

# Coprolite specimens from Pietraraja (lower Cretaceous, Southern Italy): morphological analysis by scanning electron microscopy

G. Russo,<sup>1</sup> P. Raia,<sup>2</sup> M. Lauteri<sup>1</sup>

<sup>1</sup>Consiglio Nazionale delle Ricerche (CNR), Istituto di Biologia Agroambientale e Forestale (IBAF) - Porano (TR), Italy

<sup>2</sup>Università degli Studi Napoli Federico II, Dipartimento di Scienze della Terra, dell'Ambiente e delle Risorse, Naples, Italy

Corresponding author: Giuseppe Russo

Consiglio Nazionale delle Ricerche (CNR), Istituto di Biologia Agroambientale e Forestale (IBAF),

Via Guglielmo Marconi 2, 05010 Porano (TR), Italy

Tel. +39.0763.37491; Mobile: +39.320.5758929.

E-mail: giuseppe.russo@ibaf.cnr.it

## Summary

The excavation in the palaeontological site of Pietraraja (Benevento, Italy) allowed to discover a large number of coprolites, though a precise stratigraphy would be only conceivable. The microscopic analysis of the samples provided valid elements to identify the producer and the palaeoenvironment of the icno-fossilization, through the identifications of the inclusions and the possible causes of preservation. The final part of this study presents elements to identify the producer comparing the microscopical evidences of the coprolites with rests of associated fauna, in particular the plausible dental apparatus of a *Notagodus pentlandi*.

**Key words:** coprolites, cretaceous, palaeoenvironment, fossils, fish, SEM.

## Introduction

The *Civita* di Pietraraja (lat. 41.35; long. 15.55; alt. 832 a.s.l.) is a locality on the eastern Matese mountains, 70 km northeast of Naples (Southern Italy) (Figure 1). Since the 18th century, the area was known for the beautiful fossil fish, exquisitely preserved in marly limestone, that are called *itti-oliti* (Italian, for *fish-stones*). The *Civita* di Pietraraja locality is actually a fossil Lagerstätte, dated to the Lower Cretaceous. The area of the main fossil site is loosely fenced, several buildings have been built on the fringes of the 'official' fossil site, including a football field and a never completed hospice for elder persons and two water reservoirs. All of these buildings were built upon layers of fossiliferous limestone, thus hampering research and recovery of fossils (Signore, 2004).

The coprolites are icnofossils, members of a group of fossil traces that includes *regurgitalites* (dejections from the oral cavity) and *colalites* (intestinal content fossilized *in situ*). Hunt (1992) utilized the term bromalites to include all these

fossil traces. Though found in beyond 20 countries and from a wide temporal range (Palaeozoic-Quaternary), often the coprolites without inclusions of bones were ignored in spite of the high paleoecological information about the environment related to. Most of the literature is about cretaceous coprolites derived from dinosaurs, probably herbivorous, as in the cases of India (Prasad *et al.*, 2005) or the Two Medicine formation in Montana, USA (Chin, 2007). In the European Cretaceous some cases evidenced small coprolites as in Portugal (Friis *et al.*, 2004) or the termite coprolites in France (Colin, 2011). Fish coprolites from Morocco were studied (Lamboy *et al.*, 1994) and, considering the paleogeography of Cretaceous, this represents the nearest scenario to Pietraraja site: a tropical lagoon with the contribution of a submarine channel (Carannante *et al.*, 2006).

## Materials and Methods

The specimens from the Pietraraja site are not

linked to a precise stratigraphy; unfortunately, the excavation of the site has presented many problems (Signore, 2004) and the coprolites are a sort of remains, both for technical and preconception reasons, in spite of the potential palaeo-informations that they can provide. It is more plausible that these fossils came from the more massive *catastrophic* layer, most possibly developed by rapid and catastrophic events of submarine slides (Signore, 2004) because the specimens including coprolites were often associated with dental batteries coming from durophagous fish, most possibly *Coelodus* sp. (Signore, 2004). The specimens analyzed, about 1-4 cm long, are several but the only considered were those without macroscopic inclusions (bones, unidentified rests) and including eventual microscopic details. Observations by Scanning Electron Microscope (SEM) were made on specimens, previously metalized by a vacuum evaporator JEE 4B (Jeol USA Inc.), and coated by 10-20 mg of gold. Specimens were subsequently observed under a Quanta 200 ESEM (FEI

Corporate, Oregon, USA), at the CISME center of the Federico II University of Naples.

## Results and Discussion

The first question could be: are the samples coprolites? In fact, the samples could be simple sedimentary phosphates. The nature of coprolites is suggested by their large quantities in a paleoenvironment identified as water-based, the probable nature of inclusions (Figures 2-5) as vegetables (the coprolites of herbivorous presents an elevated degree of conservation; Hill, 1976) and the presence of phosphate: actually the causes of phosphatization process are not known but the coprolites represent the main site of enucleation for the precipitation of the apatitic sediment (Ece, 1990). The external morphology of the observed coprolites does not permit to identify the fecal producer or to classify easily the icnofossil according to a discrimination about polarity. In



Figure 1. The Lagerstätte of Pietraroja (Italy).

fact no kind of morphology or remark of lamination is identifiable; then the analysis of inclusions only can be predictive. In spite of these considerations, the preservation of the fossil gives general information about the producer: the preferential preservation of the coprolites is the diet. Bradley (1946) suggested that calcium phosphate of the carnivorous diet can be the best permineralizant agent. Ca and Si are the most frequent elements in the samples as recognized by the SEM probes, most likely these elements compose the grain and the matrix of the specimens; the same instrument

cannot detect carbon and nitrogen. Moreover the association in the fossil record allows the coprolites to be related to the fish-fauna, because dental apparatuses from durophagous and carnivorous fish were discovered in the same paleoenvironment. The group of the observed fibers (Figure 2a; Figure 3; Figure 4) exhibiting a spiral pattern is consistent with materials digested and dejected by evolved fish groups (Kapoor *et al.*, 1975), though the inclusion would be considered as compressed. The pores, identified at higher magnification (Figures 2b; 5 a-d), appear bended if compared

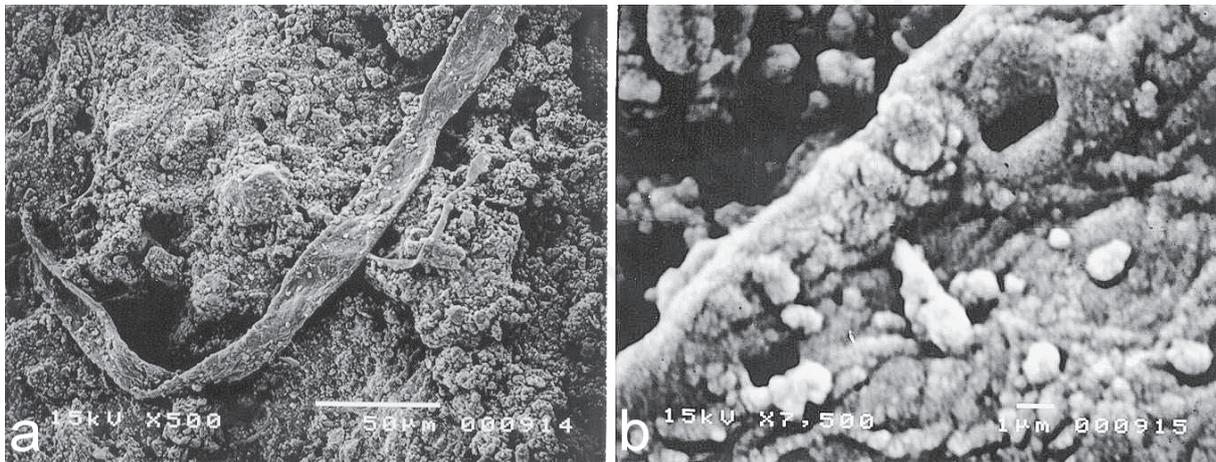


Figure 2. Single vegetable fiber (a); detail of a presumptive stomatal evidence (b). Bar: 50 µm (a); bar: 1 µm (b).

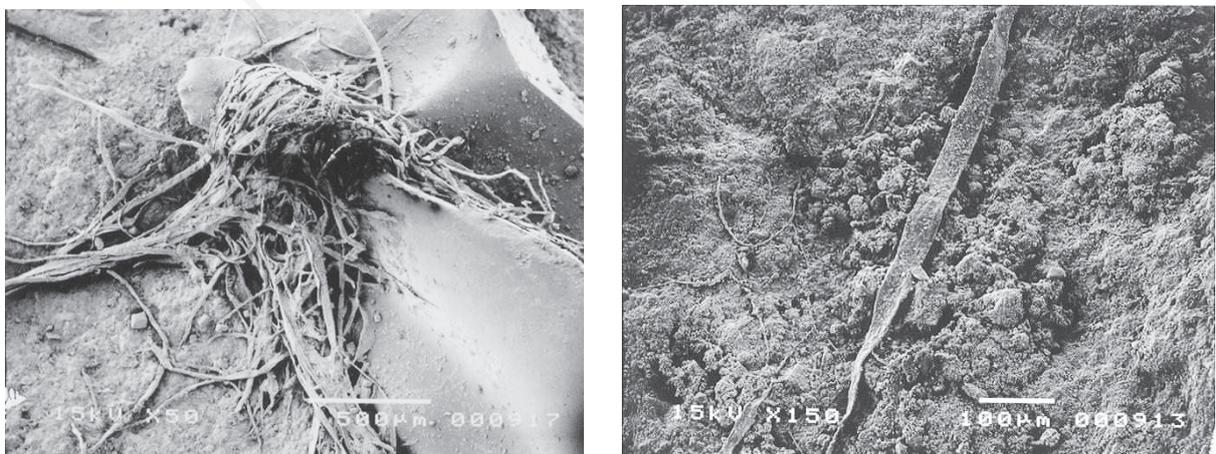


Figure 3. High concentration of vegetable fibers. Bar: 500 µm.

Figure 4. Single vegetable fiber present in the matrix of a coprolite. Bar: 100 µm.

with the length of the structure and in a serial succession. This could implicate that the pores are stomata, thus excluding that the inclusions might be algae; furthermore algae were never found in the fossil record of Pietraraja site. Initially, these vegetable fragments were supposed to belong to the Gymnosperm group (which is present in the Pietraraja paleoflora), but the traits observed would place the inclusions among the primitive Angiosperms, whose presence is not well documented in the fossil record though rests are cited in literature (Bartirromo *et al.*, 2006). This could be consistent with the large diffusion of Angiosperms since the lower Cretaceous at lower latitudes (Hughes, 1994). The only doubtful feature is the too small size of stomata (about  $3\ \mu\text{m}$ ), though an epidermal accretion could make them less visible and the typical sunken structure is similar as in

some higher plants adapted to xeric climate (Schimper, 1903). The great quantity of preserved fishes in a lagoon of lower Cretaceous, the morphology of dejection and the absence of residual bones suggest that the coprolite producer were herbivorous fishes.

Another hypothesis deals with the entomologic nature of inclusions as tracheas of insect, dorsal vessels or crustacean esocuticle. The tracheas of insects have a typical spiral structures (Strelin, 2012), but they do not resemble the observed inclusions, whereas the vessels which are made of a soft tissue could hardly be fossilized or preserved; the inclusions found actually exhibit the rhomboidal forms as the scales of some crustaceans (Euphasiacea, Anfibodia) with pores resembling stigma, but these crustacean taxa are absent in the fossil record of Pietraraja. Thus, this

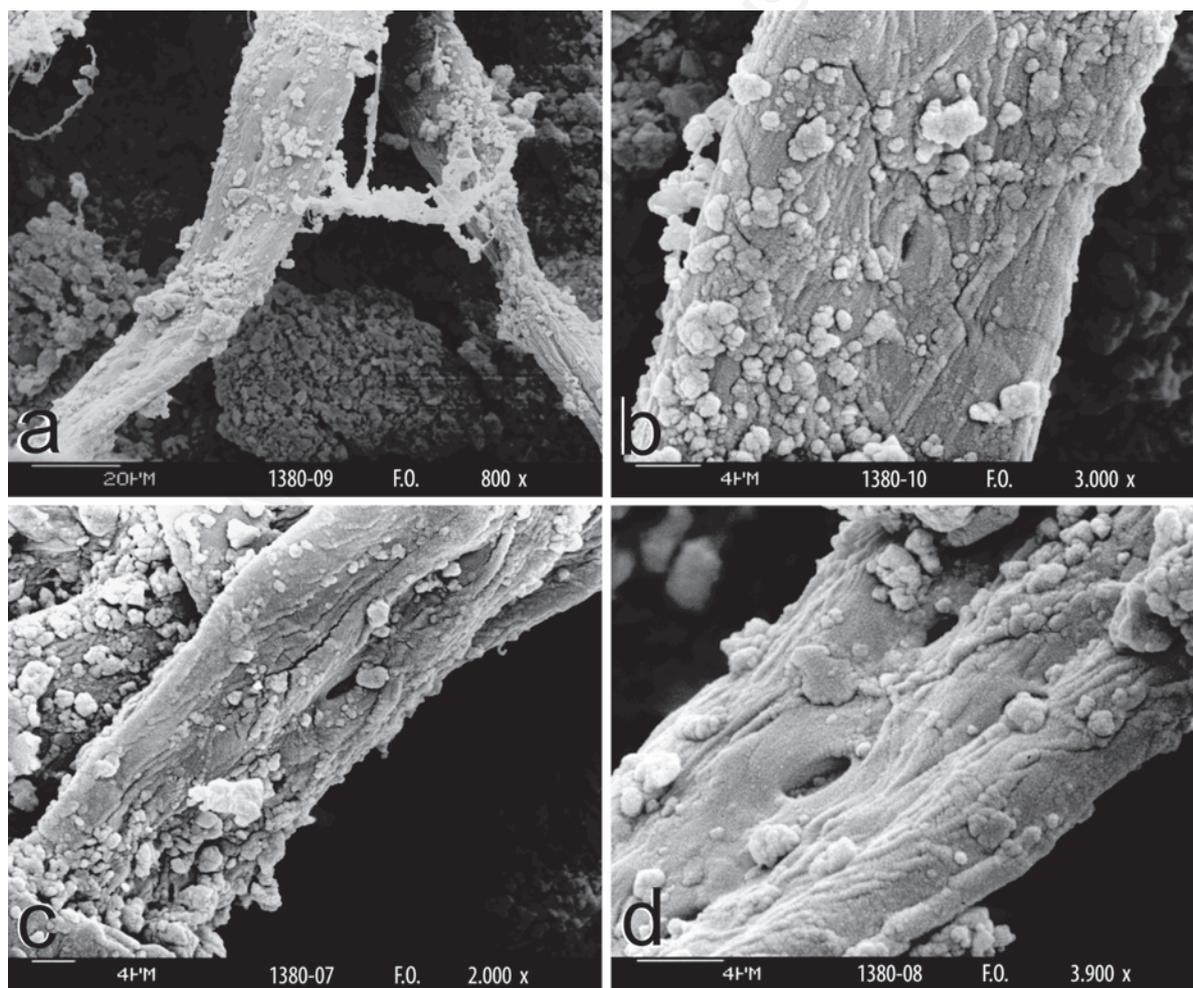


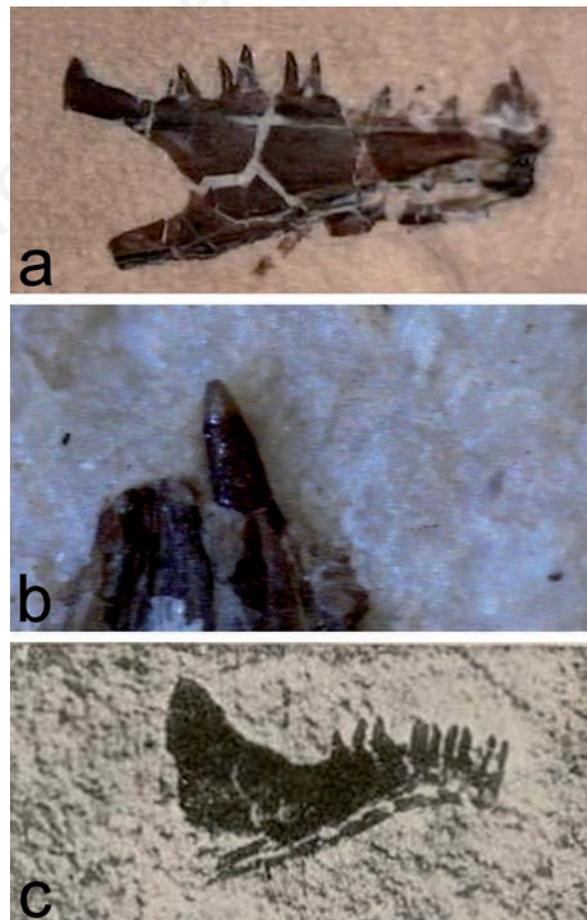
Figure 5. Details of a vegetable fiber with stomatal evidence. Bar:  $20\ \mu\text{m}$  (a); bar:  $4\ \mu\text{m}$  (b-d).

hypothesis is unlikely. Furthermore, studies on the activity of fish digestive enzyme demonstrated that the chitinous material is completely degraded (Gutowska, 2002), while less than half of the plant material ingested by an herbivorous fish is assimilated (Targett and Targett, 1990). The mechanisms of fossilization are attributable to permineralization process with tridimensional conservation of tissues, if early diagenesis occurs, the precipitation of mineral takes place in the first phases of anaerobic fermentation, without the complete obliteration of the structures of vegetable cells (Perkins, 1976). The presence of Si and Ca, as demonstrated by SEM, would confirm this process during which the permineralized inclusions were not compressed by the  $\text{NH}_3$ -releasing decomposition. The presence of a more basic environment would facilitate  $\text{CaCO}_3$  precipitation inside the decomposing tissue as in the case of Santana Formation (Brasil), where the francolite permineralized the tissues (Martill, 1988).

The fish digestive systems differ according to the diet: it is short and rectilinear in the carnivorous species or long and convoluted in the herbivorous ones (Helfman *et al.*, 2009); the presence and orientation of the vegetable fibers in the coprolites seem to be more compatible with herbivorous fish. This makes it likely that hypothetical producer could be an herbivorous fish living in a changeable water-based environment with primitive Angiosperms (riparian?). The fossil record, though rich of examples, does not permit to recognize the changes in the marine environment or the interactions and the behaviour of predator and herbivorous fishes. The hypothesized riparian ecosystem, in which the producer deposited its dejection, is plausibly characterized by turbidity due to the grazing activity and floods, with a reduced photosynthesis. In coastal settings there is a great complexity if compared to basin and riverine wetlands: the latter is subjected to inundation with salt water, disturbance regimes and other environmental gradients (Hunter, 2000). Because of fluctuating water regimes, there is a dynamic interface between aerobic and anaerobic conditions, causing leaf abscission and the consequent favorable conditions for fossilization. The presence of vegetable inclusions is linked to a certain abundance and probably a lower index of biodiversity. These aspects characterize the riparian as extreme salty zone, favorable to species able to adapt their habits to a specific diet.

#### Elements to hypothesize *Notagogus pentlandi* Agassiz as the coprolite producer

A remarkable case of association with the coprolite samples is constituted by the discovery of a dental apparatus (Figure 6a-b); a similar structure was never found in the fossil record of Pietraraja site and, although the correspondence producer-production can only be hypothesized, it offers the basis for an investigative approach. The most similar dental apparatus present in the fossil record of Pietraraja belongs to *Notagogus pentlandi* (Agassiz, 1835; Figure 6c) that presents about 10 teeth on the mandible (Bravi, 1994). The analyzed dental apparatus (about 0.5 cm in length, eterodontic and with a small number of teeth)



**Figure 6.** Dental apparatus of discovered in association with the coprolite specimens (a). Detail of a conic tooth (b). Rest of dental apparatus of *Notagogus pentlandi* found in Pietraraja site (c).

would indicate *rubble* habits of the fish. Two groups of teeth are present: long incisive (number not well defined) and three triangular teeth, almost conical, with a large basis (Figure 6a). The groups are equidistant, including the final teeth, characterized by three groups of conical teeth of two each. It is plausible an increase of thickness of dental basis in the distal position of the apparatus. The dimensions of the sample could be too low than cited in literature, but D'Erasmus (1914), describing *Notagogus*, attributes a certain intraspecific variability, that previously caused mistakes in the classification. The presence of a white extremity at the top of a conical tooth would establish that the fish belong to the Osteichthyes, and recent studies in a Morocco area similar to Pietraraja site clearly suggest the family Macrosemiidae (Murray and Wilson, 2009); in particular the observed specimen could be premaxillary teeth. Bartram (1977) describes the teeth of *Notagogus* as mammaliform, and evidence of barbed or promiscuous teeth in the species discovered at the Tlayua Quarries site in Central Mexico (Gonzalez-Rodriguez *et al.*, 2004) would indicate a similarity with the present case of study. The characteristics of the Mexican site are similar to Pietraraja (fish limestones), with presence of *Notagogus pentlandi* and also, in a lower number, *Notagogus helenae*. Stratigraphic information suggests a double migration of the family from its original distribution in Tethys Ocean to the west, following the aperture of the northern part of the Atlantic Ocean until Mexico (Gonzalez-Rodriguez and Reynoso, 2004). It is the only European species of Macrosemiidae present in South America. In Spain, the Macrosemiidae as *Notagogus* are often associated to both cliff and fresh-water environments. The specimens of

Tlayua Quarries would be associated to cliff environment, though a massive presence of fresh-water fauna. Furthermore in the Mexican specimens of *Notagogus* a spiral digestive tract was fossilized, except for two species with straight one, confirming the herbivorous habits of the fish. The presence of animals in the intact enterolites (crustaceans, mollusks and rests of fishes) identifies the genera as carnivorous but it was hypothesized that *Notagogus* can vary its diet according to the period in which trophic resources were poor: it was hypothesized that *Notagogus* could even eat plancton (Gonzalez-Rodriguez and Reynoso, 2004).

The lower Cretaceous site of Pietraraja was therefore comparable with an actual tropical lagoon with fresh-water channels, i.e. an environment favorable to the preservation of herbivorous coprolites. The nature of the coprolite producer is identifiable with an herbivorous fish of a plausible riparian paleoenvironment, maybe a species able to change the diet according to the environmental scenery at the moment. SEM analysis of coprolites and their inclusions can provide useful evidence about the paleoenvironment (producer species, products and trophic dynamics), as in the case of the present study, even when referring stratigraphic data are lacking. Further investigations about the coprolites in Pietraraja will include chemical analyses, a more precise stratigraphy and a statistical analysis on the quantitative data.

## Acknowledgements

The authors thank all the researchers that supported the present study.

## References

- Bartiromo A, Barone Lumaga MR, Bravi S. First finding of a fossil fern (Matoniaceae) in the paleontological site of Pietraraja (Benevento, Southern Italy). *B Soc Paleontol Ital* 2006;45:29-34.
- Bartram AWH. The Macrosemiidae, a Mesozoic family of holostean fishes. *Bull Br Mus Nat Hist Geol* 1977;29: 137-234.
- Bradley WH. 1946. Coprolites from the Bridger Formation of Wyoming: their composition and microorganisms. *Am J Sc* 1946;244:215-39.
- Bravi S. New observations on the Lower Cretaceous fish *Notagogus pentlandi* Agassiz (Actinopterygii, Halecostomi, Macrosemiidae). *B Soc Paleontol Ital* 1994;33:51-70.
- Carannante G, Signore M, Vigorito M. Vertebrate-rich Plattenkalk of Pietraraja (Lower Cretaceous, Southern Apennines, Italy): a new model. *Facies* 2006;52:555-77.

- Chin K. The palaeobiological implications of herbivorous dinosaur coprolites from the upper cretaceous Two Medicine formation of Montana: why eat wood? *Palaios* 2007;22:554-66.
- Colin JP. Termite coprolites (Insecta: Isoptera) from the Cretaceous of western France: a palaeoecological insight. *Revue de Micropaléontologie* 2011;54:129-39.
- D'Erasmus G. La fauna e l'età dei calcari a ittioliti di Pietraroia (provincia di Benevento). *Palaeontogr Ital* 1914;20:29-86.
- Ece OI. Geochemistry and occurrence of authigenic phosphate nodules from the Desmionesian cyclic Excello epeiric sea of the Midcontinent, USA. *Mar Petrol Geol* 1990;7:298-312.
- Friis EM, Pedersen KR, Crane PR. Araceae from the Early Cretaceous of Portugal: evidence on the emergence of monocotyledons. *P Natl Acad Sci USA* 2004;101:16565-70.
- Gonzalez-Rodriguez K, Applegate SP, Espinosa-Arraburena L. A new world Macrosemiid (Pisces: Neopterygii-Halecostomi) from the Albian of Mexico. *J Vertebr Paleontol* 2004;24:281-9.
- Gonzalez-Rodriguez K, Reynoso VH. A new Notagogus (Macrosemiidae, Halecostomi) species from the Albian Tlayúa Quarry, Central Mexico. *Mesozoic Fishes 3 - Systematics, Paleoenvironments and Biodiversity*. In: Arratia G, Tintori A, editors; 2004. p. 265-78.
- Gutowska M. Chitinase activity of fishes with varying depth distributions. MBARI publications, Annual Report; 2002.
- Helfman G, Collette B, Facey BE, Bowen BW. *The Diversity of Fishes: Biology, Evolution, and Ecology*. Wiley; 2009.
- Hughes NF. *The enigma of angiosperm origins*. Cambridge University Press; 1994.
- Hunt AP, Chin K, Lockley MG. The palaeobiology of vertebrate coprolites. *The Palaeobiology of Trace Fossils*. 2004. p. 221-40.
- Hunter ML. *Maintaining biodiversity in forest ecosystems*. University of Cambridge; 2000.
- Kapoor, BB, Smit H, Verighina IA. The alimentary canal and digestion in teleosts. *Adv Mar Biol* 1975;13:109-239.
- Martill DM. Preservation of fish in the Santana Formation of Brazil. *Palaeontology* 1988;31:1-18.
- Murray AM, Wilson MVH. A new late Cretaceous Macrosemiid Fish (Neopterygii, Halecostomi) from Morocco, with temporal and geographical range extensions for the family. *Palaeontology* 2009;52:429-40.
- Perkins TW. Textures and conditions of formation of middle Pennsylvanian coal balls, Central United States. *The University of Kansas Paleontological Contributions* 1976;82:1-13.
- Prasad V, Stromberg CAE, Alimohammadian H, Sahni A. Dinosaur coprolites and the early evolution of grasses and grazers. *Science* 2005;310:1177-80.
- Schimper AFW. *Plant-geography upon a physiological basis*. Oxford: Clarendon Press; 1903.
- Signore M. Sample excavations in Pietraroia (lower Cretaceous, Southern Italy) in 2001 and notes on the Pietraroia palaeoenvironment. [www.PalArch.nl](http://www.PalArch.nl), *Vertebrate Palaeontology* 2004;2:2.
- Strelin GS. Patterns in the spiral formations of insects. *Morfologija* 1993;105:104-14.
- Targett TE, Targett NM. Energetics of food selection by the herbivorous parrotfish *Sparisoma radians*: roles of assimilation efficiency, gut evacuation rate, and algal secondary metabolites. *Mar Ecol Prog Ser* 1990; 66:13-21.