

Changes in the benthic algal flora of lake Ganzirri, north-eastern Sicily (Italy)

CLARA BERTUCCIO¹, GIUSEPPA GENOVESE^{1*}, ANTONIO MANGHISI¹, CORINNE CRUAUD³, ARNAUD COULOUX³, LINE LE GALL² & MARINA MORABITO¹

¹Dept. Biological and Environmental Sciences - Botany - University of Messina - Salita Sperone, 31 - 98156 Messina (Italy)

²Muséum National d'Histoire Naturelle (MNHN) - UMR 7138/USM 603 - Systématique, Adaptation Evolution - Paris, France

³Genoscope - Centre national de séquençage - Evry, France

* Corresponding Author: ggenovese@unime.it

Abstract. We produced the first DNA barcode assisted floristic list for the Mediterranean Sea, censusing the macroalgae of lake Ganzirri (Sicily) performed on collections from the last 15 years. The presence of alien species and the consistent reduction in species number found in comparison to previous works could be attributed to the violent impairment that the territory has suffered over the last 50 years. Nowadays monitoring of macroalgal diversity cannot transcend to couple molecular tools with traditional morphoanatomic observations in order to perform a more conscious environmental management.

Keywords: Benthic algal flora, DNA barcoding, lake Ganzirri, Cape Peloro lagoon; transitional environment, Sicily.

INTRODUCTION

Lake Ganzirri usually called “pantano grande” (large marsh) is part of Cape Peloro lagoon (northeastern Sicily), a transitional water system which is a complex ecosystem under significant anthropogenic pressure, a site of notable interest for its peculiar morphological, biological and landscape features. Together with lake Faro, or “pantano piccolo” (small marsh), the lake is located in the Oriented Natural Reserve of Cape Peloro (A.D. 437/44, 21/06/2001 of the Region Sicily), which is also a Site of Community Importance (C.D. 92/43/EEC, 21/05/1992) and a Special Area of Conservation (C.D. 79/409/EEC, 02/04/1979).

Cape Peloro lagoon is placed in a NNE-SSW direction along the extreme northeastern tip of Sicily, parallel to the Ionian shore of the Strait of Messina, and reaches the Tyrrhenian sea in its northern part.

Lake Ganzirri is a small, brackish shallow coastal basin, with a surface of 0.34 Km² area (maximum depth: 7 m; estimate water volume: 106 m³). It is characterized by two sub-basin: a southern, with average depth of 3 m and muddy sediments, and a northern, which accounts for one quarter of the total surface area, shallower (maximum depth 1 m) and with sandy bottoms (Manganaro *et al.*, 2011).

The lake is linked to the Ionian Sea by two channels: “Catuso”, located in the southern basin of the lake, and “Carmine” or “Due Torri”, situated in the northern. It is located in the North of the village of Ganzirri, where the coastal plain is approximately 500 m wide and it

is separated from the sea by a strip of Holocene alluvium deposits formed by silt, sand and gravel (Gargano, 1994) on which aeolian deposits outcrop (Bonfiglio & Violanti, 1983). It is connected by the “Margi” channel (about 1 Km long and 12 m large) with lake Faro, which in its turn communicates with the open sea by the channel “degli inglesi”.

The salinity of lake Ganzirri varies between 21 and 37 psu (Vanucci *et al.*, 2005). However, in particularly warm and dry summers a maximum of 39.1 psu was registered (Bergamasco *et al.*, 2005). The water temperature ranges between 10°C in winter and 30°C in summer.

For the macroalgal flora a total of 32 taxa at a specific and intraspecific level, comprising 15 Rhodophyta (46.9%), 5 Phaeophyceae (15.6%), and 12 Chlorophyta (37.5%), are reported in lake Ganzirri in the most recent floristic list (Serio *et al.*, 2009). However, the only records actually based on a field survey are much older, dating back to the work of Cavaliere (1963), who reported almost all of the species (29) presently considered, followed by sporadic reports on particular taxa (Gargiulo *et al.*, 2006; Polifrone *et al.*, 2006; Manghisi *et al.*, 2010; Manghisi *et al.*, 2011; Gargiulo *et al.*, 2013).

The aim of the present study has been the census of macroalgae in lake Ganzirri and their identification by DNA barcoding in order to produce an updated floristic list.

Systematic field observations covering two years (2009-2011), coupled with observations on the algal collection, from the last 15 years, housed in the Phycological Lab Herbarium of the University of Messina, and a comprehensive review of literature resulted in the present work.

The COI-5' region, the official DNA barcode for Rhodophyta, in association with the *rbcL* gene, used as a secondary marker for selected species (Saunders & McDevit, 2012), were sequenced within a cooperative project with the Muséum National d'Histoire Naturelle of Paris (France) and Genoscope, Centre national de séquençage, Evry (France).

The *tufA* gene, the proposed DNA barcode region for Chlorophyta (Saunders & McDevit, 2012), was sequenced within a cooperative project with the Center for Environmental and Molecular Algal Research, at the University of New Brunswick (Canada).

MATERIAL AND METHODS

Macroalgae were sampled monthly in lake Ganzirri (38° 15' N, 15° 37' E, Cape Peloro lagoon, northeastern Sicily, Italy), at seven sites along the coast and one in middle of the pond (Fig. 1), from April 2009 to November 2011. Samples collected were transported in seawater to the laboratory, where they were washed thoroughly with seawater to eliminate debris. For each sample, a voucher specimen was prepared by pressing a single individual on a herbarium sheet with a subsample preserved in 4% formalin in seawater for morpho-anatomical observations and another dried in silica gel and stored at -20° C for molecular analyses.

All specimens are housed in the Phycological Lab Herbarium (PhL) of the University of Messina, Italy. Additional samples from the last 15 years included in the algal collection of the PhL Herbarium were also considered.

Anatomical observations were made on hand sections of fresh or formalin preserved thalli stained with 1% aniline blue solution and observed under a Diaplan Leica light microscope (Leica Microsystems, Italy).

For all Rhodophyta samples, DNA was isolated from silica dried thalli or from freshly

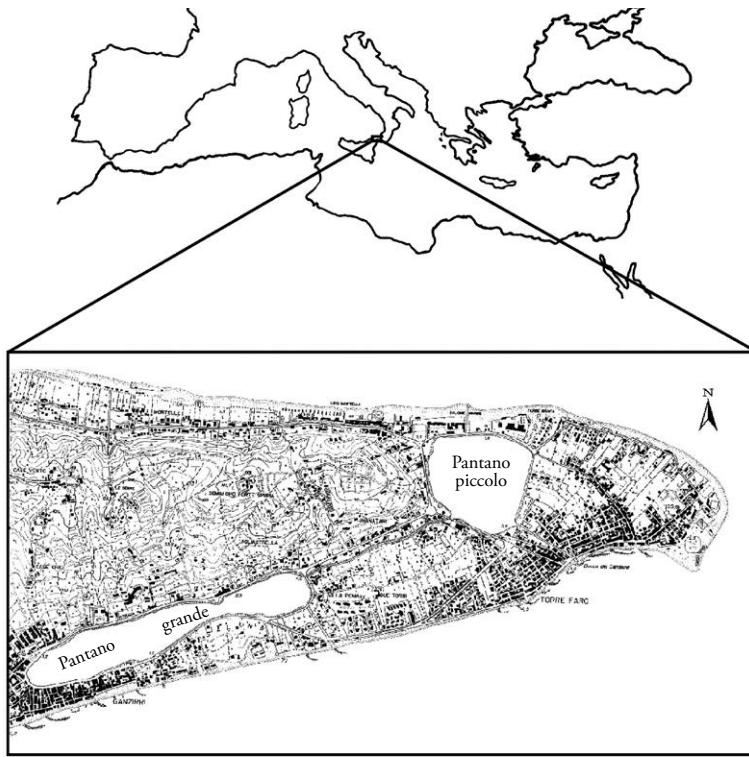


Fig. 1 – Lakes Ganzirri (Pantano grande) and Faro (Pantano piccolo), Cape Peloro lagoon, Sicily, Italy.

released spores (Morabito *et al.*, 2005), and the mitochondrial COI-5' region was PCR amplified as described in Manghisi *et al.* (2010). The plastidial *rbcL* gene was also PCR amplified for problematic taxa as described in Manghisi *et al.* (2010). Sequencing reactions were performed by Genoscope (Centre National de Séquençage, www.genoscope.fr, Evry, France).

For the Ulvales (Chlorophyta), DNA extractions, PCR and sequencing were performed at the Center for Ecological and Molecular Algal Research (CEMAR) of the University of New Brunswick, Canada. The plastid elongation factor gene (*tufA*) was sequenced according to Saunders and Kucera (2010).

Specimen data and sequences were deposited in the Barcode of Life Data Systems (BOLD, <http://www.boldsystems.org>).

Forward and reverse sequence reads were assembled with the software ChromasPro (v. 1.41, Technelysium Pty Ltd) and multiple sequence alignments were constructed in Seaview (v. 4.3.3, Gouy *et al.*, 2010), including sequences of related taxa downloaded from GenBank (Benson *et al.*, 2012). In addition, COI-5' sequences were contrasted with BOLD, with the "identify specimen" interface. Sequence alignments were subjected to distance analysis with a Neighbor-Joining algorithm under a general time reversible model of nucleotide substitution (GTR) (Lanave *et al.*, 1984) as performed in PAUP* 4b10 for the Macintosh (Swofford, 2002) to visualize clusters of genetic species.

RESULTS and DISCUSSION

The present paper aimed to update the floristic list of lake Ganzirri, in Cape Peloro lagoon, by means of DNA barcoding performed on new collections from the last 15 years. The actual consistence of the algal flora is reported in Tab. 1.

The previous field work of Cavalieri (1963) highlighted the particular abundance of species belonging to *Gracilaria*, *Ulva* (including *Enteromorpha*), *Chaetomorpha* and *Cladophora*. After, macroalgal species were occasionally reported in papers dealing with specific taxa (Gargiulo *et al.*, 2006; Polifrone *et al.*, 2006; Manghisi *et al.*, 2010; Manghisi *et al.*, 2011; Gargiulo *et al.*, 2013).

The macroalgal flora of the Mediterranean Sea has changed since 1970s and is still changing (Zenetas *et al.*, 2012). Such changes resulted, on the whole, in a slight increase of the biodiversity and more significantly in an enlargement of the distribution area of species with warm water affinity (both native and introduced) and a reduction of the distribution area of cold water affinity species, with at least 128 species of alien macrophytes present, all that due to both global climatic change and an increasing input from exotic flora by several means (Zenetas *et al.*, 2012). Coastal lagoons, such as Thau lagoon in France, Venice lagoon and Cape Peloro lagoon in Italy, are well-known points of introduction for marine macrophytes, especially because they are under strong anthropogenic pressure, among all due to the practice of shellfish aquaculture.

In the past, shellfish activity has been common in Cape Peloro lagoon. Nowadays, the activity is reduced almost exclusively to the marketing of shellfish depending on importation from various farming sites both Italian and foreign (Spain, France, Netherlands). Adult livestock are re-immersed in water for a stabulation period before being sold in local markets.

In the present paper, among identified taxa (Fig. 2), significant was the presence of the alien species *Agardhiella subulata* (C. Agardh) Kraft et M.J. Wynne (Solieriacae, Rhodophyta, Fig. 2B) (Manghisi *et al.*, 2010) and *Hypnea "cornuta"* (Kützing) J.Agardh (Cystocloniaceae, Rhodophyta, Fig. 2H) (Manghisi *et al.*, 2011).

Agardhiella subulata has been found in lake Ganzirri in 2007 and, since then, all reproductive stages has been collected and a large population has been observed all year round (Manghisi *et al.*, 2010). Hence, according to the criteria of Ribera and Boudouresque (1995), it could be in either its settlement or naturalization phase.

The species is mainly distributed along the eastern American coast (Yarish *et al.*, 1984), but it is also present in the Indian Ocean (Silva *et al.*, 1996). In the Mediterranean, it is reported in Thau lagoon (Hérault, France; Ben Maiz, 1986) as *Solieria chordalis* (C. Agardh) J. Agardh, in Mar Piccolo (Taranto, Italy) and in Pantano d'Arci (Catania, Italy) (Perrone & Cecere, 1994). However, the species has never been mentioned for Sicily in later publications (Furnari *et al.*, 2003; Cormaci *et al.*, 2004; Furnari *et al.*, 2010). More recently, *A. subulata* was reported in Yerseke, Netherlands (Stegenga, 1999), and in Venice lagoon, Italy (Curiel *et al.*, 2005; Sfriso & La Rocca, 2005). The population found in Cape Peloro has been introduced by means of oyster importation as proved in Manghisi *et al.* (2010) by means molecular analyses.

Hypnea "cornuta" was observed in the pond since 1998 (Manghisi *et al.*, 2011). From the comparison of *rbcL* sequences of *H. "cornuta"* from lake Ganzirri and others available from GenBank, two species are reported under the name *H. "cornuta"*: one from lake Ganzirri and

the second from Japan, Australia, Malaysia and Mar Piccolo of Taranto (Yamagishi *et al.*, 2003; Cecere *et al.*, 2004; Geraldino *et al.*, 2009).

Hypnea "cornuta" has been reported from various localities in the world (Guiry & Guiry, 2013). The first Mediterranean report was from Rhodes island, Greece, as *H. valentiae* (Turner) Montagne, shortly after the opening of the Suez Canal in 1869 (Reinbold, 1898). Successively, *H. cornuta* has been reported from Egypt in 1948 (Aleem, 1948), Israel since 1964 (Nemlich & Danin, 1964). In 2000, it has been discovered in Mar Piccolo of Taranto (Cecere *et al.*, 2004).

The mean of introduction of the species is at present only speculative, waiting for the clarification of the status of the *Hypnea cornuta*-complex and its biogeographic pattern. Hypothesizing that the Mar Piccolo isolate is of western Pacific origin, it is likely to be one of the many oyster-driven taxa, while the actual origin of the Sicilian isolate is less clear. It might be introduced as migrant through the Suez canal (Lessepsian migrant) or by a secondary introduction from the eastern Mediterranean basin.

This study highlights the presence of another species of *Hypnea* in lake Ganzirri, *H. musciformis* (Wulfen) J.V. Lamouroux, a native species for the Mediterranean but not yet reported in the lake (Fig. 2G), although already recorded from the Strait of Messina (Furnari *et al.*, 2003). It is a common species widely distributed in the warm and temperate seas. More DNA data and a careful revision of all reports together with the comparison with material from type areas are needed to assess the actual consistence of the genus *Hypnea* in the Mediterranean.

Changes of macroalgal diversity in the Mediterranean Sea due to introduction of alien species were frequently highlighted since 1970s (Nemlich & Danin, 1964; Cecere *et al.*, 2004; Occhipinti-Ambrogi *et al.*, 2011; Zenetos *et al.*, 2012). In the last decades, the problem of the occurrence of alien species in the Mediterranean Sea was brought to public attention mostly because of the invasive behaviour of some introduced green algae, *Caulerpa taxifolia* (M.Vahl) C. Agardh and *C. racemosa* (Forsskål) J. Agardh, interacting with *Posidonia oceanica* (Linnaeus) Delile meadows, and the occurrence of several alien species of Asian origin in Thau lagoon (France) mainly due to mollusc culture. Despite the collective effort of experts in their work, the number of introduced species remains probably underestimated.

Cavaliere (1963) reported from lake Ganzirri *Gracilaria compressa* (C.Agardh) Greville (Gracilariaeae, Rhodophyta), presently synonym of *G. bursa-pastoris* (S.G. Gmelin) P.C. Silva, and *G. confervoides* (Linneus) Greville, a species with a cylindrical thallus under which, due to the morphological similarity, at least three distinct species can be confused: *Gracilaria longissima* (S.G.Gmelin) Steentoft, L.M. Irvine *et al.*, *Gracilaria gracilis* (Stackhouse) M. Steentoft, L.M. Irvine *et al.* W.F. Farnham and *G. longa* Gargiulo, De Masi *et al.* Tripodi.

The species can be distinguished by fine anatomical details (Fredericq & Hommersand, 1989; Gargiulo *et al.*, 1992) and, therefore, often there is confusion about the precise identity of collected thalli. According to (2009) the report of *G. confervoides* of Cavaliere (1963) should be attributed to either *G. gracilis* or *G. longa* or to both species.

More recently (Gargiulo *et al.*, 2006) revised the status of the family in the Mediterranean Sea and reported *G. bursa-pastoris* and *G. gracilis* in Cape Peloro lagoon excluding the presence of *G. longa*.

In the present paper DNA barcodes revealed cryptic diversity within the *Gracilaria* complex and allowed us to recognize three distinct taxa: *G. bursa-pastoris* (Fig. 2E), *G. gracilis* (Fig.

Tab. 1 – List of macroalgal species recorded in lake Ganzirri. Abbreviations: A = Atlantic; Abt = Atlantic boreo-tropical; C = Cosmopolite; CB = Circumboreal; IA: Indo-Atlantic; IP = Indo-Pacific; P: Pantropical; SC: Sub-cosmopolite; BOLD #: BOLD process ID; GB # = GenBank accession number.

Species	Phytogeographic element	Reference	DNA barcode
Chlorophyta			
<i>Bryopsis plumosa</i> (Hudson) C. Agardh	SC	Cavaliere 1963	-
<i>Chaetomorpha linum</i> (O. F. Müller) Kützing	C	Cavaliere 1963	-
<i>Cladophora prolifera</i> (Roth) Kützing	IA	Cavaliere 1963	-
<i>Cladophora vagabunda</i> (Linnaeus) C. Hoek	SC	Cavaliere 1963, as <i>Cladophora penicillata</i> Kützing	-
<i>Cladophora</i> spp.	-	present study	BOLD # (GB #):
<i>Ulva fasciata</i> C. Agardh (consistent with usage in Kirkendale et al., 2013)	P	Cavaliere 1963, as <i>Ulva lactuca</i> Linneus	ITGREF003-11 (KM212023); ITGREF016-11 (KM212025); ITGREF017-11 (KM212022); ITGREF018-11 (KM212026)
<i>Ulva flexuosa</i> Wulfen subsp. <i>pilifera</i> (Kützing) M. J. Wynne	IA	present study	BOLD # (GB #):
			ITGREF020-11 (KM212027); ITGREF021-11 (KM212024); ITGREF023-11 (KM212021)
Rhodophyta			
<i>Agardhiella subulata</i> (C. Agardh) Kraft et M. J. Wynne	IA	Manghisi et al. 2010	BOLD # (GB #):
			ITRED001-10 (KF714838); ITRED002-10 (KF714840); ITRED003-10 (KF714841); ITRED004-10 (KF714842); ITRED005-10 (KF714843); ITRED006-10 (KF714844); ITRED007-10 (KF714845); ITRED008-10 (KF714846); ITRED009-10 (KF714839)
<i>Dermacorynus dichotomus</i> Gargiulo, M. Morabito et Manghisi (J. Agardh)	Abt	Gargiulo et al. 2013	GB #: JX070628
<i>Gracilaria bursa-pastoris</i> (S. G. Gmelin) P. C. Silva	SC	Cavaliere 1963, as <i>Gracilaria compressa</i> (C. Agardh) Greville	GB #: AV651031

continued

Species	Phytogeographic element	Reference		DNA barcode
<i>Gratularia gracilis</i> (Stackhouse) Steentoft, L. M. Irvine et Famham	IA	Cavaliere 1963, as <i>Gratularia coniformis</i> (Linnaeus) Greville p.p.	GB#:AY651053; BOLD # (GB #):	
			ITREDD099-13 (KF714847); ITREDD098-13 (KF714848); ITREDD093-13 (KF714849); ITREDD092-13 (KF714850); ITREDD097-13 (KF714851); ITREDD096-13 (KF714852); ITREDD095-13 (KF714853); ITREDD094-13 (KF714854)	
<i>Gratulariopsis longissima</i> M. Steentoft, L. M. Irvine et W. F. Famham	C	present study	BOLD # (GB #):	
<i>Gymnogongrus griffithiae</i> (Turner) Martius	CB	present study	ITRED100-13 (KF714856); ITRED101-13 (KF714855)	
<i>Hypnea cornuta</i> (Kürzing) J. Agardh	IP	Manghisi et al. 2011	BOLD # (GB #): ITREDD102-13 (KF714857)	
			ITREDD081-13 (KF714858); ITREDD083-13 (KF714859); ITREDD091-13 (KF714860); ITREDD090-13 (KF714861); ITREDD089-13 (KF714862); ITREDD088-13 (KF714863); ITREDD084-13 (KF714864); ITREDD087-13 (KF714865); ITREDD085-13 (KF714866); ITREDD086-13 (KF714867); ITREDD080-13 (KF714868)	
<i>Hypnea musiformis</i> (Wulfen) J. V. Lamouroux	P	Manghisi et al. 2011	BOLD # (GB #): ITREDD082-13 (KF714869)	

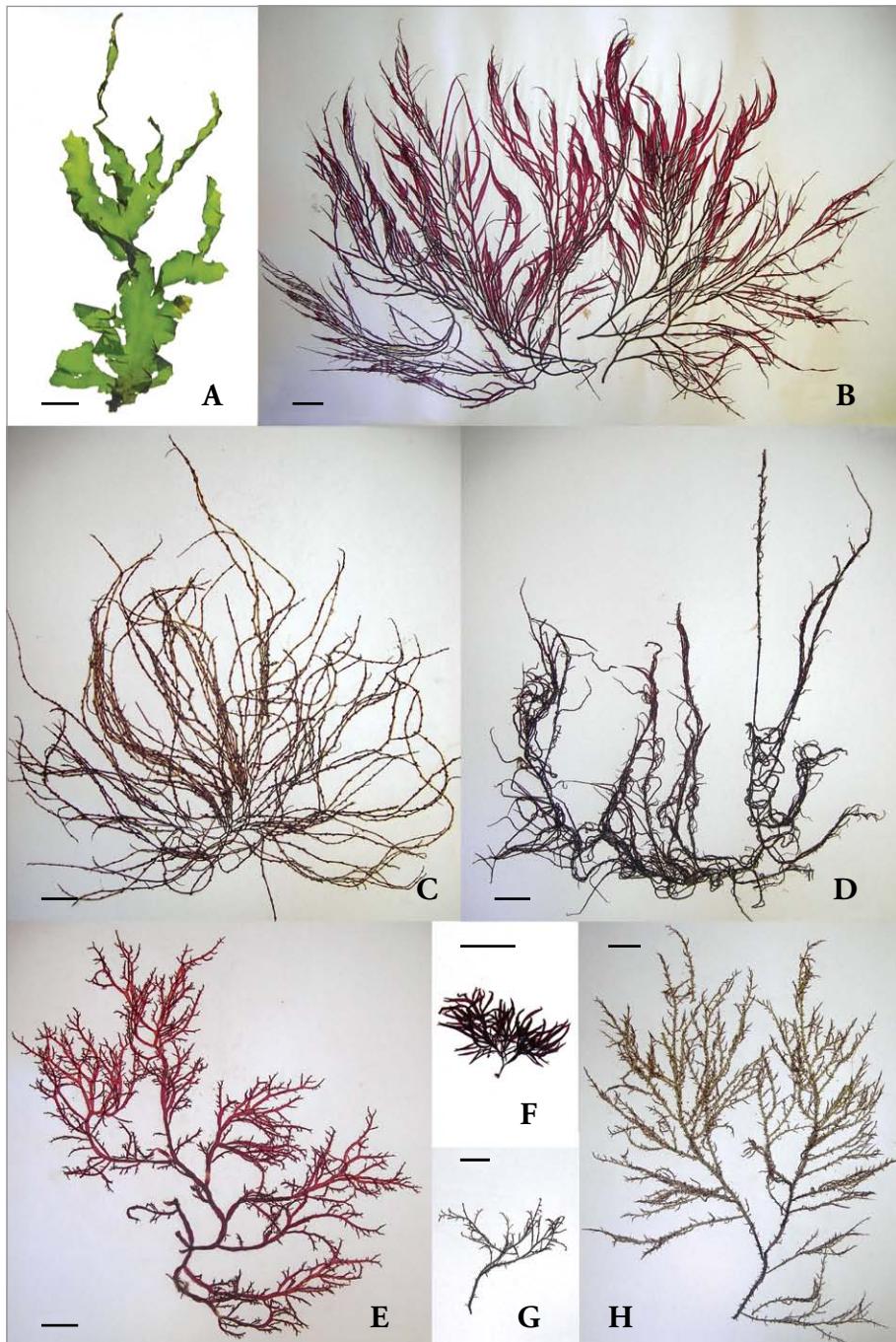


Fig. 2 – Herbarium specimens of algae collected in Lake Ganzirri: A, *Ulva fasciata* (CB141); B, *Agardhiella sulcata* (CB303); C, *Gracilaria gracilis* (CB005); D, *Gracilaria longissima* (CB100); E, *Gracilaria bursa-pastoris* (CB017); F, *Dermocorynus horridus* (CB083); G, *Hypnea musciformis* (CB115); H, *Hypnea cornuta* (CB151E).

2C) and the previously unreported *Gracilariaopsis longissima* (S.G. Gmelin) M. Steentoft, L.M. Irvine *et al.* W.F. Farnham (Fig. 2D).

G. bursa-pastoris, *G. gracilis* and *Gracilariaopsis longissima* were reported from various localities in the world and are widely distributed in the Mediterranean (Guiry & Guiry, 2013). Cavaliere (1963) reported *Ulva lactuca* Linnaeus as the only laminar Ulvacean species (Chlorophyta) and six tubular species (*Enteromorpha clathrata* (Roth) Greville, *E. compressa* (Linnaeus) Nees, *E. intestinalis* (Linnaeus) Nees, *E. linza* (Linnaeus) J. Agardh, *E. prolifera* (O.F. Müller) J. Agardh and *E. plumosa* (Kützing) in Cape Peloro lagoon.

Members of the Ulvaceae comprise a major macroalgal assemblage found growing in transitional environments and coastal habitats of the Mediterranean Sea for which species discrimination is a highly demanding task.

Morphological and culture studies revealed that these species have a wide range of environmentally influenced blade forms (Hayden *et al.*, 2003). Laminar species growing without exposure to wave action, desiccation or other environmental factors may not develop normally beyond the juvenile tubular stage. Therefore great caution is needed when using morphological characters in comparative, taxonomic or systematic studies in this and other groups of morphologically simple algae. According to molecular phylogenies (Hayden *et al.*, 2003), the genera *Ulva* Linneus and *Enteromorpha* Link are not distinct evolutionary entities and should not be recognized as separate but both included under the older name *Ulva*.

Samples of *Ulva lactuca* reported by Cavaliere (1963) were considered as *Ulva laetevirens* Areschoug by Serio *et al.* (2009). In the present paper DNA barcodes pointed out that this entity should be referred to as *Ulva fasciata* Delile (Fig. 2A, consistent with usage in Kirkendale *et al.*, 2013).

Moreover, we found that tubular forms (*Enteromorpha* like) grow mixed with laminar form (*Ulva* like) both having the same DNA barcode and, therefore, conspecific and assigned to *Ulva fasciata*. Genetically distinct tubular forms were also collected, belonging to *Ulva flexuosa* Wulfen subsp. *pilifera* (Kützing) M.J. Wynne.

In light of the present knowledge, we are not able to assess whether one of the *Enteromorpha* spp. reported by Cavaliere (1963) could be the same entity we found recently or the latter represents a subsequent introduction.

A careful taxonomic and biogeographic worldwide study is needed to clarify the *Ulva* complex.

Cladophora (Cladophorales, Chlorophyta) is a large and common green genus with a cosmopolitan distribution in marine as well as freshwater habitats. The taxonomy of the genus *Cladophora* is problematic as shown by molecular phylogenetic studies (Boedeker *et al.*, 2012). At the species level, taxonomic problems arise from intraspecific morphological variability and cryptic genetic diversity (Leliaert *et al.*, 2009). Specimens collected in the present study form a morphologically diverse species complex, rather difficult to determine using morpho-anatomical identification keys. At present, all DNA barcodes proposed for green algae failed to work properly for the Cladophorales (Saunders & McDevit, 2012). By morphological identification we tentatively confirm the presence of *Cladophora vagabunda* (Linnaeus) Hoek and *Cladophora prolifera* (Roth) Kützing and notice the further presence of two undefined morph types which could represent distinct species.

We further point the presence of two small Rhodophyta, *Dermocorynus dichotomus*

(J.Agardh) Gargiulo, M. Morabito *et al.* Manghisi (Gargiulo *et al.*, 2013) (Halymeniaceae, Fig. 2F) and *Gymnogongrus griffithsiae* (Turner) Martius (Phyllophoraceae), newly reported.

Dermocorynus dichotomus is widely reported in various sites in the world including the Mediterranean, even if its actual presence is under investigation (Gargiulo *et al.*, 2013).

Gymnogongrus griffithsiae is a cosmopolitan species, previously reported for several sites in the Strait of Messina and in other Mediterranean coastal lagoons (Verlaque, 2001; Furnari *et al.*, 2003; Sfriso, 2009).

CONCLUSIONS

The consistent reduction in species number that we found in comparison to the work of Cavaliere (1963) could be attributed to the violent impairment that the territory has suffered over the last 50 years due to both the intensification of shellfish marketing and the huge urbanization extended to all the area of Cape Peloro (Manganaro *et al.*, 2011).

The present study produced the first DNA barcode assisted floristic list for the Mediterranean coasts. Molecular tools have revolutionized our understanding of algal phylogeny and diversity and have resulted in major restructuring of systems of classification. In macroalgae, simple morphology and anatomy, rampant convergence, remarkable degrees of phenotypic plasticity in response to environmental factors tend to confound attempts at identification. Presently, a new approach has been developed, DNA barcoding (Hebert *et al.*, 2003; Saunders & McDevit, 2012), which is the use of a short gene sequence from a standardized region of the genome that can be used as a molecular diagnostic for species-level identification. The ability to discriminate quickly and accurately an unknown specimen benefits not only biodiversity and basic taxonomic studies, but the potential applications for DNA barcoding include enabling the prompt identification of new invasive taxa, monitoring of endangered species and population size and growth, exploring marine biodiversity, monitoring environmental quality.

Nowadays monitoring of macroalgal diversity cannot transcend to couple molecular tools with traditional morphoanatomic observations in order to perform a more conscious environmental management.

Acknowledgements. Dr. Gary Saunders and the Center for Environmental and Molecular Algal Research, at the University of New Brunswick, are greatly acknowledged for the sequencing of green algal specimens within the ALGA (Algal Life Global Audit) project under the WG1.8 Marine Bio-surveillance of the iBOL initiative. Molecular data from red algae were generated at the Service de Systématique Moléculaire du Muséum National d'Histoire Naturelle (CNRS - UMS 2700) thanks to funds provided by the ATM "Taxonomie moléculaire: DNA Barcode et gestion durable des collections". Sequencing was performed by the Genoscope through the projects entitled 'Speed ID' and "Bibliotheque du Vivant". The Authors would like to thank Mr. Marco Vicinanza and Dr. Simona Armeli Minicante for their support in collecting algal material.

This study was supported by grants from the University of Messina to G.G. and M.M.

RIASSUNTO

Cambiamenti della flora bentonica del lago di Ganzirri, Sicilia nord orientale (Italia)

Il lago di Ganzirri è parte del sistema lagunare di Capo Peloro (Sicilia nord-orientale), un ecosistema complesso sotto notevole pressione antropica, Riserva Naturale Orientata, Sito di Importanza Comunitaria e Zona a Protezione Speciale. Lo scopo del presente studio è sta-

to il censimento delle macroalghe nel lago di Ganzirri e la loro identificazione mediante analisi di DNA al fine di produrre il primo elenco floristico per le coste del Mediterraneo basato su DNA barcoding. Il lavoro ha previsto raccolte sistematiche in campo nell'arco di due anni (2009-2011), osservazioni su una collezione algale relativa agli ultimi 15 anni e una revisione della letteratura. Il sequenziamento del DNA è stato svolto nell'ambito di progetti di collaborazione con il Muséum National d'Histoire Naturelle di Parigi (Francia), con Genoscope, Centre national de séquençage, Evry (Francia) e con il Center for Environmental and Molecular Algal Research presso l'Università di New Brunswick (Canada). La precedente lista floristica, basata su osservazioni in campo, risale al 1963, seguita da report occasionali di specifici taxa tra il 2006 e il 2013. È significativa la presenza delle specie aliene *Agardhiella subulata* e *Hypnea "cornuta"*, i cui vettori di introduzione sono da attribuire rispettivamente alla molluscoltura e a migrazioni lessepsiane. Inoltre è da segnalare una diversità critica all'interno del genere *Gracilaria*, in cui abbiamo riconosciuto tre taxa distinti, *G. bursa-pastoris*, *G. gracilis* e, come nuova segnalazione, *Gracilaria longissima*, e all'interno delle Ulvaceae, un gruppo di macroalghe tipico di ambienti di transizione e habitat costieri, tra cui segnaliamo per la prima volta *Ulva fasciata* e *Ulva flexuosa* subsp. *pilifera*. La consistente riduzione nel numero di specie (15) che abbiamo trovato in confronto alla lista del 1963 (29 specie) potrebbe essere attribuita alla violenta alterazione che il territorio ha subito negli ultimi 50 anni a causa sia dell'intensificazione del commercio dei molluschi che all'enorme urbanizzazione estesa a tutta l'area di Capo Peloro. Al fine di effettuare una gestione ambientale più consapevole, il monitoraggio della diversità delle macroalghe non può trascendere da strumenti molecolari, quali il DNA barcoding, associati con osservazioni morfoanatomiche, soprattutto considerando che tali organismi hanno morfologia e anatomia semplici, elevata plasticità fenotipica, fattori che tendono a confondere i tentativi di identificazione.

REFERENCES

- Aleem, A. A. 1948. The recent migration of certain Indo-Pacific algae from the Red Sea into the Mediterranean. *New Phytologist* **47**: 88-94.
- Ben Maiz, N. 1986. Flore algale (Rhodophyta, Phaeophyceae, Chlorophyceae, Bryopsidophyceae) de l'étang de Thau (Hérault). Thesis Écologie Université d'Aix-Marseille II, Aix-Marseille.
- Benson, D. A., Karsch-Mizrachi, I., Clark, K., Lipman, D. J., Ostell, J. & Sayers, E. W. 2012. GenBank. Nucleic Acids Research **40**: D48-D53.
- Bergamasco, A., Azzaro, M., Pulicanò, G., Cortese, G. & Sanfilippo, M. 2005. Ganzirri Lake, north-eastern Sicily. In: G. Giordani, P. Viaroli, D.P. Swaney, C.N. Murray, J.M. Zaldivar and J.I. Marshall Crossland (eds) Nutrient fluxes in transitional zones of the Italian coast. *LOICZ, Texel, the Netherlands*: ii+1-157.
- Boedeker, C., O'Kelly, C. J., Star, W. & Leliaert, F. 2012. Molecular phylogeny and taxonomy of the aegagropila clade (Cladophorales, Ulvophyceae), including the description of *Aegagropilopsis* gen. nov. and *Pseudocladophora* gen. nov. Journal of Phycology **48**: 808-25.
- Bonfiglio, L. & Violanti, L. 1983. Prima segnalazione di Tirreniano ed evoluzione Pleistocenica del Capo Peloro (Sicilia Nord-orientale). *Geografia Fisica e Dinamica Quaternaria* **6**: 3-15.
- Cavaliere, A. 1963. Biologia ed ecologia della flora dei laghi di Ganzirri e Faro, sua sistematica e distribuzione stagionale. *Bollettino di Pesca, Piscicoltura e Idrobiologia* **39**: 169-86.
- Cecere, E., Petrocelli, A. & Verlaque, M. 2004. Morphology and vegetative reproduction of the introduced species *Hypnea cornuta* (Rhodophyta, Gigartinales) in the Mar Piccolo of Taranto (Italy, Mediterranean Sea). *Botanica Marina* **47**: 381-88.
- Cormaci, M., Furnari, G., Giaccone, G. & Serio, D. 2004. Alien macrophytes in the Mediterranean Sea: a review. *Recent Research Developments in Environmental Biology* **1**: 153-202.

- Curiel, D., Bellemo, G., Checchin, E., Dri, C., Miotti, C. & Marzocchi, M. 2005. Segnalazione di nuove macroalghe per la Laguna di Venezia. Lavori- Società Veneziana di Scienze Naturali **30**:41-44.
- Fredericq, S. & Hommersand, M. H. 1989. Proposal of the Gracilariales ord. nov. (Rhodophyta) based on an analysis of the reproductive development of *Gracilaria verrucosa*. Journal of Phycology **25**:213-27.
- Furnari, G., Giaccone, G., Cormaci, M., Alongi, G., Catra, M., Nisi, A. & Serio, D. 2010. Macrophytobenthos. Biologia Marina Mediterranea **17**:801- 28.
- Furnari, G., Giaccone, G., Cormaci, M., Alongi, G. & Serio, D. 2003. Biodiversità marina delle coste italiane: catalogo del macrofitobenthos. Biologia Marina Mediterranea **10**:3-482.
- Gargano, C. 1994. Carta geologica di Messina e del settore nord-orientale dei Monti Peloritani (Sicilia N.E.), scala 1:25000. SELCA, Firenze.
- Gargiulo, G. M., De Masi, F. & Tripodi, G. 1992. Morphology, reproduction and taxonomy of the Mediterranean species of *Gracilaria* (Gracilariales, Rhodophyta). Phycologia **31**:53-80.
- Gargiulo, G. M., Morabito, M., Genovese, G. & De Masi, F. 2006. Molecular systematics and phylogenetics of Gracilariaeacean species from the Mediterranean Sea. Journal of Applied Phycology **18**:497-504.
- Gargiulo, G. M., Morabito, M. & Manghisi, A. 2013. A re-assessment of reproductive anatomy and postfertilization development in the systematics of *Grateloupia* (Halymeniales, Rhodophyta). Cryptogamie, Algologie **34**:3-35.
- Geraldino, P. J. L., Yang, E. C., Kim, M. S. & Boo, S. M. 2009. Systematics of *Hypnea asiatica* sp. nov. (Hypnaceae, Rhodophyta) based on morphology and nrDNA SSU, plastid *rbcL*, and mitochondrial *cox1*. Taxon **58**:606-16.
- Gouy, M., Guindon, S. p. & Gascuel, O. 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Molecular Biology and Evolution **27**:221-24.
- Guiry, M. D. & Guiry, G. M. 2013. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>; searched on 02 June 2013.
- Hayden, H. S., Blomster, J., Maggs, C. A., Silva, P. C., Stanhope, M. J. & Waaland, J. R. 2003. Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. European Journal of Phycology **38**: 277-94.
- Hebert, P. D. N., Ratnasingham, S. & deWaard, J. R. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London Series B: Biological Sciences **270**: S96-S99.
- Kirkendale, L., Saunders, G. W. & Winberg, P. 2013. A molecular survey of *Ulva* (Chlorophyta) in temperate Australá reveals enhanced levels of cosmopolitanism. Journal of Phycology **49**: 69-81.
- Lanave, C., Preparata, G., Sacone, C. & Serio, G. 1984. A new method for calculating evolutionary substitution rates. Journal of Molecular Evolution **20**: 86-93.
- Leliaert, F., Boedeker, C., Peña, V., Bunker, F., Verbruggen, H. & De Clerck, O. 2009. *Cladophora rhodolithicola* sp. nov. (Cladophorales, Chlorophyta), a diminutive species from European mael beds. European Journal of Phycology **44**: 155-69.
- Manganaro, A., Pulicanò, G. & Sanfilippo, M. 2011. Temporal evolution of the area of Capo Peloro (Sicily, Italy) from pristine site into urbanized area. Transitional Waters Bulletin **5**: 23-31.
- Manghisi, A., Armeli Minicante, S., Bertuccio, C., Morabito, M., Fiore, V., Genovese, G. & Gall, L. L. 2011. Identifying alien macroalgae through DNA barcoding: the case of *Hypnea cornuta* (Cystocloniaceae, Rhodophyta). Transitional Waters Bulletin **5**: 42-49.
- Manghisi, A., Morabito, M., Bertuccio, C., Le Gall, L., Couloux, A., Cruaud, C. & Genovese, G. 2010. Is routine DNA barcoding an efficient tool to reveal introductions of alien macroalgae? A case study of *Agardhiella subulata* (Solieriaceae, Rhodophyta) in Cape Peloro lagoon (Sicily, Italy). Criptogamie Algologie **31**: 423-33.
- Morabito, M., Genovese, G. & Gargiulo, G. M. 2005. A simple and rapid technique to PCR amplify plastid genes from spores of *Porphyra* C. Agardh (Bangiales, Rhodophyta). Journal of Applied Phycology **17**: 35-38.
- Nemlich, C. & Danin, Z. 1964. Marine algae of the coast of Israel. Hakibbutz Hameuchad, 199 pp.
- Occhipinti-Ambrogi, A., Marchini, A., Cantone, G., Castelli, A., Chimenz, C., Cormaci, M., Froglia, C., Furnari, G., Gambi, M. C., Giaccone, G., Giangrande, A., Gravilli, C., Mastrototaro, F., Mazzotti, C., Orsi-Rolini, L. & Piraino, S. 2011. Alien species along the Italian coasts: an overview. Biological Invasions **13**: 215-237.

- Perrone, C. & Cecere, E. 1994. Two solieriacean algae new to the Mediterranean: *Agardhiella subulata* and *Solieria filiformis* (Rhodophyta, Gigartinales). *Journal of Phycology* **30**: 98-108.
- Polifrone, M., De Masi, F. & Gargiulo, G. M. 2006. Alternative pathways in the life history of *Gracilaria gracilis* (Gracilariales, Rhodophyta) from north-eastern Sicily (Italy). *Aquaculture* **261**: 1003-13.
- Reinbold, T. A. 1898. Meeresalgen von der Insel Rhodos. Gesammelt von Lehrer J. Nemetz. Bestimmt von Th. Reinbold-Itzehoe. *Hedwigia* **37**(Beiblatt 3/4): 87-90.
- Saunders, G. W. & Kucera, H. 2010. An evaluation of *rbcL*, *tufA*, UPA, LSU and ITS as DNA barcode markers for the marine green macroalgae. *Cryptogamie Algologie* **31**: 487-528.
- Saunders, G. W. & McDevit, D. C. 2012. Methods for DNA Barcoding Photosynthetic Protists Emphasizing the Macroalgae and Diatoms DNA Barcodes. In: Kress, W. J. & Erickson, D. L. (eds.), *DNA barcodes: methods and protocols*. *Humana Press, Methods in Molecular Biology*, 858: 207-22.
- Serio, D., Cormaci, M. & Furnari, G. 2009. The Lakes Faro and Ganzirri In: Cecere, E. Petrocelli, A. Izzo, G. and Sfriso, A. (eds), *Flora and Vegetation of the Italian Transitional Water Systems*, p. 229-38.
- Sfriso, A. 2009. Segnalazione di macroalghe nuove per la Laguna di Venezia. *Lavori - Società Veneziana di Scienze Naturali* **34**:65-71.
- Sfriso, A. & La Rocca, B. 2005. Aggiornamento sulle macroalghe presenti lungo i litorali e sui bassofondali della laguna di Venezia. *Lavori - Società Veneziana di Scienze Naturali* **30**: 45-56.
- Silva, P. C., Basson, P. W. & Moe, R. L. 1996. Catalogue of the benthic marine algae of the Indian Ocean. *University of California Press, Berkeley*, XIV+1259.
- Stegenga, H. 1999. Het roodwier *Agardhiella subulata* in Nederland. *Zeepaard* **59**: 54-57.
- Swofford, D. 2002. PAUP* 4.0 b10: Phylogenetic analysis using parsimony. *Sinauer Associates, Sunderland, MA, USA*.
- Vanucci, S., Bruni, V. & Pulicanò, G. 2005. Spatial and temporal distribution of virioplankton and bacterioplankton in a brackish environment (Lake of Ganzirri, Italy). *Hydrobiologia* **539**: 83-92.
- Verlaque, M. 2001. Checklist of the macroalgae of Thau Lagoon (Hérault, France), a hot spot of marine species introduction in Europe. *Oceanologica Acta* **24**: 29-49.
- Yamagishi, Y., Masuda, M., Abe, T., Uwai, S., Kogame, K., Kawaguchi, S. & Phang, S. M. 2003. Taxonomic Notes on Marine Algae from Malaysia. XI. Four Species of Rhodophyceae. *Botanica Marina* **46**: 534-47.
- Yarish, C., Breeman, A. M. & van den Hoek, C. 1984. Temperature, light, and photoperiod responses of some northeast American and west European endemic rhodophytes in relation to their geographic distribution. *Helgoland Meeresunters* **38**: 273-304.
- Zenetas, A., Gofas, S., Morri, C., Rosso, A., Violanti, D., Raso, J. E. G., Çınar, M. E., Almogi-Labin, A., Ates, A. S., Azzurro, E., Ballesteros, E., Bianchi, C. N., Bilecenoglu, M., Gambi, M. C., Giangrande, A., Gravili, C., Hyams-Kaphzan, O., Karachle, P. K., Katsanevakis, S., Lipej, L., Mastrototaro, F., Mineur, F., Pancucci-Papadopoulou, M. A., Esplá, A. R., Salas, C., Martín, G. S., Sfriso, A., Streftaris, N. & Verlaque, M. 2012. Alien species in the Mediterranean Sea by 2012. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part 2. Introduction trends and pathways. *Mediterranean Marine Science* **13**: 328-52.