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ELABORATO DI LAUREA

DNA isolation from century-old specimens of *Fucus virsoides* for the valorization of the historical collections of the Herbarium Patavinum

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ABSTRACT

In recent years, herbaria and historical collections have become important tools for dissemination, historical and scientific research, resources with numerous applications in various fields such as teaching, open communication among scholars, taxonomic stability and studies in different disciplines (botanical, ecological, anatomical and morphological, evolutionary, paleobotanical research). Particularly important is the discipline of herbariomics that allows, through the process of DNA extraction from herbarium specimens, followed by high-throughput DNA sequencing and bioinformatics analysis, to conduct investigations about several topics such as pollution, habitat change, invasive species and climate change. However, DNA damage caused by time and conservation methods in old herbarium samples, in addition to external DNA contamination, makes nucleic acid extraction and amplification difficult.

Here we present the results of DNA extraction (CTAB method) and amplification (PCR) conducted on six samples of the endemic Adriatic alga *Fucus virsoides* J.Agardh, 1868, collected between 1908 and 1932 and now conserved in the Padua Botanical Garden Herbarium.

Furthermore, since the amplification failed, the study focused on the analysis of the putative problems that led to this result. Although DNA amplification failed, the adopted protocol has allowed the extraction of a more than adequate amounts of DNA with a concentration between 113.75 and 565.20 ng/μL.

Our results highlight the possibility of applying this extraction method to these specimens, so that it will be feasible in the near future to sequence their DNA. A future study comparing the DNA of ancient and fresh samples may verify the presence of deleterious mutations accumulated during the demographic decline that the species has suffered along the northern Adriatic coast in recent years, possibly due to climate change.

1. **INTRODUCTION**

1.1 **Importance of herbaria**

In recent years, ancient herbaria have garnered widespread recognition as invaluable repositories of historical and scientific information, owing to their multifaceted applications. Professor Vicki Funk of The Yale University Herbarium has extensively documented the myriad uses of this precious resource, identifying 72 distinct applications across diverse fields (Funk, 2003). Given the breadth of these applications, this introduction will present them under five thematic areas, accompanied by relevant examples.

Primarily, herbaria serve as testamentary artifacts of both scientific and historical significance. They bear witness to the presence of species in specific locations and time periods, while also providing insights into the movements of collectors. Furthermore, by taking a broader perspective, herbaria aid in reconstructing the history of scientific exploration and conservation practices (Clementi, 2014; Funk, 2003).

Additionally, herbaria play a crucial role in ensuring nomenclature stability. Each name in botanical taxonomy necessitates a type specimen, which is a physical sample serving as a reference (Clementi, 2014).

Moreover, herbaria allow more open communication among scholars who can share information about the samples contained in it especially if they are publicly accessible (Clementi, 2014; Funk, 2003). To this end, recent advancements in technology have enabled the digitization and online availability of herbaria worldwide, thereby facilitating widespread access for individuals equipped with devices and internet connections. This trend is expected to continue, giving rise to a "global metaherbarium," a shared, digitally interconnected, and openly accessible resource (Davis, 2023).

Furthermore, the educational value and outreach potential of herbaria should not be underestimated. Exploiting their substantial communicative power, educators can employ herbaria to demonstrate the morphological characteristics of plants. Communicators can leverage these resources to produce illustrations and other materials for effective dissemination (Clementi, 2014). Moreover, not only

botanical gardens can be destination of scientific tourism but also herbaria, which could give life to museums such as "The Botanical Museum" of Padua University ("Apre al pubblico il Museo botanico dell'università di Padova," 2023; Funk, 2003).

Lastly, herbaria provide a wealth of material suitable for diverse research studies, including investigations into pollution, habitat change, climate change, and invasive species. For instance, analysis of ancient algae samples allows the determination of historical ocean temperatures and chemical concentrations (Funk, 2003). Additionally, herbarium samples offer valuable insights into the evolution of vascular plants and facilitate comparisons with fossil specimens in the field of paleobotany. The possibilities for research using ancient herbarium samples are virtually boundless, encompassing botany, animal ecology (e.g., identification of plants consumed by animals), environmental ecology, plant anatomy and morphology, pollination ecology, evolution, entomology (in instances where insects have been incidentally collected with plants), paleobotany, paleoecology, phylogeography, and numerous other disciplines (Funk, 2003) (**Table 1**).

Topic	Articles
Climate change	(Exposito-Alonso, 2023) (Davis et al., 2015)
Evolutionary history	(Cozzolino et al., 2007)
Extinction	(Nic Lughadha et al., 2019) (Exposito-Alonso, 2023) (Albani Rocchetti et al., 2021)
Habitat loss	(Exposito-Alonso, 2023)
Pathogens found in samples	(Martin et al., 2013) (Yoshida et al., 2014) (Yoshida et al., 2015)

Table 1 *Examples of studies on herbaria*

DNA extraction and analysis is one of the practices that can be conducted on herbarium samples (**Figure 1**). Extracting old DNA from herbarium samples allows researchers to conduct many others analyses depending on the study's aim, that could be evolutionary, systematics or genetics (Funk, 2003).

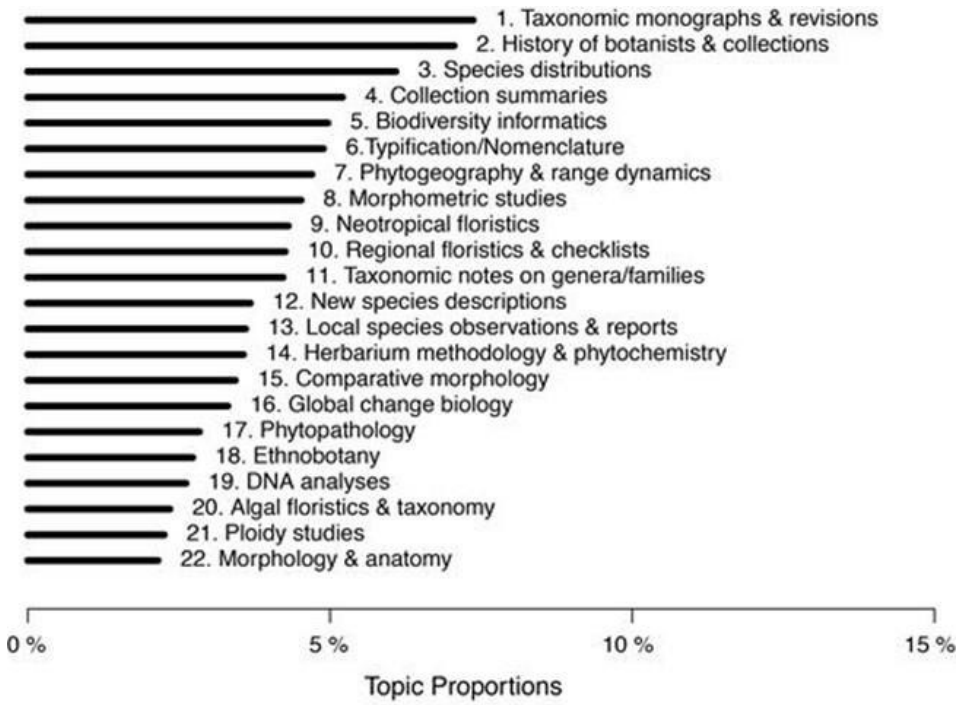


Figure 1 *Major topics in the herbarium-based studies (Heberling et al., 2019)*

1.2 **Herbariomics**

The term "herbariomics," coined by combining "herbarium" and "genomics," refers to the process of DNA extraction from herbarium specimens, followed by next-generation DNA sequencing and bioinformatics analysis (Davis, 2023). The workflow of herbariomics involves several key steps, namely sampling, processing, extraction, DNA manipulation, high-throughput sequencing library preparation, sequencing, and bioinformatics analysis (Kistler et al., 2020).

When it comes to **sampling**, there are a huge amount of material and diversity: there are more than 3000 herbaria in the world (Funk, 2003) containing more than 387 million specimens collected during the last 400 years (Kistler et al., 2020). However not every sample is a good candidate for herbariomics studies since there are a lot of difficulties working with ancient DNA (see paragraph 1.3).

Following sampling, herbarium specimens require distinct **processing** procedures compared to fresh plant tissues, with more attention and strict protocols (Kistler et al., 2020).

There are several DNA **extraction** protocols which differ in terms of input requirements (algae, fungi, vascular plants) and tissue (leaves, sclerenchyma strands, mucilaginous tissues, needles, seeds) (Záveská Drábková, 2021). In some cases, scientists have used procedures originally developed for fresh plant tissues such as the cetyl-trimethyl ammonium bromide method (Cubero et al., 1999). In other studies, commercial kits, such as the Qiagen DNeasy Plant Mini Kit (Marinček et al., 2022) or Macherey-Nagel Nucleospin 96 Plant II kit (Alsos et al., 2020), have been used.
The most frequently used are DNeasy Plant Mini Kit and modified CTAB protocols (Záveská Drábková, 2021).

After being extracted, DNA can be **manipulated** with uracil-DNA glycosylase to remove uracil residues, formed because of DNA corruption (Kistler et al., 2020) (paragraph 1.3).

Before sequencing, the raw DNA must be converted to **DNA libraries** and, considered the low concentration of available DNA, special care must be taken to obtain a high sequencing yield (Kistler et al., 2020; "Library preparation | IDT," n.d.). The creation of these libraries marks the initial step in high-throughput (HT) Sequencing, and similar to extraction protocols, there are various methods for library preparation (Kistler et al., 2020; "Library preparation | IDT," n.d.). Sequencing libraries consist of DNA fragments with adapter sequences compatible with a specific sequencing platform, along with indexing barcodes that facilitate sample identification ("Library preparation | IDT," n.d.). The essential steps of library preparation are as follows:

- Fragmentation (into 100–300 bp segments) and end repair
- Sample indexing through the addition of adapters
- PCR amplification (optional)

When libraries are ready, DNA can be sequenced. **DNA sequencing** allows to determinate the exact sequence of nucleotides, or bases, in a DNA molecule ("DNA Sequencing," 2022). Over the past three years HT sequencing has been increasingly adopted, reducing the cost of DNA sequencing and speeding up sequencing times. In fact, compared to Sanger sequencing, it does not have the limitation of sequencing only one DNA fragment at a time. In a single reaction millions of

individual fragments can be sequenced ("Next Generation Sequencing | IDT," n.d.; Shendure and Ji, 2008).

Finally, **software tools** are used to give a quality score, make alignments of sequence reads to a reference, detect polymorphism, assembly and annotate the genome and release data (Shendure and Ji, 2008).

1.3 **Problems connected to ancient DNA extraction**

When working with older samples, the steps described above present many more difficulties than fresh specimens; it is a challenging task that necessitates meticulous precautions.

Primarily, when working with ancient DNA, it is imperative to carry out the extraction and preparation of PCR in a laboratory specifically designated for handling such DNA so that it can be strictly separated from work involving modern DNA (Hofreiter et al., 2001). This segregation is essential to prevent contamination, which remains a persistent concern.

Notably, contamination from human sources is a frequent occurrence during the amplification of old specimens, particularly if they have been handled for a long time by museum curators (Thomas et al., 1989). Consequently, the conditions to which the specimens were exposed during collection and storage substantially impact the quality and quantity of DNA recovered from herbarium specimens (Marinček et al., 2022).

Any form of contamination is directly correlated with the amount of endogenous DNA, with more pronounced effects observed in samples containing less DNA (Gutaker and Burbano, 2017).

Secondly, in addition to contamination, the extraction of ancient DNA from herbarium specimens is further complicated by inherent challenges compared to the extraction of fresh DNA. This is primarily due to the typically degraded and fragmented nature of DNA in herbarium specimens (Marinček et al., 2022).
This is especially true for plant DNA, as herbaria-stored plant DNA showed a six-fold higher fragmentation rate than animal DNA preserved in bones (Weiß et al.,

2016). Remarkably, plant specimens aged up to 170 years possess DNA fragments of similar length to those of archaeological bones hundreds to thousands of years old (Weiß et al., 2016).

Moreover, DNA fragmentation can be aggravated by storage techniques. For instance, air-dried specimens yield less fragmented DNA sequences compared to alcohol-dried specimens (Särkinen et al., 2012). Additionally, among air-dried specimens, slower drying processes result in higher levels of fragmentation (Záveská Drábková, 2021).
It is also crucial to consider that historical pests and insect control methods involved chemical treatments, which further damage DNA (Albani Rocchetti et al., 2021).

Apart from physical fragmentation, other challenges associated with ancient DNA extraction include:

- Hydrolysis and oxidation that occurs to the DNA after the organism's death, leading to the possibility of Taq polymerase incorporating incorrect nucleotides during the elongation step of PCR (Hofreiter, 2001).
- Post-mortem cross-linking, which impedes polymerase bypass and blocks DNA denaturation (Yeates et al., 2016).
- Cytosine deamination, where cytosine nucleotides tend to spontaneously lose an amine group and convert to deoxyuracil (Briggs et al., 2007). This results in misincorporations during sequencing, where the uracil residue is erroneously complemented by adenine instead of guanine on the opposite strand and is subsequently identified as thymine during sequencing (Kistler et al., 2020). Consequently, an erroneous DNA strand is replicated during the first cycle of PCR (Wandeler et al., 2007).
- Oxidative stress and/or heating can induce damage to DNA nucleotide, leading to the formation of a-puric sites (loss of A and G bases) or oxidized guanine residues (Záveská Drábková, 2021).

Even if the ancient DNA is not degraded, PCR may be inhibited by secondary compounds, such as polyphenolics and polysaccharides, that covalently bind to DNA or coprecipitate with it (Kistler, 2012).

1.4 **Fucus virsoides**

Fucus virsoides J.Agardh, 1868 is a species of brown alga endemic to the Adriatic Sea (Munda, 1979).

Kingdom	Chromista
Phylum	Ochrophyta
Class	Phaeophyceae
Order	Fucales
Family	Fucaceae
Genus	Fucus
Species	*Fucus virsoides*

Table 2 *Scientific classification*

1.4.1 Description

This species has a very dark brown pedunculated thallus, ramified in a dichotomous way with 1-3 cm nastriform laminae, showing a narrow median rib in relief (**Figure 2**). The extreme part of the foil ends bifurcated or pointed. In this point there are bulges in relief with reproductive formations called conceptacles. The colour fades from brown to yellow in proximity of margins and apices, near the floating vesicles. The surface of the frond, which can be even 30 cm long, appears dotted with tiny hairs, visible even to naked eyes (La Rocca, 2002, n.d.)

Figure 2 *Fucus virsoides ©FreckLes (06.08.2008), photo, Trieste, UTI Giuliana, Italy, https://inaturalist.ca/observations/70265525, (accessed 06.12.23)*

1.4.2 Habitat

The species grows in the upper coastal plane within 40 cm above the average level of the Adriatic Sea (La Rocca, 2002, n.d.). It occupies a tidal environment, specifically within the transitional zone between the high and low tide. The upper limit of its vegetation zone corresponds to the highest level of the high tide, while the lower limit aligns with the lowest level of the low tide (Linardić, 1949) (**Figure 3**).

Figure 3 *Fucus virsoides ©Edi Gljušćić, (March 2021), photo, https://inaturalist.ca/observations/70999104, (accessed 06.12.23)*

Consequently, it can easily bear very prolonged periods of emergence during which the thallus temporarily withers and undergoes desiccation-induced darkening and rigidity (**Figure 3**). However, upon the return of the high tide, it immediately recovers (La Rocca, 2002, n.d.).

While emergence might initially appear to pose a problem for the algae, it is actually fundamental to its survival since *Fucus* dies in a constant culture in the water (Linardić, 1949). Nevertheless, this characteristic presents limitations in an era of rising sea levels, potentially leading to a decrease in the distribution of the species. In fact the global average sea level has risen 21–24 cm since 1880 (Lindsey, 2022) and projections estimate a further increase in the Adriatic sea ranging from 14 cm and 49 cm by 2100 (Scarascia and Lionello, 2013). Furthermore, the northern Adriatic is more at risk than other areas due to eustatic, tectonic and isostatic components, which add up to climate change making areas such as Venice, Grado and Marano Lagoons particularly vulnerable to future sea level rise (Lambeck et al., 2011).

1.4.3 Distribution

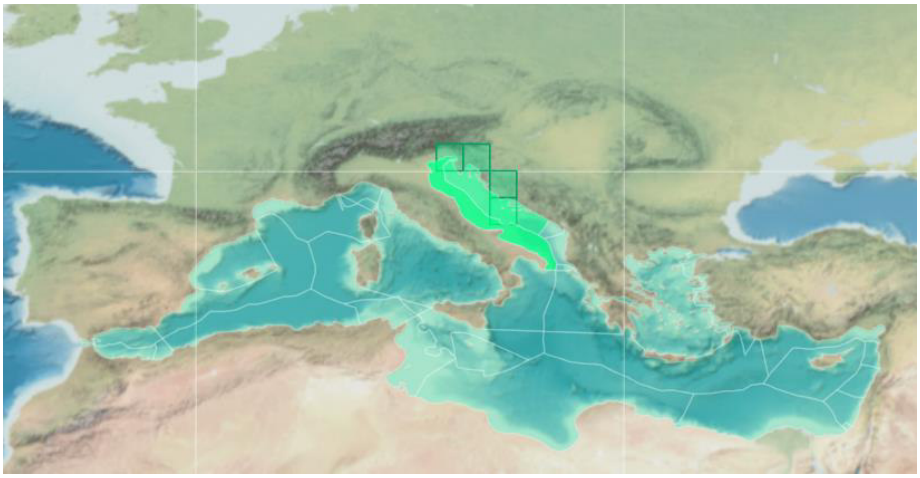


Figure 4 *Fucus virsoides distribution*
WoRMS - World Register of Marine Species,
[https://www.marinespecies.org/aphia.php?p=taxdetails&id=145549#attributes
(accessed 5.20.23).

This species occurs only in the Adriatic Sea, mostly in the northern part from the Venice Lagoon to Dalmatia (**Figure 4**). While its presence along the Albanian coastline is not entirely precluded, it remains infrequent (Cormaci et al., 2012). Being endemic to this area, it is crucial to monitor its abundance to ensure its conservation status.

Throughout the year, the species displays a wide distribution and high fertility, in the coastal stations facing the sea, as well as along the arcades, in urban centres, in the fishing valleys and inside the lagoon to the Brenta Valley near the Lombard canal, along the Treporti Canal up to that of S. Felice. It is also present in some salt valleys, demonstrating its adaptability to varying salinity levels (La Rocca, 2002).

1.4.4 Reproduction

The algae are fertile between January and March. The reproduction takes place by gametes, produced by meiosis and carried by anterids and oogons, found within the male and female apical conceptacles, belonging to distinct and diploid plants (**Figure 5**). Fertilization occurs in water and the zygote will generate diploid plants again. The cycle is therefore monogenetic diploid (La Rocca, 2002, n.d.).

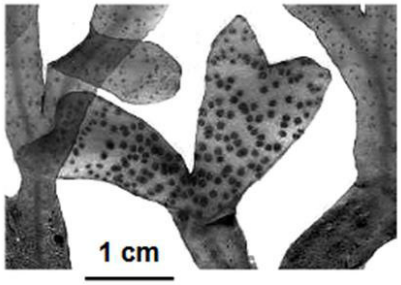


Figure 5 *Distribution pattern of conceptacles in Fucus virsoides (Cormaci et al., 2012)*

1.4.5 Ecology of the algae

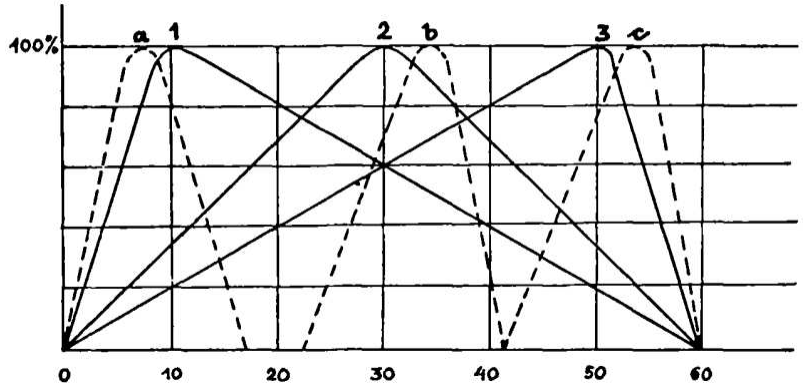
According to Pérez-Ruzafa (2001) the genus *Fucus* exhibits a notable degree of morphological plasticity in response to various environmental factors such as hydrodynamism, salinity, substrate types and others (Cormaci et al., 2012).

In terms of the influence of sea movements on vegetation, it has been observed that samples from sheltered areas tend to display larger size compared to those exposed to the impact of ocean waves, which *Fucus* actively avoids (Linardić, 1949).

Regarding salinity, *F. virsoides* is classified as an euryhaline species, capable of tolerating significant fluctuations in water salinity levels. However, its optimal conditions tend more toward the average minimum, as it prefers places with reduced salinity. Based on this characteristics, this alga can be defined as mikroeuryhalynic typus in the sence of Vouk's theory of valences (Linardić, 1949) (**Figure 6**).

Concerning the temperature, the Adriatic *Fucus* is an eurythermal algae with its optimum at the lower temperatures (again, mikroeurythermal types) (**Figure 6**). The importance of air temperature should not be underestimated, because *Fucus* is partly terrestrial. Air temperature is a limiting factor and determines its distribution to the south-western Mediterranean Sea (Linardić, 1949).

Figure 6 *Microeuryvalent types with low optimum (Vouk, 1948)*



With regards to light requirements, the Adriatic *Fucus* can be classified as an inherently euryphotic macroeuryphotic algae, as its ecological optimum is in closer proximity to the maximum light intensity.

These findings highlight the adaptability and responsiveness of *Fucus* to its environment, with specific traits and behaviours influenced by factors such as hydrodynamics and salinity variations. Such knowledge contributes to a deeper understanding of the species and its ecological dynamics, underscoring the significance of further investigations in this field.

2. **AIM OF THE THESIS**

This thesis aims to evaluate an extraction method of ancient DNA for the alga *Fucus virsoides* and in particular to investigate the problems related to the extraction of ancient DNA, which is much more complicated in comparison to fresh sample extraction. For this study we chose six samples of the alga *Fucus virsoides* J.Agardh, 1868, collected between 1908 and 1932 and conserved in the Padua Botanical Garden Herbarium. Being ca. 100 years old, these specimens represent a perfect target to evaluate the difficulties of extracting DNA from

ancient samples. The method chosen for the extraction of DNA from tissue was a CTBA method (Forin et al., 2018), which has been shown to achieve appropriate DNA quality from small quantities of herbarium material. The thesis wants to assess whether the PCR approach is feasible to sequence these old specimens and if not, propose alternative approaches.

This thesis is part of a broader project organized by the botanical garden of Padua within the context of the "EU strategy on biodiversity for 2030", more specifically in the area of *genetic diversity through space and time*. The project, organized by the botanical garden in collaboration with the professor and historian of science Elena Canadelli and the Kew Gardens (UK), aims to enhance the value of historical herbariums of the Garden for scientific and historical research: "*Plant biodiversity between history and genomics: to know, promote and digitize the collections of the historical herbariums of the Botanical Garden of the University of Padua*". Thanks to their temporal dimension, herbaria provide fundamental data to follow the ecological and evolutionary changes in this period of climate change. The ability to sequence the DNA of conserved plant material makes herbaria an invaluable resource for understanding species ecological responses to climate change.

In this perspective, this thesis constitutes the beginning of a larger project that, starting with the extraction of DNA of *Fucus virsoides,* arrives to sequence its genome and compare it with the genome of current samples allowing to reconstruct the changes and evolution that *Fucus* has displayed in the last century, especially in relation to climate change. For this reason, the choice fell on *Fucus virsoides* samples: not only because they are old enough to allow to deepen the problems of extraction, but also because being an alga of the intertidal zone, it directly faces problems related to climate change such as rising sea levels. In fact, at the end of 2010, a comparison between historical and current data (in situ surveys and from literature) highlighted a significant decline in populations of *Fucus virsoides* in the Slovenian coast (Gulf of Trieste, northern Adriatic) (Battelli, 2016).

3. MATERIALS AND METHODS

3.1 **Algarium samples**

In this thesis a total of six samples were examined, sourced from the historical Algarium at the Padua Botanical Garden. These specimens hold immense scientific and historical significance, representing a valuable resource for research. The samples under investigation were collected during the period spanning 1908 to 1932, by four distinct collectors (**Table 3**). A brief summary of their biographies can be found in the subsequent pages.

Sample	Collector	Date	Locality	Institute	Species
0101	A. Forti	15.5.1910	Lido of Venice	Padua BG Algarium	*Fucus virsoides* J.Agardh, 1868
0102	A. Forti	24.8.1908	Ancona	Padua BG Algarium	*Fucus virsoides* J.Agardh, 1868
0103	A.Vatova	10.3.1932	Rovinj	Padua BG Algarium	*Fucus virsoides* J.Agardh, 1868
0104	J. Schiller	1.3.1925	Adria, Istria, Capodistria	Padua BG Algarium	*Fucus virsoides* f. *normalis* Schiffner 1938
0105	V. Schiffner	6.4.1928	Mid Dalmatia, Bua Island, N coast	Padua BG Algarium	*Fucus virsoides* f. *normalis* Schiffner 1938
0106	A. Vatova	2.10.1930	Dam Lido	Padua BG Algarium	*Fucus virsoides* var. *subvesiculosus* Forti 1931

Table 3 *Information about the six samples analysed in the project*

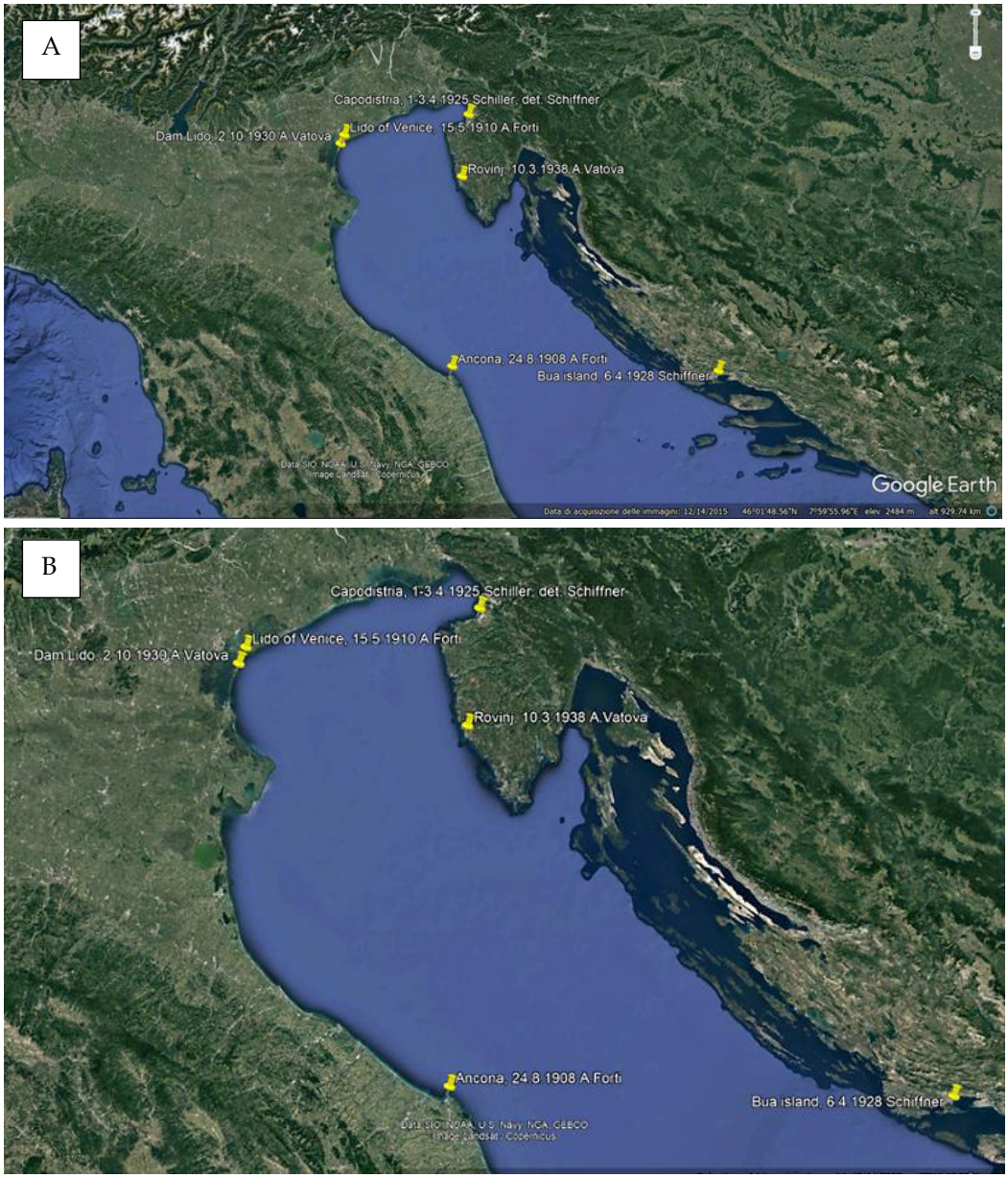
Note: Due to the high degree of polymorphism in the species *virsoides* that, like the other species of the genus, shows a morphology depending on environmental factors, it is highly probable that many infraspecific taxa are only "ecads" with no taxonomic value (Cormaci et al., 2012). In fact, nowadays the variant *subvesiculosus* Forti 1931 is considered a synonym of *Fucus virsoides* J.Agardh, 1868 (Guiry, 2015). Also *Fucus virsoides* f. *normalis* Schiffner 1938 is now

considered a synonym of *Fucus virsoides* J.Agardh 1868 ("*Fucus virsoides* J.Agardh :: AlgaeBase," n.d.).Therefore, all six samples were exactly the same taxonomic unit.

Note: in this thesis the names of the samples have sometimes been shortened (e.g., 0101 → 1; 0102 → 2…)

The following images from Google Earth are indicative of where the 6 samples were collected. They do not represent specific coordinates as this information was not available (**Figure 7.1**) (**Figure 7.2**).

Figure 7.1 *(**A**) Sampling localities of the six samples analysed in the project; (**B**) Sampling localities of the six samples analysed in the project more zoomed* ©GoogleEarthPro



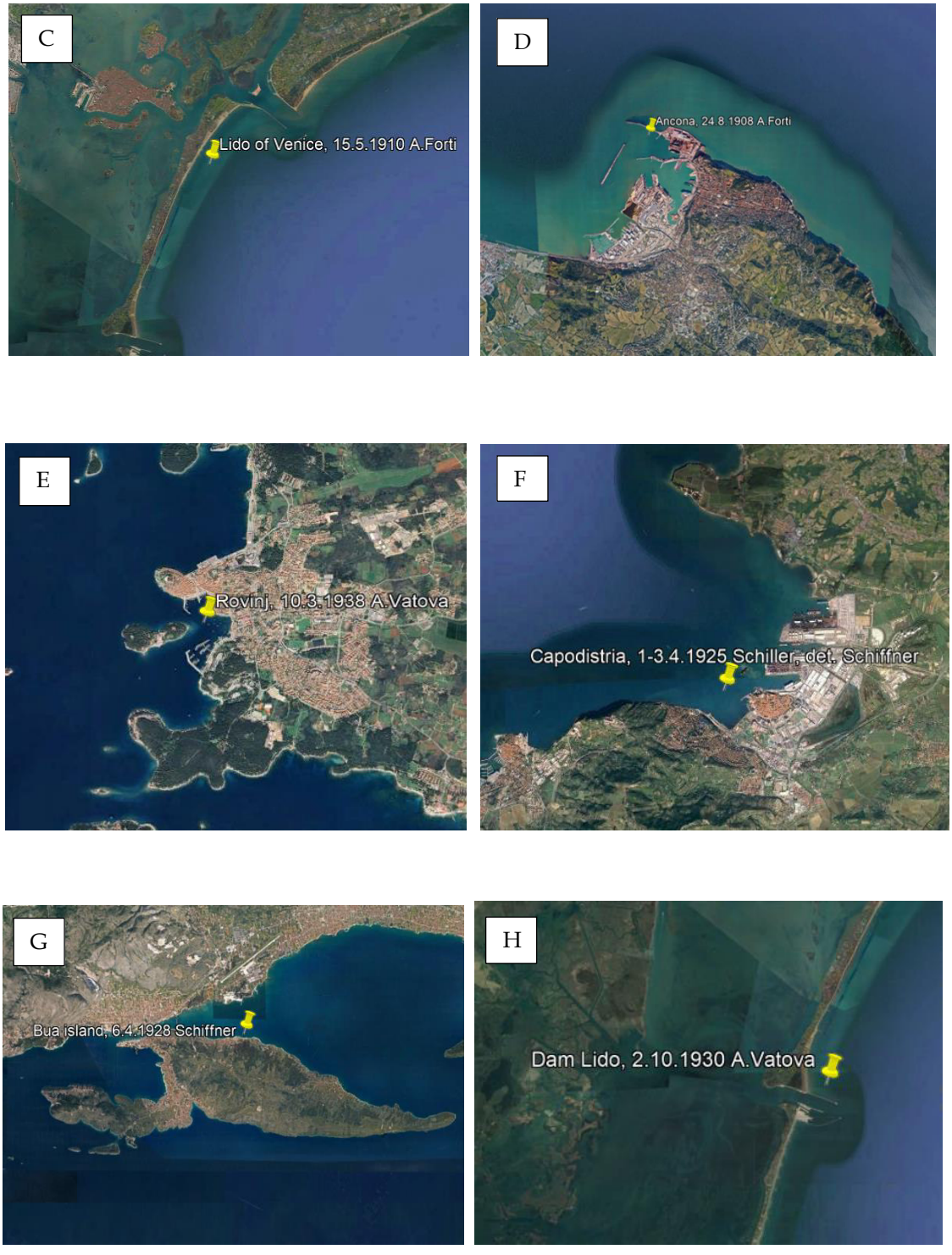


Figure 7.2 *(**C**) Sampling locality of sample 0101; (**D**) Sampling locality of sample 0102; (**E**) Sampling locality of sample 0103; (**F**) Sampling locality of sample 0104; (**G**) Sampling locality of sample 0105; (**H**) Sampling locality of sample 0106*
©GoogleEarthPro

3.2 **A look at the life of collectors**

3.2.1 Achille Forti

Israele Achille Italo Forti (Verona, 1878 – Verona, 1937) was a botanist and an art patron ("Achille Forti," 2022) (**Figure 8**). In fact, not only he was an important personality of science but also of history and art because he collected throughout his life numerous works of arts mainly of the nineteenth and early twentieth century and published numerous biographies of naturalists such as Angelo Mazza (1844-1929) and Ettore De Toni (1858-1925) ("Achille Forti - Le alghe di Achille Forti," n.d.).

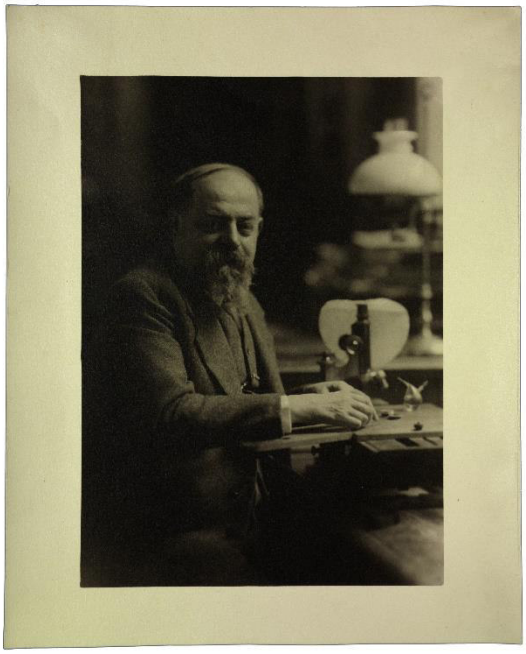


Figure 8 *Forti, Achille Italo : 1878-1937 Anon (n.d.), 298 x 240 mm., ©Bib. dell'Orto botanico IB.NN.61: Condizionamento – 2007, https://phaidra.cab.unipd.it/view/o:4383, (accessed 06.12.23)*

In 1900 he graduated in natural sciences at the University of Padua, where he came back in 1926, after having taught at the University of Modena (1916), to be a researcher in the algae field. His passion for algae accompanied him already from the bachelor degree in Padua, when he published several reviews of algological treatise, and it increased over the years making him a leading figure in this field for many scientists and scholars. To witness this passion, in addition to a room dedicated exclusively to algological studies in his palace in Verona, there are his botanical collections ("Achille Forti - Le alghe di Achille Forti," n.d.).

These are composed by samples harvested in many of his trips (in the Balcans, the Mediterranean area, Norway) and acquired from libraries of other collectors with whom he entertained a network of correspondence: Giacomo Doria (1840–1913), Swiss botanist Levier (1839–1911), the Italian Ardissone (1837–1910), the Hungarian Pantocsek (1846–1916), Lodovico Caldesi (1821–1884), Carlo Spegazzini (1858–1926), Angelo Mazza (1844–1929) and Antonio Piccone (1844–1901). This explains why some specimens are from different parts of the world

(America, Asia, Africa, Australia) ("Achille Forti - Le alghe di Achille Forti," n.d.; "L'Algario Forti: un archivio di biodiversità e storia - Le alghe di Achille Forti," n.d.).

Figure 9 *Fucus virsoides collected in Rovinj (Croatia), Herb. A. Forti, ©Bib. dell'Orto botanico, https://mostre.cab.unipd.it/forti/it/58/l-algario-forti-un-archivio-di-biodiversita-e-storia (accessed 4.21.23)*

The Forti algarium counts over 100 packages each of which contains about 100 sheets for a total of 10,000 specimens, from both fresh and salt water. The specimens are mounted on sheets or inserted in stapled envelopes and then assembled based on the kind of belonging: there are over a thousand different genera of Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae)("Le collezioni di Forti al Museo Botanico - Le alghe di Achille Forti," n.d.) (**Figure 9**).

Besides the algarium, Forti assembled about 7900 slides with specimens of unicellular algae (diatoms), sorted by gender and species, 10.000 specimens of vascular plants from Veneto, samples of calcareous algae and an interesting collection of 1705 photographic plates with silver bromide gelatine preserved in cardboard boxes and mostly related to the algological collections ("Le collezioni di Forti al Museo Botanico - Le alghe di Achille Forti," n.d.).

At his death Forti donated all of this precious archive of biodiversity and history, his collections and library, to the University of Padua, while he donated his collection of artistic works to city of Verona and are now stored at Palazzo della Ragione (Verona) in the "Galleria d'Arte Moderna Achille Forti" ("Achille Forti - Le alghe di Achille Forti," n.d.).

3.2.2 Aristocle Vatova

Domenica Aristocle Vatova (1897, Capo d'Istria – 1992 Venezia) was a marine biologist who dedicated his life to the study of benthonic flora and fauna and to saving the Rovinj Institute, reopening it in Venice (Casellato, 2019).

Originally, he studied philosophy in Graz and Vienna but, after the passage of Istria to Italy, he came in Italy (University of Turin) to study natural sciences: in 1919 he graduated with a botanical thesis: "*Flora delle brughiere dei terreni glaciali in Piemonte, studio fitogeografico*" (Flora of glacial heaths in Piemonte, phytogeography study) (Casellato, 2019).

Right after his graduation he became teacher of agriculture and chemistry in Capodistria and, between 1919 and 1923, collected specimens for his *Herbarium Vatuense,* which consists of 2700 species and is now property of "The Italian Central Herbarium" in Florence. In 1923 he began to teach mathematics and German in Rovinj (Rovigno, Croatia) where he attended the Institute of the Rovinj Marine Biology for Adriatic and also reorganized the Algological Herbarium that the Rovinj Institute owned (Casellato, 2019).

He after gave up teaching to devote himself actively to benthic marine fauna of the area with hydrographic campaigns and cruises, monthly records of parameters and physical-chemical research. For instance, in 1930-32 the International Commission for the Study of the Mediterranean commissioned him an analysis in the Venice Lagoon with a focus in benthic fauna and algal species. These samples were described in "*La Monografia della Laguna di Venezia*" (The Monograph of the Venice Lagoon, Magrini, 1933) and were donated to the Civic Museum of Natural History of Venice (Casellato, 2019).

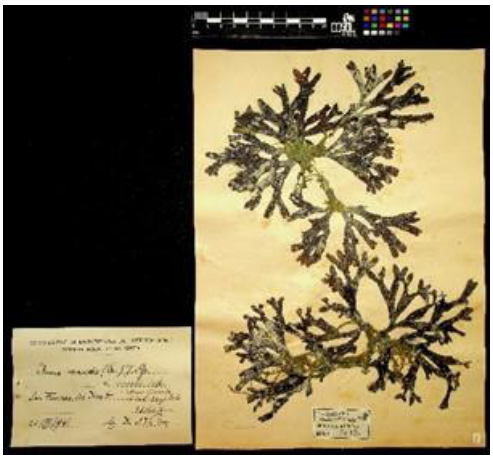


Figure 10 *Fucus virsoides (Don.) J. Ag. a normalis*
Herb. Vatova- Schiffner 26/3/1931, San Francesco del Deserto (Ve), ©Natural History Museum (Venice)

Today, the venetian museum still preserves the Vatova-Schiffner algarium, consisting of 1406 sheets with dried samples of algae taken in 77 lagoon stations. This vast work was accomplished thanks to the collaboration with Schiffner, who identified all the algae that Vatova collected ("Digitalizzazione dell'algario di Aristocle Vatova e Victor Schiffner," n.d.) (**Figure 10**).

Then, from 1934 to 1936, Vatova enriched its marine collection with other oceanographic campaigns in the upper Adriatic, where he covered 723 nautical miles, in Kvarner (Croatia) for 240 nautical miles, and in the middle Adriatic, where he travelled 1,434 nautical miles (Jeromela, 2018).

3.2.3 Josef Schiller

Josef Schiller (16 June 1877, in Ringelsheim (Bohemia) – 1960) was a renowned Austrian phycologist and hydrobiologist who graduated in natural sciences at the University of Vienna, where he also earned his doctorate in 1905. Shortly thereafter, from 1905 to 1010, he served as an assistant at the zoological station in Trieste where he was able to deepen the study of phycology, in particular he was interested in benthic algae and phytoplankton ("Josef Schiller," 2020). During this period, Schiller conducted numerous expeditions in the Adriatic to collect specimens. His involvement with the *Society for the Support of Adriatic Research*, led him to undertake frequent research trips, as the society organized four ten-day expeditions each year for this purpose (Schiller, Josef (1877-1960), 2013)

As well as being a researcher, Schiller had been a professor. Initially, he taught at middle schools, and starting from 1918, he held a position at the University of Vienna in the field of *botanical hydrobiology*. In 1927 he became an associate professor ("Josef Schiller," 2020).

However, Vienna was not the only place where he gave lessons of botanical hydrobiology. He combined research and teaching while visiting several marine stations such as Bergen (1910, Norway), Helgoland (1923, 1930, Germany), Rovigno (1937) and Spalato (Croatia) as well as the zoological station of Napoli (1925) and Ragusa in Italy ("Josef Schiller," 2020; "Schiller, Josef (1877-1960)," 2013).

3.2.4 Victor Schiffner

The Austrian Victor Félix Schiffner (10 August 1862, Böhmisch-Leipa – 1 December 1944, Baden bei Wien) was a botanist specialised in bryophytes (**Figure 11**).

He graduated in natural sciences at the University of Prague and after that he remained as an assistant and lecturer at the botanical garden of the university until 1893, when he left for a two year-scientific trip in Java and Sumatra. During this period, he worked at the Buitenzorg herbarium in the Dutch East Indies, collecting many samples, especially liverwort ("Victor Félix Schiffner," 2023).

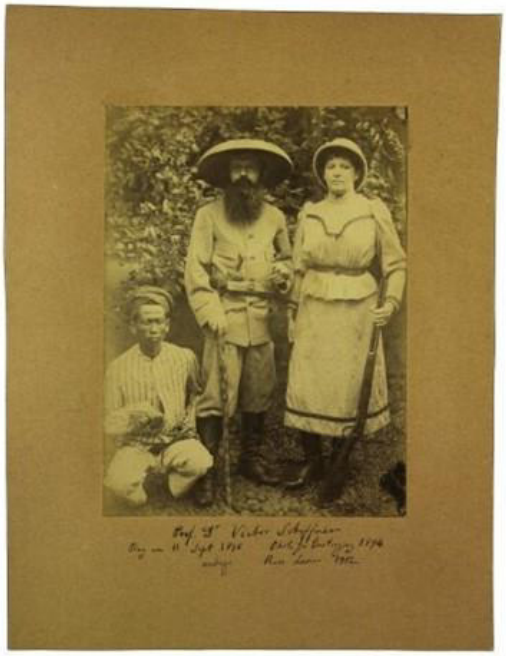


Figure 11 *Prof. Dr. Victor Schiffner, Anon (1894) 159 x 118 mm, ©Bib. dell'Orto botanico IB.PP.19: Iconoteca dei botanici. Racc. [Raccolta] Levier, 1912, https://phaidra.cab.unipd.it/view/o:4481, (accessed 06.12.23)*

In 1895 he became professor of botany at Prague, where he remained for seven years, the end of which, in 1901, he left for another expedition (this time in southern Brazil) which helped to expand his bryophytes collection ("Schiffner, Victor Félix (1862-1944)," 2013).

Once back in Austria he became professor at the University of Vienna, where he remained until his retirement in 1932, just one year after the acquisition of his personal herbarium by Harvard University; an important compendium of 50,000 hepatics and mosses. Beside its specialization in bryophytes Schiffner was also interested in algae as witnessed by a 70 pages book he wrote in 1915: *Studien über Algen des adriatischen Meeres* (Studies on algae of the Adriatic Sea) ("Victor Félix Schiffner," 2023).

3.3 **Specimens' collection**

The six samples have been collected from the ancient herbaria and in order to preserve the overall integrity of these specimens, which have an inestimable historical and scientific value, each small sample was taken carefully with the authorization and supervision of the herbarium curator. In fact, being the material more ancient than 50 years, the authorization to the superintendence was needed.

Given the inherent challenges associated with handling aged material, minimizing tissue usage was of paramount importance (Carl von Ossietzky et al., 2018). Subsequently, a small piece of each has been cut with a scalpel and put with tweezer in a tube. Before moving on to the next sample, the instruments were disinfected with 70% alcohol. In addition, the samples were cut by placing them on different sheets of paper to avoid any contamination.

3.4 **DNA extraction protocol**

The method chosen for the extraction of DNA from tissue was a CTBA method (Forin et al., 2018), which has been shown to achieve appropriate DNA quality from small quantities of herbarium material. The protocol has been modified as indicated below (modifications are given in *italics*).

CTAB extraction buffer: 2% CTAB, 100 mM Tris-HCl pH 8, 20 mM EDTA, 1.4 M NaCl. Add 2% PVP and warm buffer at 65° C.

1. Put in a sterile Eppendorf tube (1.5 mL) a piece of dry sample
2. Grind material into a fine powder with plastic pestle adding quartz sand *and leave the plastic pestle inside the Eppendorf*
3. Add 800 µL of CTAB buffer and 10 µL Proteinase K (1 mg/mL)
   Grind the material again after the addition of CTAB, then remove the pestle and add Proteinase K
   Vortex before incubation
4. Incubate at 65°C overnight
5. Centrifuge at max speed *(14000 rpm)* for 10 min *(for 15 min)*
6. Recover the supernatant in a new 2 mL eppendorf tube without disturbing the pellet
7. Add an equal volume (800 µL) of phenol/chloroform/isoamylalcool (25:24:1) and invert the tubes several times
8. Centrifuge at 10000 rpm for 10 min
   repeat step 7 and 8 if samples aren't clear enough (in this case for samples 2,3,4,6)
9. Recover only the upper phase in a new 2 mL sterile tube
10. Add 600/800 µL *(700 µL)* of chloroform (or chloroform/isoamylalcool 24:1) and *invert the Eppendorf*
11. Spin at 10000 rpm for 10 min

12. Recover the supernatant in a 1.5 mL Eppendorf tube. In this case:

Table 4 supernatant recovered from each eppendorf	*1*	*2*	*3*	*4*	*5*	*6*
	400 µL	*400 µL*	*500 µL*	*400 µL*	*600 µL*	*600 µL*

13. Precipitate the DNA adding 2/3 of the recovered volume of 2-propanol. Mix by inversion and incubate at -20°C for 2 hours. In this case:

Table 5 2-propanol added in each eppendorf	*1*	*2*	*3*	*4*	*5*	*6*
	260 µL	*260 µL*	*350 µL*	*260 µL*	*400 µL*	*400 µL*

14. Spin at 10000 rpm to pellet DNA for 10 min
15. Discard the supernatant
16. Wash the pellet with 250 µL of ethanol 70%
17. Spin at 10000 rpm to pellet DNA for 10 min
18. Discard the ethanol and dry the pellet *(for 40 minutes)*
19. Resuspend the pellet in H_2O DNAse free and incubate at 4 °C
    35 *µL of H_2O DNAse free for 2,3,4,6 and 30 µL for 1 and 5 (because the samples of DNA were smaller compared to the others)*

3.5 **DNA concentration**

After the extraction, DNA concentration in each sample was measured with a NanoDrop spectrophotometer (absorbance at 230, 260, and 280 nm).
This instrument measures the amount of radiation absorbed by a substance that is proportional to the amount of the substance itself. It detects absorbance at different wavelengths from which information about the quantity and quality of DNA extracted can be inferred (Bannò, 2021).

- Absorbance at 260 nm = this provides information on DNA concentration since it is the wavelength corresponding to the peak absorption of the nitrogen bases that make up the nucleotides (Teare et al., 1997).

- Absorbance at 280 nm = wavelength corresponding to the peaks of absorption of proteins and phenols present in the sample (Teare et al., 1997).
- Absorbance at 230 nm = wavelength providing information about the degree of contamination by buffer salts, solvents, carbohydrates, aromatic compounds, phenols and EDTA (Teare et al., 1997; Pachchigar et al., 2016).
- Ratio A260/A280 = pure DNA samples show more than 1,8 while sample contaminated with proteins, phenol or surfactants show less than 1,8 (Teare et al., 1997).
- Ratio A260/A230 = this provides an indication of the level of salt contamination of purified DNA. The lower the ratio, the lower the purity of DNA so ideally this ratio should be greater than 1.5, ideally close to 1.8 (Pachchigar et al., 2016).

Calculating the DNA concentration in each sample was crucial to determine how to properly dilute the samples for the amplification phase. In fact, if the extracted DNA is pure it will give good PCR products while DNA with lower purity gives good PCR products only after several dilutions (Turaki et al., 2017).

After the DNA calculation the six samples were placed in the refrigerator at -20°C.

3.6 **Dilution**

Before diluting, DNA samples have been homogenized with the vortex and spinned down. Apart from sample 0105, the other values were high (**Table 6**), and they needed a strong dilution for PCR.

Sample	
0101	330.60
0102	362.85
0103	398.10
0104	273.80
0105	113.75
0106	565.20

Table 6 *Content of DNA extracted from each sample (ng/ µL)*

Samples 0101, 0102, 0103, 0104 have been diluted 1:5, sample 0105 has been diluted 1:2 and sample 0106 has been diluted 1:10. For sample 0105 2 µL of H_2O (DNA free) and 2 µL of the sample DNA were used in order to have enough volume.
After being diluted DNA was vortexed and spinned down again.

3.7 **PCR amplification**

PCR has allowed the retrieval of DNA sequences from plant's specimens and animals that have been collected under controlled conditions and housed in museums over the past 200 years (Hofreiter et al., 2001). The reason why PCR is so used for ancient DNA extraction is that it can synthesize numerous copies of a few intact DNA molecules in the presence of a vast excess of damaged molecules, which it is a frequent condition in old specimens (Thomas et al., 1989).

The PCR reaction was carried out in a total volume of 25 µL including 12.5 µL of master mix, 1 µL of forward primer, 1 µL of reverse primer, 9.5 µL of DNA free H_2O, 1 µL of DNA. This mix was prepared for the six DNA samples and for one negative control (**Table 7**).

Master mix	12.5 × 7 = 87.5 µL
Forward primer	1× 7 = 7 µL
Reverse primer	1× 7 = 7 µL
H2O DNA free	9.5× 7 = 66.5 µL
DNA	1 µL

Table 7 *Mix preparation*

The forward primer used was *rbcl_aF* (sequence 5′-ATG TCA CCA CAA ACA GAG ACT AAA GC-3′) (Levin et al., 2003). The reverse primer used was *rbcL_724R* (sequence 5'-TCG CAT GTA CCY GCA GTT GC-3') (Meerow et al., 1999).
The target loci to amplify was *rbcl*, a marker 1428 bp long (Nickrent and Soltis, 1995).

Before making the cocktail two steps were needed: Master mix and primers required to be vortex and microfused and primers needed a dilution with H2O DNA free in rate 1:10. The cocktail was prepared under the hood, apart from DNA, which was added last outside the hood.

The profile of amplification used is reported here:

Stage	Phase	Temperature °C	Time	Cycles
1		95.0	3 minutes	1
2	Denaturation	94.0	30 seconds	35
	Annealing	55.0	30 seconds	
	Elongation	72.0	1 minute	
3		72.0	10 minutes	1

Table 8 *PCR profile*

To have qualitative feedback of the success of amplification, all products of PCR were subjected to a DNA concentration test and an electrophoretic run on agarose gel. The 1.2% agarose gel was prepared using 25 mL of TAE 1X, 0.3 g of agarose and 1 µL of gel safe.

4. **RESULTS**

4.1 **DNA extraction**

The adopted protocol has allowed the extraction of a more than adequate amounts of DNA with a concentration between 113.75 and 565.20 ng/µL (**Table 9**) (**Figure 12**).

Sample	Content of DNA extracted from each sample (ng/ µL)	A260/A280	A260/A230
0101	330.60	1.855	1.323
0102	362.85	1.551	0.780
0103	398.10	1.379	0.792
0104	273.80	1.335	0.901
0105	113.75	1.538	0.490
0106	565.20	1.628	0.770

Table 9 *Content of DNA extracted from each sample (ng/ µL) and purity ratios*

The purity ratio 260/280 ranged between 1.3 and 1.8 while the ratio 260/230 ranged between 0.4 and 1.3. Except for 0101, these results show a high level of contamination (**Table 9**). However, the A260/A280 values were not markedly below 1.8, thus indicating no contamination by proteins, phenols or other contaminants that absorb strongly near 280 nm. Instead, the A260/A230 values were significantly below 1.5, which indicates a contamination with EDTA, carbohydrates and/or other phenols that absorb near 230 nm (Carl von Ossietzky et al., 2018).

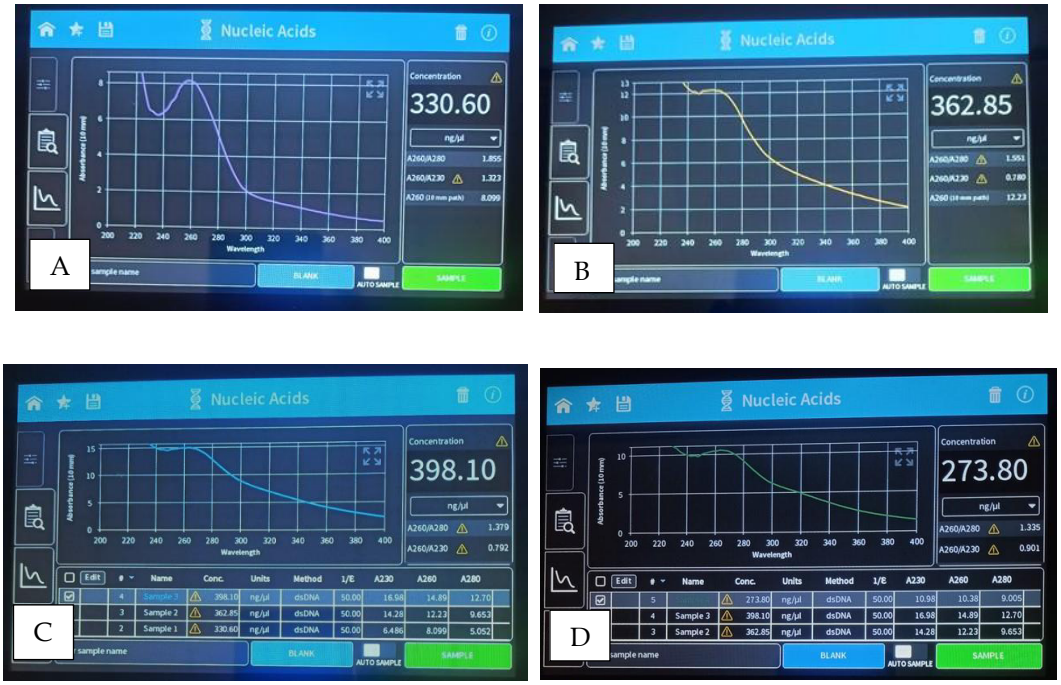


Figure 12 *DNA concentration measured with NanoDrop spectrophotometer (**A**) sample 0101; (**B**) sample 0102; (**C**) sample 0103; (**D**) sample 0104*

4.2 **PCR**

After PCR, DNA concentration has been measured again with the NanoDrop spectrophotometer (**Table 10**) (**Figure 13**).

Sample	Content of DNA after PCR (ng/ µL)	A260/A280	A260/A230
0101	1008.4	1.953	2.460
0102	1030.8	1.942	2.458
0103	1012.2	1.963	2.248

0104	977.75	1.940	2.630
0105	1032.6	1.974	2.249
0106	1099.8	1.974	2.164

Table 10 *Content of DNA extracted from each sample (ng/ µL) and purity ratios after PCR*

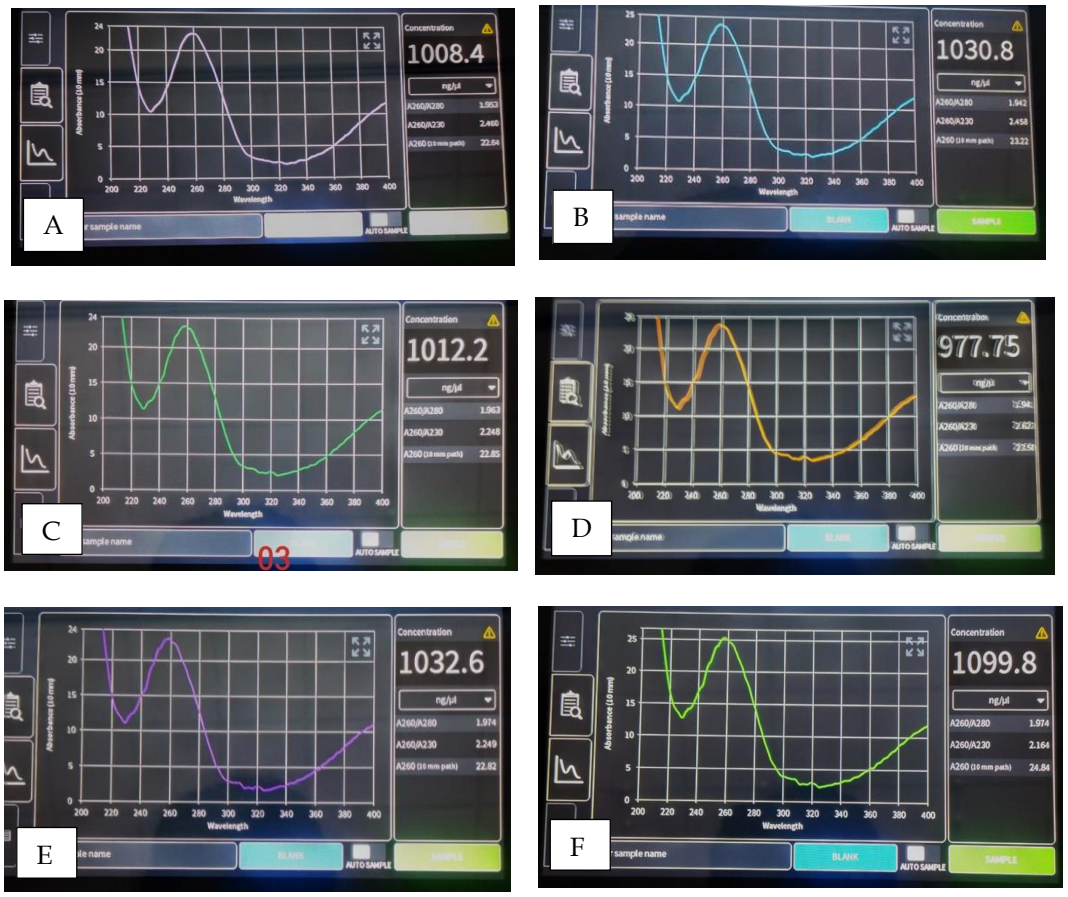


Figure 13 *DNA concentration measured with NanoDrop spectrophotometer after PCR (**A**) sample 0101; (**B**) sample 0102; (**C**) sample 0103; (**D**) sample 0104; (**E**) sample 0105; (**F**) sample 0106*

4.3 **Electrophoretic run**

The electrophoretic run on agarose gel done to have qualitative feedback of the success of amplification failed since no DNA was visible for any sample.

5. **DISCUSSION**

Despite having extracted a good amount of DNA, the failure of the post-PCR electrophoretic run has shown the difficulties of amplifying ancient DNA samples via PCR. It is challenging to identify the exact problem responsible for this failure as it could be a series of problems, all related to the limits of PCR when working with ancient DNA. Considering the limited results of this study, further analysis of the material is required to deepen the issue, but until then, some putative reasons can be discussed.

A first problem may concern the quality of DNA since it has been shown that there is a close connection between PCR success and DNA purity (Särkinen et al., 2012). As discussed in the Results section the CTAB method used extracted more than adequate amounts of DNA however with a hight rate of contamination, especially, as seen from the A260/A230 values, carbohydrates and/or other phenols. The presence of polyphenols and polysaccharides can lead to negative results of PCR, due to the inhibitory properties of PCR of primary and secondary chemicals, even in non-degraded DNA samples (Särkinen et al., 2012).

These results are in line with those obtained by another study which demonstrated that the purity of the extracted DNA is more important than the quantity when it comes to amplifying it with PCR (Särkinen et al., 2012): in this study eight extraction methods were compared and it was found that CTAB, despite being the best protocol in terms of extracted DNA quantity, was also the one that produced the worst quality of DNA among the eight protocols, with 0 positive amplicons for the *rbcL* locus. There is a compromise between the DNA amount that can be extracted and its quality as quantitatively performing protocols show little purity (low A260/ A280 ratios) whilst protocols with low DNA yield tend to show purer DNA (Särkinen et al., 2012).

A possible solution to this problem would be combining a CTAB method with a silica binding approach as shown in Särkinen et al. (2012) in order to have high purity and high DNA yield simultaneously.

A second problem might have been the target amplicon size. As written before, ancient DNA is highly fragmented and, although PCR can synthesize numerous copies of a few intact DNA molecules in the presence of a vast excess of damaged molecules (Thomas et al., 1989), fragments could be smaller than then amplifiable size. It has been proven that PCR of smaller target regions have higher success rate so it is advisable to aim for regions shorter than 300 bp when working with ancient DNA (Särkinen et al., 2012). To get an idea of how much the target size choice affects PCR success we report the data of the study by Särkinen et al. (2012) that used herbarium samples from 52 to 92 years old: PCR success rates are close to 100% for 100 bp long region, 24% for the 260 bp region, and 10% for the longest region (670 bp). A solution to this problem would be to choose a smaller target region to amplify and other primers for PCRs. For example, some candidate loci could be Mpv17-like protein (HS544) *JF788328* or *JF788329* both 321 bp mRNA linear both already been used with *Fucus virsoides* (Cánovas et al., 2011). More information about these loci can be found in GenBank ("*Fucus virsoides* isolate Fvirs2 Mpv17-like protein (HS544) mRNA, partial cds," 2016).

We will need to measure mean fragment size to understand whether we can use more specific primers or directly find an alternative method that avoid directly PCR.

For example, PCR could be replaced by a capture approach using oligonucleotides (=capture probes) that target conserved regions. The probability of finding appropriate capture probes is much higher than that of finding suitable PCR-based metabarcodes. This is because with capture probes there is not the need of having pairs separated by short distances to design metabarcodes, as instead is the case with PCR (Taberlet et al., 2012).

Another option to avoid PCR would be to directly sequence the DNA extract with high-throughput (HT) platforms, which can produce several billions sequence reads per run (e.g. using the Illumina platform) (Taberlet et al., 2012). The innovative Illumina NovaSeq 6000 Sequencing System performs whole-genome sequencing efficiently and cost-effectively. An advantage of this approach is that it allows for a direct quantification of the endogenous DNA amount ("NovaSeq 6000 Applications & Methods | Scalable, flexible sequencing power," n.d.).

A third problem could be linked to the method used for collecting, drying storing the samples over these 100 years, considering that specimens dried using alcohol yield lower amounts and more fragmented DNA compared to air-dried specimens (Särkinen et al., 2012). Moreover herbarium specimens were generally treated with chemical procedures to kill insects and other pests and this causes additional DNA damage (Albani Rocchetti et al., 2021). These problems could be solved directly by changing the sample to be analysed. However, information about storage techniques, as in this case, is not always available, therefore the prediction of whether a sample is a viable candidate for DNA extraction is difficult.

Although the *Fucus virsoides* DNA amplification failed, one interesting aspect that emerged from the analysis is that the CTAB extraction method is valid for these samples so that in the near future it will be feasible to sequence their DNA.
We could argue what is the benefit of extracting DNA from old samples given all the related difficulties but the advantages are unquestionably greater than the costs. In fact, it is not just about accessing the DNA of a historical specimen but being able to use this information for many different purposes. For example, an application for the extracted DNA is taxonomic research for species delimitation and phylogenetics, to link type specimens to specific members of morphologically indistinguishable species group or even to find new species (Yeates et al., 2016). Sequencing the DNA of these samples may be useful to reconstruct history of biological invasions, investigate changes in population size and connectivity between populations as can be seen in **Table 11** by numerous articles: (Brunet et al., 2016), (Cozzolino et al., 2007), (Devey et al., 2013), (Muniz et al., 2019), (Nakahama et al., 2015). In general, ancient DNA extraction allows to travel through time by building a bridge between the past and the present, since allows comparisons among time points,

Species	Scope	Genetic diversity parameter estimated	Citation
Ulmus rubra	USA	Estimate of past genetic bottlenecks	Brunet *et al.* (2016)
Anacamptis palustris	Italy	Distribution of haplotypes	Cozzolino *et al.* (2007)
Eligmocarpus cupuron	Madagascar	Distribution of haplotypes	Devey *et al.* (2013)
Dimorphandra exaltata	Brazil	Fragmentation; declines due to climate change	Muniz *et al.* (2019)
Vincetoxicum pycnostelma	Japan	Allelic richness; found unique alleles in seedlings grown from historical herbarium specimens	Nakahama *et al.* (2015)

Table 11 *Use of herbaria to assess the extent and provenance of genetic diversity declines (Albani Rocchetti et al., 2021)*

permitting evaluation of allelic changes across time (Yeates et al., 2016). This is relevant because low levels of genetic variation may be caused by recent population declines (Wandeler et al., 2007). However, low allelic changes across time can stand also for an ancestral state. To discriminate between the two, levels of genetic variability among ancient samples collected before a genetic bottleneck and those found in fresh samples can be compared (Wandeler et al., 2007). Discovering that a population is declining can trigger conservation projects to preserve it and knowing the historical level of loss of haplotypes can be very important for create an effective species recovery plan (Albani Rocchetti et al., 2021) (**Figure 14**).

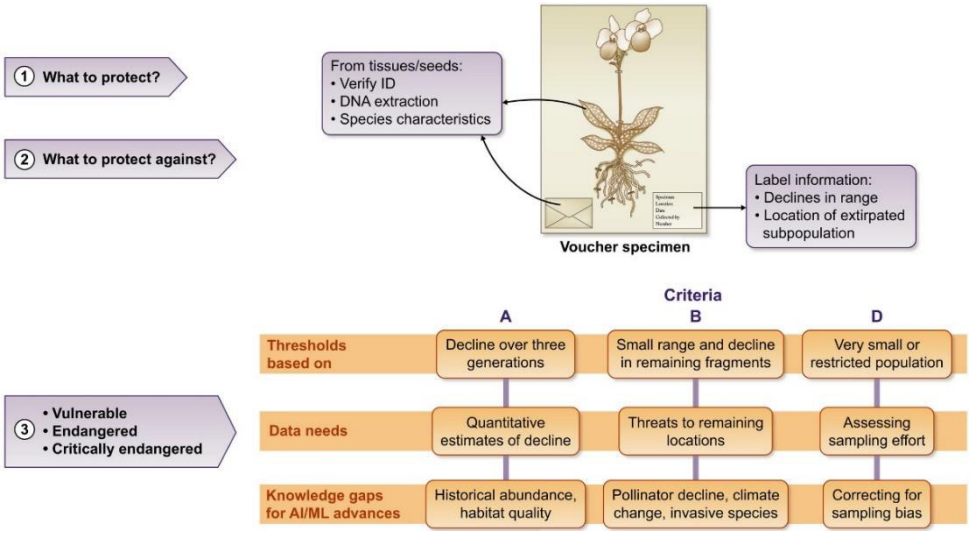


Figure 14 *Herbarium specimen components and their uses (Albani Rocchetti et al., 2021)*

6. **CONCLUSION**

This thesis provides the backbone for a project that could, through further analysis, have many implications for the future and can be further developed with *Museomics* approaches. This word, which mixes the sounds and combines the meanings of 'museum' and 'genomics', refers to the study of DNA sequences obtained from museum specimens. This emerging discipline applies omics techniques (genomics, paleogenomics and even paleoproteomics) to historical specimens allowing the retrieval of genomic data from samples belonging to many groups, from vertebrate animals to microbial communities, from bryozoa to plants

and therefore herbaria (Raxworthy and Smith, 2021; Lalueza-Fox, 2022).

Fucus virsoides is right now already part of a collection partly exhibited at the new Botanical Museum of Padua where the historical herbarium is exposed, a remarkable archive of plant biodiversity with about 800,000 specimens of plants, algae, mushrooms and dried lichens ("Apre al pubblico il Museo botanico dell'università di Padova," 2023).
The aim could be to exhibit at the museum the *Fucus virsoides* specimens that have been sequenced and to present them as a bank of historical and scientific information, as are the herbariums themselves.
On the one hand this would be a way to explain the techniques of extraction of ancient DNA and its potential to the general public. On the other hand, it would be a way to reconstruct the history of the collectors and their expeditions. Being *Fucus* an alga of the upper Adriatic, its distribution and history of sampling refers closely to our territory. The collectors have operated in the coasts of the upper Adriatic and have interacted with institutions that are part of the history of our region, such as the University of Padua.

In addition to the historical dissemination, which looks to the past, there is the scientific one that looks to the present and the future.
In fact, once the genome of the ancient samples of *Fucus* has been sequenced, it could be compared with the genome of a fresh sample to evaluate if in the last century the species has undergone genetic changes (Lang et al., 2022). For example, to get information about *Fucus virsoides* population, we could calculate *runs of homozygosity,* which are contiguous lengths of homozygous genotypes created when parents transfer to their progeny identical haplotypes (Purfield et al., 2012). ROH, which can be detected in whole-genome sequencing (WGS), reflects individual demographic history and gives information about population history and trait architecture (Purfield et al., 2012).
Comparing fresh and ancient *Fucus* DNA could be useful even to investigate if the species decline is due to climate change. For instance, future studies could explore the presence of deleterious mutations accumulated during the demographic decline (Lalueza-Fox, 2022) that the species has suffered along the Slovenian coast

(Gulf of Trieste, northern Adriatic) in last years (Battelli, 2016). From this point of view, the choice to analyse *Fucus* was far-sighted because it is an alga endemic to the Adriatic Sea that could be seriously affected by the rise of the sea due to climate change. Its monitoring over time is therefore essential to assess the impact that they have on biodiversity and the coastal system.

Additionally, a *museomics* approach would also allow to better predict future species' scenarios. Identifying and tracking genetic diversity of *Fucus* is essential for its long-term survival because it adds a temporal dimension (Lalueza-Fox, 2022). Looking into the past reveals how selection has operated in natural populations allowing to elaborate more refined models of natural selection, that consider both genomic responses and other features of natural populations, such as demographic change (Mikheyev et al., 2015) .

Given all these points, this project is one of the first steps towards creating the museum of the future, a museum that from simple describer of objects becomes an active player, with a dialogue between researchers and curators in a multidisciplinary collaborative network of museums, universities and institutions (Lalueza-Fox, 2022; Davis, 2023).

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