

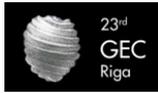


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Riga, Latvia

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Early Pleistocene charophyte flora from Dursunlu (Ilgın Basin, Central Anatolia, Turkey): Palaeoecological implications

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Key words: Central Anatolia, Charophyta, palaeoecology, palaeoenvironment, palaeolimnology, Palaeolithic.

A charophyte assemblage composed of nine species is described and illustrated, for the first time, in lower Pleistocene deposits at Dursunlu (district of Konya, Ilgın basin) in central Anatolia (Turkey). This flora has been recovered from 50 samples distributed along a 15 m thick stratigraphic section. The base of the section is constituted by organic clays and lignite beds attributed to palustrine environments. Overlying strata are composed of yellowish marls and limestones related to lacustrine facies. This sedimentary sequence ranges in age between 780–990 ka (Güleç et al. 2009).

The charophyte assemblage is dominated by *Chara hispida* Linnaeus 1753, which co-occurs in several samples with *Nitellopsis obtusa* (Desvaux in Loiseleur) Groves 1919. Other associated taxa include *Chara vulgaris* Linnaeus 1753, *Chara globularis* Thuillier 1799, *Chara* cf. *molassica notata* (Straub 1952) Soulié-Märsche 1989, *Chara* cf. *pappii* Soulié-Märsche 1979, *Chara* sp., *Lychnothamnus barbatus* var. *antiquus* Soulié-Märsche 1989, and *Sphaerochara* cf. *intricata* Trentepohl ex Roth 1797. These species occur in minor amount throughout the section. Moreover, corticated diplostichous and haplostichous thalli were found in several samples.

Several ostracod species, previously reported by Tuncer (2020), have been found associated to this flora. The ostracod assemblage is dominated by *Neglecandona angulata* G.W. Müller 1900, *Ilyocypris bradyi* Sars 1890, *Heterocypris salina* Brady 1868, *Cypris pubera* O.F. Müller 1776 and *Zonocypris membranae* Livaltal 1929. Moreover, a diverse assemblage of aquatic and terrestrial gastropods (*Valvata* sp., *Gyraulus* sp., *Islamia* sp., and *Vertigo* sp.),

bivalves (*Pisidium* sp.), fish (teeth and bones), mammal and bird bones have been extracted from several samples.

The discovery of this aquatic flora and the associated fauna sheds new light on the palaeolimnological conditions that prevailed in the lake during the early Pleistocene (lower Palaeolithic), in a glacial context (Günz glaciation).

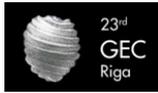
The dominance of *Chara hispida* associated to *Nitellopsis obtusa* in the lacustrine deposits suggest that permanent and stable freshwater conditions (alkaline and oligo/mesotrophic) prevailed in the palaeolake. Moreover, the presence of *Nitellopsis obtusa* in Dursunlu represents the first occurrence of this well-known boreal species in Anatolia.

Acknowledgements

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Charophytes and other subrecent microfossils of Lake Liman (Kızılırmak Delta, Samsun, Northern Turkey)

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Key words: Bafra Plain, Charophytes, Kızılırmak Delta, mollusc, Northern Turkey, ostracod.

Lake Liman is a lagoon located very close to the Black Sea coast at the northernmost part of the Kızılırmak Delta (Bafra Plain, Samsun) where Kızılırmak River disembogues to Black Sea. The lake is separated from the Black Sea by a very narrow dune barrier. With the drainage channel located to the south of the lake, excess water from the agricultural areas is discharged into the lake. Due to the periodical seawater inflow from the north and the continuous freshwater input from the south, two water layers with different chemical structures lead to the emergence of a mixed mesohaline zone (Soylu et al. 2011).

Lake Liman is a triangular lake and its average surface area is approximately 1.5–2.0 km². This very shallow (1–2 m depth) lake, contains abundant freshwater and brackish water plants. The salinity of the lake water varies between 1.96 and 4.06‰ (Soylu et al. 2011). The fauna and flora assemblages of the lake and its surroundings reflect a lagoon environment.

Within this study, a total of 23 grab samples were collected on the substrate (15–20 cm bottom sediments) along the littoral margin of the lake in autumn 2016 and water samples were also taken from several points. By the investigation of these bottom samples the following flora and fauna assemblages were identified: Charophyte remains; gyrogonites (*Lamprothamnium* cf. *papulosum*, *Chara aspera*, *Chara* sp. and *Chara* cf. *hispidata*), ostracods (*Heterocypris salina*, *Neglecandona neglecta*, *Cyclocypris ovum*, *Cypria ophthalmica*, *Physocypris kraepelini*, *Cyprideis torosa*, *Limnocythere inopinata*, and *Darwinula stevensoni*), molluscs (*Gyraulus* sp., *Ecrobia* sp., *Physa* sp., *Theodoxus*

sp., *Valvata* sp., *Oxyloma* sp. and *Succinea* sp.), benthic foraminifers (*Ammonia* sp. and *Elphidium* sp.). Moreover, submerged plants (*Potamogeton perfoliatus*, *Potamogeton pectinatus* and *Potamogeton nodosus*) were also identified in previous studies (Demirkalp et al. 2010). Meanwhile water tick and fish teeth were also observed.

The determined ostracod species are known from both recent water bodies and Quaternary sediment records of Anatolia and its vicinity. *D. stevensoni* (cosmopolitan), *H. salina*, *C. torosa*, *P. kraepelini* and *L. inopinata* are widespread in faunal area while *C. ovum* and *C. ophthalmica* are only recorded from the Holarctic (Nearctic and Palearctic) and *N. neglecta* is only known from the Palearctic ecozone.

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Anthropogenic driven occurrence and disappearance of *Chara* spp. over the last 2000 years in Trikātas lake, northern Latvia

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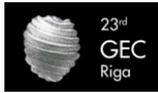
Key words: carbonates, land use, palaeoecology, palaeolimnology, soil erosion.

Anthropogenic impacts on lake ecosystems have increased substantially towards the present. However, the strength and timing in most cases are not evaluated in detail, missing valuable information on the response and recovery of an aquatic system. Here, we studied a hemiboreal lake Trikātas (Latvia, NE Europe). We analyzed lake sediment by various proxies (carbon/total nitrogen ratio, inductively coupled-optical emission spectroscopy, plant macroremains, pollen, non-pollen palynomorphs, ¹⁴C radioactive dating) and our results reveal that the anthropogenic land use driven erosion and accompanied calcium carbonate (CaCO₃) matter influx favored the abundance of *Chara* spp. in Lake Trikātas since 500 CE. Such algae form the protected specific habitat-type (H3140) of the European Union. However, currently specific submerged macrophyte *Chara* habitat-

type diminished almost entirely due to increased nutrient input, phytoplankton blooming, hypertrophic conditions and reduced light availability in the water column. The continued land use practices led to a switch in organic matter source in the lake from macrophytes to solely algal origin. The current study underlines the need of additional methods used to detect the sensitivity of lake ecosystem to external disturbances such as minor anthropogenic land use that might not necessarily be apparent in more traditional analyses such as modern ecological monitoring actions.

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Comparison of *Chara filiformis* and *Chara contraria* oospore morphological parameters

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Key words: *Chara*, morphometry, oospores, oospores characters, stoneworts.

Chara filiformis (A. Braun ex Kütz. 1845) and *Chara contraria* (Hertsch 1855) are usually clearly morphologically differentiated species usually found growing together in the common stands. *C. filiformis* thallus morphology varies only a little, while several differing forms of *C. contraria* are found in nature, with shortened or incomplete branchlets that might lead to misidentification of species. Also, no genetic differences have been found between these two species so far (Nowak et al. 2016; Schneider et al. 2016). The recent morphometric studies on fructifications of both species are quite scarce (e.g. Urbaniak, Gąbka 2014). In order to supplement the current data and determine whether features of oospores could be useful for species differentiation, oospore morphological parameters of *C. filiformis* and *C. contraria* were studied.

Oospore specimens for study was collected from lakes and herbarized material in Lithuania and Latvia territories. Dry oospores were decalcified according to Holzhausen et al. (2015) and analysed in wet condition using a NICON SMZ800 microscope with software NIS – Elements D. The number of ridges was counted, the largest polar axis (LPA, length) and the largest equatorial diameter (LED, width) were measured, and the index of isopolarity (ISI = LPA/LED × 100) was calculated (Soulie-Marsche, Garcia 2015).

The length (LPA) of *C. filiformis* oospores examined ($n = 826$) ranged from 530 to 940 μm (mean $684 \pm 54.9 \mu\text{m}$), when the width (LED) range was 290–530 μm (mean $399 \pm 37.5 \mu\text{m}$). The ISI varied within the range of 131–293 (mean 173 ± 22.4). The number of ridges ranged from 8 to 14 (mean 10.9 ± 1.2). The length (LPA) of *C. contraria* oospores ($n = 742$) ranged from 510 to 820 μm (mean $660 \pm 51.3 \mu\text{m}$), whereas width range was 250–480 μm (mean $377 \pm 39.5 \mu\text{m}$). The ISI varied from 128 to 272 (mean 177 ± 26). The number of ridges ranged from 8 to 15 (mean 11.9 ± 1.1). According to K–W test, oospores of both species differed mainly in the number of ridges ($H = 253.5$, $p < 0.01$), less in width ($H = 113.3$, $p < 0.01$) and in length (H

$= 76.8$, $p < 0.01$) and insignificantly according to the index of isopolarity.

Significance of differences between morphological parameters of *C. filiformis* and *C. contraria* oospores from eight sampling sites, where both species were found growing together was tested using one-way ANOSIM test (Clarke 1993). In these samples tested morphometric parameters of oospores were found to be different, but overlapping only in one pair $R > 0.5$ ($p < 0.01$). According to K–W test, the oospores of both species from 6 different samples differed mainly by number of ridges ($H = 14.3$ – 57), and oospores of one sample differed by all characters.

Despite measured differences in morphological parameters of *C. filiformis* and *C. contraria* oospores these characters are strongly overlapping and therefore should not be used for species separation when oospores are the only available material for study.

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Preliminary Red List of charophytes in Serbia

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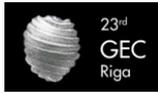
Key words: charophytes, Red List, Serbia.

According to the latest checklist, updated in December 2022, 25 species of charophyte algae are registered on the territory of Serbia. These data are gathered in a number of projects and finally integrated within the project related to the production of Red books and Red Lists of flora, fauna and fungi on the territory of the Republic of Serbia. The crown results of this work are preliminary Red Lists for all organism groups, including charophyte algae. The lists were produced in accordance with IUCN criteria, using an application especially created for the purpose of this project. According to IUCN criteria, all charophyte species registered on the territory of Serbia are Red Listed, that is, belong to one of the three categories – Critically Endangered (CR) – ten species, Endangered (EN) – seven species, and Vulnerable (VU) – seven species, except one species which

is Data Deficient. The main factors endangering the flora of charophytes in Serbia are habitat fragmentation and desiccation, which were accordingly used as the main criteria to assess charophyte species status. Here we present the process as well as the results of the assessment and discuss the main factors of endangerment.

Acknowledgements

This work was supported by the project “Data and services related to continuation of the production of Redbooks and Red Lists of flora, fauna and fungi on the territory of the Republic of Serbia” funded by Ministry of Environmental protection of the Republic of Serbia (2016-2021), as well as by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200026; Grant No. 451-03-68/2022-14/200178).



Diversity and ecology of charophytes in Vojvodina (Serbia) along the gradient of salinity

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Key words: charophytes, chlorophyll *a*, conductivity distribution, diversity, ion composition, nutrients.

In 2021 a charophyte survey was conducted in the southern part of the Carpathian Basin, the Vojvodina province (Serbia). We investigated 14 waterbodies that were different in origin (natural, natural degraded, artificial) and were chosen along the gradient of salinity. Furthermore, waterbodies were characterized according to their ion composition: cations (potassium, sodium, calcium, magnesium) and anions (carbonate, bicarbonate, chloride, sulfate). Several other environmental parameters were investigated, such as temperature, pH, conductivity, nutrient content (ammonia, nitrites, nitrates, total phosphorus) and chlorophyll *a*. A gradient of salinity, calculated from electrical conductivity (Boros et al. 2013), ranged from freshwater to mesosaline (Hammer 1986). Magnesium was the dominant cation in seven waterbodies, frequently in combination with calcium, while sodium prevailed in three waterbodies. Bicarbonate made up a major portion of anions in nine, sulfate in three, and chloride in two waterbodies. Altogether, seven charophytes species were found: *Chara canescens*, *Chara tenuispina*, *Chara connivens*, *Chara vulgaris*, *Chara globularis*, *Chara hispida* and *Chara papillosa*. All species were found in subsaline waters, except *C. papillosa*, found only in freshwaters. Only three species, *C. tenuispina*, *C. vulgaris* and *C. canescens*, were found in hyposaline waters. We found no charophytes in mesohaline

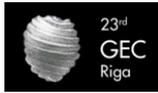
waters. Extremely rare male individuals of *C. canescens* were found in Pečena Slatina, a natural saline pond. This species showed a preference for hypertrophic and alkaline hyposaline aquatic habitats, with a different pattern of ionic predominance (sodium, magnesium, sulfate, bicarbonate, chloride). All species but *C. connivens* and *C. papillosa* were found in eutrophic waters. In addition, two species, *C. hispida* and *C. canescens*, showed tolerance to higher values of nitrates (> 3 mg L⁻¹). In conclusion, we could state that inland saline habitats, despite their high productivity, represent important habitats for charophytes, especially for rare species such as *C. canescens* and *C. tenuispina*.

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Charophyte island-biogeography in the Cretaceous Tethyan Archipelago

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Key words: Characeae, Clavatoraceae, Early Cretaceous, Mesogea, paleobiogeography, Porocharaceae.

Most of present-day Europe and North Africa formed during the Cretaceous a large archipelago in the middle of the Tethys Sea, called the Cretaceous Tethyan Archipelago. This was a large tropical sea, east-west oriented, which included many islands of different sizes and paleogeographic locations. Some were large and close to the mainland, while others were quite distant and isolated in the middle of this ocean.

This study explores the island-biogeography based in charophytes from this archipelago. For this purpose, the charophyte biodiversity from the timespan between 129.4 and 126.3 Ma (late Barremian), was documented from four islands. These were (1) Iberia (present day Spain and Portugal), a large island, ca. 250 km away from the mainland of Laurasia (parts of present-day North America and Eurasia) and 400 km far from Gondwana (in particular, most of present-day Africa); (2) the Subalpine Chains and Jura Mountains (the area limiting present-day France and Switzerland), which was a small island located 200 km away from Laurasia; (3) the Tunisian Island that was a small island located more than 600 km away from Gondwana; (4) the Lebanese Island, a small one, 100 km away from the Gondwanan continent. A reliable late Barremian charophyte fossil record is available from these four islands, which allows reducing the biases due to insufficient record that may occur in other paleo-islands, such as Moesia-Dacia, present day Romania and Hungary.

The results show that the largest biodiversity corresponds to Iberia, with up to 17 charophyte species recorded, some of them unique from this island, i.e., possibly endemic. This contrasts with the smaller islands: i.e., the Subalpine Chains and Jura Mountains and the Tunisian islands that recorded up to nine species, while the Lebanese Island recorded only five (Pérez-Cano et al. 2020 and references therein).

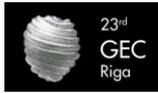
These numbers fit well with the generally well-accepted rule of MacArthur and Wilson (1963) that large islands hold a larger biodiversity. However differences between isolated islands and islands close to continents are not well-defined here. Possible explanations for that could be related to charophyte paleoecology, the number of depositional environments recorded in each island, the paleoclimate, or the differential dispersal routes.

Acknowledgements

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Double triplostichous cortication, a new type of cortication found in fossil charophytes

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Key words: Charophyta, *Charaxis*, Clavatoraceae family, Early Cretaceous, Iberia.

Living charophytes show three main types of cortication of their internodes: haplostichous, diplostichous and triplostichous. Primary cortical cells usually depart from nodal cells beneath the branchlets, while secondary cortical cells develop from the primaries at a distal position of the

internode. However, these three types of cortication have not always been the rule in charophyte evolution. A new type of cortication was described after studying tens of thin sections from charophyte-rich limestones deposited in ancient shallow temporary lakes from the Barremian (ca. 125 Ma) of the Maestrat Basin, Iberian plate.

The thallus of *Echinochara lazarii*, known in the fossil record as the parataxon *Charaxis spicatus* (Fig. 1), presents two types of primary cortical cells. Large primary cortical cells (lp) depart directly beneath the branchlet, while thinner and shorter primary cortical cells (sp) depart at each side of the former. Large primary cortical cells form distally an expanded area, called a cortical node (cn), from which two secondary cortical cells grow distally (dgs), and two smaller secondary cortical cells develop upwards, i.e., proximally (pgs), covering the larger primaries. This results in a triplostichous isostichous cortication distally from the cortical node, while proximally it is triplostichous anisostichous. The name double triplostichous cortication was proposed for this type of cortication (Pérez-Cano et al. 2020). This new type of cortication has been only described in the extinct family Clavatoraceae, showing that fossil charophytes were much more diverse than extant representatives in terms of Bauplans of their thalli, as it is long known for fructifications.

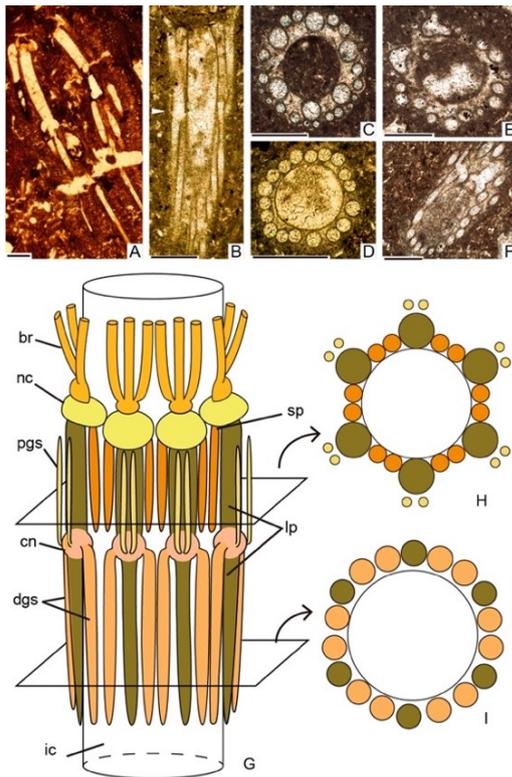


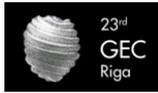
Fig. 1. A–F, reconstruction of the *C. spicatus* thallus. A–B, longitudinal sections, cortical node arrowed. C–E, transversal sections. F, tangential section. Scale bar 250 μ m. G–I, reconstruction of the double triplostichous cortication (modified from Pérez-Cano et al. 2020).

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Pérez-Cano J, Bover-Arnal T, Martín-Closas C. 2020. Barremian charophytes from the Maestrat Basin (Iberian Chain). *Cretaceous Res.* 115: 10454.



Early Cretaceous charophyte-rich wetlands from Iberia

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Key words: Barremian, Charophyta, charophyte paleoecology, lacustrine carbonate facies, Maestrat Basin.

Iberia (present day Spain and Portugal) was a large island from the Cretaceous Tethyan Archipelago with a paratropical climate. In a general context of tectonic rifting lowland wetlands developed, which hold one of the most biodiverse charophyte floras in the Early Cretaceous World. A study through of one of the most complete sections of the non-marine record from the Barremian stage (129.4–121.4 Ma) in the Maestrat Basin (East Spain) resulted in the characterization of three large types of wetlands that succeeded one each other.

(a) Freshwater and alkaline coastal lakes and ponds with poor clastic inputs developed in the earliest Barremian. They hold a vegetation dominated by the Clavatoraceae, with up to eight species recorded, but also including rare Porocharaceae and early Characeae. Shallower lake margins, submitted to certain wave energy were dominated by *Munieria grambastii thalli*, probably corresponding to a *Clavator* species. Relatively deeper lacustrine belts showed a diverse vegetation formed of species belonging to genera *Atopochara*, *Clavator*, and *Asciadiella*. Locally, *Hemiclavator*-dominated assemblages occurred within this wetland.

(b) Freshwater lakes with high clastic input and brackish coastal lakes developed mainly during the late early Barremian in a context of marine transgression. In this type of wetlands freshwater lakes hold a vegetation formed by *Echinochara lazarii*, *Globator maillardii trochiliscoides*, *Atopochara trivolvis triquetra*, *Hemiclavator neimongolensis neimongolensis* and *Clavator calcitrapus*. Two types of brackish floras occurred. Those developed in lakes with low clastic influence were exclusively formed by *Porochara*

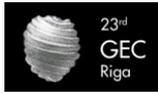
maestratica. In contrast, the brackish floras formed under conditions of high clastic influence were wholly formed by *E. lazarii*.

(c) A coastal mudflat system was developed during the late Barremian in the context of a marine regression. Brackish lakes with high clastic influence mainly contained *E. lazarii*, *A. trivolvis* and *C. harrisii*. Small populations of biostratigraphically-interesting species, such as *Clavator grovesii jiuquanensis*, *Asciadiella cruciata*, and *Pseudoglobator paucibracteatus* did also occur. *E. lazarii* formed occasionally monospecific assemblages. Temporary freshwater lakes with low clastic input mainly contained *E. lazarii*. Locally, *A. cruciata* was recognized.

The evolution of these wetlands basically depended on climatic constraints probably related to orbital cycles. Water table oscillations, enhancing the development of shallower or deeper lakes with distinct charophyte floras, depended mainly on short-term climatic cycles. Medium-scale cycles provoked changes in the precipitation and seasonality in the hinterland that controlled the erosion and clastic inputs into the lakes. The largest-scale cycles were related to sea-level oscillations that controlled marine transgressions and regressions and the extension of freshwater or brackish settings.

Acknowledgements

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Charophytes in the Permian of the Pyrenees

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Key words: floodplain-lacustrine settings, Late Palaeozoic, non-marine red-beds.

The Permian (299–252 Ma) record from the Southern Pyrenees (Catalonia, NE Iberian Peninsula) is composed of a succession of non-marine red-bed deposits. During the last decades the research upon these rocks has mainly focused on sedimentological, stratigraphic, and geochemical analysis, as well as vertebrate palaeontology (both ichnites and body fossils), and palynology. However, micropalaeontological studies, including charophytes, have not been performed so far.

Here we present the first description of charophyte remains from the Pyrenees. The samples come from the Cadí subbasin of the Pyrenean rift system and were found in the Upper Red Unit (URU). It is composed of fluvial to lacustrine mudstones, sandstones, and carbonates (the latter representing short-lived ponds) which are interpreted as fluvial floodplains and in playa-lakes (e.g., Mujal et al. 2017).

The charophyte remains described herein were found on top of 0.2–0.5 cm-thick carbonates and consist of casts of small portions of thalli and a single gyrogonite. Thalli portions are corticated, but their assignation to a specific cortication is not possible. A structure resembling a gyrogonite is found with these thalli portions. It consists of a lateral impression that shows a prolate morphology, being 510.13 µm long and 466.00 µm wide, having an isopolarity index (ISI) of 109.47. It also has a large and wide apical neck. Spiral cells are partially preserved. The basal plate is unknown.

Similar gyrogonites were associated to the genus

Leonardosia Sommer, also being associated with thalli portions similar to those described herein (Faria, Ricardi-Branco 2009). However, more, and better-preserved gyrogonites are needed to confirm the occurrence of *Leonardosia* in the Pyrenees. The small size of the thalli portions and the scarcity of gyrogonites suggest that charophytes were deposited after transport, which provoked the disarticulation of the different plant organs.

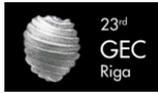
The searching and characterization of the Permian charophytes from the Pyrenees will continue in the following years. The aim of these studies is to gather biostratigraphical and palaeoenvironmental information.

Acknowledgements

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Evolution of a novel mechanism for high rates of Na⁺ export from cells in salt-tolerant *Chara*

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Key words: ATPase, evolution, Na⁺ transport, salt tolerance.

Chara species vary in their salt tolerance, from obligate freshwater species to the hypersaline tolerant. A critical aspect of salt tolerance is preventing accumulation of Na⁺ in the cells. In comparing salt-sensitive *Chara australis* (RBr) and salt-tolerant *Chara longifolia* (Rob), we showed that while *C. longifolia* has a lower Na⁺ entry rate (Hoffmann et al. 1989), the main difference between the two species is the its high rate of Na⁺ export (Kiegle, Bisson 1996). This ability is inducible; freshwater *C. longifolia* exports Na⁺ export at a rate three times that of *C. australis*, but when cultured in high salinity, that factor increased to 30 times. Physiological experiments indicated the mechanism of transport was Na/H antiport (Yao et al. 1992; Kiegle, Bisson 1996).

We tested this by measuring expression of genes associated with relevant functions in land plants: HKT (Na⁺ permeability), NHA (Na⁺ antiport) and AHA (H⁺ pump). We predicted that *C. longifolia* cultured in salt water would have lower HKT and higher NHA and AHA expression.

These predictions were at best only weakly supported (Phipps et al. 2021). The only clear correlation with salinity tolerance was the gene ENA, an ATPase that exports Na⁺ directly. This gene does not occur in angiosperms, and was previously only known in two moss species and the alga *Tetraselmis* (Phipps et al. 2021). It does not occur in

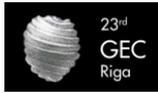
C. australis or *C. braunii* (Phipps et al. 2021; Nishiyama et al. 2018). Its expression increases 16 000 times in salt-cultured plants, compared to FW-cultures. To understand its evolution, we need to know whether it occurs in other salt-tolerant *Chara* spp.

Acknowledgements

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Are the values of stable carbon and nitrogen isotope composition of *Nitella flexilis* C. Agardh, 1824 differ in softwater and hardwater lakes?

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Key words: $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, charophytes, macrophytes, organic matter, water chemistry.

The values of stable carbon and nitrogen isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of the aquatic plant have a huge range along with different aquatic ecosystems, especially when we focus on $\delta^{13}\text{C}$ (from -50 to $+0.4\text{‰}$; Herzsuh et al. 2010). The $\delta^{15}\text{N}$ of macrophytes also varies considerably with the range from -15 to $+20\text{‰}$ (Douglas et al. 2022). Those observed in different aquatic ecosystems values might be related to multiple variables of water and sediment chemistry, such as pH and nutrient concentration.

Nitella flexilis C. Agardh, 1824 has a broad ecological amplitude but often creates underwater meadows in oligo-mesotrophic softwater lakes with low water pH. However, the well-developed communities of this charophyte species might be found in more hardwater and eutrophic lakes (Urbaniak and Gąbka, 2014). Due to this environmental plasticity of *N. flexilis*, there is an excellent opportunity to check if the differences in the water chemistry of softwater lakes and lakes with higher Ca^{2+} concentration have an impact on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *N. flexilis* organic matter. Thus, (i) the main aim of this study was to check if the values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ differ in soft and hardwater lakes. Additionally, (ii) we wanted to check which environmental variables have the most considerable influence to obtained isotopic values of *N. flexilis*. To achieve the studied aims, we compared the results of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *N. flexilis* investigated in 23 lakes in Poland (mainly from the Pomerania region) in light of other water variables. The study included hardwater lakes (Ca^{2+} concentration from 21.9 to 47.4 mg L^{-1}) investigated in the middle of the growing season of 2008–2010 and 11 softwater lakes (Ca^{2+} concentration from 2.2 to 12.4 mg L^{-1}) investigated in 2020. The classification of softwater lakes followed the recommendation of Murphy (2002).

In our study, we did not find statistically significant differences between obtained $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *N. flexilis* from those two types of lakes. Furthermore, the

$\delta^{13}\text{C}$ in softwater lakes varied more (ranging from -33.12 to -14.75‰) compared to hardwater lakes (-30.06 to -20.05‰). The $\delta^{15}\text{N}$ values slightly differ in investigated groups of lakes and varied from -7.69 to $+2.88\text{‰}$ in hardwater and -5.49‰ to $+3.18\text{‰}$ in softwater lakes. The Principal Components Analysis (PCA) was performed to check the differences between both groups of lakes in the light of the other water physicochemical parameters. The results of the PCA analysis clearly segregated two groups of investigated lakes. Among investigated variables, pH, Ca^{2+} , conductivity, dissolved inorganic carbon (DIC), and total phosphorus (TP) were the most differentiating parameters in the two groups of lakes studied. The observed differences in those variables were statistically significant (U Mann-Witney test $p < 0.05$). When comparing all the obtained $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with water's physicochemical parameters, we found several relationships. The values of *N. flexilis* $\delta^{13}\text{C}$ were positively related to photosynthetic active radiation (PAR) and negatively correlated with depth and concentration of dissolved organic carbon (DOC). The $\delta^{15}\text{N}$ values had a moderate positive relationship with total nitrogen (TN) and a low negative relationship with Ca^{+2} concentration.

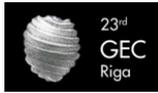
To sum up, our findings indicated that the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are rather lake-specific than follow some clear pattern across two different lake types. These findings might enhance the need for more profound studies to determine which factors not included in this study have great importance in shaping the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *N. flexilis* and other submerged plants. This knowledge might be helpful, e.g., in studies where we want to estimate the potential source of the sediments by applying source mixing models or for the better paleolimnological studies of lacustrine sediments, especially those from low productivity as softwater ones.

Acknowledgements

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Seasonal and regional variability of carbonate precipitation in charophytes and vascular plants

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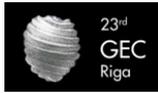
Key words: carbonate precipitation, charophytes, seasonal variability, vascular plants.

A majority of aquatic macrophytes use soluble bicarbonates as a source of CO₂ in photosynthesis. As a result, calcium carbonate is precipitated and stored in sediments. This way a significant amount of carbon may be sequestered in freshwater ecosystems. We aimed to compare carbonate production by charophytes and vascular plants in two Polish regions differing in environmental and climatic conditions, Lubuskie Lakeland (W Poland, lower trophic and milder climate) and Masurian Lakeland (NE Poland, higher trophic and cooler climate). In each region, six hardwater lakes were selected, three with vegetation dominated by charophytes and three by vascular macrophytes. Each lake was sampled four times, in three sites: in summer and autumn of one year, then in spring and summer of the following year. In each site, the biomass samples were analysed for dry weight and calcium carbonate content. Additionally, physical and chemical parameters were analysed in water taken from above the plants.

Charophytes were significantly more effective in dry weight production and CaCO₃ precipitation than vascular plants. Although a season-to-season variability of dry weight and calcium carbonate content was similar in both regions studied, for both the charophytes and vascular plants significantly higher values were found in lakes of W Poland. Most of the water parameters analysed differed between the regions. Moreover, in each region, a different set of significant correlations between the calcium carbonate content and water properties was found. The results obtained showed that not only the physical and chemical parameters of water but also the climatic conditions are of great importance for differentiation of the biomass structure of aquatic vegetation.

Acknowledgements

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Charophyte palaeoecology of the Middle Miocene Vallès-Penedès and Vilanova basins (Catalonia, Spain)

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Key words: Characeae, Neogene, palaeoecology, palaeoenvironment, palaeolake, Vallès-Penedès Basin.

The Vallès-Penedès Basin is a well-known Miocene basin located at the eastern Iberia, famous by its dense and continuous land mammal record (Casanovas-Vilar et al. 2016). This study describes and illustrates for the first time a diverse charophyte assemblage from the Vallès-Penedès and the nearby Vilanova basins.

Three outcrops have been systematically sampled for charophytes and facies analysis: els Casots (Subirats), Vilobí del Penedès and Mas de l'Alonso-el Pi Gros (Vilanova i La Geltrú). Rocks at these localities range in age from the Langhian to the Serravallian (~16–11 Ma). The charophyte assemblage is constituted by 10 well-preserved species showing a characteristic distribution in three palaeoenvironmental settings.

(1) Shallow lake claystones occur in the three studied localities and have yielded *Sphaerochara ulmensis* Straub 1952) Grambast 1962, *Chara vulgaris* Linnaeus 1753, *Chara molassica* var. *notata* (Straub 1952) Soulié-Märsche 1989, *Lychnothamnus barbatus* var. *antiquus* Soulié-Märsche 1989, *Lychnothamnus* sp., *Nitellopsis (Tectochara) merianii* (Al. Braun ex Unger 1852) Grambast and Soulié-Märsche 1972, and *Nitellopsis* sp. This charophyte flora is unevenly distributed owing to local palaeolimnological conditions. Mesotrophic to eutrophic conditions dominated the Langhian palaeolake at els Casots, while more oligotrophic and stable conditions might have prevailed at the upper part of the Vilobí del Penedès (Langhian) section and at Mas de l'Alonso (Serravallian). Abraded tests of benthic foraminifera occur associated to this flora suggesting that these freshwater lakes were located near the coastline.

(2) Facies related to coastal salinas (gypsum beds alternating with claystones) are found at Vilobí del Penedès (Langhian) and have yielded monospecific populations of *Lamprothamnium papulosum* (Wallroth 1833) Groves 1916 indicating the prevalence of waters with fluctuating salinities from 20 to 40‰.

(3) The charophyte flora extracted from permanent lake facies (marls and limestones) at el Pi Gros (Serravallian) is dominated by *Chara hispida* Linnaeus 1753 which occurs associated to *Chara* sp. and ostracods of *Leptocythere* gr. *psammophila* Guillaume 1976 and *Heterocypris salina* Brady 1868. This assemblage indicates the prevalence of oligohaline and well oxygenated waters.

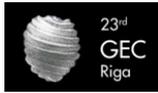
This study sheds new light on the palaeolimnology of wetlands in the coastal basins of NE Iberia during the Middle Miocene.

Acknowledgements

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Charophytes in warm springs on Svalbard (Spitsbergen): DNA barcoding identifies *Chara aspera* and *Chara canescens* with unusual morphological traits

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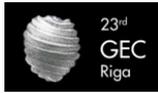
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Key words: *Chara*, DNA barcoding, Svalbard, warm springs.

The Troll springs are extremely remote warm springs on the arctic island of Svalbard (Spitsbergen). Charophytes were collected in the years 1910, 1912, 1958, 1992/1993, and 2018. Since 1910, the samples were identified by different experts and believed to belong to either *Chara aspera* or

Chara canescens. However, since the *Chara* samples showed very unusual morphological traits, there were doubts with respect to species identity. We used DNA barcoding and found that there actually occur two *Chara* species in the Troll springs: *C. aspera* and *C. canescens*.



Species concepts for charophytes – still a battlefield or some hope for agreements?

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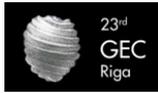
Key words: charophytes, species concepts.

For charophytes, two concepts for delineation of recent species are in use, complicating taxonomy when compiling large-scale data. Often cited as Wood's macrospecies concept and classical microspecies concept they differ for far more than just the level a taxon is ranked to the species level. Moreover, the fast development of molecular techniques allowed for analysis of population genetics, which are increasingly used for supporting species delineation hypotheses.

The talk will introduce into the world of species concepts, explaining the scientific basis for the recently accepted three basic horizontal as well as the phylogenetic

approach. With regard to the fact, that in practice pluralistic concepts are applied, the options and limitations of the different concepts for charophyte systematics will be discussed, illustrated by examples. The main focus will be "species delineation" and "lower taxonomic ranks" in order to address the still ongoing debate about micro- vs. macrospecies concept.

The talk is thought as a supporting statement for application of the "species-are-hypothesis" concept for charophyte taxonomy, hopefully paving the way to case-specific agreements instead of fundamental debates.



Status report “European charophytes”

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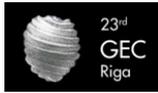
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Key words: charophyte biogeography, survey project.

Since > 5 years, charophytologists are compiling the first pan-European multi-authored publication about all aspects of European charophytes. Irrespective of many obstacles, the project is now in its final stage and being clearly a product of GEC, the status as well as the timeline for the

very last steps will be presented briefly. This presentation, however, will not include any details with respect to the content of the chapters. Such unpublished material maybe in part presented by the respective authors themselves during the conference.



Interactions between Charales (Stoneworts) and waterbirds in Europe

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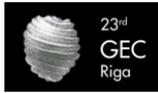
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Key words: Charophytes, grazing, oospore dispersal, water transparency, wildfowl.

The Charales is an order of multicellular green algae, numbering about 300 species worldwide. Their growth form comprises distinctive cylindrical stems, rooted in substrates, with whorls of lateral side branches, with thalli that can range from a few centimetres to two metres long, frequently dominating the sediments of lake-beds and slow flowing rivers down to 60 m where light penetration allows. Their nature, density, extent and biomass often make them important constituents of aquatic systems, as substrate, habitat and sources of food for other aquatic organisms. Preferring clear fresh- or occasionally brackish waters of low nutrient status, the Charales have therefore been adopted as useful indicators of the quality of oligo- to mesotrophic aquatic systems, because they are among the most sensitive of submerged macrophytes to nutrient enrichment, disappearing with eutrophication and from turbid water columns. Charales have a long growing season and half the fibre content of most other submerged angiosperm macrophytes, as well producing abundant oospores, all of which make them highly attractive to herbivorous waterbirds that both graze thalli and consume the reproductive disseminules of the plants. Yet other waterbirds exploit the biomass of aquatic invertebrates that live within their submerged canopy. Their often dense, compact growth forms offers high densities of both above substrate green biomass and habitat for associated

aquatic invertebrate communities, attracting conspicuous aggregations of browsing, dabbling, up-ending and diving waterbird species exploiting these rich feeding opportunities. In this review, we attempt to document the range and nature of waterbird species showing aggregative responses to Charales-dominated systems in Europe and the relationships between the species involved and their specific feeding ecologies. From a nature conservation perspective, we look for examples of how local restoration of aquatic ecosystems have resulted in the re-establishment of local Charales dominated communities and the concomitant effects on associated waterbird abundance and community composition. We also review the nutritional and energetic value of Charales to herbivorous waterbirds relative to other sympatric angiosperm macrophytes to try to understand their apparent disproportional value to supporting local waterbird densities. Given that locally abundant migratory grazing waterbirds can apparently rapidly remove all above ground biomass of Charales in autumn, we also review the documented impacts of waterbirds on the dynamics of these important food plants in aquatic systems. Finally, we consider the value of waterbirds as dispersive agents to the Charophytes, given the range of studies that apparently confirm the ability of endozoochorous dispersal of these iconic algal species by often long-distance migratory waterbirds.



Middle Jurassic charophytes. New data for a poorly known period in charophyte evolution

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Key words: biogeography, Characeae, Clavatoraceae, evolution, Middle Jurassic, Porocharaceae.

Micropalaeontological investigation of Middle Jurassic continental strata from north Gondwana (Tunisia and Morocco) and south Laurasia (France) revealed new data deciphering an enigmatic stage in the evolutionary history of Mesozoic charophytes, between Triassic radiation of 'Porocharaceae' and the Late Jurassic–Early Cretaceous radiation of Clavatoraceae in the Tehyan domain.

The charophyte assemblages from the Middle Jurassic of Tunisia, which are composed of 13 taxa (*Porochara fusca*, *Porochara schudackii*, *Porochara kimmeridgensis*, *Porochara westerbeckensis*, *Porochara douzensis*, *Porochara obovata* var. *gondwanensis*, *Auerbachichara saidakovskiyi*, *Auerbachichara tataouinensis*, *Stenochara* sp., *Stellatochara* aff. *subsphaerica*, *Aclistochara longiformis*, *Aclistochara africana*, and *Mesochara voluta*), challenged previous thoughts that considered this time interval as uniformly species poor (Tiss et al. 2019). On the contrary this assemblage showed that at that time some of the last occurrences and first appearances of genera of the Porocharaceae and Characeae occurred, which suggests that the Middle Jurassic witnessed a great deal of active evolutionary change for charophytes.

New evidence from the Middle Jurassic of Morocco and France, yielding rich and diverse charophyte assemblages with species belonging to genera *Porochara*, *Feistiella*, *Mesochara*, *Aclistochara*, *Auerbachichara* and *Echinochara*, give further arguments to support this hypothesis (work in progress). Accordingly, it can be concluded that the genera *Porochara* and *Auerbachichara* have been the main components of this flora, overcoming the Early Jurassic Crisis, which is a major gap in the history of post-Paleozoic

charophytes. Also the occurrence of *Feistiella*, *Mesochara* and *Echinochara* in the Middle Jurassic represents the oldest records of these genera and of the family Clavatoraceae. It is worth noting that the primitive form of *Echinochara* discovered in the Bathonian of France, would predate ca. 10 Ma the origin of this family in comparison with what was previously thought (upper Oxfordian according to Schudack 1993).

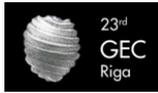
From the palaeobiogeographic point of view, the new data show that the Peri-tethyan palaeogeographic domain (present-day north Africa and south Europe) was an area of active speciation and biogeographic exchange between Gondwana and Laurasia, with species that migrated later to reach the West (North America), East (China) of Laurasia, and South of Gondwana (India).

Acknowledgements

This is a contribution to project IBERINSULA (PID2020-113912GB-I00) funded by MCIN/AE/10.13039/501100011033 and the European Regional Development Fund (ERDF), and the project I+D+i (PID2020-117289GB-I00) funded by MCIN and CERCA Programme /Generalitat de Catalunya.

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Charophytes across the Jurassic–Cretaceous boundary from the Middle Atlas, Morocco (North Africa)

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Key words: biogeography, biostratigraphy, charophytes, Gondwana, Late Middle Jurassic–Early Cretaceous red beds, Middle Moroccan Atlas, North Africa.

Litho-biostratigraphic analysis of the Late Middle Jurassic–Early Cretaceous marginal-marine red beds called “Aït Bazza Formation” (composed of three Members: AB 1 Mb, AB 2 Mb and AB 3 Mb), from the Marmoucha syncline in the eastern part of the folded Middle Atlas of Morocco, yield rich and diverse charophyte flora of higher biostratigraphic and palaeogeographic interest :

The lower part of the Middle AB 2 Member, Callovian–Kimmeridgian in age is mainly dominated by the porocharacean species *Feistiella bijuensis*, *Feistiella atlasensis*, *Porochara kimmeridgensis* and *Porochara fusca*, associated to the characean species *Mesochara harrisii*, as well as to a primitive form of the clavatoracean *Nodosoclavator* sp.

The upper part of the Middle AB 2 Member, Tithonian in age, was dominated by clavatoracean species *Globator maillardii praecursor*, *Dictyoclavator ramalhoi*, *Nodosoclavator bradleyi* and *Nodosoclavator* sp., associated to *Porochara kimmeridgensis*, *Feistiella bijuensis*, and *Mesochara harrisii*.

The third assemblage described from the Upper AB 3 Member, early Berriasien in age, is mainly composed of the clavatoracean species *Globator maillardii*, *Globator maillardii protoincrassatus*, *Clavator reidii pseudoglobatoroides*, *Clavator grovesii grovesii*, *Nodosoclavator bradleyi* and *Clavator* sp., associated to *Porochara kimmeridgensis*, *Feistiella bijuensis*, and *Mesochara harrisii*. This assemblage is certainly correlated with the European charophyte biozone *Globator maillardii maillardii* of Riveline et al. (1996).

The biostratigraphic results together with those from the associated ostracod fauna, will allow for a stratigraphic correlation with lateral equivalents strata from surrounding basins of the Middle and High Atlas of Morocco, which will give new insights on the tectono-sedimentary and palaeogeographic evolution of the studied area during the Jurassic–Cretaceous boundary. In turn, these results will allow correlation of the Late Jurassic–Early Cretaceous continental deposits of the old megacontinent of Gondwana (here represented mainly by charophyte assemblages from Morocco and Tunisia) with the mega-continent of Laurasia (represented by charophyte assemblages from France, Spain, Switzerland and Germany).

The occurrence of many clavatoracean taxa in the studied Aït Bazza Formation, notably *Nodosoclavator bradleyi*, *Globator maillardii praecursor*, *Globator maillardii maillardii*, *Globator maillardii protoincrassatus*, *Clavator reidii pseudoglobatoroides*, and *Clavator grovesii grovesii* reinforces the hypothesis that Late Jurassic (Tithonian) represents the first main pulse of biogeographic expansion of the Clavatoraceae, also in the southern coast of the Cretaceous Tethyan Archipelago.

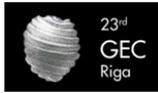
Regarding phylogenetic aspects, the presence of *Nodosoclavator* in the late Middle Jurassic (Callovian) and the occurrence of a primitive form of *Echinochara* recently described from the Middle Jurassic (Bathonian) of France (Trabelsi et al., unpublished) represent the oldest records of clavatoraceans worldwide. These new data confirm the view of Grambast (1964) and Tiss et al (2019), that the Jurassic was a time of renovation of charophyte floras.

Acknowledgements

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New findings for the charophyte flora of Sicily (Italy)

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Key words: *Chara connivens*, *Chara intermedia*, charophytes, flora, Sicily.

The charophyte flora of Sicily is surely understudied and not well known. After some occasional studies made between the end of XIX and the beginning of XX century (Ross 1905; Formiggini 1908), new investigations were reprised in the XXI century. A first contribution was made by Damino (2004), which unfortunately did not result in publications and had no continuation. However it has been used for the updated charophyte flora of Italy (Bazzichelli, Abdelahad 2009).

In 2017 the first author reprised the investigations of the Characean flora of the island. In a first contribution (Romanov et al. 2019) new findings were reported, including new species for the island and for the whole Italy, and a synthesis of the knowledge, resulting in 25 species reported (even if during a long period) for the island. In the frame of current investigations, we report here a couple of new findings; the names of the reported species follow Mouronval et al. (2015).

Chara connivens A. Braun – new for Sicily. Found in a single site in central Sicily, in a temporary pond with long hydroperiod, shallow waters and low salinity (EC = 3.6 mS cm⁻¹, TDS = 1.8 g L⁻¹). Not reported in the rest of Italy, but recently found in few sites in Sardinia (Becker 2019).

Chara intermedia A. Braun – new for Sicily. Found in two different sites in inland Sicily, in different habitats with permanent waters, this taxon (already reported for Itay and Sardinia) is considered “critical” because part of a “species complex” including *Chara baltica* Bruzelius.

The new findings are illustrated with photographs of the main morphological diagnostic characters and the habitats.

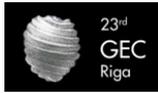
Further taxonomic investigations on these Sicilian populations are underway.

Acknowledgements

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Upper Cretaceous charophytes from the Parras Basin (Southeastern Coahuila, Mexico)

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Key words: biogeography, Characeae, Late Cretaceous, paleoecology, North America.

The Parras Basin (SE of Coahuila, Mexico) has been extensively studied from the sedimentological and paleontological viewpoints to better characterize the abundant dinosaur remains found in it. However, only a few works have been pursued in the northeastern Mexico regarding fossil charophytes, specially from the Coahuila state (Eberth et al. 2004; Aguillón-Martínez 2010). These works usually present preliminary data about the charophyte content, mainly lacking proper taxonomic identifications, which hinders the development of charophyte bibliographic studies. In order to provide valuable biostratigraphic, paleoecological and paleobiogeographical data about the Parras Basin and to improve the knowledge of Mexican fossil charophytes, two composite sections, i.e. Presa Porvenir de Jalpa (ca. 145 m thick section) and Huellas Porvenir de Jalpa (ca. 100 m thick section) were systematically sampled for microfossils and facies analysis.

A total of nine samples were obtained from the Porvenir de Jalpa section and eleven samples from the Huellas Porvenir de Jalpa section. Charophyte assemblages along with abundant gastropod and ostracod remains were found after screen-washing grey lutites and ochre siltstones. Near the sampling beds, abundant marine fauna, such as bivalves, bryozoans and ostreids were recovered. The richer charophyte content was found in the Porvenir de Jalpa section and was dominated by *Feistiella gildemeisteri* along with other accessory species such as *Hornichara* sp. and *Lychnothamnus* sp. The fructifications are usually well-preserved; however, some specimens display evidences of transport and diagenetic compression such

as fragmentation and flattening which is usually related to parautochthonous assemblages.

The charophyte flora and the associated fauna, along with the sedimentological interpretations suggest that the charophyte meadows of *F. gildemeisteri* grew in small ponds found in transitional mudflats near the coastal shore. On the other hand, charophyte-rich beds dominated by *Hornichara* sp. along with *F. gildemeisteri* and *Lychnothamnus* sp. probably grew in more continental freshwater ponds slightly influenced by marine waters. These interpretations obtained after analyzing the charophyte assemblages from Coahuila are concordant with the previous proposals of the Laramidia landmass paleogeography and provide new data about the paleoecology and paleobiogeography of Mexican charophytes.

Acknowledgements

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The impact of waterbird colonies on the charophytes vegetation in Lake Engure

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Key words: Charophytes, eutrophication, Lake Engure, waterbird colonies.

Lake Engure is an ancient sea lagoon, shallow macrophyte type lake in Latvia. It is known as a lake with a high charophyte species richness. The charophyte flora of Lake Engure was first characterized in 1937 (Transehe 1937). Charophytes grew almost everywhere in shallow regions of the lake at that time. Charophytes nearly covered the bottom of Lake Engure some decades later, too (Spuris 1960). However, in the beginning of the 1990s, charophytes disappeared from large areas of the lake. Charophytes were replaced by vascular plants or territories lacking submerged vegetation (Viksne 1997).

Colonial waterbird populations had undergone changes as well. Black-headed gull *Larus ridibundus* population had increased from 200 in 1949 to 34000 in 1986 (Viksne 1997). Following a decrease, the present breeding population comprises 3 to 5 thousand pairs. The breeding population of Cormorants *Phalacrocorax carbo* had increased from 11 pairs in 1999 to 800–1100 pairs in 2014–2018. Then due to disturbance the breeding ceased.

The lake also shelters a breeding population of 150 Mute Swan *Cygnus olor* pairs and five Whooper Swan *Cygnus cygnus* pairs as well as 500–800 moulting Mute Swans in summer. Wintering population can be from zero (lake ice-covered) to 2000.

The aim of our research was to determine the impact of bird colonies on charophyte vegetation in Lake Engure. Three sampling sites were chosen as centers of transects: (1) a *Typha* stand where over the previous four years 800–1100 Cormorant pairs had been nesting (Ciskens); (2) a stand that sheltered up to 1000 Black-headed Gull pairs and 15 Herring Gull *Larus argentatus* pairs and 200 non-breeding Cormorants rest (Viduscers); (3) a control site where colonial waterbirds have not nested during last years. Within each sampling site, four transects were laid out, facing north, east, south and west, with 1 × 1 m sample plots spaced every 25 m. In each sample plot, the percent coverage of plant species, the area of lake bottom without vegetation, and water depth were recorded. Sampling was

conducted in August or September in 2018, 2019 and 2021.

The most common species are *Chara tomentosa* and *Chara aspera*. *C. aspera* occurs commonly around Viduscers and control site, but it is rare, with low projective cover around Ciskens. *C. tomentosa*, *Chara intemedia*, *Chara rudis*, *Chara globularis* and *Chara hispida* are quite common around these sites, too, but do not appear closer than 100 m from the former *Phalacrocorax carbo* colony site (Ciskens). Other charophytes species – *Chara contraria*, *Chara delicatula*, *Chara polyacantha*, *Nitellopsis obtusa* – were quite rare at all investigated sites. Permutational multivariate analysis of variance identified significant differences amongst sampling sites ($F = 14.78$; $q = 0.0018$). Differences amongst sites were explained by charophyte coverage ($F = 48.57$; $q = 0.0018$), sampling distance from colony center ($F = 8.96$; $q = 0.0018$), water depth ($F = 3.12$; $q = 0.0129$), and year of sampling ($F = 11.13$; $q = 0.0018$). In addition to these significant explanatory variables, cardinal direction was found to be significant for both the Ciskens site ($F = 1.81$; $q = 0.002$) and the Viduscers site ($F = 1.81$; $q = 0.031$) when compared to the control site in a pairwise fashion. Interestingly, while charophyte coverage was a highly significant predictor variable amongst sampling sites, average charophyte coverage has decreased in all three sites over the study period, with decreases in average charophyte coverage from 62.8 to 39.4% at site Viduscers, 41.3 to 22.9% at site Ciskens, and from 37.7 to 26.4% at the control site. We propose that taken together our data suggest that the significant differences between our study and control sites, and the accompanying decreases in charophyte coverage suggest that proximity to bird colonies is accelerating charophyte loss in Lake Engure.

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