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On the earliest occurrence of *Tolypella* section *Tolypella* in the fossil record and the age of major clades in extant Characeae

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ABSTRACT

The genus *Tolypella* is considered the basal-most characean genus according to modern molecular phylogenies. Its fossil record, however, provides contradictory evidence since fossil *Tolypella* section *Tolypella* has its first fossil occurrence in the Late Cretaceous, about the same time as the first occurrences of other genera of living characeans, i.e. *Chara*, *Lamprothamnium*, *Nitellopsis* and *Lychnothamnus* which are considered more derived. In this study, the first occurrence of *Tolypella* sp. aff. *T. grambastii* subsp. *arctica* (*Tolypella* section *Tolypella*) is now documented from a lacustrine bed, in the Lower Cretaceous of the Garraf Massif (Catalonia, Spain), ca. 125.0 Ma old. This indicates that *Tolypella* s.s. is indeed a very old genus and that its first fossil record should be extended back at least 55 million years. The first appearance of living Characeae in the fossil record correlates well with the topology of molecular phylogenies. The basal genera *Tolypella*, *Nitella* and the ancestors of the extant Chareae represent the first radiation of the characeans during Late Jurassic–Early Cretaceous. The oldest representative of the clade of *Tolypella* in the fossil record, belonging to *Tolypella* section *Rothia*, suggests that the divergence of *Tolypella* is at least of Kimmeridgian age (157.3–152.1 Ma). The splitting of *Nitella* and the Chareae is dated as Oxfordian in age (163.5–157.3 Ma). The extant representatives of the crown group (*Chara*, *Lamprothamnium*, *Nitellopsis* and *Lychnothamnus*) thus represent the remnants of the second radiation of the Characeae during the Upper Cretaceous, at least 83.6–72.1 Ma ago.

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Charophyta; Early Cretaceous; Catalan Coastal Ranges; Iberia; phylogeny

Introduction

Living charophytes represent only a relict part of the total diversity of the group in the geological past. With about 80–90 genera recognized by Feist et al. (2005) in the fossil record, the six extant genera belong to only one family, the Characeae Agardh, and provide a very limited view of the evolution of charophytes. These genera represent only the residue of an extremely diversified ancestry. This is also true when only the extant family Characeae is considered, since it has suffered steadily from extinction during the late Oligocene and Neogene. The fossil record of this family is well documented thanks to the biomineralization (calcification) of the characean gyrogonites after fertilization. The fossil record can provide an insight into the history of extant genera, answering crucial questions such as: When did they originate? When and how did they diversify? And when and why did they begin to decline to be relicts? However, until now, the absence of *Tolypella* section *Tolypella* Wood 1962 in the fossil record has been a limitation to answering these questions. Before this study, records of *Tolypella* section *Tolypella* started in the latest Cretaceous (ca. 72.1-66.0 Ma ago) and ended up in the early Oligocene, ca. 27.82 Ma ago, when their calcified gyrogonites ceased to be found (Feist et al. 2005) probably due to an inability to calcify (Soulié-Märsche 1979). The record is extended here with a new discovery of *Tolypella* section *Tolypella* in much older deposits, the Lower Cretaceous of the Catalan Coastal Ranges (Spain). This occurrence of the genus widens significantly its previous known fossil record, allowing for a more complete comparison between first occurrences of extant genera in the fossil record and the topology of molecular phylogenies. As a result, the major branches of the living charophyte cladogram can be given an age, and the macroevolutionary interpretation of the major clades in the extant phylogeny is discussed.

Material and Methods

A 10.5 m thick coastal section of the Garraf Massif, following the railway trench, between the towns of Sitges and Vilanova i La Geltrú, south from Barcelona, has

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been sampled with base coordinates 41°13′11,55″N and 11°45′30,04″E. The locality is locally known as Cap Morro de Gos ('dog muzzle cape', in Catalan). The stratigraphy, microfacies analysis and micropaleontology of the section have been documented. The samples were prepared in the laboratory of palaeontology at the University of Barcelona. Marl containing charophytes was disaggregated in a solution of water and hydrogen peroxide and sieved with meshes of 200, 500 and 1000 µm mesh apertures. Charophyte fructifications were handpicked under a stereomicroscope Wild M5A at ×25. Measurements for biometric characterization of taxa were performed by using the software Motic Images Plus 2.0 ML in a stereomicroscope Motic BA310. Selected specimens were photographed using a Quanta 200 scanning electron microscope at the "Centres Científics i Tecnològics-CCiT" of the University of Barcelona. The material is stored in the palaeontological collections of the Departament de Dinàmica de la Terra i de l'Oceà (University of Barcelona). The geochronological ages given in this study are those provided by Cohen et al. 2013, (updated version 2017/02).

Geological setting

The Garraf Massif represents the northernmost part of the Lower Cretaceous outcrops in the Catalan Coastal Ranges (Fig. 1). It has been considered to be a small basin forming part of the Lower Cretaceous rift system of NE Iberia, along with other larger basins, such as the Maestrat Basin, Cameros Basin or the South Iberian Basin, which share a common basin evolution (Salas 1987; Salas et al. 2001). The main features of the Lower Cretaceous record in the Garraf Massif (Fig. 2) have been summarized by Albrich et al. (2006) and modified by Salas and Moreno (2008). The succession covers a thick dolomitic unit of Jurassic age, the Les Talaies Formation, that passes vertically and laterally to La Pleta limestone Formation. This unit contains the Jurassic–Cretaceous boundary, is up to 50 m thick, and is built up by laminated limestones with abundant fenestral porosity, attributed to a tidal flat deposit by Rosell-Ortiz (1978–79). The La Pleta Formation is overlain by the upper Berriasian Mangraners Formation, up to 50 m thick, formed by mainly non-marine palustrine carbonates rich in clavatoracean charophytes such as *Globator maillardii nurrensis* Pecorini, *Atopochara trivolvis micrandra* (Grambast) and *Atopochara trivolvis ancora* (Grambast). The lower Valanginian Polacos Formation, up to 50 m thick, overlies the previous unit. It is formed by shallow marine carbonates with the early orbitolinid foraminifer *Valdanchella milianii* Schroeder. The top of this unit is deeply karstified, marking a significant sedimentary discontinuity and a stratigraphic gap that includes a period of ca. 10 Ma, including the upper Valanginian and the whole Hauterivian. This gap is very common at the margins of all Iberian basins and represents a period of decreased subsidence rate. Upon

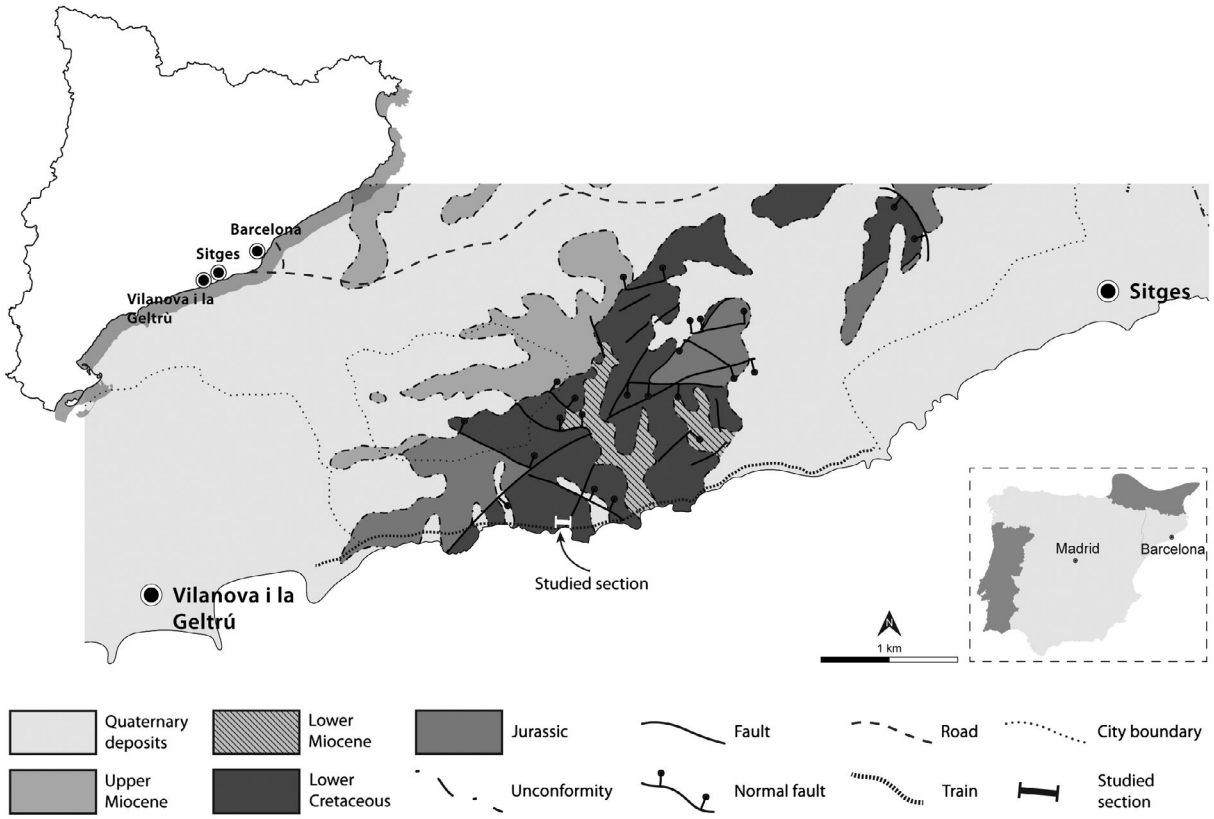

Figure 1. Geological map of the SE Garraf Massif with indication of the section studied (modified from Institut Cartogràfic de Catalunya 2005).

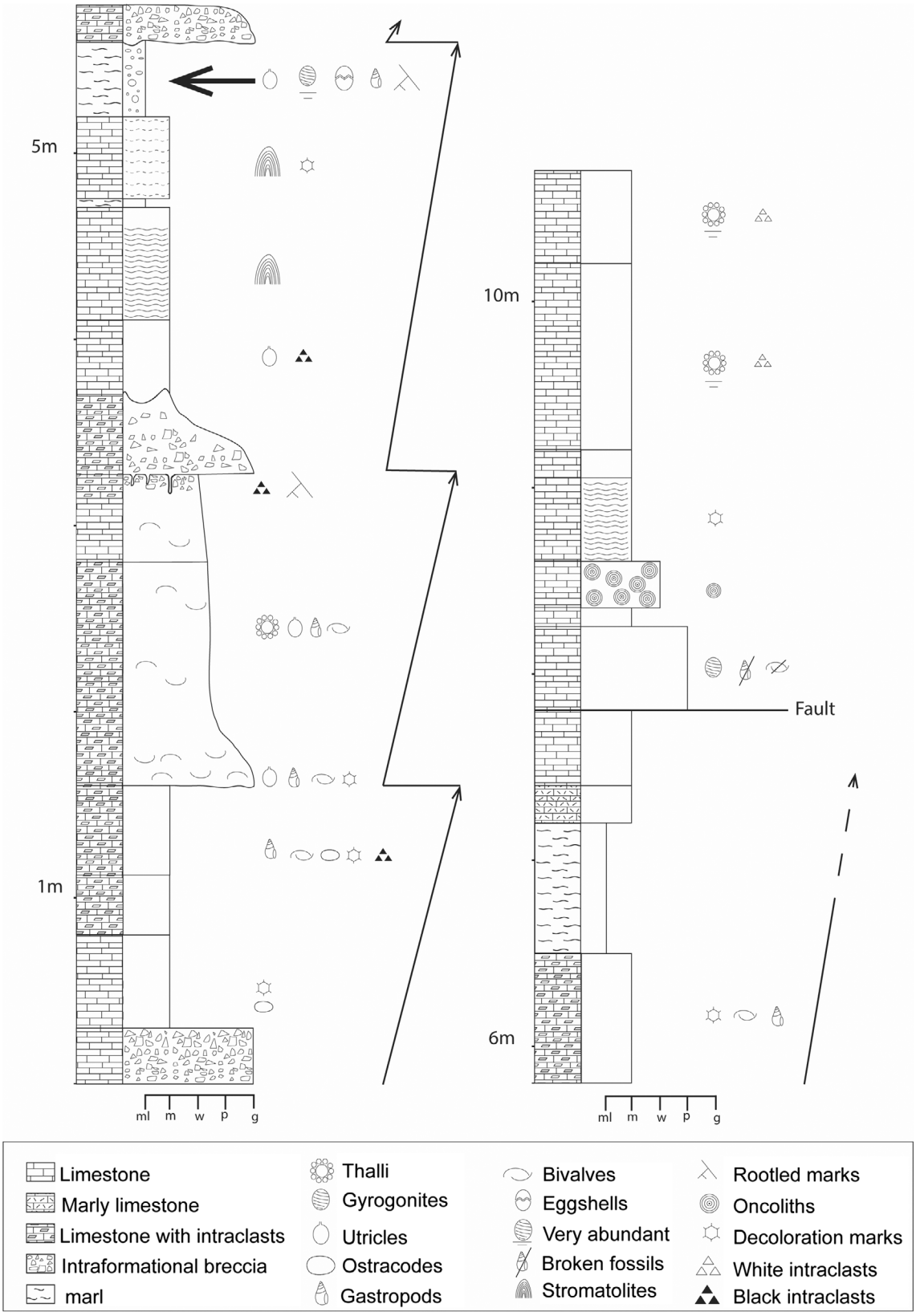

Figure 2. Stratigraphic section of Morro de Gos cape, with indication of the charophyte bed studied (horizontal bold arrow) and sedimentary cycles described in the text (vertical arrows at right of columns). Abbreviations: ml, marl; m, mudstone; w, wackestone; p, packstone.

the karstification surface, a very thick Barremian succession, up to 500 m thick, starts.

The base of the Barremian succession is formed by ca. 40 m of brackish palustrine limestone and marl, rich in edaphic features, that contains monospecific assemblages of *Porochara* sp. and oysters. It has been assigned to the Cantaperdius Formation (Albrich et al. 2006). This basal unit is overlain by the Artoles Formation, ca. 400 m thick,

formed of shallow marine carbonates, rich in dasycladacean chlorophytes and benthic foraminifera, especially orbitolinids. These shallow marine limestones are mostly lower Barremian in age, based on the benthic foraminifera *Palaeodictyoconus* sp. and *Paracoskinolina maynci* Chevalier (Albrich et al. 2006). The top of the Artoles Formation is again deeply karstified and locally covered by lateritic pockets, for instance near the farm called Les Piques. Upon the karstified surface, a mainly non-marine succession formed by 50 m of charophyte limestones, progressively passes to shallow marine carbonates, 75 m thick, rich in rudists, chondrodontid bivalves and benthic foraminifera, mainly *Palorbitolina lenticularis* Blumenbach, which would indicate an upper Barremian to lower Aptian age. This unit, formerly called La Mola Formation (Albrich et al. 2006), was redefined by Salas and Moreno (2008) as Peu de la Serra Formation. The regional lithostratigraphic framework suggests that the basal charophyte limestones may correspond with the top of the Barremian, i.e. a deposit slightly older than 125.0 Ma old.

The upper part of the Lower Cretaceous sedimentary record at the Garraf Massif includes deeper marine deposits of lower Aptian age, known as the Vallcarca Marl Formation (up to 250 m thick) and Farreny Limestone Formation (40 m thick), with a rich ammonite fauna described and biostratigraphically analysed by Moreno (2007), Moreno-Bedmar and García (2009) and Moreno-Bedmar et al. (2009). The ammonite biozones represented are *Deshayesites forbesi* Casey, *Deshayesites deshayesi* Leymerie and *Dufrenoyia furcata* Sowerby, *Epicheloniceras martini* d'Orbigny and *Parahoplites melchori* (Anthula). Finally, the upper Aptian Pujol Florit Formation, up to 110 m thick, is formed by shallower marine facies with rudists and orbitolinids (*Mesorbitolina texana* Roemer) and represents the highest Lower Cretaceous unit recorded in the Garraf Massif.

Stratigraphy, biostratigraphy and palaeoenvironments of the studied deposits

The section studied at cape Morro de Gos, near Vilanova i La Geltrú, shows a succession of non-marine limestones that are assigned here to the basal part of the Peu de la Serra Formation (Fig. 2). However, the coastal outcrop studied here cannot be cartographically correlated with the stratotype of this formation because it is bounded by faults, making it difficult to attest its vertical and lateral relationships. Therefore, the allocation of the studied deposits to the Peu de la Serra Formation is based only on facial and biostratigraphic criteria.

Description. The section studied, up to 10.5 m thick, is mainly formed by limestones intercalated with charophyte-rich marls. The limestones, with wackestone and packstone fabrics, display abundant edaphic features (nodules, rootlet marks, black pebbles). The limestones and marls are rich in well-preserved fossil remains of charophytes, ostracodes, bivalves, gastropods and tetrapod eggshells. Stromatolitic and oncolitic limestones occur as well. One of the marl beds sampled provided an assemblage of clavatoracean utricles and characean gyrogonites formed by *Globator maillardii trochiliscoides* (Grambast), *Globator maillardii biutricularis* Vicente & Martín-Closas, *Atopochara trivolvis triquetra* Grambast (advanced morphotype), *Clavator harrisii* Peck and *Tolypella* sp. aff. *T. grambastii* subsp. *arctica* Feist & Brouwers 1990 (Fig. 3A-H). The three first taxa allow assigning this bed to the Cruciata-Paucibracteatus charophyte biozone, of upper Barremian to lower Aptian age (Riveline et al. 1996; Martín-Closas et al. 2009).

The studied deposits are organized in a number of lacustrine sequences, up to 2.5 m thick with fining-upward granulometry. They begin with a 20–50 cm thick intraformational breccia or conglomeratic limestone (pseudomicrokarst in the sense of Freytet and Plaziat 1982) that changes upward to a 1–2 m thick massive limestone with charophytes, and ends with a charophyte-rich marl (Fig. 2). The basal term (pseudomicrokarst limestone) is sometimes substituted by a bivalve lumaquella or an oncolite-rich limestone, while the middle term (massive limestone with charophytes) is replaced in part by a stromatolitic limestone.

(b) Interpretation. The sedimentary sequences described are attributed to the development of deepening-upward lacustrine cycles, possibly controlled by synsedimentary faults. The basal pseudomicrokarst would represent a palustrine environment with development of hydromorphic soils and subaerial exposition (Freytet and Plaziat 1982). Sometimes this marginal area had a higher hydrodynamism, resulting from lake waves, which led to deposition of more energetic facies such as a bivalve lumaquella or oncolitic limestones. These facies grade laterally and vertically to shallow, freshwater and alkaline lake facies with abundant charophytes. Finally, sedimentation at the deeper part of the cycles is represented by the charophyte-rich marl indicating a lacustrine belt with limited carbonate production and with deposition of the suspended lime mud. Cyanobacterial mats developed on the lake bottom when conditions were not appropriate for the growth of macrophytes, for instance when the water became too rich in nutrients.

The charophyte assemblage studied here, including *Tolypella* sp. aff. *T. grambastii* subsp. *arctica*, is, in general, well preserved and is considered autochthonous in the depositional site. It would correspond to a charophyte meadow growing at the shallower parts of a freshwater alkaline lake, although some plants would colonize the deeper parts, where only the gyrogonites were preserved.

Systematic palaeontology

Division Charophyta Migula 1897
Class Charophyceae Smith 1938
Order Charales Lindley 1836
Family Characeae Richard. ex C.A. Agardh 1824

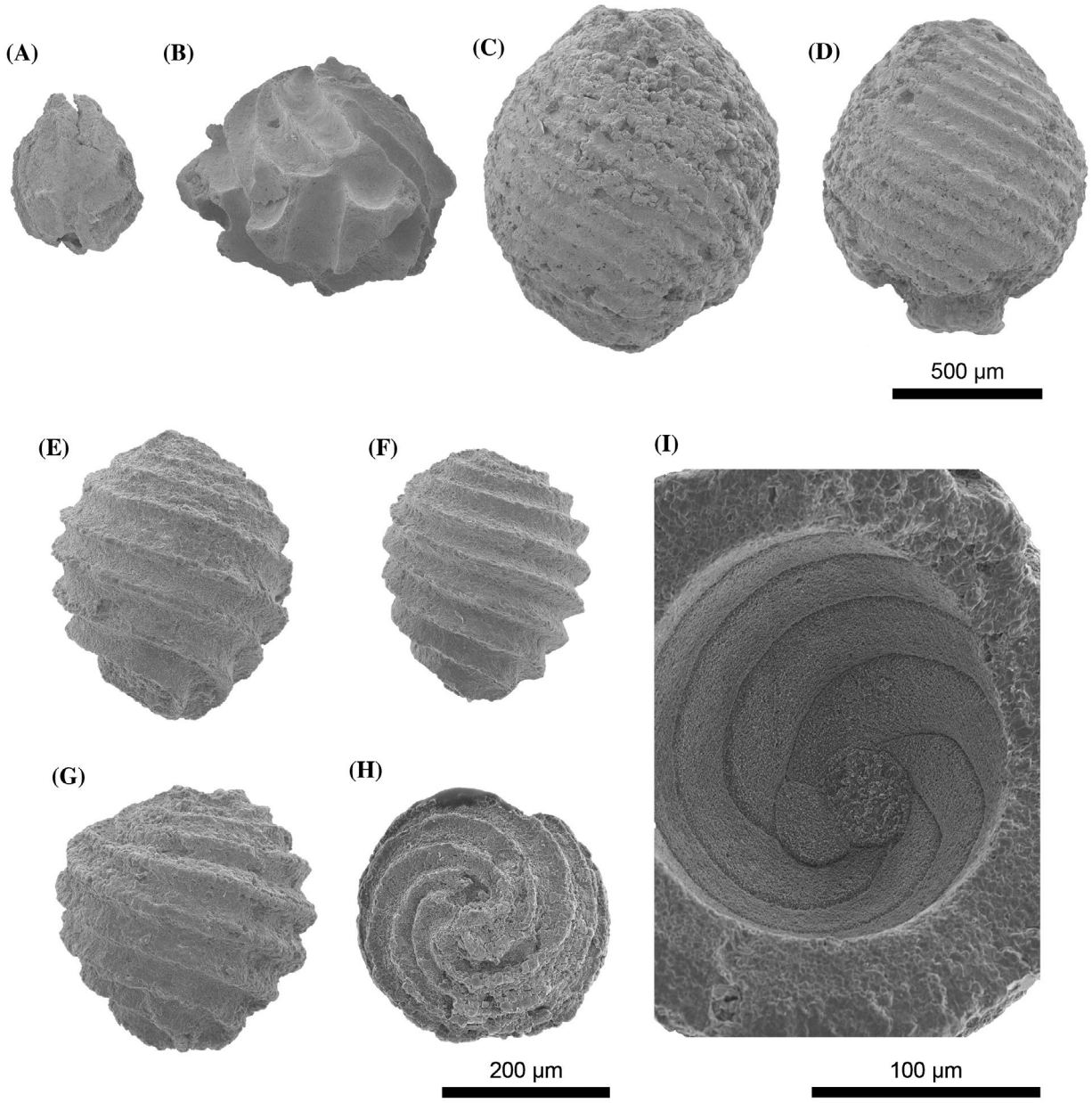

Figure 3. Charophytes from the upper Barremian–lower Aptian of the Garraf Massif. A. *Clavator harrisii*, lateral view of utricle; B. *Atopochara trivolvis triquetra* (advanced morphotype), lateral view of utricle; C. *Globator maillardii trochiliscoides*, lateral view of utricle; D. *Globator maillardii biutricularis*, lateral view of utricle; E–I. *Tolypella* sp. aff. *T. grambastii* subsp. *arctica*; E–G. lateral views of gyrogonite, H. apical view of gyrogonite, I. multipartite basal plate as seen from the inside of an empty gyrogonite.

Genus *Tolypella* (Braun 1849) Braun 1857 emend. Soulié-Märsche 1989

Section *Tolypella* Wood 1962

sp. aff. *Tolypella grambastii* subsp. *arctica* Feist and Brouwers 1990

Figure 3E-3I

1990 – *Tolypella grambasti* ssp. *arctica* n. ssp., Feist and Brouwers, US Geol. Surv. Bull. 1990-F: F5-F6, pl. 1, figs 1-6.

Material. 20 gyrogonites

Description. Gyrogonites are very small, 260–350 µm high and 240–330 µm wide, with an ISI of 95–110, and prolate spheroidal, sometimes ovoidal in shape. The apex is round or slightly pointed, without any apical modification. The base is slightly tapering with a small basal pore. In lateral view, 7–9 convolutions are visible. Spiral cells are concave, with a prominent intercellular ridge. The basal plate is multipartite and formed by two almost regular pentagonal truncated pyramids, one slightly smaller than the other. The low number of gyrogonites available makes a precise specific attribution difficult.

Remarks. The gyrogonites of *Tolypella* sp. aff. *T. grambastii* subsp. *arctica* studied here are, in external view, very similar to gyrogonites from *Mesochara harrisii* (Mädler 1952) Shaïkin 1967. This species can only be distinguished from *Tolypella* sp. aff. *T. grambastii* subsp. *arctica* by its more rounded apex, its regular ellipsoidal shape and especially by its basal plate, which is unicellular. However, this latter character is difficult to observe

when the inside of the fossil gyrogonite is full of sediment or calcite.

Comparisons. *Tolypella peckii* Feist and Brouwers 1990 displays gyrogonites about the same size as the Garraf material; however, the number of convolutions is larger (9–12) and the shape is more elongate (ISI 109–127). The studied gyrogonites are closer to *Tolypella grambastii grambastii* Uliana and Musacchio 1978; however, the gyrogonites of this species and subspecies are more ellipsoidal and show more convolutions in lateral view. The size, shape and number of convolutions of the material studied here are much closer to *Tolypella grambastii* subsp. *arctica* Feist and Brouwers 1990 from Alaska. The Catalan gyrogonites differ from the Alaskan material in the shape of the two cells forming the basal plate. In *T. grambastii arctica* one of the two cells is small and triangular, while in the studied gyrogonites both cells are approximately equal in size and shape (a pentagonal pyramid). However, differences in shape and size of the oosphere sister cells in basal plates of *Tolypella* cannot be given the status of a diagnostic character, since they are variable within species, as shown by Soulié-Märsche (1989, plate XVII).

Gyrogonites from other fossil *Tolypella* differ more from the material of the Garraf Massif. *Tolypella suprastriata* (Holifield 1964) Feist and Brouwers 1990 gyrogonites are twice as large as the material studied here and display 10–13 convolutions. *Tolypella pumila* Grambast 1958 differs in its more spheroidal shape with a clearly pointed apex and especially because its basal plate is visible from the outside. Finally, *Tolypella caudata* Grambast 1958 is clearly larger, and displays a short basal column.

Discussion

First occurrence of genus Tolypella s.s. in the fossil record

The fossil record of genus *Tolypella* section *Tolypella* is relatively poor in comparison to that of most characeans. In addition, a number of reports of this genus are dubious, probably flawed, since they were based only on the gyrogonite shape and size without providing any information on the multipartite basal plate, which is a crucial character to determine the *Tolypella* s.s. gyrogonites. Leaving aside these insecure determinations, *Tolypella* s.s. had so far its oldest fossil record in the Maastrichtian (72.1–66.0 Ma ago) from the two end points of America, since it is represented respectively by gyrogonites of *Tolypella grambastii* Uliana and Musacchio 1978 from El Zampal, Neuquén basin, Argentina and *Tolypella grambastii* subsp. *arctica* Feist and Brouwers 1990 from Ocean Point at North Slope, Alaska. Two more species occurred in North America from the end of the Cretaceous to the beginning of the Cenozoic (Palaeocene, 66.0–56.0 Ma ago), and were described by Feist and Brouwers (1990). These are *Tolypella suprastriata* (Holifield 1964) Feist and Brouwers 1990 from several localities in the United States and Canada, such as Wales Canyon (Utah), Jackson Hole (Wyoming) and Castle River (Alberta), and *Tolypella peckii* Feist and Brouwers (1990), from the Flagstaff Member of the Green River Formation at Colton (Utah).

Genus *Tolypella* is not reported again in the fossil record until the early Oligocene (ca. 33.9–27.82 Ma ago), when its youngest calcified gyrogonites occur. In this epoch, *Tolypella pumila* Grambast 1958 and *Tolypella caudata* Grambast 1958 are known from Belgium (Grambast 1958; Stockmans 1960) and the Rhine Graben in Germany (Breuer and Feist 1986; Schwarz and Griessemer 1992, 1994; Schwarz 1997). Neogene and Quaternary are devoid of records of *Tolypella* based on their gyrogonites, probably indicating the beginning of its present inability to biomineralize (Soulié-Märsche 1979). In the Holocene the fossil record of this genus resumes but is represented only by organic oospores (e.g. García 1996).

The new occurrence of *Tolypella* sp. aff. *T. grambastii* subsp. *arctica* in the upper Barremian-lower Aptian of the Catalan Coastal Ranges, ca. 125.0 Ma old, widens up to 55 Ma the previously known reports of a representative of *Tolypella* section *Tolypella*, setting it among the earliest fossil records known from the living Characeae.

Dating the phylogeny of living Characeae from the fossil record

The first phylogenetic trees of living Characeae based on both molecular and morphological characters were proposed by McCourt et al. (1996, 2000) and Karol et al. (2001) with contrasting results about the phylogenetic position of some genera, especially within the Nitelleae Von Leonhardi (genera *Tolypella* and *Nitella* Agardh). Later works reached a deeper consensus on this issue and the basic pattern of the characean cladogram is now largely stable. The recent molecular phylogeny proposed by Pérez et al. (2014) will be followed here, since this study was especially devoted to the phylogeny of genus *Tolypella*. According to this phylogeny *Tolypella* would represent the basal-most genus followed in a comb-like pattern by *Nitella* and then by a crown group including the living Chareae Von Leonhardi. This crown group is in turn organized in two branches represented by *Chara* Linnaeus and *Lamprothamnium* Groves vs. *Nitellopsis* Hy and *Lychnothamnus* (Ruprecht) respectively.

The study by Pérez et al. (2014), however, presents an interesting variation in comparison with former studies concerning the relative position of the two sections of genus *Tolypella* in the cladogram. Hence, *Tolypella* section *Rothia* Wood 1962 is poorly resolved as a monophyletic clade and may be paraphyletic, while *Tolypella* section *Tolypella* appears as a well-supported monophyletic clade. This result suggests that the ancestral condition of the Characeae as regards the basal plate (i.e. the oosphere sister cell) might be a unicellular basal plate,

while the multipartite basal plate (3 oosphere sister cells) would have been developed independently by *Tolypella* s.s. and *Nitella*. This latter situation agrees with the presently known oldest basal plate of charophytes, which is a unicellular basal plate of the Permian *Leonardosia langei* Sommer from Brazil (Souza de Faria et al. 2013).

The Lower Jurassic (ca. 201.3–174.1 Ma ago) coincides with the first occurrence of the family Characeae in the fossil record. *Aclistochara* Peck aff. *jonesi* Peck 1937 reported by Feist, Bhatia, and Yadagiri (1991) is the earliest gyrogonite to show the apical pore closed by the junction of the spiral cells, which is the character used by palaeontologists to recognize the characeans (Grambast 1956). This fructification cannot be attributed to any extant charophyte; however, the gyrogonite of *Aclistochara* Peck bears a multipartite basal plate (Schudack 1993) and was thus hypothesized to represent a species belonging to the Nitellaceae Von Leonhardi by Martín-Closas (2000). Its position in the cladogram from molecular data is uncertain, but *Aclistochara* might occupy a very basal position, perhaps close to the ancestor of the characeans (Fig. 4).

Genus *Tolypella* is subdivided in two genera, which are well characterized in the fossil record. *Sphaerochara*

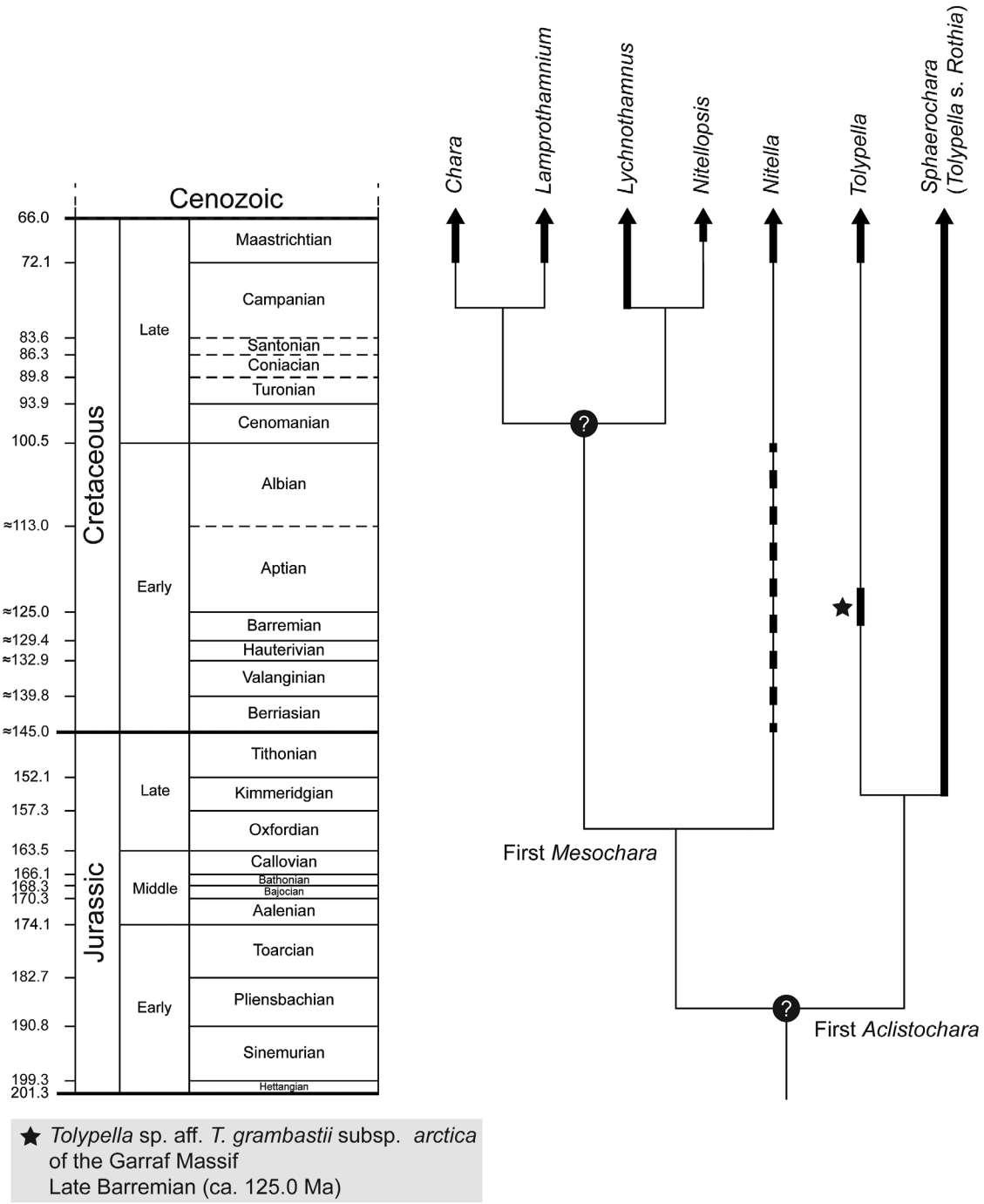

Figure 4. Phylogeny of extant characeans with ages of the main nodes as provided from fossils. The presently known fossil record of extant genera is marked with a bold line on the cladogram. Question marks at nodes indicate uncertainty in the age of the node. Phylogeny modified from Pérez et al. (2014).

Mädler (i.e. *Tolypella* section *Rothia*) represented by *Sphaerochara verticillata* (Peck 1937) appears first in the Upper Jurassic of the Morrison Formation, United States (Kimmeridgian, 157.3–152.1 Ma ago), according to Schudack (1993). Claims of older representatives of *Sphaerochara* by Horn af Rantzien (1954) and Saidakovsky (1968) are based on gyrogonites with poorly characterized basal plates and cannot be considered here. In contrast the oldest representative of *Tolypella* section *Tolypella* is documented now with *Tolypella* sp. aff. *T. grambastii* subsp. *arctica* from the Lower Cretaceous of Catalonia (upper Barremian–lower Aptian, ca.125.0 Ma ago). The age provided by *Sphaerochara* is taken here as the most probable minimum age for the first branching of genus *Tolypella*, while the age of *Tolypella* section *Tolypella* found in the Garraf Massif is hypothesized to represent an early representative of this branch of the cladogram (Fig. 4).

The node corresponding to the branching of *Nitella* and the crown group of Chareae represents the next node in the molecular phylogeny of extant Characeae. Genus *Nitella* is also one of the characeans with a limited fossil record. The reason for this is that their gyrogonites never calcify and are only represented in the fossil record either by their organic oospores or their silicified remnants. The first possible record of *Nitella* was reported by Horn af Rantzien (1957) and is a silicified oospore called *Nitellites sahnii* Horn af Rantzien 1957; from the Rajmahal series of Nipania, Bihar province, India. The age of this fossil, which was originally placed within the Lower or Middle Jurassic by Horn af Rantzien (1957), appears to be Lower Cretaceous according to Feist, Bhatia, and Yadagiri (1991). The ornamentation pattern of this fossil looks convincingly as belonging to genus *Nitella*, and is particularly similar to *Nitella mucronata* (Braun) according to Horn af Rantzien (1957). However, there is no information on its basal plate. The first undoubted record of *Nitella* appears in the Maastrichtian of El Zampal, Neuquén Basin (Argentina), where Musacchio (2010) reported abundant, well preserved and complete silicified oospores of *Nitella* sp. In consequence the first possible occurrence of the genus is documented within the Early Cretaceous (145.0–100.0 Ma ago), but this should be taken with care until the basal plate of the organic fossil from India is not described.

The Chareae themselves are thought to be derived from a plant bearing a completely plesiomorphic charean gyrogonite, which is known as *Mesochara* Grambast in the fossil record (Martín-Closas 2000). The oldest record of this genus is in the Upper Jurassic (Oxfordian, 163.5–157.3 Ma ago) of Germany (Schudack 1993), and this age is proposed here as the minimum age of the node containing the branching of *Nitella* and the Chareae (Fig. 4).

The Upper Cretaceous (100.0–66.0 Ma) contains the earliest occurrences of the living Chareae. The first well-documented *Lamprothamnium* occurs in the Maastrichtian of China (Feist et al. 2005; Li et al. 2016). However, Soulié-Märsche (1994) reported an older occurrence of this genus in the Lower Cretaceous of Texas (United States), with a poorly characterized basal plate. Also De Sosa Tomas, Vallati, and Martín-Closas (2017) reported recently a possible occurrence of *Lamprothamnium* in the Lower Cretaceous of Argentina, but again without characterizing the basal plate. *Chara* occurs first in the Late Cretaceous (Feist et al. 2005). *Lychnothamnus*, with *L. begudianus* (Grambast), is known first from the ca. 83.6–72.1 Ma old Campanian of South France (Grambast 1971). This gyrogonite was originally ranged within the fossil genus *Amblyochara* Grambast, which is now regarded as a younger synonym of *Lychnothamnus* (Soulié-Märsche 1989). Finally, the genus *Nitellopsis* has its first occurrences in the Maastrichtian of the South Pyrenean Basins, Catalonia (Galbrun et al. 1993). These data suggest that the uppermost branching of the crown group formed by extant Chareae occurred at least during the latest Cretaceous, most probably during Campanian or Maastrichtian times, 83.6–66.0 Ma ago. In contrast, the basal node of the crown group of the Chareae remains unresolved (Fig. 4).

The crown group formed by the modern Chareae contains in fact only a relict part of the diversity this group reached during the Upper Cretaceous and Palaeogene, particularly during the Eocene and early Oligocene (56.0–27.82 Ma ago). By this time some species of the living genera of Chareae, such as *Nitellopsis*, became really diverse (e.g. Grambast and Soulié-Märsche 1972) and biogeographycally well distributed (e.g. Sanjuan and Martín-Closas 2015). The Palaeogene diversification and ecological success of the Chareae, particularly of genera *Nitellopsis* and *Lychnothamnus*, probably guaranteed their survival across the Neogene, when many characean genera became extinct (Riveline 1986).

Conclusions

The first occurrence of genus *Tolypella* section *Tolypella* in the ca. 125.0-Ma-year-old upper Barremian of the Garraf Massif (Catalonia, Spain) confirms that *Tolypella* s.s. is among the oldest living characeans, i.e. those that radiated already during the Late Jurassic–Early Cretaceous. However, the oldest occurrence of the extant genus is represented by *Sphaerochara*, equivalent to extant *Tolypella* section *Rothia*, which is documented as early as the Late Jurassic (Kimmeridgian, 157.3–152.1 Ma ago). This result appears to support the hypothesis of Pérez et al. (2014) that *Sphaerochara* (=*Tolypella* section *Rothia*) is more basal than *Tolypella*.

The fossil record of *Nitella* is poor, but the node representing its branching with the charean crown group possibly occurred at least in the Late Jurassic (Oxfordian, 163.5–157.1 Ma ago), based on the first occurrence of the completely plesiomorphic charean gyrogonite *Mesochara*.

The crown group of the characean cladogram itself is formed by extant *Chara*, *Lamprothamnium*, *Lychnothamnus* and *Nitellopsis*, and represents the second radiation of the Characeae by the Late Cretaceous. This age corresponds to the branching of the upper nodes of the crown group. The lower nodes, previous to the first occurrences of the living Chareae, may be older, probably from the end of the Early Cretaceous or the beginning of the Late Cretaceous (Cenomanian–Santonian, i.e. 113.0–83.6 Ma ago), and may be represented by a number of fossil genera such as *Microchara* Grambast, *Peckichara* Grambast or *Platychara* Grambast.

The first occurrences of all extant genera of Characeae in the fossil record correspond well with the branching pattern provided by molecular phylogenies, showing the usefulness of independent testing between molecular and palaeontological data. Furthermore, testing of the hypothesis presented herein may be carried out with molecular clocks.

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