



ATTI
DELLA
SOCIETÀ TOSCANA
DI
SCIENZE NATURALI

MEMORIE • SERIE A • VOLUME CXXII • ANNO 2015



Edizioni ETS



Con il contributo del Museo di Storia Naturale dell'Università di Pisa



e della Fondazione Cassa di Risparmio di Lucca

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NICOLETTA BEDOSTI (*), WALTER LANDINI (**), RUGGERO D'ANASTASIO (***)

THE INCREASE OF BONY MASS IN A SMALL CYPRINODONTIDAE FROM THE MESSINIAN DEPOSIT OF MONTE TONDO (RAVENNA, ITALY); PALEOECOLOGICAL IMPLICATIONS

Abstract - *The increase of bony mass in a small Cyprinodontidae from the Messinian deposit of Monte Tondo (Ravenna, Italy); paleoecological implications.* The ichthyofauna of the late Messinian succession of Monte Tondo (Ravenna, Italy) is examined. Forty *Aphanius crassicaudus*, one clupeid and five goby specimens are described. Morphometric and meristic characteristics are determined, and a pachyostosis index is presented for the small cyprinodontid (*A. crassicaudus*) specimens. *Aphanius crassicaudus* in the Monte Tondo assemblage is represented by the two well-known Messinian phenotypes (non-pachyostotic or normal and pachyostotic). The increase in bone mass in the pachyostotic specimens is generally homogeneously extended throughout the body, but in the Monte Tondo assemblage, one young specimen of *A. crassicaudus* exhibits a peculiar characteristic distribution of increasing bone mass in the skeleton from head to caudal fin. *A. crassicaudus* was a dominant constituent of Mediterranean estuarine/lagoon teleost assemblages during the Messinian. The composition and ecological structure of the ichthyic association indicates that the lagoon of the Monte Tondo basin was a brackish water body that was occasionally influenced by seawater influx.

Keywords - Pachyostosis, Teleostei, Messinian, lagoon, Italy.

Riassunto - *Incremento della massa ossea in un piccolo Cyprinodontae proveniente dai depositi del Messiniano superiore di Monte Tondo (Ravenna, Italia): implicazioni paleoecologiche.* Viene studiato un nuovo giacimento di pesci fossili provenienti dalla successione del Messiniano superiore di Monte Tondo. Vengono descritti quaranta esemplari di *Aphanius crassicaudus*, cinque di *Gobius sp.* ed uno di *Clupeonella sp.* (Clupeidae). Vengono misurati i caratteri morfometrici e meristici degli esemplari di *A. crassicaudus* meglio conservati e ne viene calcolato l'indice di pachiosiosi. Gli esemplari di *A. crassicaudus* di Monte Tondo sono rappresentati dai due fenotipi messiniani ben conosciuti (il non pachiosiotico, o normale e il pachiosiotico). Negli esemplari pachiosiotici l'ipertrofia ossea è in genere omogeneamente estesa a tutto il corpo, ma un giovane esemplare di *A. crassicaudus* proveniente da Monte Tondo è caratterizzato da una distribuzione particolare dell'ingrossamento scheletrico lungo il corpo. Nel bacino del Mediterraneo durante il Messiniano, *A. crassicaudus* rappresentava il costituente dominante delle associazioni di Teleostei negli ambienti lagunari e salmastri. L'analisi della composizione ecologica dell'ittiofauna di Monte Tondo indica che questo ambiente era costituito da acqua salmastra, occasionalmente interessata dall'influsso di acqua marina.

Parole chiave - Pachiosiosi, Teleostei, Messiniano, laguna, Italia.

INTRODUCTION

During the Messinian, brackish and hyperhaline lagoons were inhabited by characteristic oligotypic teleost communities dominated by the small cyprinodontid *Aphanius crassicaudus* (Gaudant, 1979; Landini & Sorbini, 1989). The broad dominance of *Aphanius* in these fossil assemblages throughout the Mediterranean basin is indicative of specific, frequently extreme environmental conditions (Landini & Sorbini, 1989). In the Messinian fossil record, *A. crassicaudus* is represented by two different phenotypes: one with a thin skeleton and the second with an enlarged, pachyostotic skeletal structure that was previously denoted "*Pachylebias crassicaudus*" (Agassiz) (Gaudant, 1979 a). Pachyostosis in *A. crassicaudus* is a bony hypertrophy that is homogeneously extended throughout the entire skeleton (Meunier & Gaudant, 1987) but is also occasionally present as small, globose structures (Bedosti, 1999). These structures are occasionally referred to as "Tilly bones" in numerous marine teleost fishes (Smith-Vaniz *et al.*, 1995). Pachyostosis *sensu stricto* is a hyperplasy (increase in thickness) of periosteal cortices that leads to an alteration of bone morphology by causing an increase in bone volume (Houssaye, 2012). Histological analysis has indicated that, in *A. crassicaudus*, the hypertrophic bone is acellular (absence of osteocytes, osteoblasts and osteoclasts), avascular and compact (Meunier & Gaudant, 1987; D'Anastasio, 2004). By contrast, the hypertrophic bony structure of marine Teleostei affected by pachyostosis exhibits bone remodeling centers, such as in the haversian canals or involving osteoblasts and osteoclasts (Meunier & Gaudant, 1987; D'Anastasio, 2004), and becomes spongy (Driesch, 1994). Pachyostosis in *Aphanius crassicaudus* appears as enormous skeletal thickening homogeneously extended throughout the entire skeleton. Pachyostosis has been observed whether in other euryhaline teleosts from other Messinian deposits, such as Gobiidae, but is unknown among

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extant actinopterygians, which instead may exhibit “hyperostosis”. A similar condition of pachyostosis has been observed in an extraordinarily thick-boned cyprinid teleost, *Hsianwenia wui*, gen. et sp. nov., from Pliocene salt lake deposits of Qaidam Basin in the northern Tibetan Plateau (Chang M. *et al.*, 2008). *A. crassicaudus* and *H. wui* both occur in deposits rich in carbonates (CaCO_3) and sulfates (CaSO_4) and therefore were well adapted to the extreme conditions caused by the aridification of their environment (Chang M. *et al.*, 2008). However, whereas in *H. wui* the thickening of bones appears at a certain adult size, in *A. crassicaudus* from Cherasco, (Piemonte) (Gaudant, 1979 b) and from Monte Castellaro, (Marche), (Bedosti, 1999), pachyostosis is present and is occasionally very accentuated in even very small (young) specimens. The *A. crassicaudus* assemblage of the Monte Tondo Messinian evaporitic succession studied herein includes specimens of different sizes, allowing us to examine the trend of pachyostosis in relation to their ontogenetic development. We investigate whether pachyostosis is homogeneously extended to the whole skeleton also in young individuals. In the debate on the physicochemical evolution of the Mediterranean during and after

the main evaporitic event, the analysis of fossil teleost remains represents an innovative approach (Carnevale *et al.*, 2008). We describe the distribution and relationships between pachyostotic, non-pachyostotic individuals and the presence of teleost fishes from others environment inside the same community of the Monte Tondo deposit; the aim is to analyze the degree of stress present in these brackish and hyperhaline lagoons, as has been studied in the Cherasco (Piemonte) deposit (Gaudant, 1979 b).

GEOGRAPHIC AND GEOLOGICAL SETTINGS

The specimens described herein were obtained from the outcrops of the Monte Tondo quarry (Figure 1A) located at Borgo Rivola, Riolo Terme, near Ravenna, Italy; the section is located inside the Vena del Gesso Regional Park (Sami & Teodoris, 2013). The Messinian succession of the Vena del Gesso basin consists of 16 climatic-controlled gypsum-sapropel cycles (Figure 1B), each of which is composed of a stratum of gypsum (dry period with strong evaporation – regressive facies) and a stratum of sapropel (wet period with

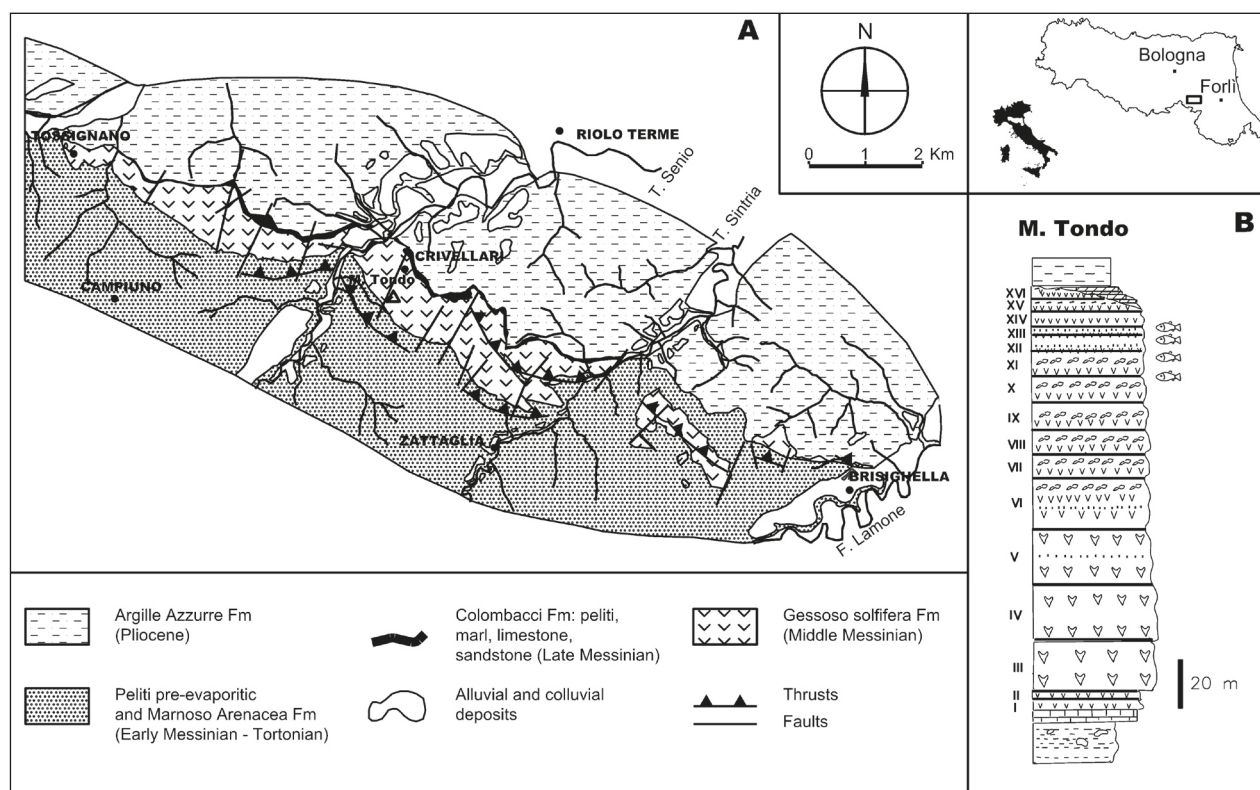


Figure 1 - A. Geographic overview of Italy – Emilia Romagna region; geological outline of Vena del Gesso; B. Schematic profile of the “Gesso – solfifera” Formation at Monte Tondo, showing thicknesses, lithofacies and occurrence of fish fossils (modified after Marabini & Vai, 1985, Bassetti *et al.*, 2004, Montanari *et al.*, 2007, Carnevale *et al.*, 2008).

freshwater input and high precipitations – transgressive facies) (Marabini & Vai, 1985; Marabini & Vai, 1988; Carnevale *et al.*, 2008). The paleontological investigation exploited the excavations that extensively cover the Gessoso-Solfifera Formation outcrops (from the second to the sixteenth evaporitic cycle). The fossils are not distributed uniformly in the levels; only an interlayer belonging certainly at the upper end (probably between the tenth and thirteenth cycle) is rich in fossils. It has not been possible to position this interlayer stratigraphically with greater accuracy because it is involved in the large end of a fault. The interlayer rich in fossils (with a maximum thickness of approximately 3 m) is fragmented in narrow polyhedral blocks in some parts and is yellow because of the intense oxidation of organic matter and sulfides of iron (Sami & Teodoris, 2013). The studied faunae originate from the sapropel interstrata (Gessoso-Solfifera Formation) X to XIII cycle (Sami & Teodoris, 2013), (Figure 1 B) and consist of three taxa representing three families (*Aphanius crassicaudus*, *Gobius sp.* and *Clupeonella sp.*). These thin sapropel interlayers are intercalated with thicker selenite gypsum benches and are the only levels that preserve the remains of organisms (teleost, insects, leaves and other plant parts) that lived on the edge of the basin. These cyclical deposits of the Vena del Gesso basin (Sami & Teodoris, 2013) represent approximately 340,000 years (deposited between 6 million and 5 million and 660,000 years ago).

MATERIALS AND METHODS

The teleost fossil assemblage consists of 46 specimens belonging to *Aphanius crassicaudus* (40), *Gobius sp.* (5) and *Clupeonella sp.* (1). All specimens are housed at the Natural Sciences Museum of Faenza with the provisional code number MT 01 to MT 046. Osteological, meristic and morphometric characteristics were studied under a stereomicroscope equipped with a photo camera (MICROSCOPE ZEISS: STEMI 2000 – C; Camera: CANON AE 1; they are both located at the “Laboratorio di Biologia Marina di Fano” – Pesaro, Italy). Complete measurements were obtained for only 37 specimens to the nearest 0.1 mm using a caliper (software free Image J – Image Processing and Analysis in Java). For the pachyostosis analysis of the *Aphanius* assemblage, only 25 well-preserved specimens were utilized to calculate the pachyostosis index. The index of pachyostosis (\emptyset/l) is defined as the ratio of the diameter and length of the apophyses examined (Gaudant, 1979 c). Specimens with an index value less than 0.2 are considered non-pachyostotic, whereas specimens with an index value greater than 0.2 are considered pachyostotic. Histological sections of the neural apophysis of pachyostotic individual MT 06

(*A. crassicaudus*) were embedded in Technovit® 9100 resin and cut using a microtome with a circular blade and diamond border (Leica 1600 – located at University Museum, Chieti, Italy). The thickness of longitudinal thin sections was approximately 100 μ m.

SYSTEMATIC DESCRIPTIONS

Osteichthyes

- Fam. Cyprinodontidae Myers, 1931
Aphanius crassicaudus (AGASSIZ), 1832
- Fam. Clupeidae Cuvier, 1817
Clupeonella sp. KESSLER, 1877
- Fam. Gobiidae Bonaparte 1810
Gobius sp. LINNAEUS, 1758.

Family CYPRINODONTIDAE Myers, 1931
Genus *Aphanius* NARDO, 1827
Aphanius crassicaudus (AGASSIZ), 1832.

Type material:

MT 01: a supraorbital sensory canal, a dermosphenotic, a dermopterotic, a hyomandibular and a posttemporal, a parasphenoid, a lachrymal, a hyoid arch; MT 0 5 - MT 0 14 b: a dental with a ventral hook-shaped evagination; MT 0 6: histological section of a neural apophysis; MT 0 9 – MT 0 10: a vertebral column consisting of 27 - 29 (12 + 15-17) vertebrae; MT 0 11: a maxilla and premaxilla, well preserved; MT 0 12 – MT 0 18: the caudal skeleton; MT 0 15 a, b: an almost well preserved articulated skeleton and head; MT 0 15 b: a premaxillary tooth; an opercular apparatus; MT 0 17: a caudal fin; MT 0 20: three premaxillary teeth; MT 0 22: a parasphenoid and a pectoral girdle; MT 0 23: cycloid scales; MT 0 25: epineurals positioned close to the vertebral centra (pachyostotic phenotype); MT 0 26: maximum S.L.; MT 0 31: epineurals positioned among the neural apophyses (thin-skeleton phenotype). All other specimens included between MT 0 1 and MT 0 40 are poorly preserved but were partially utilized for the systematic analyses.

Description

Aphanius crassicaudus is a small teleost whose standard length (S.L.) often does not exceed 60 mm (MT 026 S.L. = 69 mm). The body is moderately elongate, more or less rounded anteriorly and slightly compressed posteriorly, with a truncate caudal fin. The S.L. is four or a maximum of five times the body depth; the dorsal fin is anterior to the anal fin, which starts approximately at the level of the posterior rays of the dorsal fin. The caudal peduncle is short, approximately 2 or 3 times the caudal peduncle length. The head is rela-

tively long and pointed; its length is approximately a third to a quarter of the S. L. The orbit is large and located approximately at the middle of the head length. Mouth terminal. The gape is relatively straight, short, and does not reach posteriorly to the level of the anterior margin of the orbit. The neurocranium is moderately deep, with a large orbital region. The orbital diameter is one third to a quarter of the S.L. The dorsal surface of the skull is smooth. The frontals are the largest bones of the skull roof. The supraorbital sensory canal is particularly well exposed in specimen MT 01. The anterior region of the supraoccipital contacts the posterior area of the frontal. The parietals appear to be absent (Gaudant, 1979 c; Gaudant, 1981). The dermosphenotic, dermopterotic and posttemporal are recognizable in specimen MT 01; the bones of the hyoid arch and the robust hyomandibular are also adequately preserved (MT 01). A straight and robust parasphenoid is located in the lower third of the orbit and ends at the anterior part with the lachrymal (MT 01); the nasal is quadrangular. The quadrate is located under the anterior region of the orbit; the quadrate is triangular, and its posterior region is next to the symplectic, whereas its anterior part contacts the angulo-articular. The upper jaw is mainly composed of the premaxilla and of the maxilla, which is less recognizable. On the premaxilla, one tooth (MT 015b) and three teeth with tricuspid crowns (MT 020) are well exposed. The lower jaw is short and composed of the dentary, which presents a hook-shaped evagination in its posterior region (MT 014b, Figure 2). The opercular apparatus is preserved in MT 015b, in which the opercle, subopercle, preopercle and interopercle are easily recognizable. The pectoral girdle is rather large, consisting of a massive cleithrum, a postcleithrum, a well-developed coracoid, and a quadrangular scapula (MT 022). The vertebral column is slightly curved in its abdominal portion and consists of 27-29 (12 + 15-17) vertebrae (MT09, MT010). The 12 abdominal vertebrae are well conserved; their centra are cylindrical and laterally have short paraphophyses. The neural spines are conical, with an axis tilted nearly 45° backward (Gaudant, 1979 c). The anterior region of the neural spine has a small prezygophyse that articulates with the preceding neural spine. The curved pleural ribs vary in number from 9 to 11 and nearly reach the ventral margin of the abdomen. The epineurals are fusiform; they extend posteriorly to the 6th vertebra of the caudal region (MT25, MT31). In the pachyostotic individual, the epineurals are positioned at the bottom of the post-cranial skeleton, close to the vertebral centra (Gaudant, 1979 c), (MT 025), as a rebalancing of the body-mass load as explained for hypertrophy affecting other Teleostei (Driesch, 1994). By contrast, in the thin-skeleton individual, the epineurals are

positioned on the top of the abdominal region, among the neural apophyses (Bedosti, 1999), (MT031). The dorsal fin inserts at the level between the 11th and 12th vertebrae and consists of one or two spines and 9 to 11 soft rays supported by ten pterygiophores. The anal fin inserts at the level of the 14th vertebra. It consists of one spine plus nine or ten soft rays, supported by nine or ten pterygiophores. The pectoral fin inserts below along the lateral side of the body and has 10-12 rays and four basipterygia. The pelvic fin has six rays. The truncate caudal fin has 14-15 principal rays and 12-13 procurent rays. The caudal skeleton has a large triangular hypural plate fused to the last centrum, a parhypural, an epural, and three vertebrae with their haemal and neural spines (MT 012, Figure 3 and MT018). Large cycloid scales (MT 023) cover the body.



Figure 2 - *Aphanius crassicaudus*, the lower jaw: the dentary presents a ventral hook-shaped evagination in its posterior region (MT 014b).

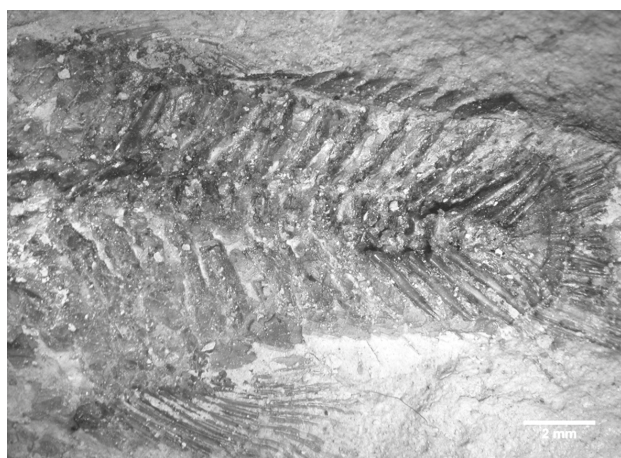


Figure 3 - *Aphanius crassicaudus*, the caudal skeleton. A large triangular hypural plate fused to the last centrum; a parhypural, an epural, three vertebrae with their haemal and neural spines (MT 012).

Family GOBIIDAE Bonaparte 1810,
Genus *Gobius* LINNAEUS, 1758
Gobius sp.

Type material:

MT O 42 a, b: a nearly well-preserved articulated skeleton and head; the dorsal and pectoral fins are visible; MT O 43: a nearly well-preserved articulated skeleton; MT O 44: a caudal skeleton; MT O 45 a, b: an articulated skeleton; MT O 46 a, b: a partially complete skeleton (lacking the caudal skeleton). The five specimens are incomplete, and thus more precise body proportions cannot be provided.

Description

The head is massive; the articulation of the jaw is located just behind the vertical through the middle part of the orbit (MT 042). There are 18 vertebrae in the post-abdominal region (MT 043). The anterior dorsal fin (MT 042) is inserted slightly behind the head and is normally composed of six well-developed spines. The posterior dorsal fin is opposite to the anal fin; it begins slightly forward of the vertical line passing through the origin of the anal fin (MT 043). The pelvic fin is positioned on the chest (MT 042). These features are very similar to those of *Gobius* sp. from Campos de Rio, Murcia, Spain (Gaudant *et al.*, 1994), and from Scaparoni, Piemonte region, Italy (Gaudant, 1979 a); therefore, we attribute this species to the genus *Gobius*. To better assess the presence or absence of pachyostosis in *Gobius* sp. from Monte Tondo, only the ribs, the vertebral centra and the apophyses were analyzed; they are pachyostotic in four specimens (MT 042 to MT 045; Figure 10 A: MT 042; Figure 10 B: MT044) and thin in one specimen (MT 046, Figure 10 C).

Family CLUPEIDAE Cuvier, 1817
Genus *Clupeonella* KESSLER, 1877
Clupeonella sp.

Type material: MT 041: an incomplete specimen lacking the head.

Description

The single available specimen is partially complete and lacks most of the skull (MT 041, Figure 10 D). Therefore, it is not possible to provide measurements and body proportions. The bones of the skull are extensively disarticulated and badly damaged. The neurocranium is not exposed at all. The anterior portion of the left premaxilla is partially exposed in the medial view; premaxillary teeth appear to be absent. In the suspensorium, the quadrate and parts of the metapterygoid and hyomandibular are preserved. The

preopercle is crescent-shaped, with the vertical limb being twice as long as the horizontal limb; the posterior border of the preopercle is smooth and gently rounded; the anterior region of this bone is thickened and encloses the latero-sensory canal. The opercle is quadrangular in shape, characterized by a shallow concavity just behind its anterodorsal corner; the outer surface of this bone is smooth, devoid of ridges and furrows. The subopercle is partially observable. The bones of the hyoid apparatus and gill arches are inadequately preserved and largely not clearly recognizable, with the exception of a number of slender gill rackets that are displaced from their original position. Because of the incompleteness of the examined specimen, it is not possible to properly estimate the original number of vertebrae; 24 vertebrae are preserved, including 17 caudal vertebrae; however, based on the associated pleural ribs, it is possible to estimate a minimum number of 38 vertebrae, implying that the original vertebral complement was likely close to 40 elements.

PACHYOSTOSIS - ANALYSIS

In Messinian teleost communities, *Aphanius crassicaudus* is represented by two distinct phenotypes: normal (thin-skeleton type) and pachyostotic. These two *Aphanius crassicaudus* phenotypes are clearly recognizable in the fossil assemblages of Monte Tondo. The *Aphanius* fossil record at this location is composed of 40 specimens; to define the structure and composition of this population and to understand the relationships between these two phenotypes, we have considered only 25 specimens that are well preserved and whose designation as either phenotype is unambiguous.



Figure 4 - *Aphanius crassicaudus*. The characteristic pachyostotic bones are clearly visible throughout the postcranial skeleton, whereas the cranial bones appear unaffected by any modification of the skeletal tissue (MT 08).

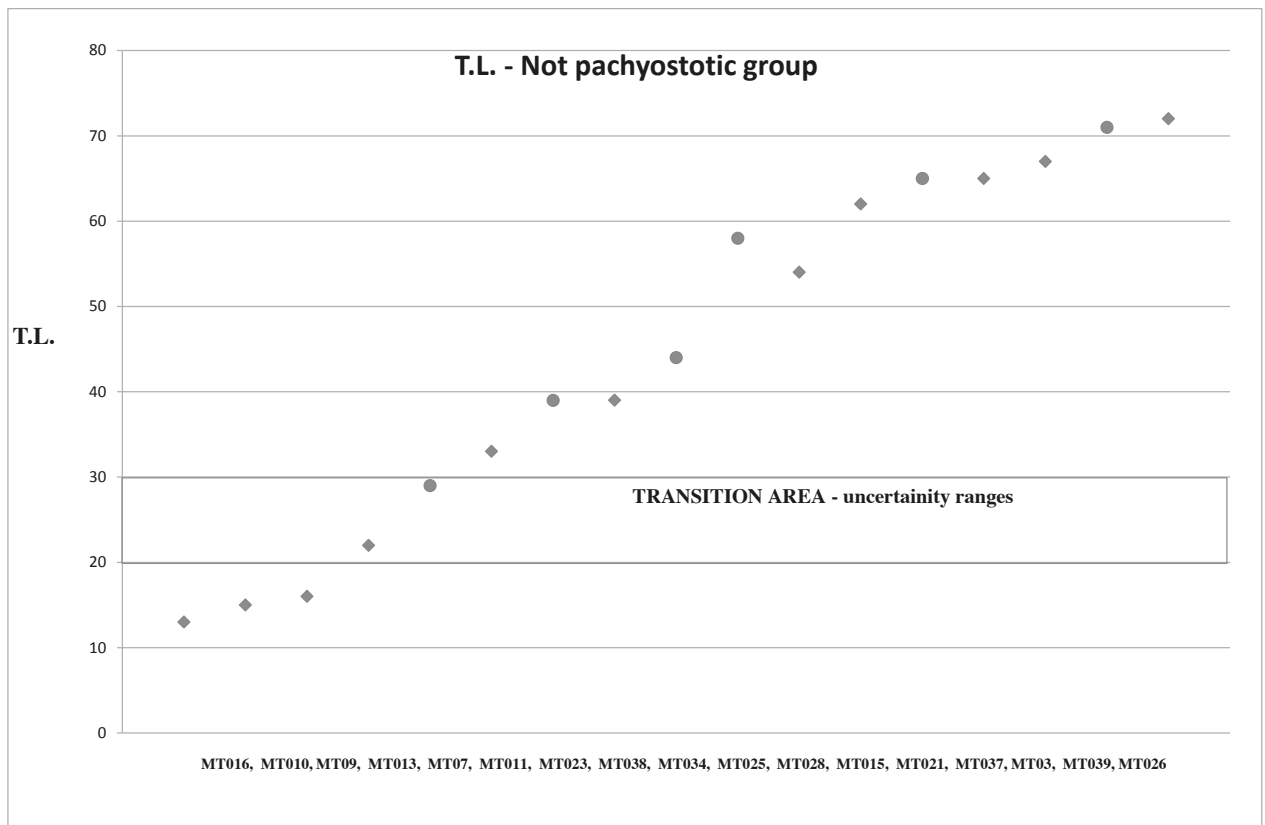


Figure 5 - Non-pachyostotic group - Total length; the transition area is between young and adult individuals; point marks indicate estimated T.L.

The non-pachyostotic phenotype is predominant, with 17 specimens, whereas only eight individuals can be attributed to the pachyostotic phenotype (ratio of non-pachyostotic/pachyostotic = 2.13). Among the non-pachyostotic individuals, the S.L. ranges between 13 and 72 mm (Figure 5), whereas in the pachyostotic individuals the S.L. ranges between 17 and 68 mm (Figure 6). This dimensional range observed in the two phenotypes probably includes young and adult stages. In fact, the extant widespread lagoon and brackish Mediterranean species *A. fasciatus*, the closest relative to the extinct *A. crassicaudus* among the living taxa of this genus, reaches maturity at approximately 20/30 mm T.L. (Zammit-Mangion & Deidun, 2010). Another species of genus *Aphanius*, *Aphanius mento*, reaches maturity at approximately those same values (Güçlü & Küçük, 2011). Because allocation to male or female is not possible in fossil specimens, we consider as immature those individuals under 20 mm (T.L.) and as mature or adults those greater than 30 mm (T.L.); therefore, we treat specimens between 20 and 30 mm (T.L.) as ambiguous in their level of development and do not consider them in the analysis. It was not possible to

measure the T.L. in all examined specimens because of their state of preservation, whereas the S.L. of each individual was recorded. The relationship between the T.L. and S.L. in the best-preserved specimens varies between 1.04 and 1.25 (average 1.16). To compare the examined fossil specimens with living *Aphanius*, we extrapolated the values of T.L. for those specimens having a known S.L. by multiplying the ratio of T.L. to S.L. (average) by each S.L. (marked with a point in the graphics, Figure 5, Figure 6, Figure 7 and Figure 8). The normal (non-pachyostotic) community comprises three specimens whose T.L. ranges between 11 and 16 mm (Figure 5); in the pachyostotic community, there is only one young specimen (MT 08, T.L. = 17 mm) (Figure 6). Within the pachyostotic community of Monte Tondo, the bone mass increases from young to adults (17 mm to 68 mm T.L.). Histological analysis of the neural apophysis of MT 06 (Figure 9) revealed osteosclerotic, compact bone tissue, confirming a bone mass increase. A general increase in density and mass is evident due to the higher compactness of the bone inner architecture (osteosclerosis) and/or from hyperplasia of the bone cortices (pachyostosis). The compact

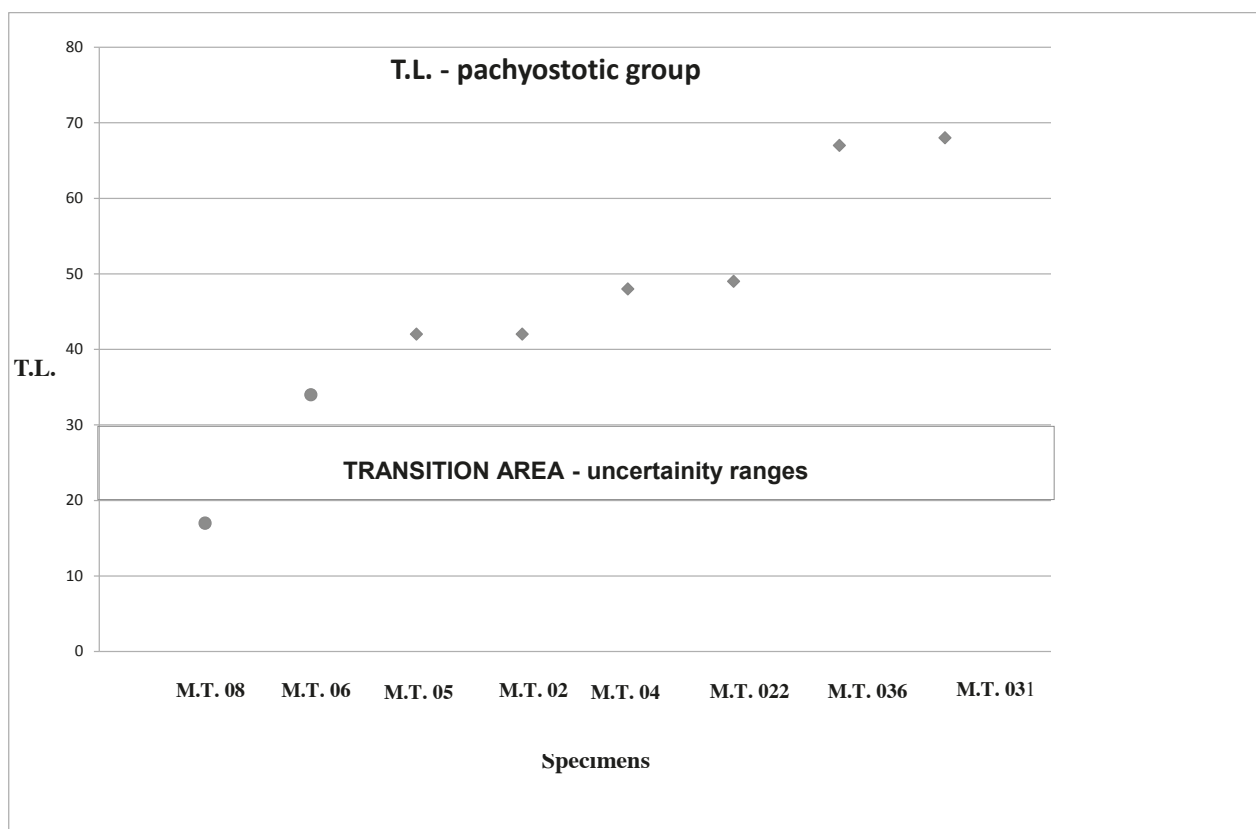


Figure 6 - Pachyostotic group - Total length; the transition area is between young and adult individuals; MT 08 (point mark): estimated T.L.

cortical bone is indistinguishable from the so-called spongy bone. The bone matrix is well mineralized, without osteocytic lacunae. A central structure, presumably a vascular channel, is located at the inner area of the histological section. However, the only pachyostotic young specimen (MT 08) exhibits a peculiar characteristic distribution of pachyostosis along the skeleton. In fact, the characteristic pachyostotic bones are clearly visible throughout the postcranial skeleton, whereas the cranial bones do not seem to be affected by any modification of the skeleton (Figure 4). Gaudant's index was calculated on the whole sample examined in the analysis of pachyostosis (25 specimens; Figure 7 and Figure 8) and ranges between 0.05 and 0.19 in the non-pachyostotic group. In the pachyostotic individuals, the index ranges between 0.2 and 0.30. These values are compatible with Gaudant's assumption. The relationships between the pachyostotic index and total length in the two phenotypic stocks of *Aphanius* are presented in Figure 7 and Figure 8. The distribution of the pachyostosis index exhibits a nearly horizontal trend (Figure 7 and Figure 8); the growth of the index of pachyostosis is not directly proportional,

and the data are highly dispersed, probably indicating a non-significant relationship, such as for *Aphanius crassicaudus* from the Monte Castellaro deposit (Bedosti, 1999). In the pachyostotic group (Figure 8), there is no statistically significant correlation between total length and the pachyostosis index; one specimen with a medium T.L. has a high value of the index of pachyostosis (MT 06; $\Theta/L = 0.30$). Moreover, pachyostosis is present also in a young individual (MT 08; T.L. = 17, $\Theta/L = 0.20$; marked by a point in the graphic) in only the postcranial region and not in the entire skeleton. We compared the two phenotype groups of *Aphanius crassicaudus* from Monte Tondo and their morphometric and meristic characteristics; the skeletons of both phenotypes consist of 27-29 vertebrae, 4-5 of which are caudal. Only the dorsal fin differs between the two phenotypes, with 10-11 rays in the pachyostotic individual but usually only nine rays in the thin skeleton. The body ratios of the two phenotypes and the position and relative lengths of the fins do not differ greatly between the two phenotypes. The only substantial difference between the two phenotypes is in the position of the epineurals. In the pachyostotic indi-

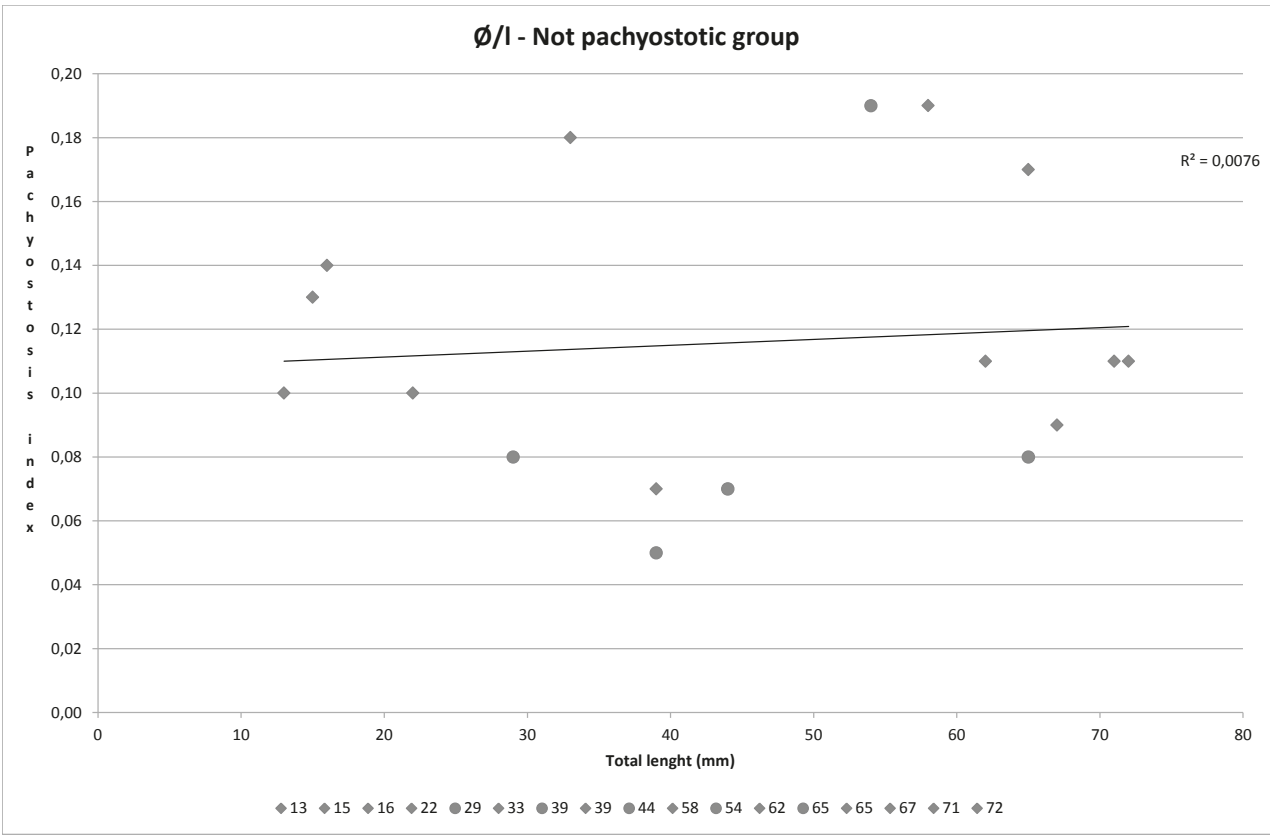


Figure 7 - Index of pachyostosis distribution - Non-pachyostotic group; the T.L. marked by a point was calculated from the known S.L.

vidual, the epineurals are positioned at the bottom of the post-cranial skeleton, close to the vertebral centra, probably as a rebalancing of the body-mass load (MT 025, MT 031), (Gaudant, 1979c, Driesch, 1994; Bedosti, 1999); by contrast, in the thin skeleton specimen, the epineurals are positioned on the top of the post-cranial skeleton, among the neural apophyses.

DISCUSSION

From a histological perspective, the compact osseous tissue in *Aphanius crassicaudus* (MT 06, Figure 9) may represent a heavy structure that prevents excessive negative buoyancy in waters that are dense because of high salinity, as in the Messinian desiccation period; by contrast, the hypertrophic bony structure of marine Teleostei affected by pachyostosis becomes spongy, probably because they did not require rebalancing in marine, non-hypersaline waters (Desse *et al.*, 1981; Meunier & Desse, 1986; Driesch, 1994; Bedosti, 1999; Houssaye, 2012). In addition, under extreme environmental conditions (hypersaline waters), an

hypertrophic bony structure may have offered a larger surface for muscle-junctions, permitting *A. crassicaudus* to swim more efficiently in such dense waters and compensating for the increased mass of the fish (Bedosti, 1999). If pachyostosis results in reduced velocity of movement compared to fish with normal skeletons, this could be detrimental to the survival of *A. crassicaudus*, which was not the only species of fish present in this hypersaline environment. However, in the Monte Tondo deposit, eurhyaline fishes of larger size, such as gobiids, which among living species are the main predators of *A. crassicaudus*, are the hypertrophic phenotype and would be slower than the thin bone phenotype. *A. crassicaudus* may have had a metabolic advantage by depositing in its bony tissue some of the very large quantity of salt absorbed from the hypersaline waters (unfavorable osmosis). This mineral homeostasis is regulated by hormonal activity (Pfeilschifter *et al.*, 1987), and the osseous tissue of *A. crassicaudus* is acellular and avascular. "Acellular" bone is a function of the calcium-regulation mechanism in teleost fishes (Lynne & Parenti, 1986) and *A. crassicaudus* occurs in marl-gypsum along the coastal

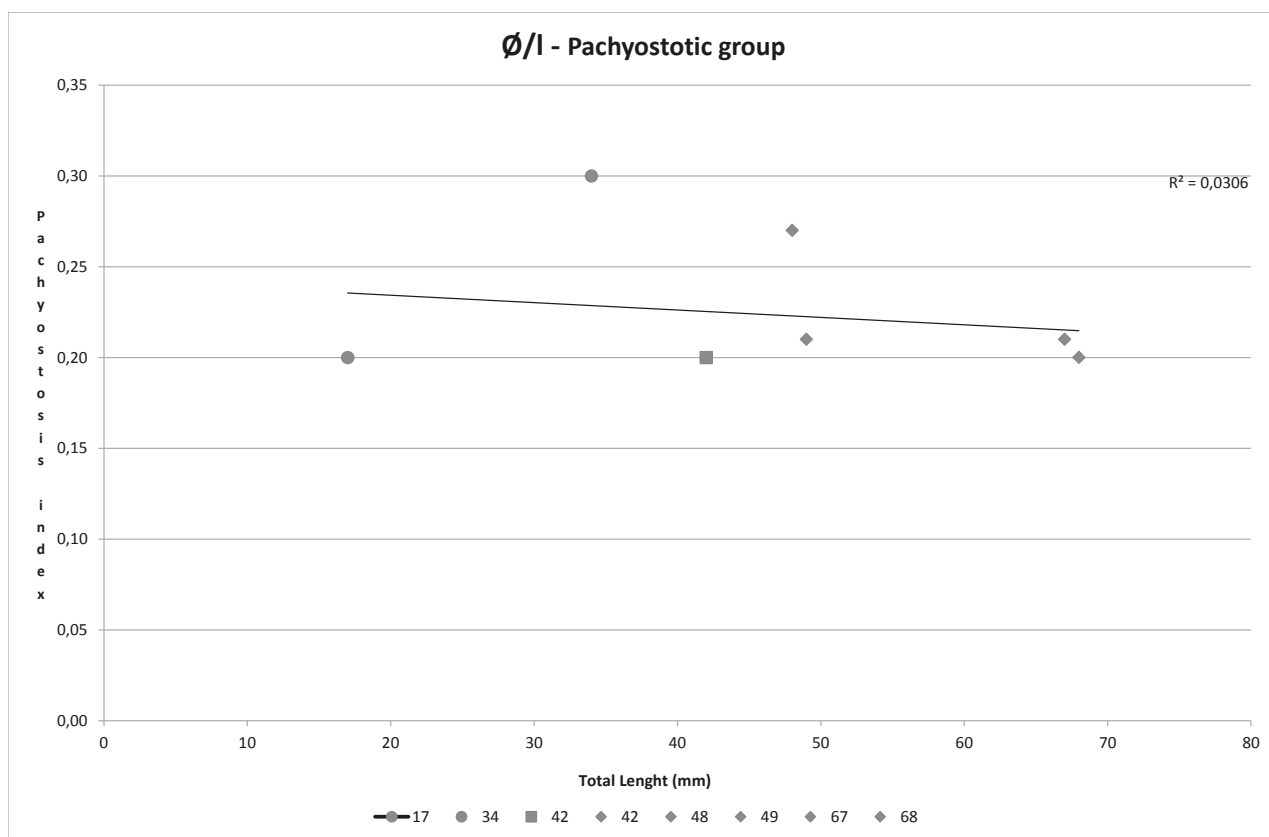


Figure 8 - Index of pachyostosis distribution - Pachyostotic group; T.L. = 17 for MT 08 and T.L. = 34 for MT 06 (point mark) were calculated from the known S.L.; T.L. = 42 (square and diamond) are two overlapping marks.

region of the Mediterranean during the Messinian Salinity Crisis, where the less soluble carbonates precipitated first; *H. wui*, from the Yahu Anticline, is also found in deposits with a high concentration of calcium (Chang *et al.*, 2008). Additional data from previous studies (Meunier & Gaudant, 1987; Bedosti, 1999) of living and fossil *Aphanius* have revealed discrepancies with the hypothesis of the origin of pachyostosis. Environmental salinity appears to be the main cause of bony hypertrophy in fossil *Aphanius* (Meunier and Gaudant, 1987; Bedosti N., 1999); an endemic cichlid with hyperostosis was observed in a tiny mineral-rich pool (185 ppm, CaCO_3) in a sabkha in the desert of Namibia (Greenwood, 1992). In addition, studies on populations of *A. fasciatus* from Sicily and from the Adriatic coasts have indicated some differences in osteological features (cartilaginous or ossified mesethmoid). Based on these differences, the populations of *A. fasciatus* have been subdivided into two groups that belong to environments of differing salinity: Adriatic populations and Sicilian populations (Tigano & Parenti, 1988). Other factor that are no longer present in the environments of living *Aphanius* may also have

been present (Chang *et al.*, 2008). As described for the extraordinarily thick-boned cyprinid fish *Hsianwenia wui*, gen. et sp. nov., chemical elements present in the environment could form a component of hyperostotic bone (Chang, *et al.*, 2008). In addition, one of the main differences between the hypertrophic bony-tissue of Teleostei with hyperostotic bone and thin bone is the presence only in the former of sodium ions (Driesch, 1994); future research should examine the presence of sodium ions in the tissue of *Aphanius crassicaudus*. The body lengths of the whole Monte Tondo *Aphanius* community (non-pachyostotic and pachyostotic populations) range between 11 and 69 mm. (S.L.). This dimensional trajectory includes the different ontogenetic stages related to juveniles and adult individuals and indicates that all of the life cycle of this population was spent entirely in the same environment. The medium and maximum values of S.L. of this community compared with those of *A. crassicaudus* from other Messinian deposits (i.e., Gessoso-Solfifera Formation) (Gaudant & d'Estevou, 1985, Gaudant, 1979b, Bedosti, 1999) suggest that this community represents a population of rather large body size.

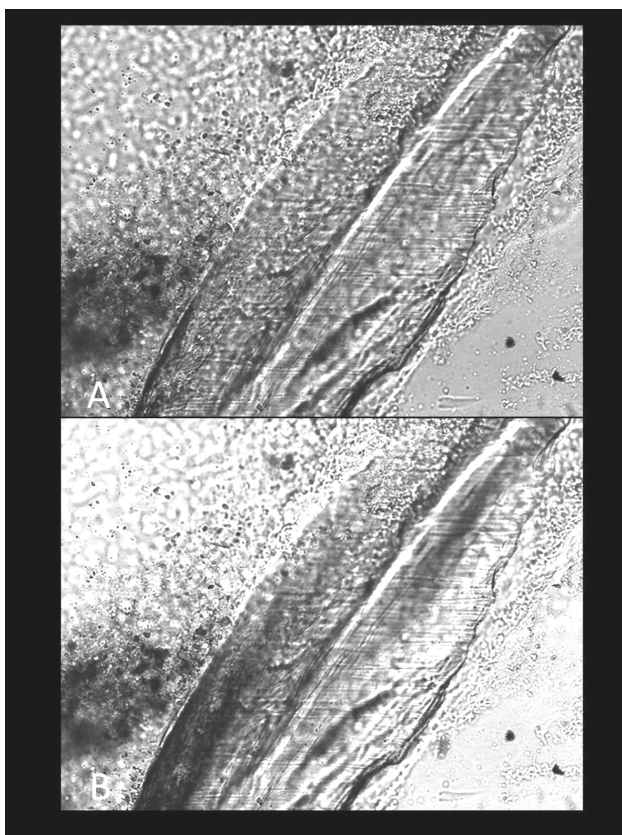


Figure 9 - Histological features of neural apophysis in *A. crassicaudus*: longitudinal sections; the medulla consists of osteosclerotic and acellular bone and is indistinguishable from the cortex. The neural apophysis is crossed by an inner structure attributable to a vascular canal (sample MT 06; A: natural transmitted light; B: polarized transmitted light; magnification: 100X).

CONCLUSIONS

The studied specimens were obtained from interstrata of clay (Gessoso-Solfifera Formation) from the 10th to 13th cycles (Sami & Teodoris, 2013). In the Vena del Gesso basin, the 6th to 15th cycles originated from a strong mixing of thalassic and continental unsaturated waters (Lugli *et al.*, 2007). The fossil teleost remains of Monte Tondo can be related to other typical oligotypic Messinian evaporitic teleost communities. The *A. crassicaudus* specimens are widely dominant (86.96%) compared to the other identified taxa (*Gobius sp.* 10.87% and *Clupeonella sp.* 2.17%). This fossil assemblage is typical of the estuarine residents (*Aphanius crassicaudus* and *Gobius sp.*) but also includes species of marine origin (*Clupeidae*). The oligotypic composition and the ecological structure of the ichthyic association indicate that the lagoons of the Vena del Gesso basin studied herein were constituted by a body of brack-

ish water that was occasionally influenced by inputs of seawater (Gaudant, 1978 b; Carnevale *et al.*, 2008). The lagoons may have been positioned distally inside an estuarine system, as indicated by the fossil remains of hydrophytic trees such as *Taxodium* (Sami & Teodoris, 2013). The paleofloristic framework, outlined mainly by phyllites, also includes the poor remains of fossil seeds and fruits, including the “Cones” *Tetralinias*, *Taxodium* and *Pinus*, *Pinus* seeds, and fruits of *Platanus*, *Ulmus* and *Acer*. More generally, based on the above statements, we can provisionally state that the Monte Tondo paleoflora overlaps with the various Messinian fossil flora of northern Italy. An even more direct comparison can be made with the contemporary paleontological site of Tossignano, which is only a few kilometers distant. Overall, the paleoflora belongs to a transitional vegetation type between “Broad-leaved Evergreen Forest (BLEF)” of subtropical climates and a “Mixed Mesophytic Forest (MMF)” of warm-temperate/subtropical climates. Some hypotheses about possible paleoclimatic parameters have also been advanced: the Messinian climate appears to have been much more rainy and mild than the current climate (Sami & Teodoris, 2013). The presence of two genera of euryhaline fishes affected by pachyostosis provides insights into the relationship between environmental conditions and bone mass increase. In the fossil assemblage of *Aphanius*, we recognize the presence of both the non-pachyostotic and pachyostotic phenotypes. Normal individuals are dominant in the Monte Tondo deposit (68%; ratio of non-pachyostotic/pachyostotic = 2.13), and both phenotypes are represented by different ontogenetic stages (young and adult). The presence of young and adults (non-pachyostotic and pachyostotic individuals) is attributable to the permanent occupation of this environment by this species. In the *A. crassicaudus* pachyostotic specimens, only one can be related to a young stage (MT 08) and is affected only in the postcranial skeleton. This peculiar diffusion of the bone mass increase has not been noted previously in other Messinian communities of *Aphanius crassicaudus* and may indicate that pachyostosis probably started in young specimens first in the postcranial portion, followed shortly thereafter by the cranial portion of the skeleton. Future studies of bony mass increase in *Aphanius crassicaudus* should include observations of skeletal tissue in young individuals (T.L. < 20 mm) to determine if there is a trend in the appearance of pachyostosis in the ontogenetic development of this small cyprinodontid. Smith Vanitz *et al.* (1995) hypothesize that hypertrophic bones are buoyancy correctors that form during fish development because of mechanical compression, such as in *Trachurus trachurus*. Smith Vanitz’s hypothesis is controversial (Taylor, 2000; Amson *et al.*, 2014), and the limited pachyostotic sample of the Monte Tondo, even

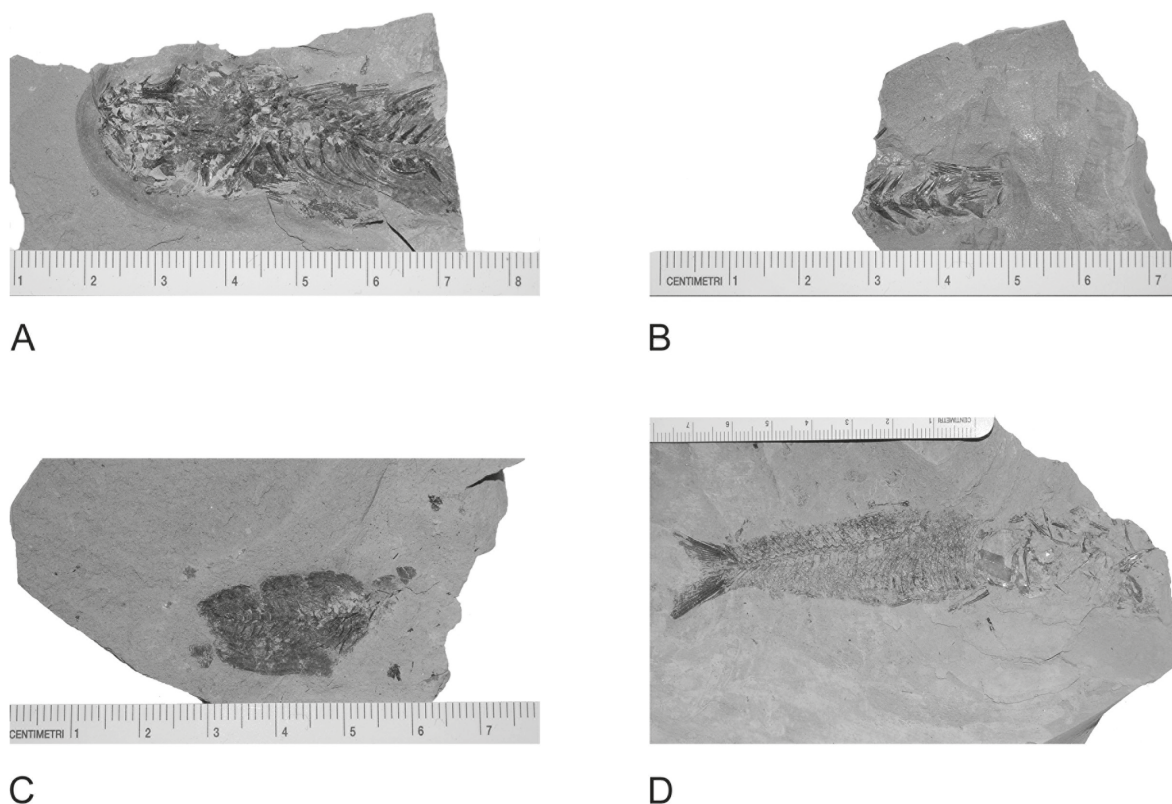


Figure 10 - (A) *Gobius* sp., specimens with pachyostosis (MT 042), skull and post-cranial region; (B) *Gobius* sp., specimens with pachyostosis (MT 044), caudal region; (C) *Gobius* sp., specimen without pachyostosis (MT 046), post-cranial and caudal region; (D) *Clupeonella* sp., specimen MT 041; it is partially complete and lacks most of the head skeleton.

though represented by different ontogenetic stages, does not allow us to support this hypothesis. In contrast to the Monte Tondo population, in the communities of *Aphanius* in several Mediterranean fossil deposits, the *Aphanius* pachyostotic phenotype prevails over the thin phenotype and, in some cases, constitutes a characteristic monotypic association (Gaudant, 1979 b; Gaudant, 1979 c; Gaudant & d'Estevou, 1985). These huge changes in the structure of the messinian communities of *Aphanius* (non-pachyostotic/pachyostotic rate) can be related to the different environmental conditions (extreme in some cases) or a more articulate environmental structure in which different eco-spaces allow for the two phenotypes to complete their life cycle. Considering all of the assumptions for the etiology of pachyostosis (genetic, mechanical, metabolic, hormonal, kinematics (specifically buoyancy control), environmental origin (salinity), a greater attack surface for strengthener muscles in denser water, excretion of some toxic elements in large quantities in the bone), future studies should investigate pachyostotic bone tissue from histological and geochemical perspectives.

ACKNOWLEDGEMENTS

We express our gratitude to Dr. Marco Sami, Mr. Antonio Benericetti, and Mr. Stagoni for the kind loan of all specimens preserved at the Natural Science Museum of Faenza. We are particularly in debt to Prof. Corrado Piccinetti and Dr. Marco Stagoni of the Marine Biology Laboratory of Fano (University of Bologna, Italy) for the use of microscopes and facilities. We also thank Professor. Giorgio Carnevale (University of Torino) for assistance with the classification of *Clupeonella* sp. and Professor James C. Tyler of the Smithsonian Institution, Washington, D.C., for improving the English of the manuscript. We thank Prof. Marco Ferraguti, University of Milano, and Prof. P. Gobbi, Prof. Andrea Minelli and Prof. Mario Zunino, University of Urbino, for their recommendations on evolutionary adaptations and the analysis of histological sections. We greatly appreciate the support of Dr. Stefano De Angelis and Dr. Sauro Teodori, University of Urbino, for their assistance with constructing the geological map.

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- (ms. pres. 19 gennaio 2015; ult. bozze 20 febbraio 2015)

Edizioni ETS
Piazza Carrara, 16-19, I-56126 Pisa
info@edizioniets.com - www.edizioniets.com
Finito di stampare nel mese di giugno 2016

