THE END OF A MYTH: *ARPYLORUS ANTIQUUS* PALEOZOIC DINOFLAGELLATE CYST

ALAIN LE HERISSE[1*], EDWIGE MASURE[2], EMMANUELLE J. JAVAUX[3] and CRAIG P. MARSHALL[4]

[1] *Université de Brest , UMR-CNRS 6538 « Domaines Océaniques », IUEM, CS 93837 □6 Avenue le Gorgeu, 29238 Brest Cedex, France*

[2] *UMR-CNRS 7207, Centre de Recherche sur la Paléobiodiversité et les Paléoenvironnements, CR2P, Université Pierre et Marie Curie, case 104, 4, place Jussieu 75005 Paris, France.*

[3] *Palaeobiogeology, Palaeobotany, Palaeopalynology, Department, Université de Liège, Bâtiment B18, 17, allée du 6 Août, 4000 Liège -1, Belgique*

[4] *Department of Geology, The University of Kansas, Lawrence, Kansas 66045-7613, USA*

e-mail: <u>alain.le.herisse@univ-brest.fr</u>

* Corresponding author

RRH: *REVISION OF ARPYLORUS ANTIQUUS*

LRH: *LE HERISSE ET AL.*

Keywords: *Arpylorus*, Paleozoic, Protodinoflagellate, revision, evolution

ABSTRACT:

***Arpylorus antiquus*, erected by Calandra (1964), was isolated from Upper Silurian sedimentary rocks from the Mechiguig-1 borehole in southern Tunisia, with other palynomorphs. The folded vesicle and the quadrangular form of the aperture breaks down into plate-like fragments, resembling the tabulation of dinoflagellates. The presence of these elements, have been used to interpret *A. antiquus* as a dinoflagellate**

cyst. The morphology and affinity of *A. antiquus* is reinterpreted herein based on investigation of larger sets of samples, including material from the type locality, together with material of Algeria, Saudi Arabia, and Brazil. More complete specimens than those previously described have been observed using gentle laboratory techniques, showing a large development of a fine membrane at the periphery of vesicles. This element was destroyed using classical palynological treatments, implying that the holotype is an incomplete specimen. The membrane at the periphery of vesicles and dorsoventral differentiation of these vesicles suggest that *A. antiquus* is a part of a more complex biological structure. We suggest a possible relationship with eurypterids, arthropods related to phyllocarids, represented by abundant fragments in the assemblages. *Arpylorus antiquus* is possibly a structure of storage. The chemical composition of *A. antiquus* using a Fourier transform infrared FTIR microspectroscopy analysis, reveals a wall composed of biopolymer that is not consistent with dinosporin. We conclude that *Arpylorus antiquus* is definitively not a dinoflagellate cyst. Although dinoflagellates may have older Paleozoic or even Proterozoic ancestors as the biomarker record may suggest, the dinoflagellate tabulation evolved only in the early Mesozoic.

INTRODUCTION

At the 1964 CIMP (Commission Internationale de Microflore du Paléozoïque) acritarch subcommission seminar in Bordeaux (France), Calandra introduced *Arpylorus antiquus*, which he interpreted as a dinoflagellate. This organic-walled microfossil was originally isolated from the upper Silurian rocks of the Mechiguig borehole (MG 1) in southern Tunisia (Fig. 1). The quadrangular form of the opening of *Arpylorus antiquus* was indeed reminiscent of an operculate plate (archeopyle), and the vesicle surface showed

possible traces of tabulation, both diagnostic characters of dinoflagellates. Evitt (1967) confirmed the attribution of this discovery to the dinoflagellates, in the commentary sent to the acritarch subcommission members after the seminar, and by reinvestigations of the type material. Later Sarjeant (1978) re-examined the type material and emended the diagnosis of the genus and species and introduced a new family, the Arpyloraceae, represented by a single fossil cyst species *Arpylorus antiquus*. Stover and Evitt (1978) suggested also a similarity in shape between *Arpylorus* and *Pyxidiella*. Evitt (1985, p. 38) mentioned again *Arpylorus,* stating the following: a very dinoflagellate-like dinoflagellate so much so in fact, if it was found in an Mesozoic assemblage, it might attract no more attention than any other distinctive species Presently, *Arpylorus antiquus* is listed in an uncertain class and order (Fensome et al., 1993).

The presence of *Arpylorus antiquus* 200 million years before the widespread presence of dinoflagellates at the end of the Triassic, however, has remained a concern for many researchers who have raised questions concerning the absence of such characters as the cingulum and sulcus (Bujak and Williams, 1981; Bujak and Davies, 1983). Evitt (1985) also suggested that, in order to definitively establish the detailed organization of this microfossil and its affinities with the dinoflagellates, better preserved material would be necessary to study by scanning electron microscopy (SEM).

The aim of this work is to evaluate the affinities of *Arpylorus antiquus* Calandra, 1964, *emend* Sarjeant, 1978, by morphological and microchemical study of new material, using optical and SEM investigations, as well as micro-FTIR (Fourier transform infrared) spectroscopy analysis. The combination of these new techniques on well-preserved material may permit to better understand the biological affinities of *Arpylorus*.

PREVIOUS STUDIES

The type material of *Arpylorus antiquus*, was firstly described by Calandra (1964), in the Silurian core section Mechiguig 1 (MG1). Mechiguig 1 (MG1) borehole was drilled in the 1960s by the Serept Company, in the south of Tunisia, north of Hammadah basin (Ghadames), and close to the Libyan border (Fig. 1). During the Paleozoic, this area located on the northern margin of the Ghadames Basin, was a part of the North African Platform, north of Gondwana plate (Fig. 2).

The investigated section penetrates a Siluro-Devonian succession beneath Triassic cover (Loboziak et al., 1992), at a depth of 1970 to 3757.70 m. Calandra (1964) mentioned a total of 22 specimens of *Arpylorus antiquus*, including the holotype, encountered at three different levels, between 3345 to 3478 m. Calandra (1964) used standard processing for the samples, with disaggregation of the rocks to small fragments, strong digestion in acids, oxidation, and centrifugation. Residues were also subjected to density separation in order to separate organic elements from heavy minerals.

Additional samples of core MG1 were collected by Massa, who described the paleontological content of the Siluro-Devonian succession by Massa (1988). Organic-walled microphytoplanktonic associations (acritarchs and other microalgae) are abundant and diverse in the upper Tannezzuft and Acacus formations, of middle and late Silurian age (Le Hérissé, in preparation).

NEW MATERIAL AND METHODS

For this study, reprocessing of samples from core section Mechiguig 1 MG1 allowed to isolate new specimens of *Arpylorus antiquus* at several depths: Core 38 at 2893□2896 m ; Core 55 at 3352 m and 3362.50 m; Core 57 at 3478.70 m; Core 59 at 3599 m; Core 61 at

3654 m and 3657 m; and Core 63 at 3757.70 m. This interval is dated late Homerian-early Gorstian to Lochkovian: with occurrence of abundant chitinozoans *Conochitina pachycephala* in the older samples (Verniers et al., 1995). Some acritarchs characteristic of Ludlow are encountered between 3352 and 3657 m, including *Baltisphaeridium areolatum* var *areolatum* (total range of the species), *Buedingiisphaeridium incertum*, some abundant *Cymbosphaeridium* spp., and some *Deflandrastum* spp. etc. They are elements of the acritarch zonation established in Libya (Buret, 1990, Le Hérissé, 2002) and are also index taxa of the G3-G5 zones in the Algerian Sahara (Jardiné et al., 1974). Few specimens have also been observed between 2893 and 2896 m, in an interval dated to the Lochkovian by miospores (Loboziak and Streel, 1992 ; Spina and Vecoli, 2009).

The material referred to *A. antiquus* is never abundant in the residues, and may breakeand fragment by classical procedure of chemical dissolution of minerals used in palynology (e.g. Wood et al., 1996). The delicate nature of *A. antiquus* is an important point. With a control of oxidation and by avoiding centrifuging (cf. description of the method later), specimens have been possible to isolate that are more complete than those previously described. Up to now, more than 100 specimens have been encountered in the material of Tunisia.

We have also found additional specimens of *A. antiquus* from various localities and other Paleozoic strata in the world. Specimens were encountered from core samples of the upper Sharawra Member of the Qalibah Formation in Saudi Arabia, dated to the upper Wenlock to lower Ludlow (Stump et al., 1995). Some specimens have been noted in core material of upper Silurian age in the Sbaa Basin in Algeria (Le Hérissé unpublished data). In Algeria however, this species was first mentioned by Jardiné et al. (1974) in the Silurian Zone G4 of the Algerian Sahara. Specimens have also been observed in Silurian core samples of the Chaco Basin in Bolivia, and in the Llandovery in the Tiangua Formation of the Parnaiba

Basin, Brazil, and issued from reworking of Silurian strata in the Frasnian-Famennian of the Amazon Basin, Brazil (Le Hérissé, 2001). Occurrences have also been established elsewhere by others researchers, e.g. in the Silurian of Syria (K.J. Dorning personal communication, 2004) and possibly in the Lower Devonian of Saudi Arabia (Breuer et al., 2005). The latter specimens noted of *?Arpylorus* spp. noted in Breuer et al. (2005), however, have a reticulate ornamentation on the vesicle, not seen in our material, and seems to be different from *Arpylorus antiquus* Calandra, 1964, *emend* Sarjeant, 1978.

At this time, the paleogeographical distribution of *Arpylorus antiquus* (Fig. 2) is extended to Western Gondwana (Brazil and Bolivia in South America) and the North Gondwana margin (Algeria, Tunisia and Syria) up to Arabia in the Middle East. The material is found in majority in an interval upper Wenlock-Ludlow, but the older specimens are in the Llandovery in western Gondwana (Brazil). The species is mainly found in shallow marine marginal marine deposits of Llandovery-Ludlow age rich in eurypterids.

In this study, all samples were subjected to gentle laboratory treatment. Relatively large fragments of rock samples (several cm^3) were immerged in hydrofluoric acids for demineralization. Neither centrifugation nor oxidation was used. Specimens were handpicked under a binocular scope using a glass microtube, mounted on rounded slides and stubs, and coated with gold for SEM study. The preparation procedure for SEM examination follows the method described by Paris (1978), with the advantage that the same specimen can be examined on the two sides by repeated SEM observations, and latter by optical microscopy.

The molecular biogeochemistry study was performed to elucidate the chemical composition of *Arpylorus antiquus*, using FTIR micro-spectroscopy analysis. This method has a great advantage, compared to other bulk analyses, to be applicable on small samples, as FTIR micro-spectroscopy can provide data on the chemical composition of individual microfossils. This is particularly useful and discriminates separate elements of a polytypic

microfossil population. FTIR microspectroscopic analyses of *Arpylorus antiquus* specimens were performed using a Bruker IFS66. The FTIR spectrometer was coupled to a Bruker microscope accessory housing a dedicated liquid nitrogen cooled (77 K), and narrowband mercury cadmium telluride detector. The microscope was fitted with an IR/ visible Cassegrainian 15X objective (numerical aperture = 0.4). A total of 8 specimens isolated from other palynomorphs in the residue, and handpicked in the material from MG1 borehole in Tunisia, were placed on an infrared transparent silicon wafer for analysis. Interferograms were acquired in the transmission mode within the range 4000-600 cm^{-1} by accumulating 256 scans at a spectral resolution of 4 cm^{-1}.

Reference slides are stored in the collections of the Laboratoire de Paléontologie et de Stratigraphie, Université de Brest, France, prefixed LPB, and in the collections of Total, Pau, France, prefixed PG.

REVISION OF THE MORPHOLOGY OF *ARPYLORUS ANTIQUUS* AND BIOGEOCHEMICAL ANALYSIS: TAXONOMIC IMPLICATIONS

Original systematic description

The genus *Arpylorus*, a contraction of *Archaeo-pylo-phorus* was proposed by Calandra (1964, footnote p. 4114) as an organic ovoidal test, with a lateral quadrangular archeopyle, upper angles sometimes rounded or truncated, bearing vestiges of polar membranous expansions. The diagnosis of the type species *A. antiquus* n. sp., was briefly stated similarly to the characteristics of the genus, ⬜brown wall, vermiculate⬜ (Transl. from Calandra, 1964, footnote p. 4114). Emended diagnosis proposed by Sarjeant for *A. antiquus* (1978, p. 174)

mentioned   a species of *Arpylorus* having a broadly ovoidal cyst. The surface of the periphragm is minutely chagrinate, a more prominent ornamentation of vermiculae, sometimes with briefly furcating ridges is also developed, with the size, distribution and complexity varying not only between individuals but also on different parts of the same cyst. Parasutures may be marked by tubercules or short spines, low ridges or (especially near the poles) more elevated membranes. Fensome et al. (1993) assigned *Arpylorus* an uncertain position in class and order, within the Division Dinoflagellata, in the family Arpyloraceae Sarjeant, 1978.

New morphological observations

The isolation of specimens attributed to *Arpylorus antiquus* in the Silurian of MG 1 borehole, using gentle processing techniques, less damaging to the organic-walled microfossils than the standard techniques, resulted in the discovery of new morphological features. They have not been considered important in the past but are of crucial significance for the taxonomic revision and reattribution: 1. The *Arpylorus antiquus* specimens are attached to a fine membrane developed around the vesicles, more or less preserved, and in most cases reduced to vestiges at the periphery of vesicles; 2. The specimens of *Arpylorus antiquus* display approximately the same size and general morphology, but have a variable shape, ranging from spherical to ellipsoidal to more elongate; 3. The microfossils are slightly compressed dorsoventrally: the ventral side (i.e. the side oriented toward the membranous surface), is flat and smooth, and the dorsal side with the opening and operculum, is more convex (but frequently exhibiting some compression); 4. The surface sculpture is limited to irregular folds with random distribution (cf. Fig. 3, reconstruction); 5. the vesicle is strongly ornamented with vermiculations aligned in subparallel rows, and the scanning electron

micrographs clarify its position on the internal face of the vesicle. The ornamentation is particularly well developed on the operculum and around the opening (cf. Fig. 8, A□C).

Scanning electron micrographs show other morphologic features of *Arpylorus antiquus*, and do not confirm the former description of a tabulation with a cingulum, with three episomal plate series and two hyposomal plate series (Fensome et al., 1993).

Attachment to a membrane □ The systematic occurrence of thin membrane developed on one side of the microfossil structures attributed to *Arpylorus antiquus* has been largely underestimated in the preceding descriptions, even though footnotes of Calandra (1964, p. 4114) mentioned vestiges of polar membranous expansions to upper angles of the ovoid test. The holotype of *Arpylorus antiquus*, illustrated by Calandra, (1964, fig. 1), consists of an incomplete specimen, which lacks the membranous extension (Calandra, 1964, Fig. 1, reproduced herein, Fig. 6, A□B).

The solid attachment of the vesicles to the thin membrane, by means of lateral *septae* around the microfossils (see e.g. Fig. 6, C□F, H etc.), suggest they are fixed on this surface, making part of it, and not only deposited on it. The wall of the vesicles is thick and robust even though it is frequently compressed, and more darker than the membrane around.

Variation in shape and size □ Specimens of *Arpylorus* are spherical to ellipsoidal or more or less elongate in shape (cf. the different illustrations, Figs. 6□8). The size of the specimens encountered ranges between 112 and 184 μm in length and 62 to 133 μm in width (Fig. 4). The opening, closed by a polygonal operculum, is between 48 and 62 μm in long. The morphological differentiation in shape and size among the different specimens analysed is not sufficient to separate different groups. We conclude that all the specimens were produced by the same kind of organisms.

Dorsoventral differentiation □ The organization of the structures attributed to

Arpylorus antiquus, shows a dorsoventral development. The dorsal side is the top of the structure. The dorsal surface is convex, bearing the opening closed with an operculum. The ventral side is the bottom, flat and in connection with the membrane. The schematic line drawing proposed for *Arpylorus antiquus* (Fig. 3), takes into account of this dorsoventral differentiation.

Biogeochemical analysis

The IR spectrum (cf. Fig. 5) shows absorptions centered at a low broad absorption at 3380 cm^{-1} assigned to alcoholic OH, phenolic OH, and/or carboxylic OH; strong narrow aliphatic absorptions centered at 2925 and 2850 cm^{-1} assigned to antisymmetric stretching vibrations from CH$_2$ and symmetric stretching vibrations from CH$_2$ methylene groups, respectively; a shoulder centered at 1700 cm^{-1} assigned to the vibration of carbonyl C=O; a strong absorption of conjugated C=C (probably aromatic) centered at 1600 cm^{-1}; moderate absorptions of deformation bending of CH$_2$ and CH$_3$ centered at 1450 cm^{-1}; minor absorptions of ether (C-O) bonding between 1200 to 1000 cm^{-1}; and weak out of plane aromatic C-H bending at 920 and 820 cm^{-1}.

The IR analysis reveals a cyst wall biopolymer consisting of moderate long chain aliphatic hydrocarbon structures with minor conjugated carbon (C-C=C-C) residing in aromatic ring structures. The macromolecule also comprises aliphatic monocarboxylic acids (C=O), and aliphatic esters (C-O) substituents. The biopolymer composition is not consistent with the composition of dinosporin, an aromatic macromolecule characterized in (only) one species of dinoflagellate cyst (Kokinos et al, 1998). The spectra also differ from spectra obtained on chitinozoan, leiosphere, cryptospore and acritarchs obtained in previous studies (Marshall et al, 2005; Steemans et al, 2010).

DISCUSSION ON THE ANATOMY AND AFFINITES OF *ARPYLORUS ANTIQUUS*

The data presently available the observations on the morphology showing an absence of paratabulation the presence of a membrane attached with septae and the biogeochemistry of *Arpylorus antiquus* wall do not support the conclusions than *Arpylorus antiquus* is a protodinoflagellate. In the following section, we discuss possible biological affinities for *Arpylorus antiquus*.

The chemical composition of specimens of *Arpylorus antiquus* and the gross morphology are important features. Since they survive the chemical treatment involved in palynological preparation, and considering their color and texture and the microchemical results, this species evidently has walls with decay-resistant macromolecular organic composition. Their small size the mean size for the sac-like body is 138μm in length and 98μm in width suggests comparison with cysts, eggs, reproductive bodies or biological structure of protist, plant, or animal clades.

The residues further contain such marine microfossils as acritarchs, chitinozoa, scolecodonts and graptolites remains, as well as such elements of terrestrial origin as trilete spores, tetrads, and vascular tissues. In several studied assemblages in Silurian and Devonian sediments, the residues with *Arpylorus antiquus* also contain abundant acid-resistant arthropod fragments of eurypterids and/or phyllocarids.

At our knowledge, the morphology of *Arpylorus antiquus,* attached to a membrane, differs from that of known protists. Possible affinities for *Arpylorus antiquus* could include eggs of small invertebrates. We have previously considered this hypothesis, with the possibility that they represent pieces of exoskeleton (Le Hérissé et al., 2000). In some cases eggs may be attached to a support, however the shape of our specimens, not completely

spheroidal but flattened on one side, is not a common morphology for small invertebrates eggs. Another possibility is a relationship with insects that lay their eggs under water, on different media. The first fossils insects are described from the uppermost Silurian (Engel and Grimaldi, 2004; Labandeira, 2007). Among the few available illustrations of fossil material are the possible insect eggs in the Aptian of Brazil (Regali and Sarjeant, 1986), showing a large opening and operculum, with a meshwork of polygonal fields defined by ridges on the surface, some elements of morphology that resemble *Arpylorus*. These possible eggs, however, are ovoidal to broadly spheroidal and do not display a flat side like our specimens. Our specimens may also show some similarities with annelid cocoons. Some of them, described in the Early Jurassic of Australia (Janson et al., 2008) have an ovoid shape, a mesh-like ornamentation and operculum, but they are larger and the position and form of the opening, differs from those of *A. antiquus*.

We conclude that *Arpylorus antiquus* might not be an egg, cyst or cocoon. The characters observed as the shape, opening, and attachment to a thin membrane to the periphery of the vesicles suggest that *Arpylorus antiquus* was possibly a storage structure. The association with eurypterid cuticles is one piece of evidence in favor of possible affinities with these arthropods or to phyllocarids crustaceans.

Eurypterids are an exclusively Palaeozoic arthropod group. They are most commonly found in nearshore, shallow water marine deposits, although deep-water forms are known (Plotnick, 1996). Among the fragments encountered in our residues several types of eurypterid fragments occur, and are the most common, but it is not excluded that remains of phyllocarid crustaceans are also represented.

Various types of cuticlelike fragments of eurypterids have been observed, particularly in the type locality of MG1 in Tunisia, with sheetlike fragments with dark broad-based spines, or lines of crescentic thickenings (Fig. 8, G) as illustrated by Mc Gregor and Narbonne

(1978). The Kiemenplatten structure term used in preference of gill-tract, described by Manning and Dunlop (1995), has also been observed in the material (Fig. 8, H). This structure is interpreted as a respiratory organ of eurypterids, and possible strategy of aerial respiration and partial terrestrial mode of life for these arthropods.

Morphological revision of *Arpylorus antiquus* and model reconstruction allow features to be described more rigorously. The possible anatomical interpretation includes mechanism of reproduction of arachnids, myriapods or scorpions. An example of a mating structure is the spermatophore (a sac for sperm storage) placed onto a suitable surface by the male for an indirect sperm transfer to the female, according the schema proposed by Braddy and Dunlop (1997, fig. 14, p. 458), for the Silurian eurypterid *Baltoeurypterus tetragonophtalmus* from Estonia. But the model that implies a spermatophore is hypothetical for fossil arthropods, and we can only suggest a relationship between *Arpylorus antiquus* and the eurypterids.

The size of these sac-like bodies may suggest an animal of a few centimeters in size. Brady and Dunlop (1997, p.459) note that eurypterids may have acquired sexual maturity in early instars and *Arpylorus antiquus* could be also the spermatophore of a juvenile. The decisive element, however, would be a potential discovery, in the future, of specimens of *Arpylorus antiquus* directly attached to an animal.

CONCLUSION

The discovery of complete specimens of *Arpylorus antiquus* in Silurian material from the type locality in Tunisia, together with new observations on additional material from elsewhere, complement Calandra□s (1964) original description, and permits revision of the morphology and affinity of these microfossils. The observations of a fine membrane developed all around the vesicles and the dorsoventral differentiation of the vesicle, reveal

major discrepancies to the former interpretation placing *A. antiquus* within the tabulate Paleozoic dinoflagellates. Biogeochemical analyzes do not support a dinoflagellate affinity. We instead propose that *A. antiquus* may represent a storage structure produced by invertebrates. A comparison to the spermatophore of the eurypterids (sea scorpions) has been discussed, but it could represent also another piece of the anatomy of the animals.

Compared to the record of *Arpylorus antiquus* in the Silurian and Lower Devonian, the report of the first fossil recognizable as dinoflagellates (based on morphological criteria) in the Trias (Fensome et al., 1996), 200 millions years latter lead to more questions:

- Were the dinosteranes (lipid biomarkers present in extant dinoflagellates) identified in Proterozoic and Paleozoic deposits been produced by proto-dinoflagellates, or other alveolates or organisms closely related to the group?

- If dinoflagellates had older ancestors among acritarchs, why did they modify their morphology by evolving the tabulation during the Triassic? Is there a physiological or morphological adaptive advantage to this biological innovation? Is there a causal link with environmental changes linked to the breaking up of the Pangea and the global transgression during the Triassic? Further investigations are required in order to fully answer these important questions.

ACKNOWLEGMENTS

REFERENCES

BRADDY, S.J., and DUNLOP, J.A., 1997. The functional morphology of mating in the Silurian eurypterid, *Baltoeurypterus tetragonophthalmus* (Fischer, 1839). Zoological Journal of the Linnean. Society, 435-461.

BREUER, P., AL-GHAZI, A., FILATOFF, J., HIGGS, K.T., STEEMANS, P., and WELLMAN, C.H., 2005. Stratigraphic palynology of Devonian boreholes from Northern Saudi Arabia. In : Pre-Cambrian to Paleozoic Paleopalynology and Paleobotany , meeting PPMB, 11 May 2005, Steemans, P. and Javaux, E. (eds.). Abstract, 1-7.

BUJAK, J.P., and WILLIAMS, G.L. 1981. The evolution of dinoflagellates. Canadian Journal of Botany, 59, 2077-2087.

BUJAK, J.P. and DAVIES, E.H., 1983. Modern and fossil Peridiniineae. American Association of Stratigraphic Palynologists, Contribution Series, N° 13, 216p.

BURET, M. B., 1990. Palynologie et planctologie de la Formation des Argiles Principales du Silurien du Bassin de Rhadames, Libye Occidentale. Unpublished Doctoral Thesis, Université Pierre et Marie Curie, Paris, 362 p.

CALANDRA, F., 1964. Sur un présumé dinoflagellé *Arpylorus* nov.gen., du Gothlandien de Tunisie. Comptes Rendus de l'Académie des Sciences, Paris, 258, 4112-4114.

COCKS, L.R.M., and TORSVIK, T.H., 2002. Earth geography from 500 to 400 million years ago : a faunal and palaeomagnetic review. Journal of the Geological Society of London, 159: 631-644.

DA SILVA PARES REGALI, M. and SARJEANT, W.A.S. 1986. Possible insect eggs in

palynological preparations from the Aptian (Middle Cretaceous) of Brazil. Micropaleontology, 32, 2, 163-168.

ENGEL, M.S., and GRIMALDI, D.A., 2004. New light shed on the oldest insect. Nature, 427, 12 february 2004 : 627-630.

EVITT, W.R., 1967. Dinoflagellate studies, II. The archeopyle. Stanford University, Publications, Geology, Sciences, 10, (3), 1-83.

EVITT, W.R., 1985. Sporopollenin dinoflagellate cysts. Their morphology and interpretation . American Association of Stratigraphic Palynologists Foundation. ISBN 0-931871-00-X.

FENSOME, R.A., TAYLOR, F.J.R., NORRIS, G., SARJEANT, W.A.S., WHARTON, D.I., and WILLIAMS, G.L., 1993. A classification of living and fossils dinoflagellates. Micropaleontology Press Special Paper, n°7, 351p.

FENSOME, R.A., MCRAE, R.A., MOLDOWAN, J.M., TAYLOR, F.J.R., and WILLIAMS, G.L., 1996. The early Mesozoic radition of dinoflagellates. Paleobiology, 22(3), 329-338.

JANSSON, I-M., MCLOUGHLIN, S., and VAJDA, V., 2008. Early Jurassic annelid cocoons from eastern Australia. Alcheringa 32, 285-296.

JARDINÉ S., COMBAZ, A., MAGLOIRE, L., PENIGUEL, G. and VACHEY, G., 1974. Distribution stratigraphique des acritarches dans le Paléozoïque du Sahara Algérien. Review of Palaeobotany and Palynology, 18 (1974), 99-129.

KOKINOS, J.P., EGLINTON, T.I., GONI, M.A., BOON, J.J., MARTOGLIO, P.A., and ANDERSON, D.M., 1998. Characterization of a highly resistant biomacromolecular material in the cell wall of a marine dinoflagellate resting cyst. Organic Geochemistry, 28, 265□288.

LABANDEIRA, C, 2007. The origin of herbivory on land: initial patterns of plant tissue consumption by arthropods. Insect Science, 14, 259-275.

LE HÉRISSÉ, A., 2001. Evolution of Devonian phytoplanctonic assemblages in the Upper Eréré Formation and Curua Group (Barreirinha and Lower Curiri Formations),

Tapajos River area, Amazon Basin, Northern Brazil. In Correlacao de sequencias Palaeozoicas sul-Americanas, J.H.G. Melo and G.J.S. Terra, eds. Cienca-Tecnica-Petroleo. Secao Exploracao de Petroleo, 20, 117-124.

LE HÉRISSÉ, A., 2002. Paleoecology, biostratigraphy and biogeography of late Silurian to early Devonian acritarchs and prasinophycean phycomata in well A161, Western Libya, North Africa. Review of Palaeobotany and Palynology, 118, 359-395.

LE HÉRISSÉ, A., MASURE, E., AL RUWAILI, M, and MASSA, D., 2000. Revision of A*rpylorus antiquus* from the Silurian : the end of a myth. Wang, W., Ouyang, S., Sun, X., Yu, G. (eds .) : 10th International Palynological Congress, June 24-30, 2000, Nanjing, China. Abstract, 88.

LOBOZIAK, S., STEEMANS, P., STREEL, and VACHARD, D., 1992. Biostratigraphie par miospores du Dévonien inférieur à supérieur du sondage MG-1 (Bassin d Hammadah, Tunisie) Comparaisons avec les données des faunes. Review of Palaeobotany and Palynology, 74, 193-205.

MANNING, P.L., and DUNLOP, J.A., 1995. The respiratory organs of eurypterids. Palaeontology, vol. 38, 2, 287-297.

MARSHALL, C.P., JAVAUX, E.J., KNOLL, A.H., and WALTER, M.R., 2005. Combined micro-Fourier transform infrared (FTIR) spectroscopy and micro-Raman spectroscopy of Proterozoic acritarchs: a new approach to Palaeobiology. Review of Palaeobotany and Palynology,138, 208 224.

MASSA, D., 1988. Paléozoïque de Libye Occidentale : Stratigraphie et Paléogéographie. Thesis, Université de Nice, Nice, 2 volumes, 514 pp.

McGREGOR, D.C., NARBONNE, G.M., 1978. Upper Silurian trilete spores and other microfossils fom the Read Bay Formation, Cornwallis Island, Canadian Artic. Canadian Journal of Earth Scienceds, vol.15, 1292-1303.

PARIS, F., 1978. Apports du microscope électronique à balayage dans l'étude des chitinozoaires opaques. Annales des mines de Belgique, 2$^\text{è}$ livraison, 193-202.

PLOTNICK, R.E., 1996. The scourge of the Silurian seas. American Paleontologist, vol. 4, 1, 1-3.

SARJEANT, W.A.S., 1978. *Arpylorus antiquus* Calandra, emend., a dinoflagellate cyst from the upper Silurian. Palynology, vol. 2: 167-179.

SPINA, A., and VECOLI, M., 2009. Palynostratigraphy and vegetational changes in the Siluro-Devonian of the Ghadamis Basin, North Africa. Palaeogeography, Palaeoclimatology, Palaeoecology, 282, 1-18

STEEMANS, P., LEPOT, K., MARSHALL, C.P., LE HERISSE, A., and JAVAUX, E.J., 2010. FTIR characterisation of the chemical composition of Silurian cryptospores from Gotland, Sweden., Review of Palaeobotany and Palynology, 162, 577-590.

STOVER, L.E., and EVITT, W.R., 1978. Analyses of Pre-Pleistocene Organic-walled Dinoflagellates. Standford University Publications, Geological Sciences, volume XV, 300 p.

StumpTUMP, T.E., AlL-HajriAJRI, S, and VanAN DerER EeemEEM, J.G.L.A., 1995. Geology and biostratigraphy of the Late Precambrian through Palaeozoic sediments of Saudi Arabia. Review of Palaeobotany and Palynology, 89, 5-17.

VERNIERS, J., NESTOR, V., PARIS, F., DUFKA, P., SUTHERLAND, S, and VAN GROOTEL, G., 1995. A global Chitinozoa biozonation for the Silurian. Geological Magazine, 132 (6), 651-666.

WOOD, G.D., GABRIEL, A.M., and LAWSON, J.C. 1996. Chapter 3. Palynological techniques □ processing and microscopy. In Jansonius, J. and Mc Gregor, D.C.(ed.), Palynology: Principles and Applications, American Association of Stratigraphic Palynologists Foundation, Vol. 1, 29-50.

FIGURE CAPTIONS

FIGURE 1☐Geographic setting and location of well MG1

FIGURE 2 ☐Geographical distribution of *Arpylorus antiquus* , plotted on middle Silurian-Early Devonian paleogeographical reconstruction (Cocks and Torsvik, 2002). 1) Bolivia; 2) Brazil; 3) Algeria; 4) Tunisia; 5) Syria; 6) Saudi Arabia.

FIGURE 3 ☐Schematic reconstruction of *Arpylorus antiquus* showing in : A) An incomplete specimen with characteristic development of a thin membrane to the periphery ; B) A most classical aspect of this microfossil with folds, residues of the membrane all around the vesicle, and vermiculate ornamentation mainly in the zone of opening; C) Another specimen (cf. Fig, 7, A, B) with a detached operculum ; D) Dorsoventral differentiation of the structure attributed to *Arpylorus antiquus*: the dorsal side is convex, and bears the opening closed with an operculum. The ventral side is the bottom, flat and in connection with the membrane.

FIGURE 4 ☐Biometrics of *Arpylorus antiquus*

FIGURE 5 ☐The micro-FTIR spectrum obtained from a single *Arpylorus antiquus*

FIGURE 6 ☐A☐B) The holotype of *Arpylorus antiquus*, designed and illustrated by Calandra, 1964, fig. 1,   p. 4113, overall length 154 μm, overall breadth 107,5 μm, MG1 Silurian of Tunisia , depth 3351 m, slide N° PG 102. A) unretouched specimen ; B) the same specimen retouched by Calandra to indicate the supposed tabulation and the opening. C) A specimen surrounded by fragments of membrane. Vesicle 122 μm in length and 95 μm in

width. Opening 45x33 µm. MG1 Silurian of Tunisia depth 3362.50 m, LPB 13186, England Finder coordinates M 33.3. D) A specimen with large fragments of membrane around the vesicle. Vesicle 140 µm in length and 100 µm in width. MG1 Silurian of Tunisia depth 3362.50 m, LPB 13191, England Finder coordinates C 56. E) A specimen with elongated vesicle and fragments of membrane attached to the apex.Vesicle 168 µm in length and 112 µm in width. MG1 Silurian of Tunisia depth 3362.50 m, LPB 13186, England Finder coordinates P 35.2. F) A rounded specimen. Vesicle 117 µm in length and 105 µm in width. MG1 Silurian of Tunisia depth 3362.50 m, LPB 13192, England Finder coordinates K 28. G) Isolated specimen after classical palynological technique, with a very reduced membrane around the vesicle. Vesicle 168 µm in length and 105 µm in width. MG1 Silurian of Tunisia depth 3478.70 m, LPB 13184, England Finder coordinates U 40.3. H □I) Specimen with well developed ornamentation of the Operculum. Vesicle 155 µm in length and 102 µm in width, operculum 65x48 µm. KAHF 1 Saudi Arabia depth 2488.50 m, LPB 13193, England Finder coordinates O 34.

FIGURE 7 □A□B) A specimen in optical and SEM view. A) Dorsal side to show the opening without the operculum ; B) ventral side. Vesicle 168 µm in length and 108 µm in width, MG1 Silurian of Tunisia depth 3362.50 m, LPB 13185, England Finder coordinates M33.1. C□H) A specimen to show the development of the membrane outside the vesicle optical and S.E.M views of the same specimen. Length of the apical fragment of membrane 358 µm. MG1 Silurian of Tunisia depth 3362.50 m, LPB 13185, England Finder coordinates M31.2. D□E) Specimen showing the dorsal side, optical and S.E.M. views. Vesicle 134 µm in length and 95 µm in width, MG1 Silurian of Tunisia 3362.50 m, LPB 13185, England Finder coordinates L 34.4. F□G) Specimen in dorsal view, optical microscope and S.E.M,

with fragments of membrane to the periphery and the opening without operculum. MG1

Silurian of Tunisia 3352 m, LPB 13187, England Finder coordinates M38.

FIGURE 8 ☐A, B, C) The specimen in optical and S.E.M view allows to confirm the

internal position of the microornementation developed on the operculum and proximity.

Vesicle 134 μm in length and 95 μm in width, MG1 Silurian of Tunisia depth 3352 m, LPB

13187, England Finder coordinates 038.1. D, E, F) A rounded specimen showing the

opening. Optical and S.E.M views; Vesicle 145 μm in length and 115 μm in width. MG1

Silurian of Tunisia depth 3599 m, LPB 13190, England Finder coordinates P 26. G)

Eurypterid fragment of cuticle with serially arranged thickenings with crescentic form.

Overall length 650 μm, overall width 320 μm, MG1 3347.90 m, LPB 13189, England finder

coordinates P32.4**.** H) Fragment of Kiemenplatten structure interpreted as an accessory aerial

respiratory organ of eurypterids. Overall length 250 μm, MG1 3354 m, LPB 13188, England
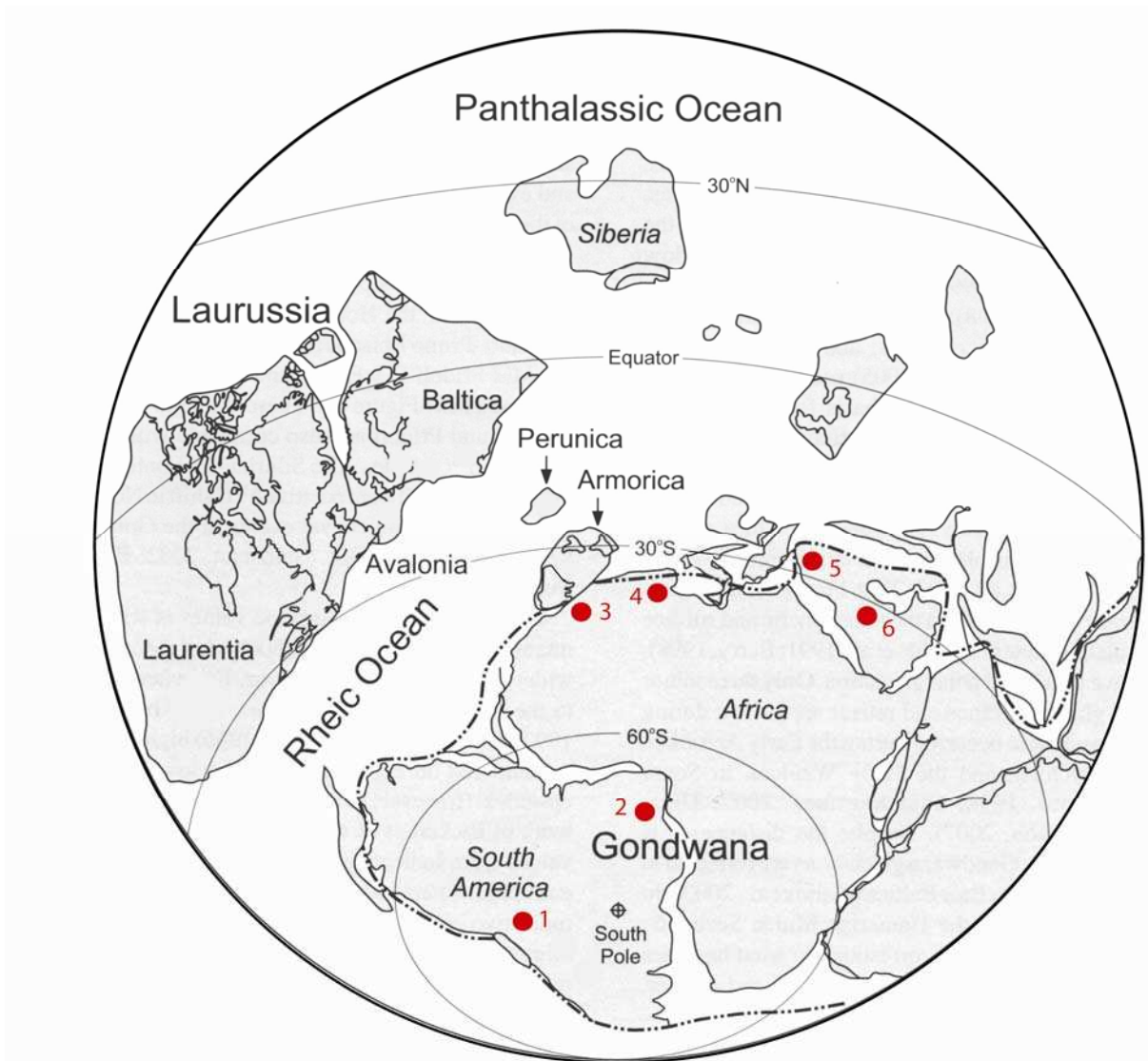
Finder coordinates R33.

Figure 1.

Figure 2.

Network of vermiculate ornaments on the internal face

vestiges of cuticle

operculum

B

100 µm

opening

Irregular folds

C

A

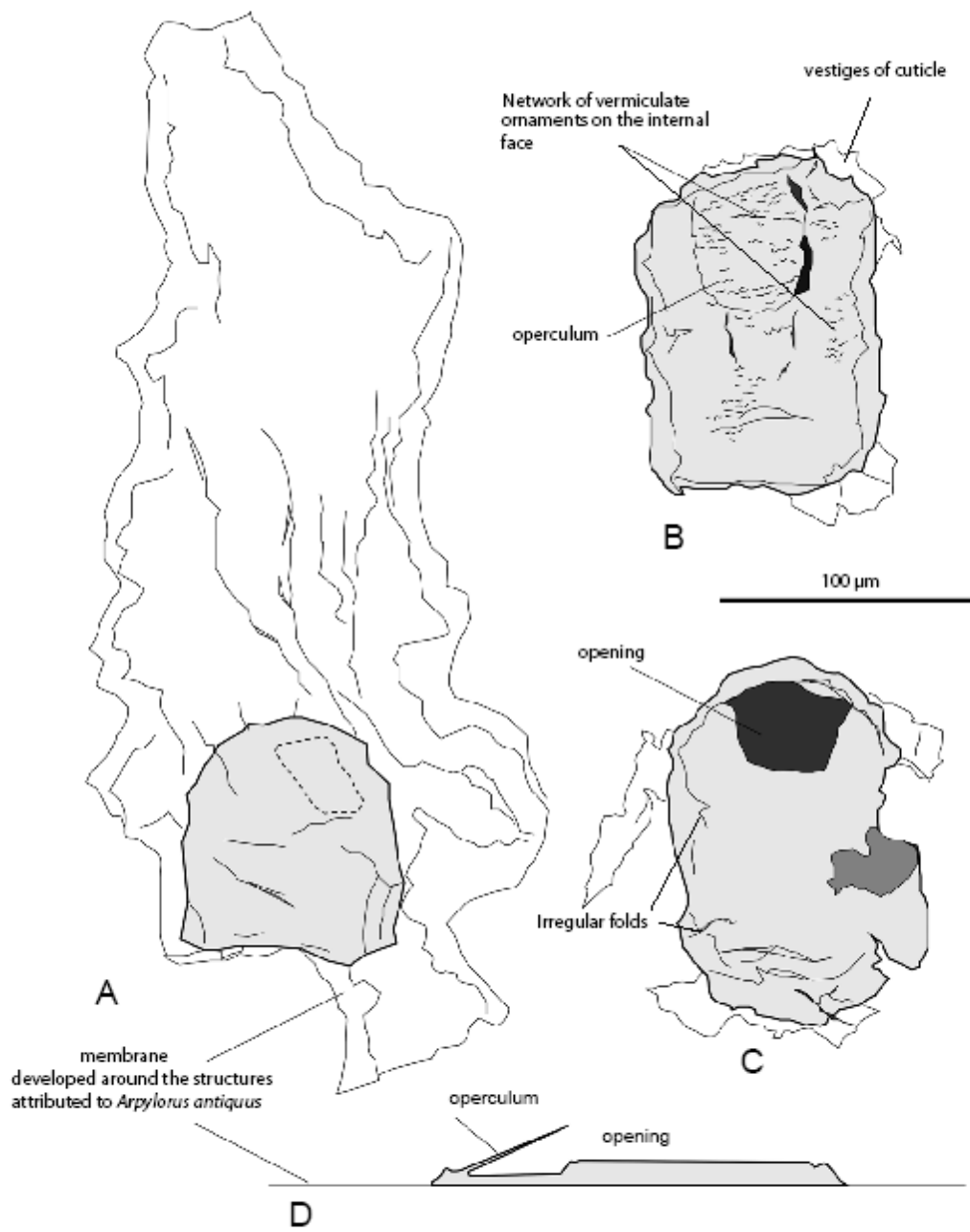membrane developed around the structures attributed to *Arpylorus antiquus*
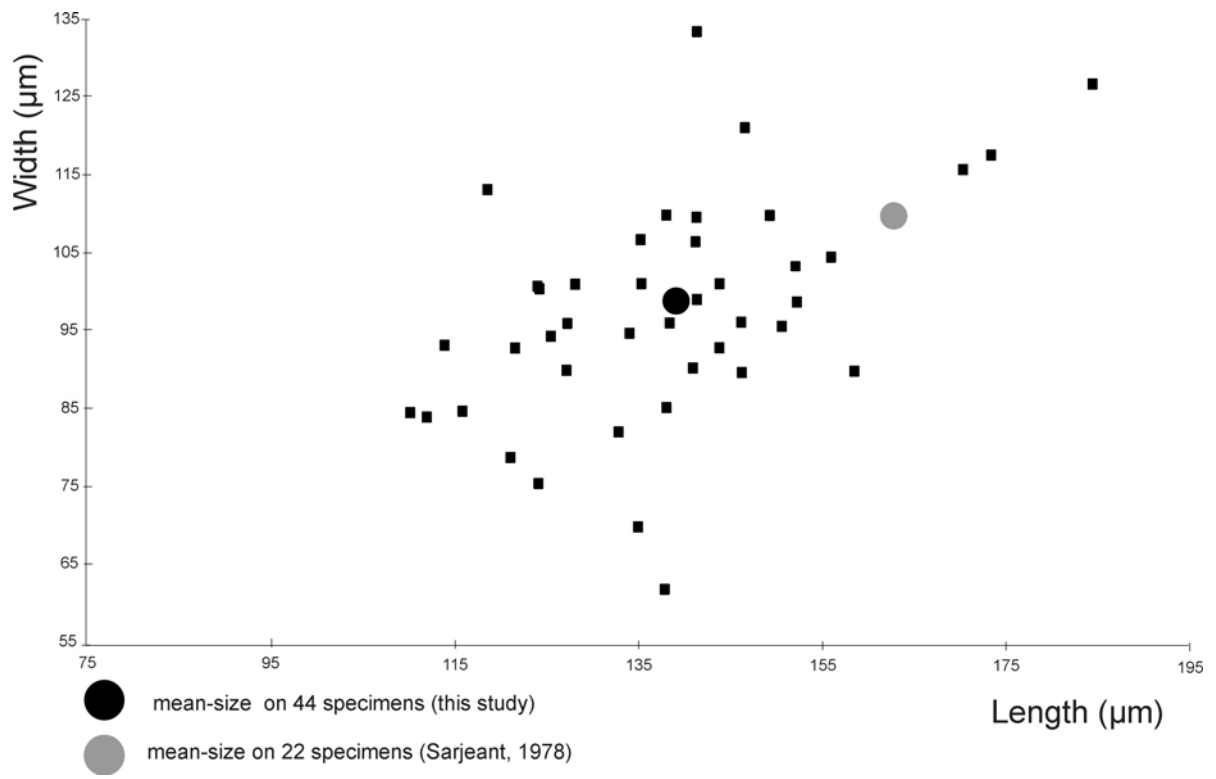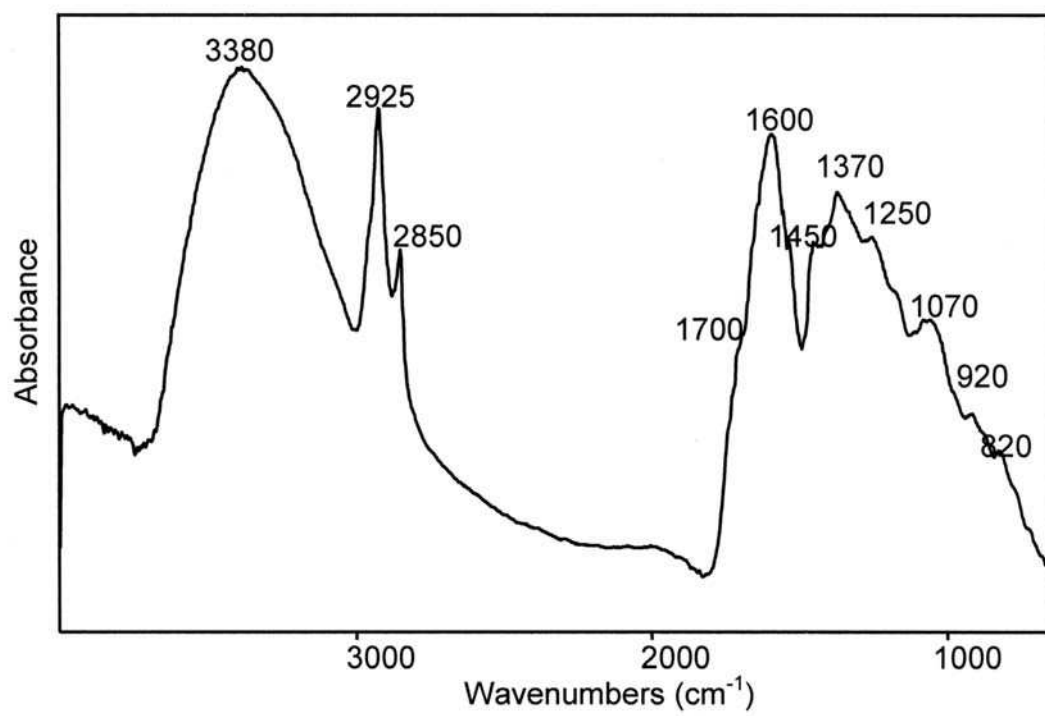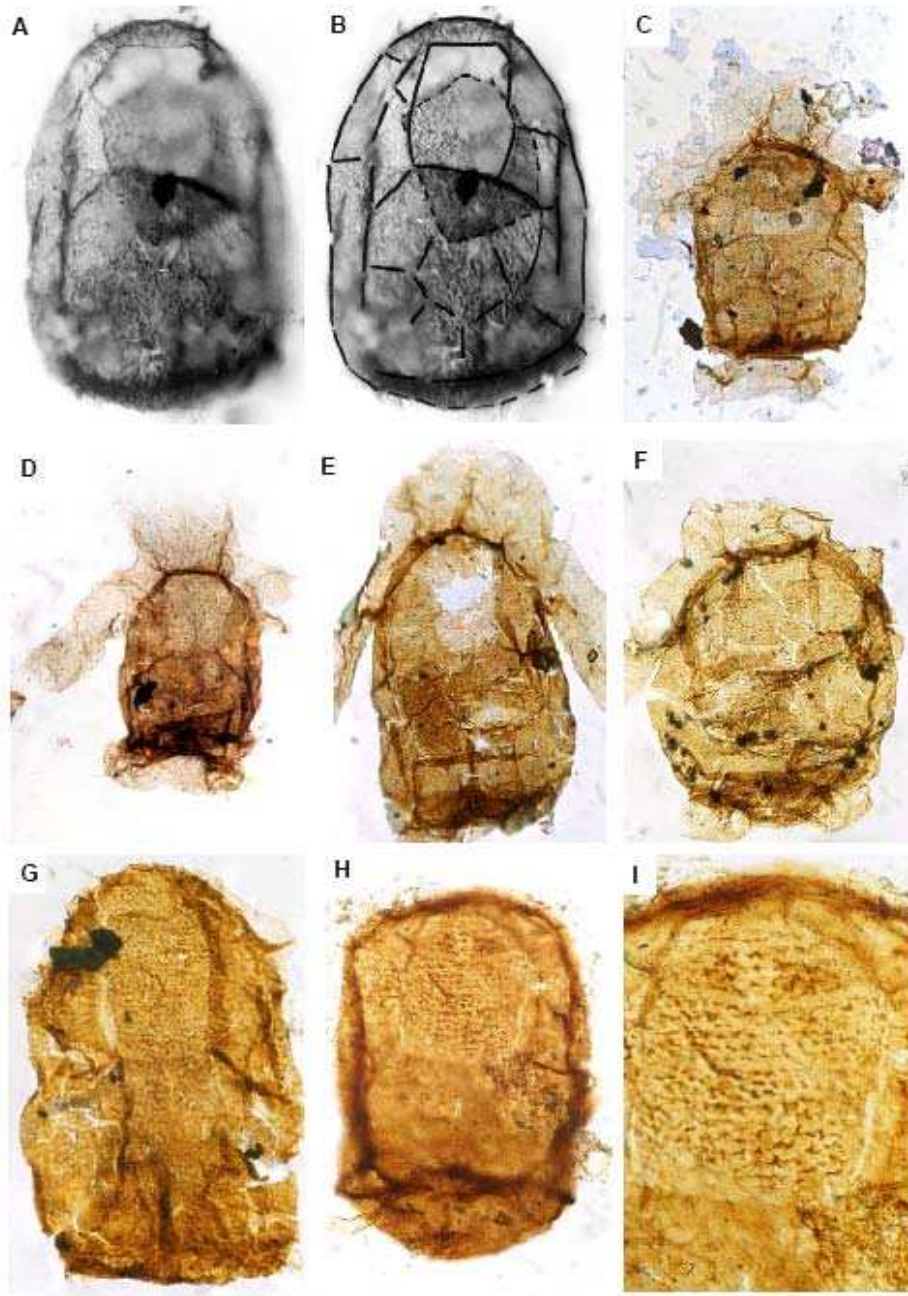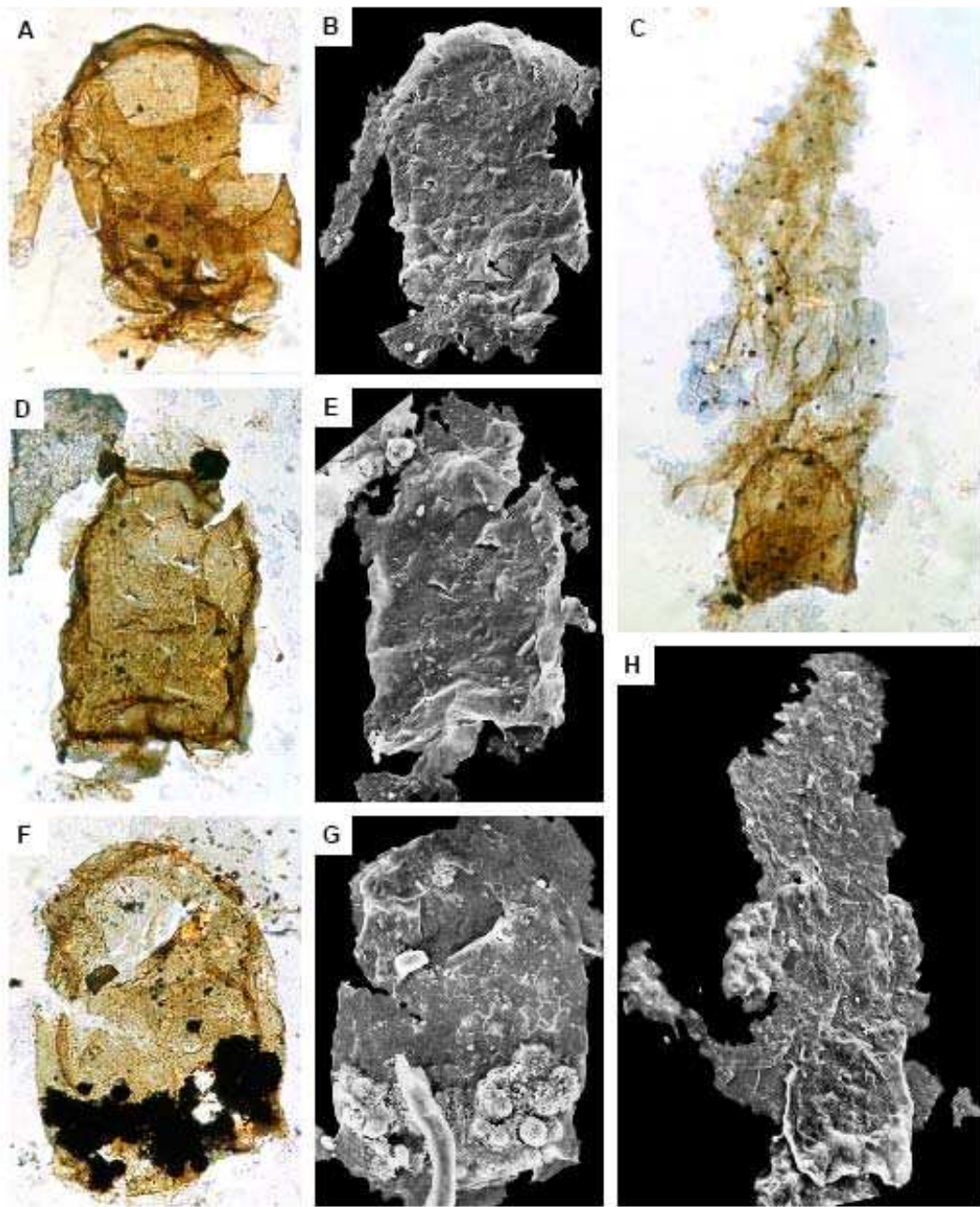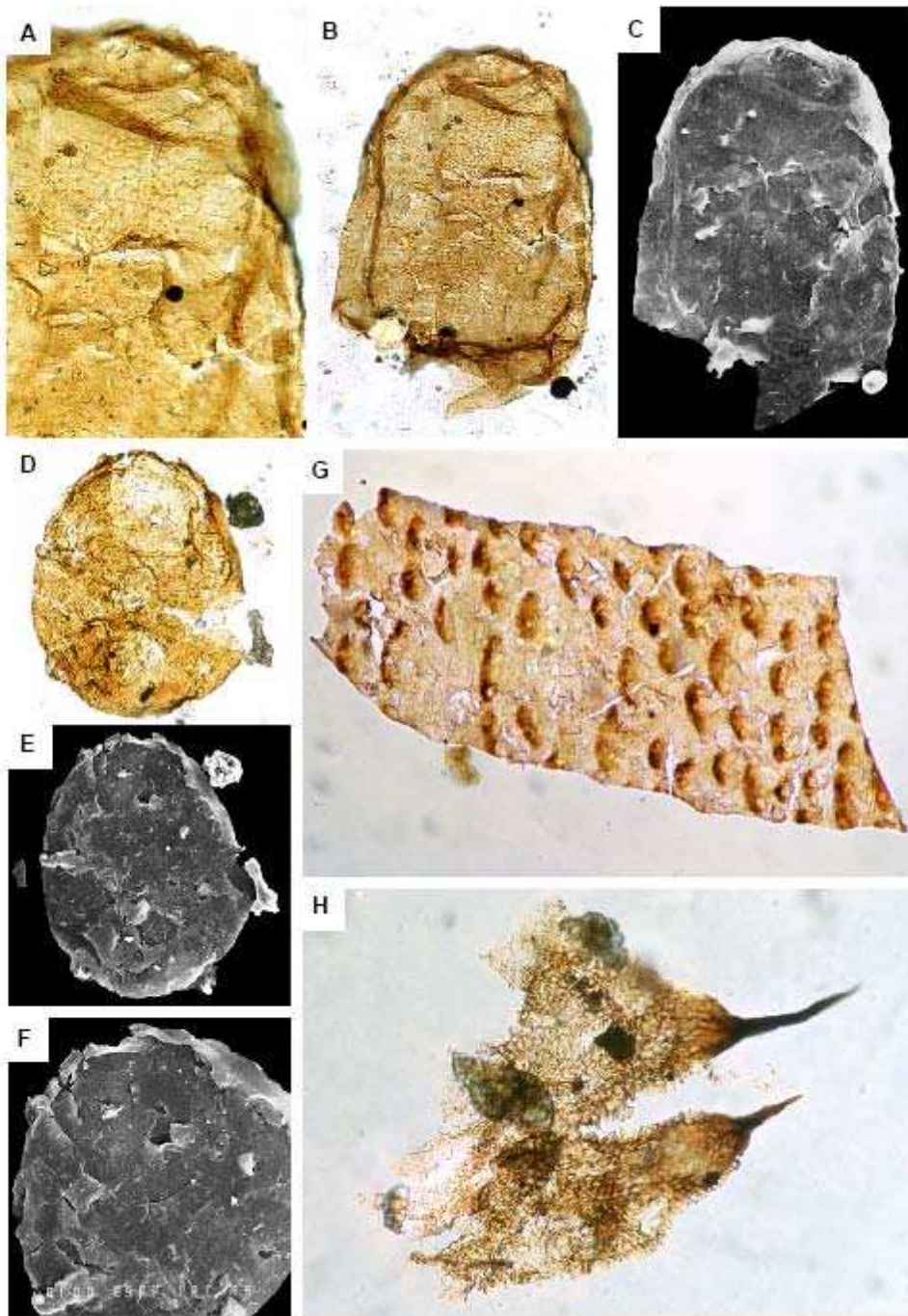
operculum

opening

D
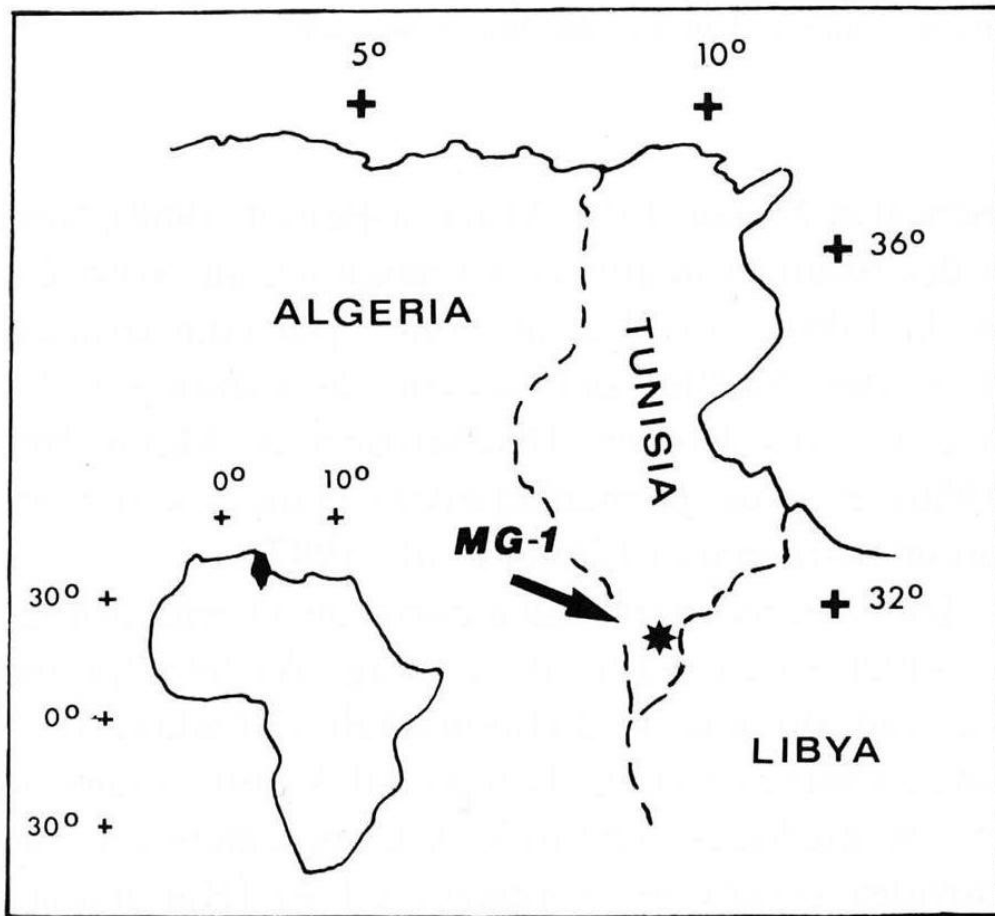
Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

Figure 8.