 June 2020; Accepted: 7 August 2020; Published: 10 August 2020

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Abstract: The Mediterranean Sea is currently experiencing a decline in the abundance of several key species, as a consequence of anthropogenic pressures (e.g., increase in human population, habitat modification and loss, pollution, coastal urbanization, overexploitation, introduction of non-indigenous species and climate change). Herbaria and natural history collections are certainly fundamental for taxonomic studies, but they are also an invaluable, if currently underestimated, resource for understanding ecological and evolutionary responses of species to environmental changes. Macroalgae herbarium collections, which are really consistent (ranging from 200,000 to approximately 500,000 specimens) in some European herbaria (e.g., Muséum National d'Histoire Naturelle in Paris, University of Copenhagen, Natural History Museum in Kensington), can be successfully used as real "witnesses" to biodiversity changes. In this respect, we report some case studies from the Mediterranean Sea which summarize well the potential of macroalgae herbarium specimens to provide useful data on biodiversity changes. Indeed, these data enable the evaluation of the responses of biota, including shifts in species ranges, the detection of the presence of introduced species, and the prediction of changes in species distributions and patterns under future climate scenarios. To increase the use of this invaluable tool of research, their curation, the digitization of collections, and specimen genomics should be even more addressed.

Keywords: biodiversity; climate change; Mediterranean Sea; herbaria; macroalgae collections

1. Introduction

The Mediterranean Sea, representing the 0.82% of the world's oceans, is inhabited by an unusually rich and diverse biota (approximately 17,000 species) which makes it a true hotspot of biodiversity [1,2], even by virtue of the high rates of endemic species it supports (25%) [3]. At the same time, the Mediterranean Sea is among the most impacted seas, as a consequence of different anthropogenic pressures that are significantly affecting biodiversity, such as habitat modification and loss, pollution, coastal urbanization, overexploitation, the intentional or unintentional introduction of non-indigenous species (NIS, i.e., organisms introduced outside of their natural range) and climate change [2,4–6]. Thus, several valuable species and/or habitats are currently experiencing a severe decline or local extinction, and these consequences are predicted to increase in the future [7,8].

Understanding how human stressors have affected biodiversity over time is crucial for understanding the biological impact they have had in the past and to make provision on how biodiversity will respond to future global environmental changes. In this context, herbarium specimens provide unique information for studying long-term changes [9–12].

In its original meaning, the term "herbarium" refers to a book on medicinal plants, whereas the beginning of the herbarium as a collection of dried specimens is attributed to Luca Ghini (1490–1556). Since then herbaria have been fundamental as taxonomic repositories [13], and nowadays, with the possibility to extract DNA from herbarium specimens for genetic studies, practiced since the 1990s [14], the importance of these repositories has increased even more. However, in the late 20th century the use of herbaria diversified a lot. As an example, in the 1960s, historical data available through the herbarium specimens were first used to study global change [15] to which they are currently considered real 'witnesses' [16]. Funk [17] reports that: "Herbaria, dried pressed plant specimens and their associated data ... are remarkable and irreplaceable sources of information about plants and the world they inhabit", emphasizing the scientific value they have for a broad range of studies (e.g., taxonomy, systematics, morphology, phenology, biodiversity, paleobiology, ecology and ethnobotany). As a veritable gold mine of information even after a long time, herbaria may also be essential and invaluable resources for studying biodiversity for conservation and ecological purposes. Thus, they can provide information about species habitat, the presence of a species in a certain locality and at a certain time, and the loss of habitat and species. They can highlight changes in species ranges, the introduction but also the expansion in space and time of NIS, and enable the prediction of future changes in species richness and distributions under future climate scenarios [16,18]. In fact, they can facilitate the detection of spatial-temporal biodiversity changes at regional, national and global scale over a longer period than field-based observation studies can do. They can also provide information to define conservation priorities, improve decisions taken on rare and/or threatened species and apply conservation efforts with as much efficiency as possible [19,20]. Even the IUCN (International Union for Conservation of Nature) Committee indicates their importance as a crucial step for the elaboration of red lists [21]. On the other hand, herbarium specimens can facilitate the identification of hotspots, ecoregions and centers of biodiversity, as well as the establishment of priority areas [22].

Recently, the increasing specimen digitization [23,24] and the development of online platforms (e.g., Encyclopedia of Life, GBIF, Map of Life, iNaturalist, JSTOR's Global Plants), which facilitate access to collections via the Internet, freely and free of charge, has greatly enhanced the use of herbarium data in scientific research [25]. One of the most important initiatives is GBIF—the Global Biodiversity Information Facility, (https://www.gbif.org/what-is-gbif)—which is an international network and research infrastructure funded by the world's governments and aimed at providing open access to data about all types of life on Earth. The final objective of this network is to support scientific research, promote biological conservation and sustainable development. Another initiative is the Federation of European Phycological Societies (FEPS) which aims to create a "European Database of Algal and Cyanobacterial Archived Materials for Analytical and Molecular Biology Research". The FEPS initiative will lead to more complete data on the algal collections present in Europe.

However, it should be noted that taxonomic errors may be present in herbaria, and information may be not detailed in very old specimens; moreover, the absence of a species in a certain locality may be explained in different ways, for instance the species may not be present, was not collected, or was not detected. Instead, a high number of specimens in a certain area may be due to a specific interest for the area, or the presence of experts working on those taxonomic groups. In addition, common species are undercollected whereas rare or unusual species are overcollected [26].

The aim of this paper is to highlight the important role of phycological herbaria not only for taxonomic research but also as witnesses to changes of macroalgae diversity, providing some case studies from the Mediterranean Sea. To reach this aim we also provide an overview of the European collections of macroalgae. Data from the European phycological herbaria, with a look also at Mediterranean specimens, were obtained by consulting the Index Herbariorum (http://sweetgum.nybg.org/science/ih) [27], the Algae Directory [28], GIBF (https://www.gbif.org/what-is-gbif), the CoRIMBo network of

Italy, e-ReColNat infrastructure (https://www.recolnat.org/en/), and the webpages and/or the curators of the different herbaria. With respect to the Italian collections, we referred also to Giaccone et al. [29].

2. Materials and Methods

The search for relevant literature, updated till June 2020, was carried out using standard electronic databases. Data from the European phycological herbaria, with a look also at Mediterranean specimens, were obtained by consulting the Index Herbariorum (http://sweetgum.nybg.org/science/ih) [27], the Algae Directory [28], GIBF (https://www.gbif.org/what-is-gbif), the CoRIMBo network of Italy, e-ReColNat infrastructure (https://www.recolnat.org/en/), and the webpages and/or the curators of the different herbaria. With respect to the Italian collections, we referred also to Giaccone et al. [29].

3. Results

3.1. Overview of European Phycological Collections

According to the Index Herbariorum, 688 herbaria are present in 33 European countries (Figure 1), with France, the UK, and Germany hosting the highest number of herbarium specimens (25.96, 22.31, and 22.16 million specimens, respectively) [27]. With respect to the phycological collections, more than one million, seven thousand specimens are currently preserved in the European herbaria. This figure is substantially smaller than that of vascular plants, as a consequence of the considerably smaller number of seaweed species, but also to the lower number of specialists on the subject, probably due also to the greater difficulty in sampling.

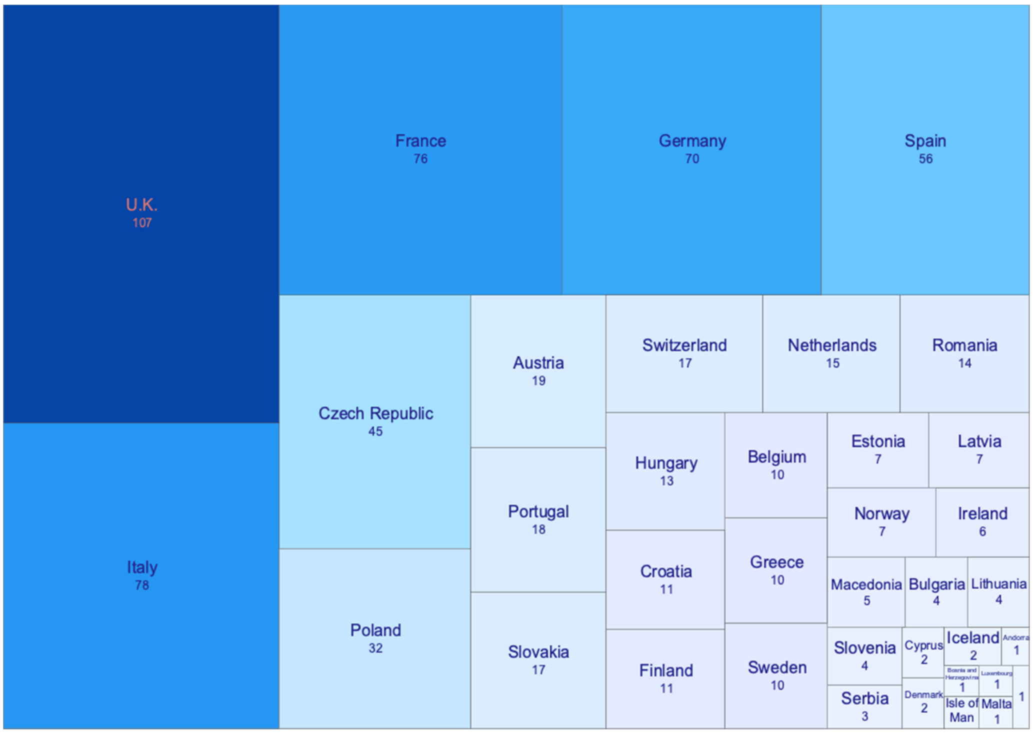

Figure 1. Number of herbaria reported for each European country [27].

In Table 1 we report the largest collections of marine macroalgae hosted in the European phycological herbaria. In particular, the biggest macroalgae collections are hosted in the herbarium of the University of Copenhagen (C), with 260,000 specimens, the Natural History Museum of London (BM), with 205,000 specimens (9000 types), the herbarium of the Naturalis Biodiversity Center at Leiden (L) with 148,000 specimens, and the herbarium of Lund University (LD), with 102,000 specimens, among which, the Agardh Herbarium (50,000 samples and 6000 types) represents one of the most important collections of algae in the world. These are also outstanding: the Muséum National d'Histoire Naturelle of Paris (PC), with 500,000 specimens (more than 10,000 types) and the Naturhistorisches Museum Wien (W), with 200,000 specimens, although these herbaria are not totally digitized (PC) or not digitized (W), and the figures provided by the herbaria curators include freshwater algae and marine Bacillariophyta.

Table 1. European herbaria hosting consistent marine macroalgae collections. The acronym of the herbaria in the Index Herbariorum [27] is also included. The number of specimens of herbaria labelled with *, includes freshwater algae and marine Bacillariophyta.

Acronym	Institution	City	Number of Specimens of Marine Macroalgae
PC	Muséum National d'Histoire Naturelle	Paris, France	500,000 *
C	University of Copenhagen	Copenhagen, Denmark	260,000
BM	Natural History Museum	Kensington, United Kingdom	205,000
W	Naturhistorisches Museum Wien	Wien, Austria	200,000 *
L	Naturalis Biodiversity Center	Leiden, The Netherlands	148,000
LD	Lund University	Lund, Sweden	102,000
GENT	Ghent University	Ghent, Belgium	42,653
HCOM	Centre d'Océanologie de Marseille - University of Aix-Marseille II	Marseilles, France	40,000
SANT	Universidad de Santiago de Compostela	Santiago de Compostela, Spain	30,218
FI	Natural history Museum	Firenze, Italy	30,000
TCD	Trinity College	Dublin, Ireland	25,000
RO	Sapienza Università di Roma	Rome, Italy	23,000
HGI	Universitat de Girona	Girona, Spain	20,600
GB	Gothenburg University	Gothenburg, Sweden	20,000
MCVE	Museo di Storia Naturale di Venezia	Venice, Italy	18,500
PAD	Università degli Studi di Padova	Padova, Italy	15,000
MS	Università di Messina	Messina, Italy	8000
BCN	Universidad de Barcelona	Barcelona, Spain	7500
CAT	Università di Catania	Catania, Italy	6193
MGC	Universidad de Málaga	Malaga, Spain	5496
TSB	Università de Triestre	Trieste, Italy	5169–5426
MA	Real Jardín Botánico de Madrid	Madrid, Spain	4105
ZA and ZAHO	University of Zagreb	Zagreb, Croatia	4000
PAL	Orto Botanico dell'Università degli Studi di Palermo	Palermo, Italy	3856
—	Herbarium J.J. Rodríguez y Femenías, Ateneu de Menorca	Menorca, Spain	2775

Few data are published on the Mediterranean macroalgae collections deposited at the European herbaria, with the latest update going back to the list reported in the Algae Directory [28]. The number of Mediterranean macroalgae specimens currently preserved and quantified in European herbaria ranges between 95,000 and 124,000. However, in addition to these, we estimate that there would be about 91,000 more specimens hosted in those herbaria that are not yet partially or totally digitized. Apart from the big Mediterranean macroalgae collection (between 20,000 and 40,000 specimens) hosted at the Muséum National d'Histoire Naturelle, Paris (PC), there are other consistent collections at the Centre d'Océanologie de Marseille (HCOM, ~15,000–20,000 specimens), at the University of Girona (HGI, 17,700 specimens), at Ghent University (GENT, 8075 specimens), and at the University of Messina (MS, 8000). Finally, besides these big collections we can find other relevant collections hosted at Universidad de Barcelona (BCN, 6500 specimens), Università di Catania (CAT, 5590 specimens), Trinity College (TCD, 4000–5000 specimens), Università di Trieste (TSB, ~4190 specimens) and University of Zagreb (ZA and ZAHO, 4000 specimens).

3.2. Case Studies from the Mediterranean Sea

Herbaria specimens can be a crucial tool for reconstructing distribution maps and variations in species ranges through time, analyzing the regression of some species and assessing their conservation status (stable, in decline, extinct), depicting the introduction and/or the geographic expansion of non-indigenous species. In this context, large brown fucoid algae (Fucales, Phaeophyceae) and kelps (Laminariales and Tilopteridales, Phaeophyceae), but also introduced species, such as *Dictyota cyanoloma* (Dictyotales, Phaeophyceae), are effective examples (Figure 2).

Figure 2. Specimens of *Carpodesmia mediterranea* (Sauvageau) Orellana and Sansón (**A**) and *Laminaria rodriguezii* Bornet (**B**) from the algae section of the herbarium of Universitat de Girona (HGI-A).

Large canopy-forming fucoids are dominant species in pristine environments along temperate rocky coasts worldwide, which provide essential ecological goods and services [30–33]. These valuable and sensitive communities are currently experiencing worldwide a severe decline and/or loss [34–36], as a result of a variety of anthropogenic stressors such pollution, coastal urbanization, overexploitation, the intentional or indirect introduction of NIS, and climate changes, i.e., acidification and warming.

Thibaut et al. [37], for instance, compared the state in 2003 of Fucales on the Albères coast, France, by means of historical records. The authors found that only five species of the fourteen reported in the first thirty years of the 20th century by Sauvageau [38] and Feldmann [39,40], or the nine species reported by Gros [41] were still present, showing a decrease in the area of all the populations since the 1940s and the total collapse of the genus *Sargassum*. Seven taxa (*Carpodesmia crinita, Treptacantha barbata*, *Cystoseira foeniculacea* f. *tenuiramosa*, *T. ballesterosii, T. ballesterosii* var. *compressa*, *S. hornschuchii* and *S. vulgare*), considered frequent or abundant by Feldmann [39,40] and some of them also dominant species in several phytobenthic communities, resulted extinct in the area. Moreover, only one of the five recorded species (*Cy. compressa*) showed no signs of regression, two species (*Ca. brachycarpa* and *Ca. zosteroides*) were considered as rare, and one (*T. elegans*) very rare. An increase in water turbidity could be responsible for the progressive replacement of *T. ballesterosii* var. *compressa* by *Ca. zosteroides* between the 1940s and the 1970s [41,42] and the total collapse of *T. ballesterosii* var. *compressa* at the late 1970s [41]. *Carpodesmia mediterranea*, a species that was reported to make a continuous belt along the shores of the Albères coast [39,40], in 2003 was less abundant and has almost disappeared from some areas.

Blanfuné et al. [43] analyzed in detail the distribution of *Ca. mediterranea* over the Gulf of Lions, France, comparing the current distribution with historical data. The authors observed two minima: 1960s–1970s and 2007, the latter being the most severe. Other minima could have occurred in the past, although they have gone unnoticed. Overall, the general trend over the past century has been a decline, but, in French Catalonia it has been marked by clear-cut ups and downs. Possible causes for the observed decline of the range of *Ca. mediterranea*, together with its disappearance at its northernmost limit, could be the anthropogenic impact and overgrazing. In the 1960s–1970s, Gros [41] described the invasion of *Ca. mediterranea* habitat by dense stands of the mussel *Mytilus galloprovincialis*, possibly due to organic pollution. Mussels could reduce the settlement and survival of *Ca. mediterranea* recruits because of the instability of the mussels' support or the lack of light for recruits that grow directly on the rocky substrate. The mussel farming spread from the 1950s to the 1970s could be responsible for the mussels' larvae invasion of the *Ca. mediterranea* habitat. Heatwaves and an exceptional storm are plausibly responsible for the 2007 decline of *Ca. mediterranea*. Between 2007 and 2012, further exceptional storms opened spaces in the mussel beds for the recruitment of *Ca. mediterranea*, which partially recovered [43].

Recently, Thibaut et al. [34] reconstructed the distribution of Fucales species along the French Riviera coast (north-western Mediterranean), comparing their distribution between 2007 and 2013 with historical data from the 19th century. Of the 18 species historically reported for this area, five were probably extinct in the zone (*T. elegans*, *Cy. foeniculacea* f. *latiramosa*, *T. squarrosa, T. ballesterosii* and *S. hornschuchii*). For instance, *T. squarrosa*, originally described by De Notaris from material collected in Nice [44] and reported till the end of the 19th century, was not found during the survey period (2007–2013). Similarly, *S. hornschuchii,* first collected in 1821 in Cannes [45], was reported till the 20th century in Cannes (Raphélis *in* Général Herbarium) and Antibes (J. Feldmann Herbarium), but the authors did not find this species during their survey. In addition, nine taxa suffered a decline (*Ca. amentacea, T. barbata* f. *barbata*, *Ca. brachycarpa*, *Ca. crinita*, *T. sauvageauana* and *S. vulgare*) or became nearly locally extinct (*Cy. foeniculacea* f. *tenuiramosa*, *T. ballesterosii* var. *compressa* and *S. acinarium*). Regressions were localized around the entrances to ports and offshore from the cities.

On the other hand, Thibaut et al. [34] showed that *Cy. compressa,* first collected in 1839 in Nice (C. Agardh *in* Requien Herbarium, Algues Vertes Herbarium), and *Ca. amentacea*, collected for the first time in 1826 in Nice by Antoine Risso (*in* Bory de Saint-Vincent Herbarium), were still abundant and common. Thibaut et al. [46] analyzed the long-term changes in *Ca. amentacea* populations along the French Mediterranean coasts, comparing the state between 2008 and 2011 with historical records (from the 18th–19th to the early 21st century). A remarkable stability over 1–5 decades was observed, except for four areas along the Western Provence, the French Riviera and Corsica, where the conspicuous decline of *Ca. amentacea* populations was highlighted. In the vicinity of the Cortiou sewage outfall at Marseilles, the largest French Mediterranean city and one of the largest cities of the whole Mediterranean Sea, a steady decrease of *Ca. amentacea* has apparently occurred since the 1960s. Likewise, in the Gulf of Fos (western Provence, French Mediterranean coast), which harbors one of the largest French ports and industrial facilities (petrochemical and chemical industries, oil refineries, steelworks, power plant, etc.), the distribution of *Ca. amentacea* has remained unchanged since 1975, but subsequently the populations were more fragmented and in competition with mussel beds. Instead, *T. ballesterosii* var. *tenuior* was first recorded during the survey by Thibaut et al. [46]. Habitat destruction, overgrazing by the sea urchins *Paracentrotus lividus* and *Arbacia lixula,* competition with the invasive macroalgae *Caulerpa taxifolia* and *Caulerpa cylindracea,* fishing activities and the increase in turbidity were considered the most severe probable causes of the decline of most Fucales in France [37,47–50].

Blanfune et al. [36] compared the current distribution of *Ca. crinita,* an ecologically important species and a regionally protected marine algae (Barcelona Convention; UNEP/MAP, 2009), over the entire French Mediterranean coast, including Corsica, with the historical data (e.g., herbarium vouchers since 1700). A drastic decline of *Ca. crinita* on the continental coast, up to local extinction (French Catalonia), near extinction (Languedoc and western Provence), or functional extinction (French Riviera) was observed, while a still stable and healthy population was found in Eastern Provence and in Corsica. The authors also assessed the conservation status of *Ca. crinita*, according to the IUCN Red List criteria [51]. On the basis of the comparison between historical and current data, and the knowledge of anthropogenic pressures, the authors propose the classification of *Ca. crinita* as 'Critically Endangered' (CR), near to 'Regionally Extinct' (RE) in French Catalonia and Languedoc, as 'Vulnerable' (VU) in the region Provence-Alpes-Côte-d'Azur, as 'Least Concern' (LC) in Corsica.

Furthermore, Žuljević et al. [52], comparing historical data (e.g., herbarium collections) [53,54] and recent records of the endemic deep-water Mediterranean kelp *Laminaria rodriguezii* in the Adriatic Sea, found that *L. rodriguezii* had three principal areas of distribution, located in the central part of the Adriatic Sea: Jabuka Pit and the islands of Biševo and Palagruža. Recent data (since 1996 data from MEDITS expeditions, ROV surveys and benthic surveys) on the distribution of *L. rodriguezii* in the Adriatic Sea revealed that the distribution range of the species has drastically declined within the last 40 years, reducing by more than 85%. Since 2000, the species was recorded only at Palagruža. It can be said with high confidence that *L. rodriguezii* is no longer present at Jabuka Pit, where it was repeatedly found in the late 1940s and 1950s, and Biševo. The most probable reason for its disappearance is the direct and indirect impact of trawling (physical collecting and decrease of water transparency).

Historic collections have played a very important role in tracing the introduction of *Dictyota cyanoloma* (Dictyotales) into the Mediterranean [55]. *Dictyota cyanoloma* was described in 2010, based on specimens from Palamós, NW Mediterranean [56], although the species was first reported from the Iberian Peninsula as *D. ciliolata* by Rull Lluch et al. [57]. The species is widely distributed in the Mediterranean Sea and recent records have proven its expansion to the European Atlantic (Portugal, Galicia, the SW England), and the Macaronesia [56,58–63], while Steen et al. [55] reported it from Australia and New Zealand. Steen et al. [55] obtained molecular sequence information from historical herbarium samples of *Dictyota* spp. which proved the presence of *D. cyanoloma* in the Adriatic Sea as early as 1935. Molecular data obtained from herbarium samples collected in 1947, 1958 and 1976 confirmed its presence in the Split area. Other herbarium records also confirmed the presence of *D. cyanoloma* from Cannes (1999) and Barcelona (2005). However, the question remains unanswered whether *D. cyanoloma* was ubiquitous but unrecognized within the Mediterranean throughout the 20th century, or whether it was an introduction that remained contained during a lag phase of several decades before expanding its range. To answer this question, it would be necessary to screen other specimens from European herbaria.

Molecular studies on herbaria specimens can also be a useful tool to understand the ancient species composition, structure and diversity of a community. Herbaria host many inadequately identified species that have been used in ecological studies to characterize marine communities. Nowadays, molecular data may allow us to distinguish these species and, consequently, improve the knowledge about the community. As an example, several deep detritic communities of the Balearic Islands were described between 2012 and 2016 [64–66]. Phylogenetic analyses of Mediterranean and European Atlantic herbarium specimens of a species usually thriving in these bottoms, *Halymenia latifolia* (Halymeniales, Rhodophyta), supported by morphological data, indicated that this taxon encompasses at least five species belonging to three cryptic genera, four of which are present in the Mediterranean Sea. Accordingly, the true *H. latifolia* was transferred to a new genus, *Nesoia,* as *N. latifolia* [67], some Mediterranean specimens were transferred also to the genus *Nesoia, as N. mediterranea* [68], other were maintained inside *Halymenia,* as *H. ballesterosii* [69], and other were transferred to a new genus, *Neofolia*, as *N. rosea* [70]. Consequently, future ecological work on macroalgae communities off deep detritic bottoms should take into account these changes.

4. Discussion

A key challenge for today is to understand how species are responding to habitat degradation, the spread of invasive species, pollution, overexploitation, and climate change. In this respect, herbarium specimens, due to their temporal and geographical extent, have great potential for the study of biodiversity changes and for the planning of conservation actions.

Within the past decade, the recognition of the role of herbaria as an invaluable tool for observing biodiversity changes has greatly increased [37,63,71]. The case studies reported here highlight well the important role of phycological herbaria to monitor both key native species changes and alien species over a long time period. As already stated, they may help in detecting shifts in species ranges and the presence of introduced species, and predicting future changes, e.g., retreat or extinction, in species distributions and richness under future climate scenarios [11,72,73]. They may help in the assessment of native and alien species distribution also thanks to the support of molecular data [54].

Since specimens can also capture trait shifts through time, historical collections can provide the opportunity to reconstruct past environments. They may also depict the past history of introduced algal species, showing for instance that they were already present many years before they were noticed, and also in reconstructing the evolutionary and biogeographical history of extant, as well as extinct taxa [74]. Certainly, reconstructing past distributions from historical records is often challenging [75,76], and herbarium records can provide biased information if particular taxa have been insufficiently or partially recorded [77]. Even though there are some limitations in using herbarium specimens as they are qualitative rather than quantitative data, herbarium specimens have a great potential for monitoring and conservation purposes.

However, the value of herbaria for monitoring study is strictly linked to their curation, continuity through time, digitization and sequencing data. All this prevents an agile and easy search and acquisition of data, also sorting them by specific fields, such as geographic area, collector, or taxonomic group. The search carried out on European phycological herbaria highlighted some gaps and scarcity of information mainly due to the partial or total lack of cataloguing and digitization of the majority of collections. Moreover, the greatest part of the digitized collections is not accessible online. Thus, for instance, some herbaria e.g., the ones of the Real Jardín Botánico from Madrid (MA) and the Natural History Museum of Kensington (BM) are already available online at GIBF, while e.g., the Herbarium of the University of Girona (HGI-A), is totally digitized, but not yet available online.

5. Conclusions

The examples presented here summarized well the potential of herbarium specimens as witnesses to biodiversity changes, one of the possible use of herbaria data. However, the potential of the herbaria collections is not fully exploited. Indeed, to increase the use of herbaria data it is essential their digitization, to ensure the continued support of herbaria and their staff. Investment is necessary to maintain the infrastructure of the herbaria, to ensure the conservation of the specimens, and to guarantee regular updating of the databases making them easily accessible to society and researchers.

Author Contributions: Conceptualization, methodology, validation, data curation, writing—original draft preparation, writing—review and editing, A.M.M., S.A.M., C.R.-P.; supervision, A.M.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: We want to thank the curators of the European phycological herbaria which provided information on Mediterranean specimens: Roser Guàrdia (BCN), Christian Lange and Nina Lundholm (C), Chiara Nepi and Lorenzo Cecchi (FI), Claes Persson (GB), Olivier De Clerck (GENT), Marc Verlaque (HCOM), Roxali Bijmoer (L), Arne Thell (LD), Margarita Dueñas (MA), Raffaella Trabucco (MCVE), José García Sánchez (MGC), Rossella Marcucci (PAD), Line Le Gall (PC), Mauro Iberite (RO), David García San Leon (SANT), John Parnell (TCD), Annalisa Falace and Marisa Vidali (TSB), Christian Bräuchler (W), Marija Gligora Udovič (ZA and ZAHO), and the Museo di Biologia Marina "Pietro Parenzan" (Anna Maria Miglietta). We thank the Ateneu de Maó for provinding the information about the Herbari d'Algues J.J. Rodríguez y Femenías. We also thank the Italian CoRIMBo (Coordinamento della Rete Italiana dei Musei Botanici) network and GIBF

for the information on BM (https://doi.org/10.15468/dl.a84xjw, https://doi.org/10.15468/dl.7wyfyb and https://doi.org/10.15468/dl.2wbahp), MA (https://doi.org/10.15468/dl.uk4xb9, https://doi.org/10.15468/dl.s2b8uv and https://doi.org/10.15468/dl.7errz3) and SANT (https://doi.org/10.15468/dl.2wa2fz, https://doi.org/10.15468/dl.75re49, https://doi.org/10.15468/dl.hynp9g).

Conflicts of Interest: The authors declare no conflict of interest.

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