

**UNIVERSITY OF PÉCS**

Biological and Sportbiological Doctoral School

**Scale Analysis of Mediterranean Teleosts**

**Ph.D. Dissertation**

**Zsuzsanna Bräger**



**PÉCS, 2016.**

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Ph.D. Dissertation

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*“It always seems impossible until it is done.”*

Nelson Mandela



*Looking at a piece of the whole and seeing the whole itself...*

*...is like immersing into the art of Nature*

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## ABBREVIATIONS

<b>A</b>	area of the scale surface
<b>ADR</b>	Adriatic Sea
<b>AF</b>	anterior field
<b>AMV</b>	Gulf of Ambracia
<b>AR</b>	aspect ratio
<b>Cir</b>	circularity
<b>Cr</b>	crenate scale
<b>CS</b>	centroid size
<b>CVA</b>	canonical variate analysis
<b>Cy</b>	cycloid scale
<b>DEA</b>	discriminant function analysis
<b>FF</b>	form-factor
<b>Fi</b>	focal index
<b>GMM</b>	geometric morphometric method
<b>GPA</b>	generalized procrustes analysis
<b>ION</b>	Inner Ionian Sea Archipelago
<b>Jsl</b>	relative scale length
<b>Jst</b>	relative scale transverse diameter
<b>Jsw</b>	relative scale width
<b>KAV</b>	Gulf of Kavala
<b>LD</b>	scale longitudinal diameter
<b>LF</b>	lateral field
<b>P</b>	perimeter of the scale surface
<b>Pe</b>	ctenoid scale with peripheral cteni
<b>PF</b>	posterior field
<b>Rec</b>	rectangularity
<b>Rnd</b>	roundness
<b>Si</b>	shape index
<b>SL</b>	standard length of the specimen
<b>Sp</b>	spinoid scale
<b>TD</b>	scale transverse diameter
<b>TL</b>	total length of the specimen
<b>Tr</b>	ctenoid scale with transforming cteni
<b>VD</b>	scale vertical diameter
<b>Wh</b>	ctenoid scale with whole cteni

# 1. INTRODUCTION

## 1.1. The history of fish scale analysis

Fish scales, besides gills and fins, are one of the major characteristics of fish-like vertebrates. Scales are present in all major fish groups and show a huge variety in their morphology, histology and ontogeny (SIRE *et al.*, 2009). This variability over all the fish groups attests fish scales to be of high value to contribute in various ichthyological issues. LOUIS AGASSIZ (1833-1843) was the first to recognize this potential and to classify fishes into four groups according to their scale types: (1) “Les Placoides” (the placoid scales of, e.g., sharks, rays and chimaeras) with spine-like denticles of enamel and dentine; (2) “Les Ganoides” (the ganoid scales of basal actinopterygians, e.g., *Polypterus senegalus* and *Lepisosteus ocellatus*) with thick plates of ganoine and bone; (3) “Les Ctenoides” (the ctenoid scales, e.g., in many perciform species) having thin plates with comb-like posterior borders; and (4) “Les Cycloides” (the cycloid scales, i.e., common throughout the teleostean lineage) having thin plates with smooth borders. The latter two scale types are typical for the teleost species. Although this classification was unnatural, the nomenclature introduced by AGASSIZ has been fully incorporated into ichthyology.

Few decades later, light microscope studies of scales by WILLIAMSON (1851), BAUDELLOT (1873), COCKERELL (1910, 1913, 1915), CHU (1935), LAGLER (1947), and MCCULLY (1961) have contributed largely to our knowledge of scale morphology and demonstrated the value of scales in fish taxonomy. The occurrence of keys, based upon scale characteristics, allowed identification more accurate at the species level (BATTS, 1964; LAGLER, 1947; MOSHER, 1969; CASTEEL, 1972, 1973; PATTERSON *et al.*, 2002). Most of these keys were limited to only some fish families comprising regional fish faunas.

More recently, the use of scanning and of transmission electron microscopy has revealed many new features of teleost scales. Studies on scale growth and development by DELAMATER & COURTENAY (1973), HUGHES (1980, 1981), and particularly by SIRE (1986) and SIRE *et al.* (1997, 2000) showed that scales contain essential phylogenetic information and highlighted that scales have great utility in systematic research. In this field, ROBERTS' work (1993) is one of the most important. ROBERTS described the scale morphology of 330 teleost species, and demonstrated that the presence of “spined” scales (crenate, spinoid and ctenoid types) can be a valuable tool not only to help in systematic studies but also in investigations dealing with teleost evolution. Others, such as COBURN & GAGLIONE (1992)

and KHEMIRI *et al.* (2001) have confirmed the utility of scale characters while acknowledging the difficulties encountered in defining character states and in dealing with problems such as variation among scales from different areas of the body.

Ever since VAN OOSTEN (1957) stated that scales had “limited use in fish systematics”, it has been proven many times that scales indeed bear valuable information on different levels of systematics: for major groups (e.g., COBURN & GAGLIONE, 1992; KHEMIRI *et al.*, 2001; PATTERSON *et al.*, 2002), at family level (e.g., LAGLER, 1947; CASTEEL, 1972, 1973; LIPPITSCH, 1990; JAWAD, 2005), and even at species level (e.g., MOSHER, 1969; KAUR & DUA, 2004; ESMAEILI *et al.*, 2007; JAWAD & AL-JUFAILI, 2007; ESMAEILI & GHOLAMI, 2011; YOKOGAWA & WATANABE, 2011; HARABAWY *et al.*, 2012).

Traditionally, species identification is based on morphological characteristics. Scale characteristics, such as the overall shape and internal features, have proven to be useful not only for species but even for population identification, e.g., for walleye *Sander vitreus* (JARVIS *et al.*, 1978), lake whitefish *Coregonus clupeaformis* (CASSELMAN *et al.*, 1981), Atlantic salmon *Salmo salar* (PONTUAL & PROUZET, 1987), and striped bass *Morone saxatilis* (RICHARDS & ESTEVES, 1997). Scale morphology was shown to discriminate among fish populations at relatively large spatial scales, e.g., within the great lakes system (JARVIS *et al.*, 1978; CASSELMAN *et al.*, 1981) or among rivers from different drainage basins (PONTUAL & PROUZET, 1987; RICHARDS & ESTEVES, 1997). Advanced technologies in image processing and analytical approaches now allow to utilize scale shape as a discriminator among local populations. Morphometric analysis, based on landmark data (i.e., geometric morphometric methods) has been used and proven to be a reliable tool in differentiating congeneric species, e.g. *Mugil* species (IBÁÑEZ *et al.*, 2007, 2012), allowing the identification of populations on a local scale (STASZNY *et al.*, 2012) as well as among sympatric phenotypes (GARDUÑO-PAZ *et al.*, 2010).

Although developing technology has been providing improved analytical approaches towards a more reliable discrimination, there is still an increasing demand for descriptive morphological studies of scales due to the large number of fish species and the lack of reference catalogues from many regional fish faunas. Comprehensive morphological studies of scales – especially the ones with ontogenetic material – are needed to add valuable information on the evolutionary scenarios of several fish groups (ROBERTS, 1993).

## 1.2. The anatomy of fish scales

### 1.2.1. The function, origin and types of fish scales

In most biological nomenclature, the word scale (Greek *λεπίς* *lepis*, Latin *squama*) refers to a small rigid plate that grows out of an animal's skin to provide protection. In ichthyology, the term "scale" is often used as a generalized term for all the hard, generally flattened, skeletal elements formed in the dermis of aquatic non-tetrapod vertebrates. Scales have a number of functions; they do not only act as a mechanical barrier to avoid water and electrolyte loss, but also provide protection against skin erosion, ectoparasites, and predation, as well as improve the hydrodynamics of the fish body (PAXTON & ESCHMEYER, 1995).

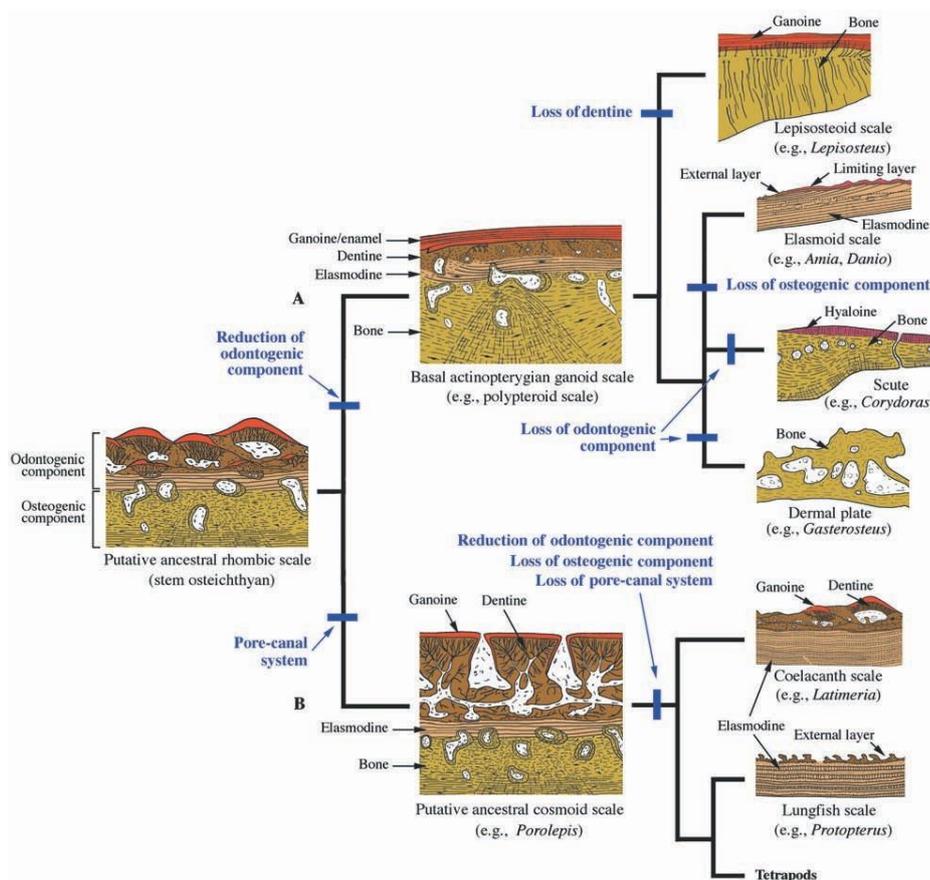
One has to bear in mind that "fish scales" differ from the superficial epidermis-derived appendages, also named as scales, in Sauropsids (i.e., reptiles and birds). Although both types of scales are distributed over the body surface in an orderly pattern, their morphology, their tissue and their evolutionary origins are distinct (SIRE & AKIMENKO, 2004).

In a recent comparative developmental study of the dermal skeleton, SIRE & HUYSSEUNE (2003) have proposed the term "**odontode**", which are considered to be the likely ancestral structure of all the elements of the dermal skeleton. Odontodes were present in some early vertebrates 500 million years ago and have a tooth-like structure (REIF & RICHTER, 2001). The scales of chondrichthyans (i.e., **placoid scales** often called "dermal denticles") have greatly retained the odontode-structure and organization in a nearly unchanged form. These ancestral elements in living chondrichthyans are composed of enameloid, dentine, a pulp cavity that is surrounded by a collagen-rich tissue, and a layer of acellular bone, called aspidin (SIRE & AKIMENKO, 2004). Each "odontode" is anchored to the fibrous stratum compactum by anchoring fibres, permitting the elements to be firmly fixed and yet shed as necessary (SIRE *et al.*, 2009).

In contrast, in the osteichthyan lineage, the odontodes have been progressively modified into various types of scales. The ancestral **rhombic scale** of the earliest actinopterygians, e.g., the acanthodian *Nostolepis* (Silurian) and *Cheirolepis* (Devonian), was composed of tissues derived from both odontogenic and osteogenic condensations. This scale type is known as the ancestor of the two structural forms, the ganoid and the cosmoid scale (FIG. 1).

Although the **cosmoid scale** of extinct sarcopterygians is clearly common among fossil forms of basal sarcopterygians, it is not known in extant taxa. Nonetheless, modifications

(i.e., reduction of odontogenic component and loss of osteogenic component) of ancestral cosmoid scale have given rise to the scale type of living sarcopterygians (coelacanth and dipnoans) known as **sarcopterygian elasmoid scale** (SIRE & AKIMENKO, 2004; SIRE *et al.*, 2009). **Ganoid scales** are found in two lineages of living actinopterygians, i.e., in the polypterids (bichirs and redfish) and lepisosteids (gars). According to the review by SIRE *et al.* (2009), three types of ganoid scales are currently recognized: **palaeoniscoid** (in extinct basal actinopterygian lineages), **polypteroid** (in polypterids), and **lepisosteoid** (in lepisosteids). Among modern actinopterygians, the diversity of scale type structure (**elasmoid scale, scute and dermal plate**) can be explained due to the loss and/or modification of the odontogenic and/or osteogenic components of the basal actinopterygian ganoid scale. Although all these types of appendages are evolutionary linked as derivatives of a common ancestral type (reviews in HUYSSSEUNE & SIRE, 1998; SIRE & HUYSSSEUNE, 2003), they have a different structure (FIG. 1).



**FIGURE 1. A revised scenario depicting the evolution of different scale types in the osteichthyan lineage (SIRE *et al.*, 2009)**

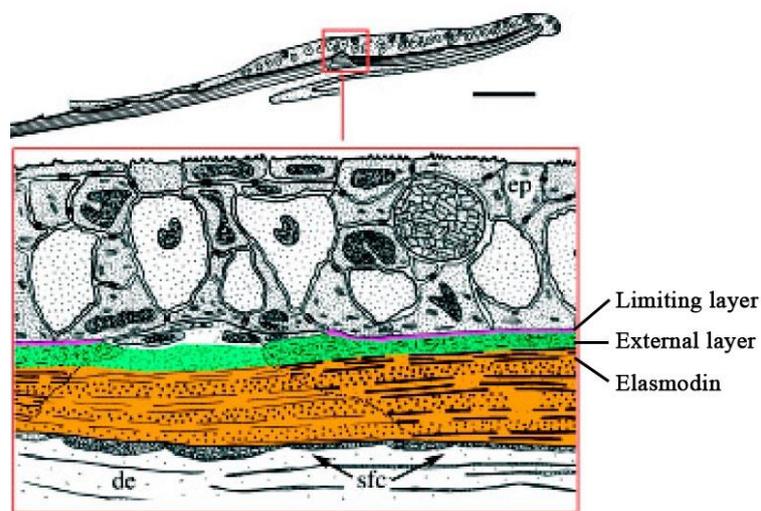
**Elasmoid scales** made of thin bony plate without any additional distal hard layer are a synapomorphy of recent teleost fishes. Two main types are traditionally distinguished within the elasmoid scale type, i.e., **cycloid scale** (generally in basal actinopterygians) and **ctenoid scale** (in advanced actinopterygians). They have the advantage of being imbricate which gives great flexibility compared with ganoid or cosmoid scales. Cycloid scales are rounded, smooth scales that lack tooth-like projections (spines) on their posterior margin. However, including all scales with spines under the term ctenoid is an oversimplification of the situation (JOHNSON, 1984; ROBERTS, 1993). Three different, general types of “spined” scales exist: (1) **crenate**, with simple marginal indentations and projections; (2) **spinoid**, with spines that have continuous connexion with the main body of the scales; and (3) **ctenoid**, with cteni formed as separate ossifications distinct from the main body of the scale (ROBERTS, 1993). Crenate scales occur in the Elopomorpha and Clupeomorpha, while spinoid scales occur widely in the Euteleostei. Peripheral ctenoid scales (whole cteni in one apical row) can be found in the Ostariophysi, “Paracanthopterygii”, and Percomorpha; and transforming ctenoid scales (cteni arising in two or three rows and transforming into truncated spines) are a possible synapomorphy of the Percomorpha (HELFMAN *et al.*, 2009).

### 1.2.2. The structure of the elasmoid scale

The elasmoid scale is an ornamented, thin, lamellar, collagenous plate located within the upper region of the dermis, close to the epidermis (SIRE & AKIMENKO, 2004). Its chemical composition is about 41-84% organic protein, mostly albuminoids such as collagen (24%) and ichthyolepidin (76%). Up to 59% is bone, mostly  $\text{Ca}_3(\text{PO}_4)_2$  and  $\text{CaCO}_3$  (HELFMAN *et al.*, 2009). The dominant mineral phase of the “bone” is a carbonated hydroxyapatite  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  with a Ca:P molar ratio of 1.85 (LIU *et al.*, 2008).

The structure of the elasmoid scales has been described for several species as belonging to the actinopterygian and the sarcopterygian lineages (see e.g., SIRE, 1990; SIRE & AKIMENKO, 2004; SIRE *et al.*, 2009). In all studied species, elasmoid scales were found to be invariably composed of three tissues, in sequence from the deep face upwards (FIG. 2): (1) **basal plate**, a thick layer of incompletely mineralised tissue composed of elasmodin (previously called isopedin), itself consisting of several layers of collagen fibrils organised into a plywood-like structure; (2) **external layer**, a thin layer of well-mineralised tissue composed of a network of interwoven collagen fibrils; (3) **limiting layer**, a hyper-

mineralised tissue devoid of collagen fibrils and deposited at the scale surface in the region close to the epidermis. The structure and organisation of this upper layer is the most variable amongst the various species. Separately, these three tissues present structural similarities with the typical dental and skeletal vertebrate tissues (enamel, dentin and attachment tissue, and cartilage and bone). Elasmodin resembles either orthodontin or lamellar bone, the external layer looks like mantle dentin or woven-fibered bone, and the limiting layer is structurally closer to enamel (or ganoine) than to any other known skeletal tissue. Based on structural comparison alone, it is difficult to determine from which tissue (dental or skeletal) the tissues of the elasmoid scale are derived (SIRE & AKIMENKO, 2004).

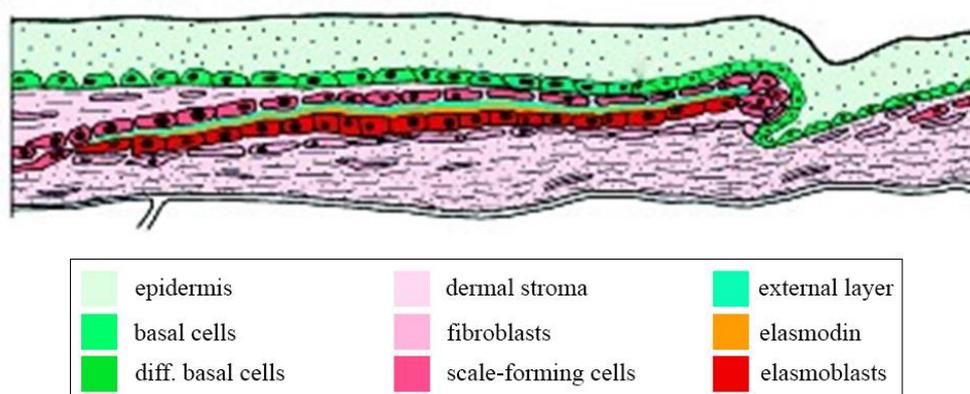


**FIGURE 2. Structural components of the elasmoid scale (SIRE & AKIMENKO, 2004)**  
 Notes: ep – epidermis; dc – dermal stroma; sfc – scale-forming cells; scale bar = 100  $\mu$ m

### 1.2.3. The development and squamation pattern of the elasmoid scale

The development of elasmoid scales, from initiation to terminal differentiation, has been studied in detail at light and electron microscopic level in a cichlid fish (SIRE & GÉRAUDIE, 1983) and, more recently, also in the zebrafish (*Danio rerio*) (SIRE *et al.*, 1997). The process starts by the invasion of fibroblasts into the primary dermal stroma, which is mainly composed of a collagenous matrix organised into a plywood-like structure. Some of these fibroblasts are the precursors of the dermal papilla, i.e., the scale-forming cells, but their precise embryonic origin is still unknown, since they do not derive from the dermal endothelium (SIRE & AKIMENKO, 2004). The suggested evolutionary origin of scales from dental elements supports the neural crest origin of the scale-forming cells (SIRE *et al.*, 2009).

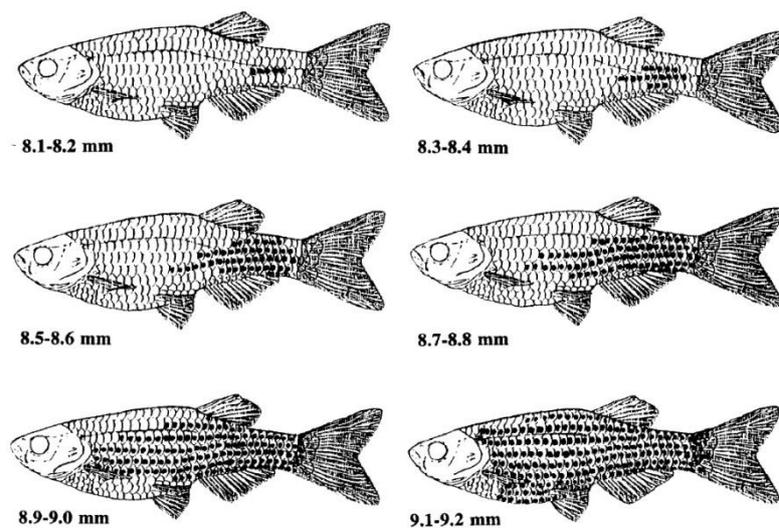
Shortly after the fibroblasts have penetrated the dermal stroma, some of them accumulate along the basal epidermal surface, adjacent to the basement membrane, and start to differentiate. During scale development in the zebrafish, SIRE & AKIMENKO (2004) distinguished five steps as follows: (1) **early morphogenesis**, i.e., the accumulation of fibroblasts along the epidermal-dermal boundary; (2) **late morphogenesis**, characterized by the differentiation of scale papillae immediately below the epidermis and at precise loci among the population of accumulated cells; (3) **early differentiation**, in which the two upper cell layers of the scale papillae differentiate into scale-forming cells and deposit the first-scale matrix of the external layer in between; (4) **late differentiation**, when the scale-forming cells located at the deep surface of the scale matrix differentiate into particular cells, the so-called elasmoblasts, responsible for the deposition of elasmodin, the plywood-like tissue; (5) **folding**, when the epidermis starts to fold around the posterior margin of the scale, a step which also corresponds to the overlap with the neighbouring scales. During these five steps the anterior region of the scales progressively withdraws from the epidermis surface and sinks in the dermal stroma (FIG. 3). The scales become obliquely oriented in the dermis whereby only the posterior region remains in contact with the epidermis. The three structural tissues mentioned earlier (i.e., basal plate, external layer, limiting layer) are deposited following an invariable sequence: first the external layer (allowing an extension in diameter), followed by the basal plate (allowing an extension in thickness) and finally the limiting layer. The latter improves scale protection and its anchoring to the epidermis.



**FIGURE 3. Interpretative drawing of the last two steps (i.e., late differentiation and folding) of elasmoid scale development in zebrafish (*Danio rerio*) (SIRE & AKIMENKO, 2004)**

In teleosts, elasmoid scales are arranged in longitudinal and vertical rows, forming a regular pattern. The establishment of the squamation pattern in the *D. rerio* has been described by SIRE *et al.* (1997) and SIRE & AKIMENKO (2004). The scales appear very late during ontogeny, i.e., after metamorphosis, when the juveniles are already miniatures of the adults. A single scale first appears as an “initiator”; scales are next added rapidly and successively in regular rows. The first scales to appear are those of the midline row, at the level of the caudal peduncle, followed by a rapid extension of the squamation anteriorly and posteriorly along this row, while new rows are added dorsally and ventrally until the final squamation pattern is reached (FIG. 4). Once the full complement of scales is attained during ontogeny, the number remains fixed. Therefore, the number of scales is a useful taxonomic character (HELFMAN *et al.*, 2009).

The developing scales are first juxtaposed but they rapidly enlarge in diameter, and progressively overlap like roof tiles. In one row, the posterior region of a scale covers the anterior region of the following scale, and part of the lateral region of the scales from the two adjacent rows. The establishment of the squamation pattern in zebrafish has been described by SIRE *et al.* (1997) and SIRE & AKIMENKO (2004). The latter study suggested that the dermal stroma of the skin is already patterned (i.e., compartmentalised) when the scales are initiated. In teleosts, the skin compartmentalisation is probably related to the complex arrangement of the muscle fibres and myosepta located below the dermis (SIRE & AKIMENKO, 2004).



**FIGURE 4.** Schematic representation of scale development pattern in zebrafish (SIRE *et al.*, 1997)

### 1.3. The multidisciplinary utilization of fish scales

Fish scales have been used for systematic ichthyology for many years as mentioned earlier. However, not only ichthyologists utilized scales in their studies. In early times, scales were retrieved and analysed by palaeontologists and archaeologists. DAVID (1944) used fish scales in palaeontological work as did COCKERELL (1919) before. Further studies also took advantage of the valuable information preserved by scales in lacustrine (e.g., PENNINGTON & FROST, 1961; DAVIDSON *et al.*, 2003) or marine (e.g., SOUTAR & ISAACS, 1969, 1974; HOLMGREN-URBA & BAUMGARTNER, 1993; PATTERSON *et al.*, 2005; VALDÉS *et al.*, 2008; DÍAZ-OCHOA *et al.*, 2009) sediments to determine ancient or historic species compositions. Fish scales as thin bony structures *per se* have a rather poor chance of becoming fossilized. However, the huge number of fish scales that occur in certain deposits makes their utilization possible. In some remarkable deposits, fish scales have accumulated to 16.000 pieces per square meter (VALLENTYNE, 1960). Archaeological works similarly benefit from the analysis of fish scales in deposits (NELSON, 1918; WALKER, 1936; CASTEEL, 1974; BROWN, 1992). Scale analysis in some archaeological studies was able to provided information on food resources of past human communities (e.g., HARDY & MONCEL, 2011).

In fisheries biology, for many years the emphasis has been placed upon the use of scales in age and growth studies (TAYLOR, 1916; JACOT, 1920; BORODIN, 1924; COOPER, 1951; CHUGUNOVA, 1959; JOHAL & TANDON, 1992; TANDON & JOHAL, 1996; GUNN *et al.*, 2008). Since the growth of fish scales happens rhythmically, growth rings, the so-called circuli, appear on the external surface of scales (OTTAWAY, 1978). This is particularly useful in temperate waters where pronounced retardation of growth of body and scales occurs in fall and winter, resulting in reduced space between the circuli and thus leaving a darker band on the scales called annulus. Scales grow in a direct relationship with body growth, making it possible to measure the distance between annuli to back-calculate the age at different body sizes (HELFMAN *et al.*, 2009). Growth rate alterations may also be caused by stress from different sources, e.g., spawning, movement from fresh to salt water, parasitism, injury, favourable or unfavourable environmental conditions or health status (CASSELMAN, 1987). Therefore, the study of annuli can be helpful in the interpretation of the life history or health status of its bearer.

In fishery science, it has been always a crucial issue how to achieve a more precise identification of fish species or populations. Recent studies using landmark-based geometric morphometric analysis were able to show that scale shape allows the reliable discrimination between congeneric species of Mugilidae (IBÁÑEZ *et al.*, 2007), sympatric phenotypes of Arctic charr (*Salvelinus alpinus*) (GARDUÑO-PAZ *et al.*, 2010), or among populations of gibel carp (*Carassius gibelio*) (STASZNY *et al.*, 2012). Scale morphometric analysis in contrast to techniques that require the sampling of the whole specimens has several advantages; it is less destructive, fast, incurs low costs and is readily available in the field compared to genetic analyses (IBÁÑEZ *et al.*, 2007). Furthermore, scale morphometry could be easily applied also on living individuals of endangered species in contrast to the traditional morphometric methods (GARDUÑO-PAZ *et al.*, 2010; STASZNY *et al.*, 2012).

One other application that has received little attention so far is to assess the bioaccumulation of pollutants such as heavy metals in scales (BASU *et al.*, 2006; RAHAMAN *et al.*, 2008). Heavy metals (LAKE *et al.*, 2006) and some trace elements (MUGIYA *et al.*, 1991) may accumulate in scales, allowing studies of pollution using fish as bioindicators.

Fish scales not only preserve information about their bearer, but they can also provide insight into the diet of piscivorous species. Scales may be present in stomach contents or can be collected directly after predatory events. Information derived from fish scales has been used for prey identification in marine vertebrates such as demersal fishes (MAUCLINE & GORDON, 1984), gulls (EWINS *et al.*, 1994), seals (COTTRELL *et al.*, 1996), and odontocetes such as killer whales (*Orcinus orca*) (FORD & ELLIS, 2006) and common bottlenose dolphins (*Tursiops truncatus*) (BRÄGER *et al.*, in press).

Given this wide interdisciplinary interest, the need to identify the scales of different fish groups often arises not only in ichthyology. Therefore, well-elaborated catalogues and atlases are needed to provide references for scale morphology and variability.

## 2. AIMS AND OBJECTIVES

The use of elasmoid scale morphology in teleost systematics generally has been confined to notations of scales being either simply “cycloid” or “ctenoid”. ROBERTS’ work (1993), however, revealed that this is an oversimplification of the situation. Some scale characteristics in teleost lineage contain a wealth of potentially valuable information that would make a significant contribution towards a better understanding of teleost systematics.

The main focus of my research is to investigate the morphological characteristics and morphometric parameters of the scales of teleost species and to reveal the key characteristic features that facilitate identification at different taxonomic levels. The primary aim is (1) to establish a system that aids scale identification of common Mediterranean teleost species. Due to the high variability and plasticity of some scales, especially within the family Clupeidae, the secondary aim (2) is to provide methodological approaches that allow a reliable differentiation among morphologically similar scales using morphometry and geometric morphometry. Last but not least, (3) a study was conducted to test practical aspects of scale geometric morphometry and to facilitate its application in ecology.

With this research I attempt to address the following questions:

**Q1:** What are the most relevant scale characteristics that aid species identification among common Mediterranean teleosts?

**Q2:** Does scale morphology provide identification at species level?

**Q3:** How can scale characteristics be transformed into numerical data that facilitate the investigation of phenotypic relations among different types of teleost scales?

**Q4:** Does scale morphometry allow the differentiation between the morphologically similar scales of clupeid species?

**Q5:** Is the landmark-based geometric morphometric approach suitable for the discrimination of sympatric clupeid species and even among their local populations?

To answer these five questions, I addressed the following 10 specific objectives in my studies:

### **1. For the scale morphology of common Mediterranean teleosts (Q1-Q3)**

- 1.1. Introduce categories of scale types and shapes to aid objective classification;
- 1.2. Define the characters of scale morphology that enable the discrimination among a large number of species;
- 1.3. Describe the discriminatory features of the scale characters in 80 species of Mediterranean teleosts;
- 1.4. Code characteristic features of scale morphology and utilize the numerical data for a cluster analysis to create a dendrogram showing the phenotypic relations among different types of teleost scales;
- 1.5. Illustrate the inter-specific and intra-individual variability of scales among 80 Mediterranean teleosts;

Clupeiform species such as herrings, shads, sardines, and their relatives possess derived cycloid scales that are easily distinguishable from other taxa by the presence of distinctive grooves and a membranous posterior field with a crenulated margin (VALDÉS *et al.*, 2008; BRÄGER & MORITZ, in press). However, the high degree of similarity in scale morphology between species and the plasticity within single individuals hinder species identification at least within families (PATTERSON *et al.*, 2002). To exemplarily reveal the inter-specific variability of scale shape in two clupeid species the following objectives were addressed:

### **2. For the scale morphometry of sympatric clupeids (Q4)**

- 2.1. Demonstrate the variability of scale shape within the European sardine (*Sardina pilchardus*) and the round sardinella (*Sardinella aurita*);
- 2.2. Describe the morphometric parameters of clupeid scales using relative size indices;
- 2.3. Apply multivariate statistical analyses in order to investigate the usefulness of scale shape indices to separate the two sympatric clupeids, as well as the scale sampling areas along the fish body.

**3. For the geometric morphometry of scales to discriminate between sympatric species and among their local populations (Q5)**

- 3.1. Apply landmark-based geometric morphometric analysis to investigate, whether the differences in clupeid scale shape are sufficient to allow a reliable discrimination between *S. pilchardus* and *S. aurita* as well as among their local populations;
- 3.2. Propose a rapid stock assessment method for clupeid species to shed light on their population segregation thus providing valuable demographic information from a conservation and fishery management perspective.

### 3. MATERIALS AND METHODS

#### 3.1. Materials

##### 3.1.1. The studied species for morphological description

Scale samples for morphological description were obtained from 80 species, belonging to 50 families and 16 orders of teleost fishes occurring in the Mediterranean Sea. The taxonomic classification of the species is given in TABLE 1. Species names and family affiliation were listed according to ESCHMEYER (2014); the ordering of the species accounts happened after NELSON (2006). Few specimens that typically occur in the Mediterranean Sea, but were not available from that region, were sampled outside of the basin, i.e., in the adjacent Atlantic ocean. All scale samples are deposited in the Deutsches Meeresmuseum in Stralsund, Germany. For most samples also the voucher specimens are deposited in the museum collection. The standard length, place of origin and the accession numbers of all specimens are provided in the figure captions in APPENDIX 1 (FIG. 1 – FIG. 89).

TABLE 1. List of species and their taxonomic classification

		Families	Species
<b>Kingdom</b>	Animalia		
<b>Phylum</b>	Chordata		
<b>Subphylum</b>	Craniata		
<b>Superclass</b>	Gnathostomata		
<b>Class</b>	Actinopterygii		
<b>Subclass</b>	Neopterygii		
<b>Division</b>	Teleostei		
<b>Image No.</b>	<b>Orders</b>		
APP. 1, FIG. 1	Albuliformes	Notacanthidae	<i>Notacanthus bonaparte</i>
APP. 1, FIG. 2	Clupeiformes	Engraulidae	<i>Engraulis encrasicolus</i>
APP. 1, FIG. 3	Clupeiformes	Clupeidae	<i>Alosa fallax</i>
APP. 1, FIG. 4	Clupeiformes	Clupeidae	<i>Clupea harengus</i>
APP. 1, FIG. 5	Clupeiformes	Clupeidae	<i>Sardina pilchardus</i>
APP. 1, FIG. 6	Clupeiformes	Clupeidae	<i>Sardinella aurita</i>
APP. 1, FIG. 7	Clupeiformes	Clupeidae	<i>Sprattus sprattus</i>
APP. 1, FIG. 8	Argentiniformes	Argentinidae	<i>Argentina sphyraena</i>
APP. 1, FIG. 9	Argentiniformes	Alepocephalidae	<i>Alepocephalus rostratus</i>
APP. 1, FIG. 10-11	Stomiiformes	Sternoptychidae	<i>Maurolicus muelleri</i>
APP. 1, FIG. 12	Aulopiformes	Synodontidae	<i>Synodus saurus</i>
APP. 1, FIG. 13	Myctophiformes	Myctophidae	<i>Myctophum punctatum</i>

APP. 1, FIG. 14	Gadiformes	Macrouridae	<i>Coelorinchus caelorhynchus</i>
APP. 1, FIG. 15	Gadiformes	Macrouridae	<i>Nezumia sclerorhynchus</i>
APP. 1, FIG. 16	Gadiformes	Macrouridae	<i>Trachyrinchus scabrus</i>
APP. 1, FIG. 17	Gadiformes	Moridae	<i>Mora moro</i>
APP. 1, FIG. 18	Gadiformes	Merlucciidae	<i>Merluccius merluccius</i>
APP. 1, FIG. 19-20	Gadiformes	Gadidae	<i>Molva molva</i>
APP. 1, FIG. 21	Gadiformes	Gadidae	<i>Trisopterus luscus</i>
APP. 1, FIG. 22	Mugiliformes	Mugilidae	<i>Chelon labrosus</i>
APP. 1, FIG. 23	Mugiliformes	Mugilidae	<i>Liza aurata</i>
APP. 1, FIG. 24	Atheriniformes	Atherinidae	<i>Atherina hepsetus</i>
APP. 1, FIG. 25	Atheriniformes	Atherinidae	<i>Atherinomorus lacunosus</i>
APP. 1, FIG. 26	Beloniformes	Belonidae	<i>Belone belone</i>
APP. 1, FIG. 27	Beryciformes	Berycidae	<i>Beryx decadactylus</i>
APP. 1, FIG. 28	Beryciformes	Trachichthyidae	<i>Hoplostethus mediterraneus</i>
APP. 1, FIG. 29	Zeiformes	Zeidae	<i>Zeus faber</i>
APP. 1, FIG. 30	Gasterosteiformes	Macroramphosidae	<i>Macrorhamphosus scolopax</i>
APP. 1, FIG. 31	Scorpaeniformes	Sebastidae	<i>Helicolenus dactylopterus</i>
APP. 1, FIG. 32	Scorpaeniformes	Scorpaenidae	<i>Scorpaena porcus</i>
APP. 1, FIG. 33	Scorpaeniformes	Triglidae	<i>Eutrigla gurnardus</i>
APP. 1, FIG. 34	Scorpaeniformes	Triglidae	<i>Lepidotrigla cavillone</i>
APP. 1, FIG. 35	Perciformes	Moronidae	<i>Dicentrarchus labrax</i>
APP. 1, FIG. 36	Perciformes	Serranidae	<i>Anthias anthias</i>
APP. 1, FIG. 37	Perciformes	Serranidae	<i>Epinephelus aeneus</i>
APP. 1, FIG. 38	Perciformes	Serranidae	<i>Serranus cabrilla</i>
APP. 1, FIG. 39	Perciformes	Serranidae	<i>Serranus scriba</i>
APP. 1, FIG. 40	Perciformes	Apogonidae	<i>Apogon imberbis</i>
APP. 1, FIG. 41	Perciformes	Epigonidae	<i>Epigonus denticulatus</i>
APP. 1, FIG. 42	Perciformes	Coryphaenidae	<i>Coryphaena equiselis</i>
APP. 1, FIG. 43	Perciformes	Carangidae	<i>Seriola dumerili</i>
APP. 1, FIG. 44	Perciformes	Carangidae	<i>Trachurus trachurus</i>
APP. 1, FIG. 45	Perciformes	Bramidae	<i>Brama brama</i>
APP. 1, FIG. 46	Perciformes	Sparidae	<i>Boops boops</i>
APP. 1, FIG. 47	Perciformes	Sparidae	<i>Diplodus annularis</i>
APP. 1, FIG. 48	Perciformes	Sparidae	<i>Diplodus vulgaris</i>
APP. 1, FIG. 49	Perciformes	Sparidae	<i>Pagellus acarne</i>
APP. 1, FIG. 50	Perciformes	Sparidae	<i>Pagellus erythrinus</i>
APP. 1, FIG. 51	Perciformes	Sparidae	<i>Sarpa salpa</i>
APP. 1, FIG. 52	Perciformes	Sparidae	<i>Sparus aurata</i>
APP. 1, FIG. 53	Perciformes	Sparidae	<i>Spondylisoma cantharus</i>
APP. 1, FIG. 54	Perciformes	Centracanthidae	<i>Spicara maena</i>

APP. 1, FIG. 55	Perciformes	Centracanthidae	<i>Spicara smaris</i>
APP. 1, FIG. 56	Perciformes	Sciaenidae	<i>Sciaena umbra</i>
APP. 1, FIG. 57	Perciformes	Mullidae	<i>Mullus barbatus</i>
APP. 1, FIG. 58	Perciformes	Mullidae	<i>Mullus surmuletus</i>
APP. 1, FIG. 59	Perciformes	Chaetodontidae	<i>Chaetodon hoeffleri</i>
APP. 1, FIG. 60	Perciformes	Cepolidae	<i>Cepola macrophthalmia</i>
APP. 1, FIG. 61	Perciformes	Pomacentridae	<i>Chromis chromis</i>
APP. 1, FIG. 62	Perciformes	Labridae	<i>Coris julis</i>
APP. 1, FIG. 63	Perciformes	Labridae	<i>Labrus viridis</i>
APP. 1, FIG. 64	Perciformes	Labridae	<i>Symphodus rostratus</i>
APP. 1, FIG. 65	Perciformes	Labridae	<i>Thalassoma pavo</i>
APP. 1, FIG. 66	Perciformes	Scaridae	<i>Sparisoma cretense</i>
APP. 1, FIG. 67-68	Perciformes	Ammodytidae	<i>Gymnammodytes cicereus</i>
APP. 1, FIG. 69	Perciformes	Trachinidae	<i>Trachinus draco</i>
APP. 1, FIG. 70	Perciformes	Tripterygiidae	<i>Tripterygion tripteronotum</i>
APP. 1, FIG. 71	Perciformes	Gobiidae	<i>Gobius bucchichi</i>
APP. 1, FIG. 72	Perciformes	Gobiidae	<i>Gobius paganellus</i>
APP. 1, FIG. 73	Perciformes	Sphyraenidae	<i>Sphyraena chrysotaenia</i>
APP. 1, FIG. 74-75	Perciformes	Sphyraenidae	<i>Sphyraena sphyraena</i>
APP. 1, FIG. 76	Perciformes	Scombridae	<i>Scomber colias</i>
APP. 1, FIG. 77	Perciformes	Tetragonuridae	<i>Tetragonurus cuvieri</i>
APP. 1, FIG. 78	Perciformes	Caproidae	<i>Capros aper</i>
APP. 1, FIG. 79	Pleuronectiformes	Psettodidae	<i>Scophthalmus maximus</i>
APP. 1, FIG. 80-81	Pleuronectiformes	Bothidae	<i>Bothus podas</i>
APP. 1, FIG. 82-83	Pleuronectiformes	Soleidae	<i>Microchirus variegatus</i>
APP. 1, FIG. 84-85	Pleuronectiformes	Soleidae	<i>Monochirus hispidus</i>
APP. 1, FIG. 86-87	Pleuronectiformes	Soleidae	<i>Solea solea</i>
APP. 1, FIG. 88-89	Pleuronectiformes	Cynoglossidae	<i>Symphurus nigrescens</i>

### 3.1.2. The studied species for morphometric analysis

Scale samples for morphometric analysis were derived from two sympatric clupeid species, the European pilchard (*Sardina pilchardus* WALBAUM 1792) and round sardinella (*Sardinella aurita* VALENCIENNES 1847), from the Gulf of Ambracia, north-western Greece (for a description of the study area see 3.1.3 and FIG. 5).

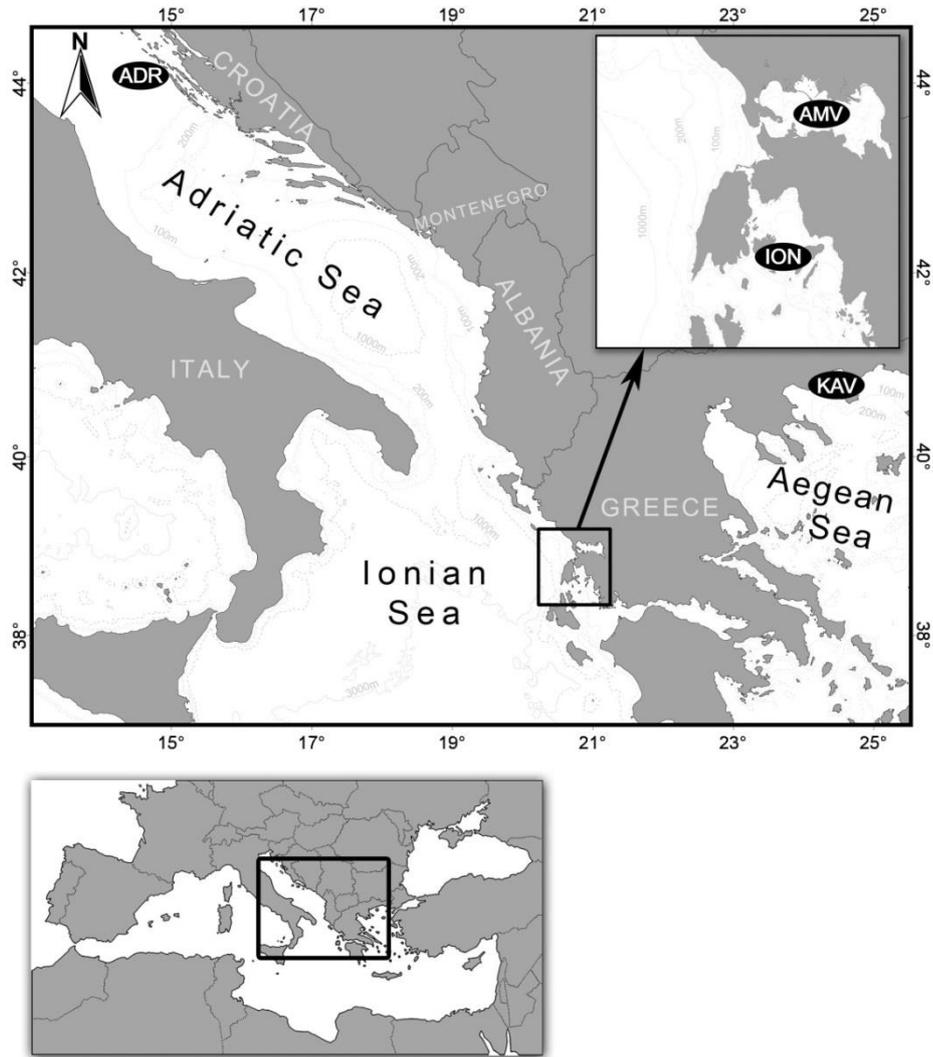
Both marine fish species belong to the family Clupeidae. *Sardina pilchardus* have a delimited distribution, inhabiting the north-eastern part of the Atlantic Ocean, from the Senegalese to the Icelandic coasts, as well as the Mediterranean and Black Seas

(WHITEHEAD, 1985). This species is one of the most exploited fishery resources throughout its distributional range, especially along the Moroccan and Spanish Atlantic coasts (FIGIS, 2004). *Sardinella aurita*, on the other hand, is widely distributed throughout the tropical and subtropical seas of the world, including the entire Mediterranean and the Black Sea. It is also commercially exploited in several southern Mediterranean countries (TSIKLIRAS *et al.*, 2005).

Both species are small, fast growing and highly fecund fishes with a pelagic lifestyle (TSIKLIRAS *et al.*, 2005; TSIKLIRAS & ANTONOPOULOU, 2006; TSIKLIRAS & KOUTRAKIS, 2013). Despite their similar life history characteristics, they exhibit important differences in reproductive strategy with *S. pilchardus* spawning from October to April (SOMARAKIS *et al.*, 2006; TSIKLIRAS & KOUTRAKIS, 2013) and *S. aurita* from May to July (TSIKLIRAS & ANTONOPOULOU, 2006; TSIKLIRAS, 2014). Their consecutive spawning and diverging feeding preferences (KARACHLE & STERGIU, 2014) enable them to coexist under favourable conditions. Although the two species can be discriminated by morphological characteristics, it is difficult to identify them by their scales alone, which may be necessary in certain studies such as food analysis of piscivorous predators (e.g., BRÄGER *et al.*, in press).

### **3.1.3. The sampling sites for geometric morphometric analysis**

The geometric morphometric scale analysis was based on six local populations of *S. pilchardus* and *S. aurita* living in four geographically different areas of the central and eastern Mediterranean Sea (FIG. 5). Among the four sampling sites, the northern Adriatic Sea and the Inner Ionian Sea Archipelago cover the largest areas, have the greatest average depths (50-150 m), and both are oligotrophic (CASOTTI *et al.*, 2003; PITTA *et al.*, 1998). The Ionian Sea, in particular, has been described as extremely oligotrophic with values of chlorophyll-a, nutrients, and particulate organic carbon among the lowest in all Mediterranean coastal waters (PITTA *et al.*, 1998). On the contrary, the Gulf of Ambracia, an enclosed embayment with a mean depth of 26 m, is highly eutrophic and increasingly hypoxic (FERENTINOS *et al.*, 2010; KOUNTOURA & ZACHARIAS, 2011). It has only a limited water exchange with the Ionian Sea through the Preveza Channel, a narrow (min. 370 m wide) and shallow (min. 5 m deep) 3 km long corridor (KOUNTOURA & ZACHARIAS, 2011). The Gulf of Kavala is a semi-enclosed shallow gulf (mean depth 34 m), which is connected to the North Aegean Sea through a wider and deeper opening in the south (20 km wide and 56 m deep) and through a secondary one in the east (7.3 km wide and 25 m deep) (TSIKLIRAS *et al.*, 2009). The North Aegean Sea is one of the most productive areas in the eastern Mediterranean (TSAGARAKIS *et al.*, 2010).



**FIGURE 5. Scale sampling sites in the central and eastern Mediterranean Sea**

Note: Adriatic Sea (ADR), Gulf of Ambracia (AMV), Ionian Sea (ION), Gulf of Kavala (KAV)

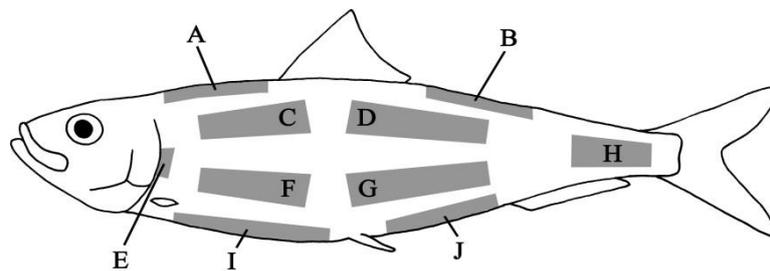
## 3.2. Methods

### 3.2.1. Scale morphology

#### 3.2.1.1. Scale sampling and preparation

Scales were sampled from museum vouchers or freshly killed specimens purchased on fish markets. If fish were sampled alive, they were anaesthetised using benzocaine before fixation of the whole fish in 4% formalin-seawater. All museum specimens were finally transferred into 70% ethanol. Fishes purchased on fish markets were sampled shortly after the purchase. Each specimen was measured and rinsed with ethanol or freshwater before scale

removal. The latter is an essential step to avoid sampling scales from foreign individuals. Scales were taken from ten body areas (FIG. 6) on the left side of each specimen. For flatfishes, scale sampling was separately performed for both sides. For some species, less than ten sampling areas are presented in this study due to the insufficient number of scales found within the given area on the available specimens. The lack of scales mainly occurred in open water and/or deep sea species that possess highly deciduous scales. This study focuses on “regular” body scales and therefore specialized scales such as lateral line scales, tiny scales on the base of fins, or keel-scutes are not investigated here.



**FIGURE 6. Schematic drawing of the ten scale sampling areas**

(A) anterior, dorsal, rostral to dorsal fin; (B) posterior, dorsal, caudal to dorsal fin; (C) anterior, dorsal, above lateral line; (D) posterior, dorsal, above the lateral line; (E) caudal to operculum; (F) anterior, ventral, below lateral line; (G) posterior, ventral, below lateral line; (H) caudal peduncle; (I) anterior, ventral, rostral to pelvic fin; (J) posterior, ventral, caudal to pelvic fin

Scale material was stored in 70% ethanol, gently brushed to remove loose tissue remains and stained with Alizarin Red S (ROTH, Germany). Images were taken either in ethanol or from mounted scales. For mounting, scales were transferred into 100% ethanol, followed by acetone and embedded into MOUNTEX (MEDITE, Germany) on object slides. Imaging was performed using a LEICA MZ75 microscope with a mounted camera DFC-425 and dedicated software (LAS, LEICA, Germany). The imaging software provided all pictures with correct scale bars. The Photoshop CS6 (ADOBE, United States) photo-editing program was used for composing the plates for each species. The scales are represented with the respective anterior (rostral) part to the top and the posterior (caudal) part to the bottom of each image in APPENDIX 1 (FIG. 1 - FIG. 89).

### ***3.2.1.2. Terminology of scale types***

Definitions of scale types follow ROBERTS (1993) with some improvements. In this study, two main scale types (FIG. 7) and six subtypes (FIG. 9) are distinguished, as follows:

(1) **Cycloid scales** – scales without additional separate ossifications; marginal indentations or spine-like projections might occur.

Cycloid scale subtypes are:

a) **True cycloid** – cycloid scale without any spine projections or marginal increments (FIG. 9A);

b) **Crenate** – cycloid scale with marginal increments termed here as crenae (FIG. 9B);

c) **Spinoid** – cycloid scale with spines that is continuous with the main body of the scale; the spines can be restricted to the posterior margin or scattered over the posterior field (FIG. 9C).

(2) **Ctenoid scales** – scales with additional separate ossifications, that form discrete spines called cteni (singular ctenus).

Ctenoid scales can occur with three types of cteni:

a) **Peripheral cteni** – ctenoid scale with separate ossifications that occur as whole spines in one row at the posterior margin (FIG. 9D); sometimes an alternating row of smaller secondary spines might be present;

b) **Transforming cteni** – ctenoid scale with separate ossifications that arise as whole spines in two or more alternating rows marginally and transform into truncated spines sub-marginally (FIG. 9E);

c) **Whole cteni** – ctenoid scale with separate ossifications that occur as whole spines marginally and sub-marginally (FIG. 9F).

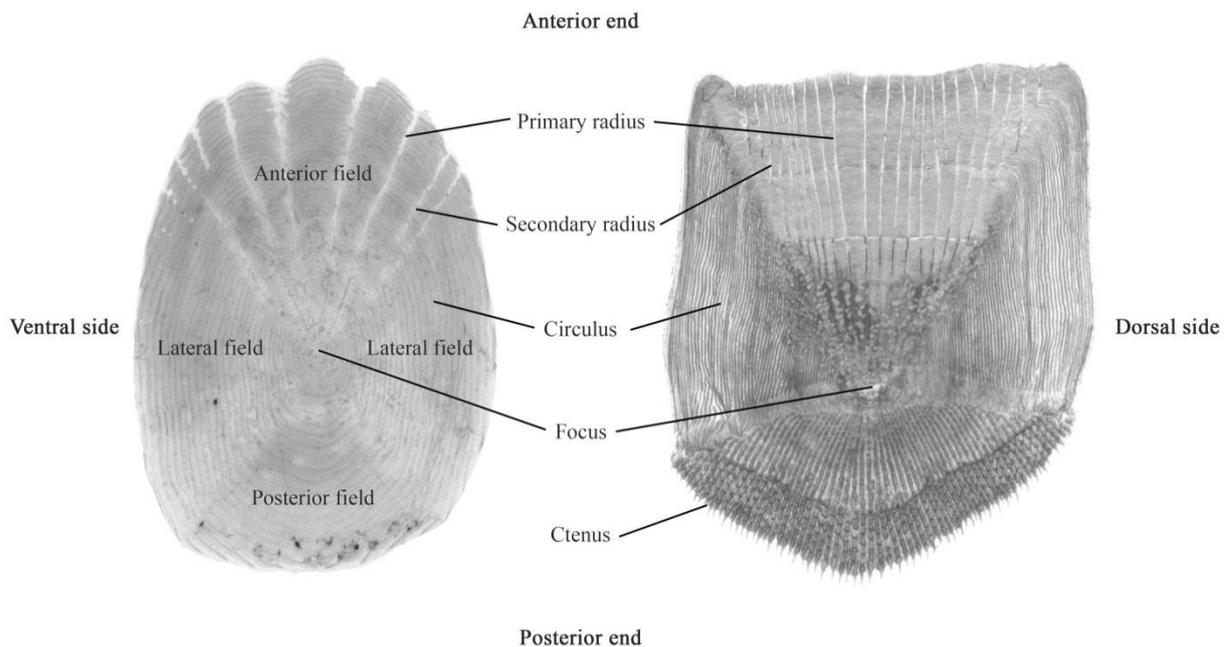
### ***3.2.1.3. Terminology of scale characteristics***

The following scale characteristics were defined as main discriminative features to aid scale identification. The definitions of scale characteristics are based on LAGLER (1947) to promote congruence with the criteria used in previous works by other scientists. FIGURES 7 to 9 illustrate the morphological characteristics, and Table 2 summarizes the discriminative features of these characteristics.

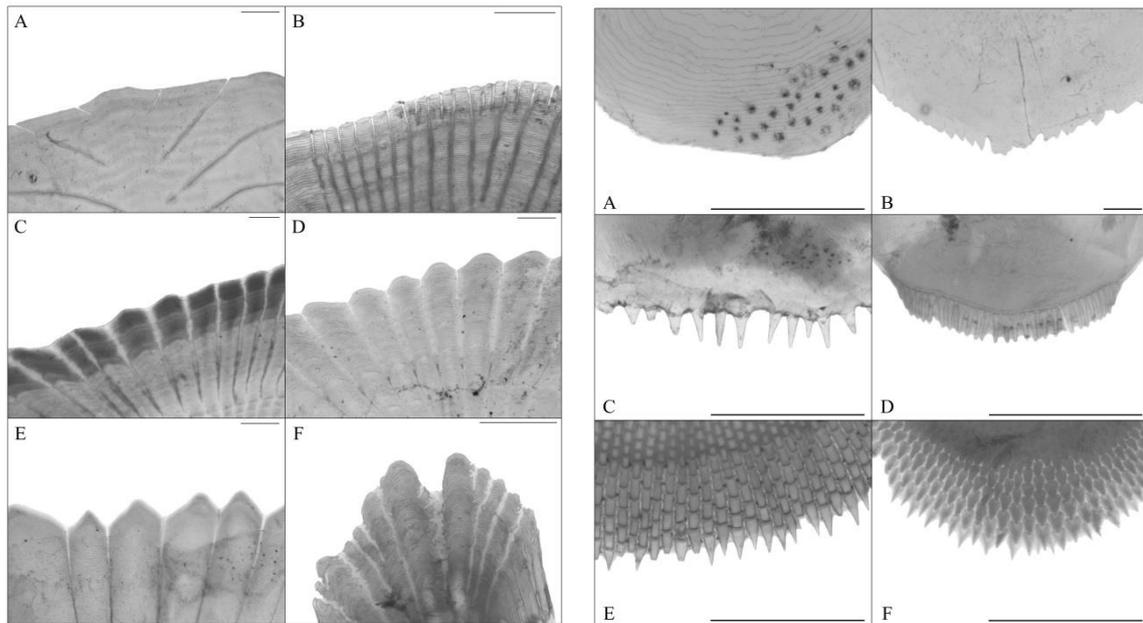
- a) **Field** – an area of the scale surface, with the focus as centre and four “corners” of the scale four fields can be distinguished: anterior, posterior, dorsal and ventral, whereas the latter two are usually much similar and summarized as lateral fields (FIG. 7). For the purpose of better discrimination, the following characteristics of these fields were described: (1) the outer curvature of the anterior field: convex, concave or flattened; (2) the margin of the anterior field: smooth – without any in- or edentations; striate – with incisions usually caused by the distal parts of radii; waved – with rounded in- and edentations; scalloped – with rounded edentations and acute indentations; dentate – with regular acute in- and edentations; fluted – with irregular rounded in- and edentations (FIG. 8); (3) the outer curvature and extension of the lateral fields: convex, concave or flattened and extended in dorso-ventral axis or elongated in antero-posterior axis; (4) the posterior end: flattened, rounded, tapered or pointed, as well as the different characteristic features of the posterior margin, as follows: smooth, membranous, crenulated, spinous, ctenous.
- b) **Focus** – also called nucleus; the first part of the scale appearing in ontogeny, forming an area inside of the first circulus. The focus can be in the geometrical centre of the scale, but is often shifted posterior or anterior of this point (FIG. 7). The focal index (Fi) was calculated as the distance from the outermost edge of the anterior field to the focus divided by the distance from the outermost edge of the anterior field to the outermost edge of the posterior field. The position of the focus was classified according to the focal index, as follows:  $< 0.20$  – anterior;  $0.21-0.40$  – antero-central;  $0.41-0.60$  – central;  $0.61-0.80$  – postero-central;  $> 0.81$  – posterior (APP. 1, TABLE 1).
- c) **Circulus** – elevated marking on the outer surface usually appearing as line following more or less the outline shape of the scale; originating from batch-wise growth (FIG. 7). Circuli generally create continuous concentric lines, however, they can be interrupted by radii or other grooves. In a few cases, circuli can be ornamented by hyper-mineralization, or disconnected by unmineralized areas. These features are described in the given species.
- d) **Radius** – groove with usually radial orientation. Commonly, radii are restricted to the anterior field; they interrupt the circuli. In general, a radius is rectilinear, positioned in the antero-posterior axis of the scale and pointing towards the focus. Primary radii reach the focus from the scale margin, whereas secondary radii start at the scale margin but do not reach the focus (FIG. 7). In some cases, radii are positioned between the focus and the scale margin. The latter are termed as tertiary radii. Other forms of grooves (e.g.,

transverse, longitudinal, vertical or irregular) can also occur and do not necessarily have an even rectilinear character nor a radial orientation. The characteristics of these grooves are described for the species concerned.

- e) **Ctenus** – tooth-like structure that ossifies separately and is more or less detached from the main body of the scale. Cteni appear in one or more rows marginally or sub-marginally at the posterior field (FIG. 9D-F).
- f) **Spine** – tooth-like ossification continuous with the main body of the scale. Spines appear at the posterior margin (FIG. 9C) or are scattered within the posterior field (e.g., *Trachyrincus scabrus*, APP. 1, FIG. 16).



**FIGURE 7. Scale characteristics shown for a cycloid scale of *Seriola dumerili* (left) and a ctenoid scale of *Dicentrarchus labrax* (right)**



**FIGURE 8. Characteristic features of the anterior margin of different scale types**

A: smooth (*Sardina pilchardus*); B: striate (*Dicentrarchus labrax*); C: waved (*Sparus aurata*); D: scalloped (*Diplodus vulgaris*); E: dentate (*Mullus surmuletus*); F: fluted (*Eutrigla gurnardus*); scale bar = 500  $\mu$ m

**FIGURE 9. Characteristic features of the posterior margin of different scale types**

A: true cycloid (*Trachurus trachurus*); B: crenate (*Alosa fallax*); C: spinoid (*Lepidotrigla cavillone*); D: peripheral cteni (*Gobius bucchichi*); E: transforming cteni (*Chaetodon hoeferi*); F: whole cteni (*Chelon labrosus*); scale bar = 1 mm

#### 3.2.1.4. Terminology of scale shapes

Scales show a high level of diversity in their shapes with considerable intra- and inter-species variation. The observed scale shapes were categorized into five main types with a total of 18 subtypes (FIG. 10). Transitions between these categories may be present and are noted in the respective descriptions, e.g., “circular to oval”. Shape variability of scales was defined according to the number of different scale shapes that occurred within a specimen: uniform – only 1 scale shape; low – 2 scale shapes; moderate – 3 scale shapes; high – 4 or more scale shapes.

The typical scale shapes are classified as follows:

**(1) Circular** – a form of or similar to a circle with rounded outline, in which the diameter varies little between all directions.

Circular subtypes:

a) **True circular** – rounded outline with equal diameter in each direction;

**b) Cordate** – rounded outline, more or less equal diameter, but slightly pointed posterior end and double (sometimes triple) rounded anterior field;

**c) Discoidal** – circular with elongated lateral fields in dorso-ventral axis; the anterior field is rounded and slightly elevated.

(2) **Oval** – a form of an elongated circle with rounded outline, in which the length of the diameter is not equal in all direction.

Oval subtypes:

**a) True oval** – rounded outline, elongated in antero-posterior axis with slightly wider (often flattened) anterior field with rounded edges and rounded, narrower posterior field;

**b) Ovoid** – rounded outline, slightly elongated in antero-posterior axis, with narrower anterior field and wider posterior field;

**c) Reversed ovoid** – rounded outline, slightly elongated in antero-posterior axis, with wider (never flattened) anterior field and narrower, slightly pointed posterior field;

**d) Oblong** – rounded outline, mostly elongated in antero-posterior axis with equally wide anterior and posterior field. The lateral fields are almost parallel;

**e) Elliptical** – rounded outline, largely elongated lateral fields in dorso-ventral axis. The anterior or posterior field is slightly flattened.

(3) **Quadrilateral** – a form of a four-sided polygon with more or less squared outline; often the sides are parallel and the edges form right angles.

Quadrilateral subtypes:

**a) Square** – quadrilateral with all sides equal in length forming more or less right angles.

**b) Rectangular** – quadrilateral, that is elongated in antero-posterior axis with parallel lateral fields; the anterior and posterior fields are shorter than the lateral fields are long. The corners are not rounded but form more or less right angles;

**c) Trapezoidal** – quadrilateral, where the length of its adjacent sides is unequal and its angles are oblique; has one pair of parallel sides;

**d) Rhomboidal** – quadrilateral, where the length of its adjacent sides is unequal and its angles are oblique; no parallel sides.

(4) **Polygonal** – multi-sided forms with somehow square outline with angled corners.

Polygonal subtypes:

**a) Pentagonal** – five-sided polygon where each of the sides are approximately equal in length;

b) **Hexagonal** – six-sided polygon where each of the sides are about equal in length;

c) **Octagonal** – eight-sided polygon where each of the sides are similar in length.

(5) **Intermediate** – typical shapes between quadrilateral, polygon and somewhat circular; corners may be square or round, i.e., not geometrical.

Intermediate subtypes:

a) **Calyx** – resembles quadrilateral, but with wide anterior field that is extended in dorso-ventral axis and lateral fields that are bulged (convex) with one pair of rounded edges;

b) **Shell-shaped** – usually resembles quadrilateral, but with wide anterior field that is extended in dorso-ventral axis and lateral fields that are concave with one pair of rounded edges;

c) **Irregular** – without a symmetrical outline.

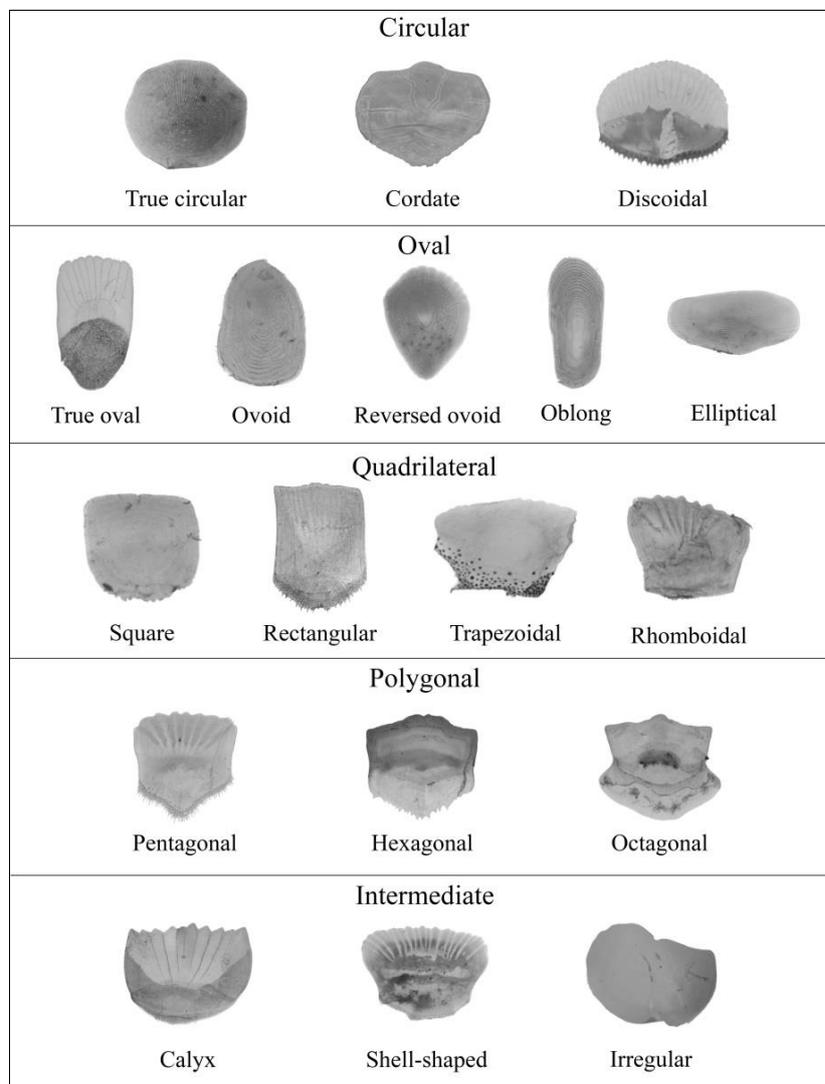


FIGURE 10. Classification of scale shapes used in this study

**TABLE 2. Summary of the morphological characters and discriminative features used in fish scale identification**

<b>Morphological characters</b>	<b>Discriminative features</b>
<b>Type</b>	cycloid: true cycloid / crenate / spinoid ctenoid: peripheral cteni / transforming cteni / whole cteni circular: true circular / cordate / discoidal oval: true oval / ovoid / reversed ovoid / oblong / elliptical
<b>Shape</b>	quadrilateral: square / rectangular / trapezoidal / rhomboidal polygonal: pentagonal / hexagonal / octagonal intermediate: calyx / shell-shaped irregular: irregular
<b>Shape variability</b>	uniform / low / moderate / high
<b>Anterior field</b>	convex / concave / flattened
<b>Anterior margin</b>	smooth / striate / wavy / scalloped / dentate / fluted
<b>Lateral fields</b>	convex / concave / flattened
<b>Extension</b>	extended in dorso-ventral axis / elongated in antero-posterior axis
<b>Posterior field</b>	flattened / rounded / tapered / pointed
<b>Posterior margin</b>	smooth / membranous / crenulated / spinous / ctenous
<b>Focus position</b>	anterior / antero-central / central / postero-central / posterior
<b>Circuli</b>	distinct / indistinct continuous / discontinuous presence / absence
<b>Radii</b>	primary / secondary / tertiary other grooves
<b>Radii orientation</b>	radial / parallel / transverse / longitudinal / vertical / irregular
<b>Spines</b>	marginal / scattered
<b>Cteni</b>	peripheral / transforming / whole

In total, 56 characteristic features of scale morphology were coded in the form of a number (TABLE 3) and served as input into a dendrogram based on the Euclidean distances as a measure of dissimilarity. Due to the large variety of species and the small sample size (number of studied species) within some orders, only those orders were selected for the analysis that consisted of more than five studied species (i.e., four orders with 15 families and 37 species, see TABLE 4). Five scales per species were selected from sampling area C (FIG. 6)

and the predominant feature was used to create the data matrix of coded characters (APP. 2, TABLE 1). The between-groups-linkage method was applied as the clustering algorithm to gather the phenotypic relationships by using the software programme PAST (PAleontological SStatistics – HAMMER *et al.*, 2001).

**TABLE 3. Coding of characteristic features of scale morphology**

<b>Characters</b>	<b>Features</b>	<b>Code</b>	<b>Characters</b>	<b>Features</b>	<b>Code</b>
<b>Type</b>	Cycloid	0	<b>Posterior margin</b>	Smooth	0
	Crenate	1		Membranous	1
	Spinoid	2		Crenulated	2
	Peripheral ctenoid	3		Spinous	3
	Transforming ctenoid	4		Ctenous	4
	Whole ctenoid	5			
<b>Shape</b>	Circular	0	<b>Focus</b>	Indistinct	0
	Oval	1		Distinct	1
	Quadrilateral	2			
	Polygonal	3			
	Intermediate	4			
	Irregular	5			
<b>Anterior field</b>	Convex	0	<b>Position of focus</b>	Anterior	0
	Concave	1		Antero-central	1
	Flattened	2		Central	2
				Postero-central	3
			Posterior	4	
<b>Anterior margin</b>	Smooth	0	<b>Circuli</b>	Indistinct	0
	Striate	1		Continuous	1
	Waved	2		Discontinuous	2
	Scalloped	3			
	Dentate	4			
	Fluted	5			
<b>Lateral fields</b>	Convex	0	<b>Radii</b>	Absence	0
	Concave	1		Radial	1
	Flattened	2		Parallel	2
				Transverse	3
			Irregular	4	
<b>Dimension</b>	Extended in D-V axis	0	<b>Spines</b>	Absence	0
	Elongated in A-P axis	1		Marginal	1
				Scattered	2
<b>Posterior field</b>	Flattened	0	<b>Cteni</b>	Absence	0
	Rounded	1		Peripheral	1
	Tapered	2		Transforming	2
				Whole	3

**TABLE 4. Selected orders and the list of species in which the predominant scale characteristics served as input for the dendrogram (FIG. 13)**

<b>Orders</b>	<b>Families</b>	<b>Species</b>	<b>Abbreviation</b>
Clupeiformes	Engraulidae	<i>Engraulis encrasicolus</i>	EngE
	Clupeidae	<i>Alosa fallax</i>	AloF
		<i>Clupea harengus</i>	CluH
		<i>Sardina pilchardus</i>	SarP
		<i>Sardinella aurita</i>	SarA
		<i>Sprattus sprattus</i>	SprS
Gadiformes	Macrouridae	<i>Coelorinchus caelorhynchus</i>	CoeC
		<i>Nezumia sclerorhynchus</i>	NezS
		<i>Trachyrinchus scabrous</i>	TraS
	Moridae	<i>Mora moro</i>	MorM
	Merlucciidae	<i>Merluccius merluccius</i>	MerM
	Gadidae	<i>Molva molva</i>	MolM
<i>Trisopterus luscus</i>		TriL	
Perciformes	Serranidae	<i>Anthias anthias</i>	AntA
		<i>Epinephelus aeneus</i>	EpiA
		<i>Serranus cabrilla</i>	SerC
		<i>Serranus scriba</i>	SerS
	Carangidae	<i>Seriola dumerili</i>	SerD
		<i>Trachurus trachurus</i>	TraT
	Sparidae	<i>Boops boops</i>	BooB
		<i>Pagellus acarne</i>	PagA
		<i>Pagellus erythrinus</i>	PagE
		<i>Sarpa salpa</i>	SarS
		<i>Sparus aurata</i>	SpaA
		<i>Spondylisoma cantharus</i>	SpoC
	Mullidae	<i>Mullus barbatus</i>	MulB
		<i>Mullus surmuletus</i>	MulS
	Labridae	<i>Coris julis</i>	CorJ
		<i>Labrus viridis</i>	LabV
		<i>Symphodus rostratus</i>	SymR
		<i>Thalassoma pavo</i>	ThaP
Pleuronectiformes	Psettodidae	<i>Scophthalmus maximus</i>	ScoM
	Bothidae	<i>Bothus podas</i>	BotP
	Solenidae	<i>Microchirus variegatus</i>	MicV
		<i>Monochirus hispidus</i>	MonH
		<i>Solea solea</i>	SolS
Cynoglossidae	<i>Symphurus nigrescens</i>	SymN	

### 3.2.2. Scale morphometry

#### 3.2.2.1. Scale sampling and preparation

Scale samples for morphometric analysis were collected in July and August 2014 from freshly caught individuals of two clupeid species. Specimens were purchased from fishermen operating in the Gulf of Ambracia. The species were identified morphologically by the presence of a faint golden spot behind the gill-opening, followed by a distinct black spot at the posterior border of the gill cover in *S. aurita*, and a series of dark spots along the upper flanks in *S. pilchardus* (WHITEHEAD, 1985). In total, 219 individuals of *S. pilchardus* (mean SL  $\pm$  SD: 81.65  $\pm$  4.68 mm) and 268 individuals of *S. aurita* (mean SL  $\pm$  SD: 155.35  $\pm$  8.51 mm) were sampled and measured. Only one scale was collected from one body area on the left body side from each specimen to avoid auto-correlation problems among scales. The number of scale samples (i.e., sampled individuals) varied for the 10 sampling areas (i.e., A-J, see FIG. 6) between 16-28 for *S. pilchardus* and 24-30 for *S. aurita* (TABLE 5). The scale preparation was followed in the same way as described under 3.2.1.1. *Scale sampling and preparation*.

TABLE 5. Number of scale samples and standard length of specimens derived from two clupeid species

Species	No. of scales collected per body area		No. of scales collected per body area		Mean SL $\pm$ SD [mm]	
<i>S. pilchardus</i>	A	21	F	20	82.10 $\pm$ 5.45	81.65 $\pm$ 5.18
	B	27	G	27	80.59 $\pm$ 4.05	80.74 $\pm$ 4.34
	C	17	H	28	83.18 $\pm$ 2.35	81.12 $\pm$ 4.68
	D	21	I	16	82.14 $\pm$ 5.50	82.94 $\pm$ 5.51
	E	16	J	26	83.00 $\pm$ 5.56	80.88 $\pm$ 3.83
<i>S. aurita</i>	A	30	F	26	153.00 $\pm$ 5.86	156.92 $\pm$ 4.15
	B	30	G	26	154.02 $\pm$ 3.99	155.18 $\pm$ 5.34
	C	30	H	25	156.16 $\pm$ 5.76	156.08 $\pm$ 5.69
	D	25	I	26	155.23 $\pm$ 6.70	154.04 $\pm$ 2.87
	E	24	J	26	151.75 $\pm$ 4.34	156.87 $\pm$ 4.03

#### 3.2.2.2. Scale morphometric measurements

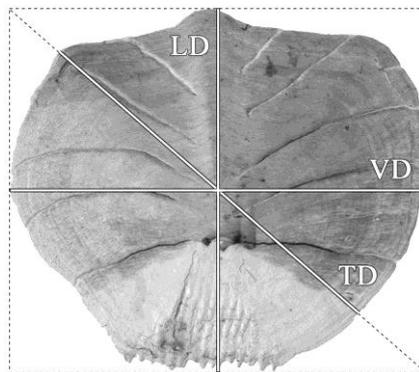
Morphometric parameters were measured to the nearest 0.1 mm on the digital images of scales (i.e., 487 scale images in total) using Photoshop CS6.

The following morphometric parameters were recorded (FIG. 11):

- a) **Longitudinal diameter (LD)** – the maximal longitudinal diameter of the scale in antero-posterior axis (i.e., scale length);
- b) **Vertical diameter (VD)** – the maximal vertical diameter of the scale in dorso-ventral axis (i.e., scale width);
- c) **Transverse diameter (TD)** – the maximal transverse diameter of the scale;

Two additional parameters were measured that are not indicated in FIG. 11:

- d) **Perimeter (P)** – the maximal length of the outline of the scale;
- e) **Area (A)** – the extent of the scale surface area within the perimeter.



**FIGURE 11. Scale morphometric parameters for an exemplary scale of *S. pilchardus***

Note: positioning of the scale: the respective anterior (rostral) part to the top and the posterior (caudal) part to the bottom of the image

**Relative scale sizes (J-indices)** for the scale length (Jsl), scale width (Jsw), and scale transverse diameter (Jst) were calculated following ESMAEILI (2001):

$$Jsl/ Jsw/ Jst = \frac{\text{length/ width/ transverse diameter of scale}}{\text{fish standard length}} \times 100$$

**Scale shape indices**, including circularity, rectangularity, roundness, form-factor, and aspect ratio, were then calculated following TUSET *et al.* (2003) (TABLE 6).

**TABLE 6. Scale morphometric parameters and resulting shape indices utilized for the shape analysis**

Morphometric parameters	Shape indices (Si)
Scale length (LD)	Circularity (Cir) = $P/A^2$
Scale width (VD)	Rectangularity (Rec) = $A/(LD \times VD)$
Perimeter (P)	Roundness (Rnd) = $(4A) / (\pi LD^2)$
Area (A)	Form-Factor (FF) = $(4 \pi A) / P^2$
	Aspect Ratio (AR) = $LD / VD$

### 3.2.2.3. *Statistical analyses*

In scale shape analysis, to remove the effect of the fish size on the morphometric data (i.e., shape indices), standardized morphometric measurements (MS) were calculated following the method of THORPE (1975) and LLEONART *et al.* (2000) for each fish as:  $MS = MO (SI/SL)^b$ , where MO – is the original morphometric measurement, SI – the mean standard length of all fish, SL – the standard length of the specimen, and b – was estimated for each morphometric character as the slope of the regression between log MO and log SL (ELLIOTT *et al.*, 1995). Correlations between transformed variables and standard length were calculated to inspect whether the data transformation was effective in removing the size effect from the data.

Owing to the nature of the data derived from the scale shape analysis, some of the shape variables did not follow a normal distribution. For this reason, a non-parametric discriminant analysis using the normal kernel density estimation was applied to investigate the usefulness of scale shape in separating the two clupeid species. Cross validation was used to estimate the accuracy of classification rules. Comparisons of scale shapes between species and among their scale sampling areas were conducted using a permutation multivariate analysis of variance (PERMANOVA; ANDERSON, 2001); a non-parametric statistical test that does not require the normal distribution of data which allows testing the simultaneous response of one or more variables to one or more factors in the ANOVA experimental design. The PERMANOVA was based on the Bray-Curtis dissimilarity measure (4999 random permutations, after BACHA *et al.*, 2014). To present the major trends in shape differences among the sampling areas in both species graphically, cluster analysis of the adjusted shape indices was used. Similarity matrices were constructed based on Bray-Curtis' similarity. Analyses and tests were carried out using SPSS and PAST.

## 3.2.3. **Scale geometric morphometry**

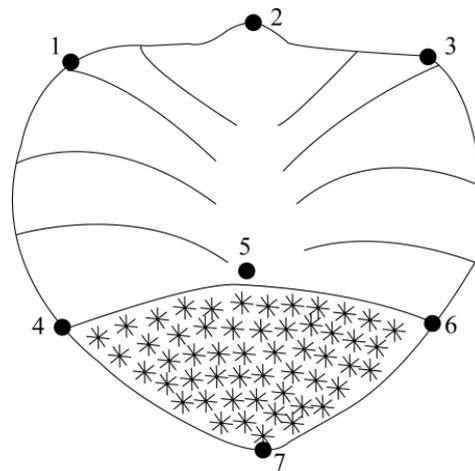
### 3.2.3.1. *Scale sampling and preparation*

The scale samples were obtained from commercially caught specimens, as mentioned earlier. Scales of *S. pilchardus* were sampled from all four study sites (i.e., 50 individuals from each site), whereas the same number of scales of *S. aurita* could be collected only from two study sites (i.e., Gulf of Ambracia and Gulf of Kavala, see FIG. 5). Scales were removed only from the left flank, below the dorsal fin above the lateral line following STASZNY *et al.*

(2012) and stored in vials with 70 % ethanol. Only one randomly selected scale per vial was used for the analysis to evaluate a rapid stock discrimination methodology (IBÁÑEZ *et al.*, 2007). Recently grown scales were discarded from the analysis as they do not show the regular growing pattern during regeneration processes (IBÁÑEZ *et al.*, 2012). In the laboratory, scales were hydrated with distilled water and placed them in a 10 % potassium hydroxide solution. After removal of the epithelial tissue by gentle brushing, the scales were placed into 70 % ethanol and stained with Alizarin Red S (Roth Co., Germany). Finally, scale images were taken in ethanol in a clear Petri dish using a Leica MZ75 stereomicroscope with a mounted DFC-425 camera and dedicated software (LAS, Leica, Germany), which resulted in digital images with scale bars for each sample.

### 3.2.3.2. Scale geometric morphometric measurements and analyses

Seven landmarks were recorded on each scale (FIG. 12) using “tpsUtil” v. 1.60 (ROHLF, 2015a) and “tpsDig2” v. 2.17 (ROHLF, 2015b) utility programs to identify the key features as suggested by STASZNY *et al.* (2013).



**FIGURE 12. Location of the seven landmarks for geometric morphometric analysis on a schematic clupeid scale**

Shape data of the scales were processed with the software program “MorphoJ” v. 1.06d (KLINGENBERG, 2011). First, group identities (by species or by sampling sites) were assigned to raw landmark coordinates. The centroid size (CS) was used as the size metric of the scales as the only mathematically shape-free size variable (ZELDITCH *et al.*, 2004). To rotate, scale and align the raw coordinates into new shape variables, a Generalized Procrustes Analysis (GPA) was performed. Furthermore, a multivariate linear regression of shape (i.e., Procrustes

coordinates) was performed on size (logCS) for each group to remove possible allometric effects. The significance of the relationship (i.e., the presence of an allometric effect) was evaluated by using a permutation test against the null hypothesis of independence (10,000 iterations). As data were free of allometric effects associated with growth, residuals of this regression provided the basis of further analyses (ELMER *et al.*, 2010). Finally, the differences between groups were assessed with a canonical variate analysis (CVA) and a discriminant function analysis (DFA) and examined the reliability of the results with permutation tests with 10,000 iterations.

## 4. RESULTS

### 4.1. Scale morphology

#### 4.1.1. Morphological descriptions of scale characteristics

The categorization of scale types, shape categories, and the scale characteristics (as described in section 3.2.1) were applied to 80 common species of Mediterranean teleosts. The description of scale morphology according to these criteria allowed the identification of a large variety of scales at species-level, which satisfies the postulated questions Q1 and Q2. To facilitate scale identification and the differentiation of closely related species, images of the scales described below are represented in APPENDIX 1 (FIG. 1 - FIG. 89) with each scale positioned with its anterior (rostral) part to the top and its posterior (caudal) part to the bottom of the image.

### Order ALBULIFORMES

*Notacanthus bonaparte* Risso, 1840

Family NOTACANTHIDAE

APP. 1, FIG. 1

**Type:** cycloid: true cycloid. **Shape:** circular: reversed ovoid to true circular. Shape variability: moderate. **Anterior field:** convex with waved margin. **Lateral fields:** convex and slightly extended in the antero-lateral section with rounded corners. These fields are gradually narrowing towards the posterior field. **Posterior field:** tapered end with smooth margin. **Focus:** antero-central. **Circuli:** distinct and discontinuous. **Radii:** primary, secondary and tertiary radii can occur in radial orientation in all four fields.

### Order CLUPEIFORMES

Scales of clupeiforms are easily distinguishable from other species, by having distinctive grooves (radii), and a membranous posterior field with crenulated margin. Grooves present on the scales of Clupeiformes were identified as “fracture lines” by PATTERSON *et al.* (2002), as “transverse striae (radii)” in the anterior field and as “longitudinal striae” in the posterior field by ROBERTS (1993). In this study, the observed grooves were classified as a special form of radii and termed according to their orientation (i.e., transverse, longitudinal, irregular). Scales within this order are highly variable in shape among the sampling areas of the fish body as well as within any given area thus hindering species identification. Scales of *S.*

*pilchardus* and *S. aurita* showed the highest similarity across species as well as a high level of plasticity in their scale characteristics.

*Engraulis encrasicolus* (Linnaeus, 1758)

Family ENGRAULIDAE

APP. 1, FIG. 2

**Type:** cycloid: crenate. **Shape:** circular: true circular to cordate or discoidal. Shape variability: high. **Anterior field:** convex to flattened with conical apex and smooth margin. **Lateral fields:** convex and well-extended in dorso-ventral axis. **Posterior field:** tapered end with crenulated margin that is subjected to fracturing. **Focus:** central. **Circuli:** distinct and discontinuous in the anterior and lateral fields, indistinct in the posterior field. **Radii:** transverse, longitudinal and irregular grooves occur in all four fields. Grooves are radial, parallel or transverse in orientation and run across the scale in an irregular manner.

*Alosa fallax* (Lacepède, 1803)

Family CLUPEIDAE

APP. 1, FIG. 3

**Type:** cycloid: crenate. **Shape:** circular to oval: true circular to true oval, or slightly polygonal. Shape variability: high. **Anterior field:** convex to flattened with conical apex and smooth margin. **Lateral fields:** flattened and only moderately extended in dorso-ventral axis. **Posterior field:** rounded to tapered end with crenulated margin that is subjected to fracturing. Crenae form a teeth-like formation. **Focus:** postero-central. **Circuli:** distinct and discontinuous in the anterior and lateral fields, indistinct in the posterior field. **Radii:** transverse grooves are present in the anterior and lateral fields. In some cases (e.g., in area B), longitudinal grooves can occur within the posterior field that run across the scale in an irregular manner.

*Clupea harengus* Linnaeus, 1758

Family CLUPEIDAE

APP. 1, FIG. 4

**Type:** cycloid: crenate. **Shape:** circular to oval: cordate to discoidal, or true oval to reversed ovoid. Shape variability: high. **Anterior field:** rather flattened than convex with conical apex and smooth margin. **Lateral fields:** flattened to convex and extended in dorso-ventral axis. **Posterior field:** tapered end with crenulated margin that is subjected to fracturing. Crenae show an irregular and membranous appearance. **Focus:** central. **Circuli:** distinct and discontinuous in the anterior and lateral fields, indistinct in the posterior field. **Radii:**

transverse grooves are present in the anterior and lateral fields. In areas A, B, H, I, and J, longitudinal grooves can occur that run across the scale in an irregular manner.

*Sardina pilchardus* (Walbaum, 1792)

Family CLUPEIDAE

APP. 1, FIG. 5

**Type:** cycloid: crenate. **Shape:** circular to oval: generally cordate to discoidal. Shape variability: high. **Anterior field:** flattened with prominent conical apex and smooth margin. **Lateral fields:** convex and extended in dorso-ventral axis. **Posterior field:** rounded to tapered end with crenulated margin that is subjected to fracturing. **Focus:** postero-central. **Circuli:** distinct and discontinuous in the anterior and lateral fields, indistinct in the posterior field. **Radii:** transverse grooves are present in the anterior and lateral fields. Longitudinal grooves can occur, especially in areas A and B that run across the scale in an irregular manner.

*Sardinella aurita* (Valenciennes, 1847)

Family CLUPEIDAE

APP. 1, FIG. 6

**Type:** cycloid: crenate. **Shape:** circular to oval or slightly quadrilateral: generally cordate to discoidal. Shape variability: high. **Anterior field:** flattened with low conical apex and smooth margin. **Lateral fields:** flattened to convex and extended in dorso-ventral axis. **Posterior field:** rounded to tapered end with crenulated margin that is subjected to fracturing. Crenae form a teeth-like formation. **Focus:** postero-central. **Circuli:** distinct and discontinuous in the anterior and lateral fields, indistinct in the posterior field. **Radii:** transverse grooves are present in the anterior and lateral fields. Longitudinal grooves can occur, especially in area H that run across the scale in an irregular manner.

*Sprattus sprattus* (Linnaeus, 1758)

Family CLUPEIDAE

APP. 1, FIG. 7

**Type:** cycloid: crenate. **Shape:** circular to oval: true circular to true oval. Shape variability: high. **Anterior field:** convex to flattened with smooth margin. The conical apex is virtually absent. **Lateral fields:** convex to flattened and generally elongated in antero-posterior axis. **Posterior field:** rounded end with crenulated margin. Crenae form a teeth-like formation. **Focus:** postero-central. **Circuli:** distinct and discontinuous in the anterior and lateral fields, indistinct in the posterior field. **Radii:** transverse grooves are present in the anterior and lateral fields. Longitudinal grooves rarely occur (e.g., in area B) and are not typical.

## Order ARGENTINIFORMES

APP. 1, FIG. 8

*Argentina sphyraena* Linnaeus, 1758

Family ARGENTINIDAE

**Type:** cycloid: true cycloid. **Shape:** irregular. Shape variability: high. **Anterior field:** asymmetric, can be flattened or convex in shape with uneven but smooth margin. **Lateral fields:** convex and generally extended in the antero-lateral section. **Posterior field:** rounded, irregular or sometimes tapered end with smooth and membranous margin. **Focus:** indistinct. **Circuli:** distinct and continuous to indistinct in the anterior and lateral fields, indistinct in the posterior field. **Radii:** absent.

*Alepocephalus rostratus* Risso, 1820

Family ALEPOCEPHALIDAE

APP. 1, FIG. 9

Scales are highly deciduous, and it was not possible to obtain them from all sampling areas. Therefore, the description refers to scales found within areas A, B and C only.

**Type:** cycloid: true cycloid. **Shape:** oval: true oval. Shape variability: uniform. **Anterior field:** flattened to slightly incline or concave end with smooth margin. Due to the dominance of the posterior field, this field is less prominent. **Lateral fields:** flattened and elongated in antero-posterior axis. **Posterior field:** convex with smooth margin. **Focus:** anterior. **Circuli:** distinct; continuous in the anterior and lateral fields, discontinuous in the posterior field. **Radii:** primary and secondary radii are present in the posterior field in radial orientation.

## Order STOMIIFORMES

*Maurolicus muelleri* (Gmelin, 1789)

Family STERNOPTYCHIDAE

APP. 1, FIG. 10-11

Scales are highly deciduous, and it was not possible to obtain them from all sampling areas. Therefore, the description refers to scales found within areas B, F, I, J only. **Type:** cycloid: true cycloid. **Shape:** circular to quadrilateral: true circular to square. Shape variability: moderate. **Anterior field:** flattened or slightly convex with striate margin. **Lateral fields:** flattened to convex and slightly extended in the dorso-ventral axis. **Posterior field:** flattened to rounded end with smooth margin. **Focus:** central. **Circuli:** distinct and discontinuous. Numerous elevated structures align among circuli that start as square-like structures and create uneven ridges in radial orientation towards the anterior margin. **Radii:** absent.

## Order AULOPIFORMES

*Synodus saurus* (Linnaeus, 1758)

Family SYNODONTIDAE

APP. 1, FIG. 12

**Type:** cycloid: true cycloid. **Shape:** intermediate: calyx to shell-shaped. Shape variability: low. **Anterior field:** flattened with deeply scalloped margin creating three to four lobes. **Lateral fields:** convex and extended in dorso-ventral axis, except in areas B and H, where these fields are rather flattened and elongated in antero-posterior axis. **Posterior field:** rounded to tapered end with membranous margin that is subjected to fracturing. **Focus:** postero-central. **Circuli:** distinct and discontinuous in the anterior field, continuous in the lateral fields, indistinct in the posterior field. **Radii:** primary and secondary radii are present in the anterior field in radial orientation.

## Order MYCTOPHIFORMES

*Myctophum punctatum* Rafinesque, 1810

Family MYCTOPHIDAE

APP. 1, FIG. 13

Scales are deciduous, and it was not possible to obtain them from all sampling areas. Therefore, the description refers to scales found within areas A, B, C, D, E, F, G, and H only.

**Type:** cycloid: true cycloid. **Shape:** intermediate to quadrilateral: calyx to shell-shaped or slightly trapezoidal. Shape variability: high. **Anterior field:** rather flattened than convex. The margin of this field is deeply scalloped creating a lobular appearance. **Lateral fields:** flattened to convex and extended in dorso-ventral axis. **Posterior field:** rounded or irregular end with smooth margin. **Focus:** central. **Circuli:** distinct and discontinuous in the anterior field, continuous in the lateral fields, indistinct in the posterior field. **Radii:** primary and secondary radii are present in the anterior field in radial orientation.

## Order GADIFORMES

*Coelorinchus caelorhinchus* (Risso, 1810)

Family MACROURIDAE

APP. 1, FIG. 14

**Type:** cycloid: spinoid. **Shape:** circular to polygonal: cordate to hexagonal. Shape variability: low. **Anterior field:** convex with pointed apex and smooth margin. The antero-lateral corners are extended and pointed. **Lateral fields:** convex and extended in dorso-ventral axis.

**Posterior field:** rounded to tapered end with smooth margin but spinous posterior field. **Focus:** antero-central. **Circuli:** distinct and continuous in the anterior and lateral fields, distinct and discontinuous in the posterior field. **Radii:** absent. **Spines:** several large, thorn-shaped spines are scattered within the entire posterior field. The length of the longest spine on scale C is 21% of the total scale length.

*Nezumia sclerorhynchus* (Valenciennes, 1838)

Family MACROURIDAE

APP. 1, FIG. 15

**Type:** cycloid: spinoid. **Shape:** circular to intermediate: cordate to calyx. Shape variability: low. **Anterior field:** convex with smooth margin. The antero-lateral corners are extended and pointed. **Lateral fields:** convex and extended in dorso-ventral axis. **Posterior field:** generally rounded end with smooth margin but spinous posterior field. **Focus:** central. **Circuli:** distinct and continuous in the anterior and lateral fields, indistinct in the posterior field. **Radii:** absent. **Spines:** numerous small, leaf-shaped spines are scattered within the entire posterior field. The length of the longest spine on scale C is 13% of the total scale length.

*Trachyrincus scabrus* (Rafinesque, 1810)

Family MACROURIDAE

APP. 1, FIG. 16

**Type:** cycloid: spinoid. **Shape:** circular: true circular to discoidal. Shape variability: low. **Anterior field:** convex or flattened with smooth but irregular margin. **Lateral fields:** convex and extended in dorso-ventral axis, except in area H, where these fields are rather elongated in antero-posterior axis. **Posterior field:** rounded end with smooth margin but spinous posterior field. **Focus:** central. **Circuli:** distinct and continuous in the anterior and lateral fields, distinct and discontinuous to indistinct in the posterior field. **Radii:** absent. **Spines:** few (2-5) robust, stout-shaped spines are scattered within the posterior field. The length of the longest spine on scale C is 44% of the total scale length.

*Mora moro* (Risso, 1810)

Family MORIDAE

APP. 1, FIG. 17

**Type:** cycloid: true cycloid. **Shape:** oval: ovoid to oblong. Shape variability: moderate. **Anterior field:** strongly convex with prominent apex and smooth margin. The antero-lateral corners are slightly pointed. **Lateral fields:** flattened and elongated in antero-posterior axis. **Posterior field:** mostly rounded, except in areas A, B and J, where the end of this field is

rather pointed; in area H, it is concave. The posterior margin is smooth. **Focus:** postero-central. **Circuli:** distinct and continuous. **Radii:** absent.

***Merluccius merluccius*** (Linnaeus, 1758)

Family MERLUCCIIDAE

APP. 1, FIG. 18

**Type:** cycloid: true cycloid. **Shape:** oval: ovoid to oblong. Shape variability: low. **Anterior field:** strongly convex with rounded apex and smooth margin. **Lateral fields:** flattened to convex and elongated in antero-posterior axis. **Posterior field:** rounded end with smooth margin. **Focus:** central. **Circuli:** distinct and continuous. **Radii:** absent.

***Molva molva*** (Linnaeus, 1758)

Family GADIDAE

APP. 1, FIG. 19-20

**Type:** cycloid: true cycloid. **Shape:** oval: oblong. Shape variability: uniform. **Anterior field:** convex with smooth margin. **Lateral fields:** flattened or concave and elongated in antero-posterior axis. These fields are slightly narrowing towards the posterior field. **Posterior field:** rounded end with smooth margin. **Focus:** central. **Circuli:** distinct and discontinuous. Circuli are densely ornamented by elevated, brick-like structures on the scale surface. **Radii:** absent.

***Trisopterus luscus*** (Linnaeus, 1758)

Family GADIDAE

APP. 1, FIG. 21

**Type:** cycloid: true cycloid. **Shape:** oval: ovoid to irregular. Shape variability: moderate. **Anterior field:** convex with prominent apex and smooth to striate margin. **Lateral fields:** flattened to inclined or convex and generally elongated in antero-posterior axis. **Posterior field:** rounded end with smooth margin. **Focus:** antero-central. **Circuli:** distinct and discontinuous. **Radii:** numerous primary, secondary and tertiary radii are present in radial orientation in all four fields.

## Order MUGILIFORMES

Scales of the two species from the herein investigated family Mugilidae show a high level of morphological similarity. Therefore, only one description is given for the two species. Scale images are provided for both species in APPENDIX 1.

*Chelon labrosus* (Risso, 1827) APP. 1, FIG. 22

Family MUGILIDAE

*Liza aurata* (Risso, 1810) APP. 1, FIG. 23

**Type:** ctenoid: whole cteni. **Shape:** intermediate to quadrilateral: calyx to square or rectangular. Shape variability: moderate. **Anterior field:** flattened with striate margin. **Lateral fields:** flattened to convex, and in general, equally extended in both axes. **Posterior field:** rounded end with ctenous margin. Within this field a distinct vertical cleavage often occurs in both species. **Focus:** postero-central. **Circuli:** distinct and discontinuous in the anterior field, continuous in the lateral fields, indistinct in the posterior field. **Radii:** primary and secondary radii are present in the anterior field in parallel to radial orientation. **Cteni:** whole cteni.

## Order ATHERINIFORMES

*Atherina hepsetus* Linnaeus, 1758

Family ATHERINIDAE

APP. 1, FIG. 24

**Type:** cycloid: true cycloid. **Shape:** polygonal: octagonal. Shape variability: uniform. **Anterior field:** flattened with pointed apex and smooth margin. The antero-lateral corners are extended and strongly pointed. **Lateral fields:** strongly concave. **Posterior field:** rounded end with smooth margin. **Focus:** central. **Circuli:** distinct and continuous in the anterior field, indistinct in the lateral and posterior fields. **Radii:** absent.

*Atherinomorus lacunosus* (Forster, 1801)

Family ATHERINIDAE

APP. 1, FIG. 25

**Type:** cycloid: true cycloid. **Shape:** circular: true circular to cordate or discoidal. Shape variability: moderate. **Anterior field:** flattened to convex with smooth margin. The antero-lateral corners are extended and rounded. **Lateral fields:** strongly convex and extended in the dorso-ventral axis. Scales are pierced by a varying number of small hollows that are arranged in line in the middle of the scale in dorso-ventral axis. **Posterior field:** rounded to tapered end

with smooth margin. **Focus**: central. **Circuli**: distinct and continuous in the anterior and lateral fields, indistinct in the posterior field. **Radii**: absent.

## Order BELONIFORMES

*Belone belone* (Linnaeus, 1761)

Family BELONIDAE

APP. 1, FIG. 26

**Type**: cycloid: true cycloid. **Shape**: circular to oval: true circular or discoidal to elliptical. Shape variability: high. **Anterior field**: flattened to slightly convex with smooth margin. **Lateral fields**: convex and extended in dorso-ventral axis. This extension is extreme in areas E, F, and G. **Posterior field**: rounded end with smooth margin. **Focus**: central. **Circuli**: generally distinct and continuous; indistinct in the posterior field in areas A, B, C, and D; and indistinct in the lateral fields in areas E, F, G, and H. **Radii**: absent.

## Order BERYCIFORMES

*Beryx decadactylus* Cuvier, 1829

Family BERYCIDAE

APP. 1, FIG. 27

**Type**: cycloid: spinoid. **Shape**: polygonal: pentagonal to hexagonal. Shape variability: low. **Anterior field**: flattened to convex with waved margin. The antero-lateral corners are extended and pointed. **Lateral fields**: flattened to concave. **Posterior field**: tapered to pointed end with spinous margin. **Focus**: central. **Circuli**: distinct and continuous in the anterior and lateral fields, indistinct in the posterior field. **Radii**: absent. **Spines**: teeth-like spines emerge from the posterior margin and several tiny spines are scattered within the posterior field.

*Hoplostethus mediterraneus* Cuvier, 1829

Family TRACHICHTHYIDAE

APP. 1, FIG. 28

**Type**: cycloid: spinoid. **Shape**: circular to polygonal: true circular to discoidal or pentagonal. Shape variability: high. **Anterior field**: flattened or slightly convex with smooth margin. **Lateral fields**: flattened to convex and generally extended in dorso-ventral axis. **Posterior field**: rounded end with spinous margin. **Focus**: central. **Circuli**: distinct and continuous in the anterior and lateral fields, indistinct in the posterior field. **Radii**: absent. **Spines**: numerous, tiny spines are present marginally as well as scattered throughout the posterior field. In areas A and I, smaller scales have long spines that are both marginal and scattered.

## Order ZEIFORMES

*Zeus faber* Linnaeus, 1758

Family ZEIDAE

APP. 1, FIG. 29

**Type:** cycloid: true cycloid. **Shape:** circular to oval: true circular to ovoid. Shape variability: low. **Anterior field:** slightly convex with smooth margin. This field is less extended in dorso-ventral axis than the posterior field. **Lateral fields:** convex or inclined, widening towards the posterior field. **Posterior field:** rounded end with smooth margin. **Focus:** central. **Circuli:** distinct and continuous. **Radii:** absent.

## Order GASTEROSTEIFORMES

*Macrorhamphosus scolopax* (Linnaeus, 1758)

Family MACRORAMPHOSIDAE

APP. 1, FIG. 30

**Type:** cycloid: spinoid. **Shape:** circular to oval: cordate to reversed ovoid. Shape variability: moderate. **Anterior field:** convex with smooth margin. A distinct diamond- or triangular-shaped structure emerging from the scale surface characterizes this field. **Lateral fields:** convex and narrowing towards the posterior field. **Posterior field:** strongly pointed end with spinous margin. **Focus:** antero-central. **Circuli:** indistinct. **Radii:** absent. **Spines:** long spines originate from the anterior field, forming longitudinal ridges that run across the entire scale surface and terminate as marginal spines.

## Order SCORPAENIFORMES

*Helicolenus dactylopterus* (Delaroche, 1809)

Family SEBASTIDAE

APP. 1, FIG. 31

**Type:** ctenoid: transforming cteni. **Shape:** intermediate to oval: calyx to true oval. Shape variability: moderate. **Anterior field:** flattened with scalloped margin. **Lateral fields:** flattened to slightly convex and elongated in antero-posterior axis. **Posterior field:** rounded end with ctenous margin. **Focus:** posterior. **Circuli:** distinct; discontinuous in the anterior field, continuous in lateral fields, discontinuous in the posterior field. **Radii:** primary and secondary radii are present in the anterior field in radial orientation. **Cteni:** transforming cteni.

*Scorpaena porcus* Linnaeus, 1758

Family SCORPAENIDAE

APP. 1, FIG. 32

**Type:** cycloid: spinoid. **Shape:** oval: true oval. Shape variability: low. **Anterior field:** slightly convex with scalloped margin. **Lateral fields:** flattened and elongated in antero-posterior axis. **Posterior field:** rounded end with spinous margin. **Focus:** postero-central. **Circuli:** distinct and discontinuous in the anterior field, continuous in the lateral fields, indistinct in the posterior field. **Radii:** primary and secondary radii are present in the anterior field in radial orientation. **Spines:** long, root-like spines originate within the posterior field, overstretching the entire field, and end in tiny spines on the posterior margin.

*Eutrigla gurnardus* (Linnaeus, 1758)

Family TRIGLIDAE

APP. 1, FIG. 33

**Type:** cycloid: true cycloid in areas I, J; and ctenoid: transforming cteni in areas A, B, C, D, E, F, G, H. **Shape:** oval to quadrilateral: ovoid to square or trapezoidal. Shape variability: moderate. **Anterior field:** flattened to convex with fluted margin. **Lateral fields:** flattened to convex and elongated in antero-posterior axis. Slightly bulging towards the posterior field. **Posterior field:** flattened to rounded end with smooth or ctenous margin. **Focus:** postero-central. **Circuli:** distinct; discontinuous in the anterior field, continuous in the lateral fields, discontinuous in the posterior field. **Radii:** primary and secondary radii are present in the anterior field in radial orientation. **Cteni:** transforming cteni.

*Lepidotrigla cavillone* (Lacepède, 1801)

Family TRIGLIDAE

APP. 1, FIG. 34

**Type:** cycloid: spinoid. **Shape:** quadrilateral: square or rectangular to rhomboidal. Shape variability: moderate. **Anterior field:** flattened or inclined outline with fluted margin. **Lateral fields:** flattened to convex or inclined and extended in dorso-ventral axis. **Posterior field:** flattened end with spinous margin. **Focus:** postero-central. **Circuli:** distinct and discontinuous in the anterior field, continuous in the lateral fields, indistinct in the posterior field. **Radii:** primary and secondary radii are present in the anterior field in radial orientation. **Spines:** thorn-like marginal spines are present, restricted to the posterior margin.

## Order PERCIFORMES

*Dicentrarchus labrax* (Linnaeus, 1758)

Family MORONIDAE

APP. 1, FIG. 35

**Type:** ctenoid: transforming cteni. **Shape:** quadrilateral to polygonal: square or rectangular to pentagonal. Shape variability: moderate. **Anterior field:** flattened with striate to slightly scalloped margin. The antero-lateral corners are slightly pointed. **Lateral fields:** flattened and elongated in antero-posterior axis. **Posterior field:** rounded to tapered end with ctenous margin. **Focus:** postero-central. **Circuli:** distinct; discontinuous in the anterior field, continuous in the lateral fields, discontinuous in the posterior field. **Radii:** primary and secondary radii are present in the anterior field in radial orientation. **Cteni:** transforming cteni.

*Anthias anthias* (Linnaeus, 1758)

Family SERRANIDAE

APP. 1, FIG. 36

**Type:** ctenoid: peripheral cteni. **Shape:** circular to intermediate: discoidal to calyx or shell-shaped. Shape variability: moderate. **Anterior field:** flattened to slightly convex with waved to scalloped margin. **Lateral fields:** vary from flattened to convex or concave. In general, these fields extend in dorso-ventral axis. **Posterior field:** rounded to tapered end with ctenous margin. **Focus:** posterior. **Circuli:** distinct and discontinuous in the anterior field, continuous in the lateral fields, distinct to indistinct in the posterior field. **Radii:** primary, secondary and tertiary radii are present in the anterior field in radial orientation. **Cteni:** peripheral cteni.

*Epinephelus aeneus* (Geoffroy St. Hillaire, 1817)

Family SERRANIDAE

APP. 1, FIG. 37

**Type:** cycloid: true cycloid in areas A, B, C, D, H, I, J; and ctenoid: transforming cteni in areas E, F, G. **Shape:** oval to quadrilateral: true oval to rectangular. Shape variability: low. **Anterior field:** flattened with scalloped margin. **Lateral fields:** flattened to slightly convex and elongated in antero-posterior axis. **Posterior field:** rounded end with smooth or ctenous margin. **Focus:** central. **Circuli:** distinct; discontinuous in the anterior field, continuous in the lateral fields, discontinuous in the posterior field. **Radii:** primary, secondary and tertiary radii are present in the anterior field in radial orientation. **Cteni:** transforming cteni.

*Serranus cabrilla* (Linnaeus, 1758)

Family SERRANIDAE

APP. 1, FIG. 38

**Type:** ctenoid: transforming cteni. **Shape:** intermediate to polygonal: calyx to pentagonal. Shape variability: moderate. **Anterior field:** flattened with scalloped margin. **Lateral fields:** flattened, and in general, equally extended in both axes. **Posterior field:** rounded to tapered end with ctenous margin. **Focus:** postero-central. **Circuli:** distinct; discontinuous in the anterior field, continuous to discontinuous in the lateral fields, discontinuous in the posterior field. **Radii:** primary and secondary radii are present in the anterior and lateral fields in radial orientation. **Cteni:** transforming cteni.

*Serranus scriba* (Linnaeus, 1758)

Family SERRANIDAE

APP. 1, FIG. 39

**Type:** ctenoid: transforming cteni. **Shape:** quadrilateral: square to rectangle. Shape variability: moderate. **Anterior field:** flattened with wavy margin. **Lateral fields:** flattened and elongated in antero-posterior axis. **Posterior field:** rounded end with ctenous margin. **Focus:** postero-central. **Circuli:** distinct; discontinuous in the anterior field, continuous in the lateral fields, discontinuous in the posterior field. **Radii:** primary and secondary radii are present in the anterior field in radial orientation. **Cteni:** transforming cteni.

*Apogon imberbis* (Linnaeus, 1758)

Family APOGONIDAE

APP. 1, FIG. 40

**Type:** ctenoid: transforming cteni. **Shape:** circular to intermediate: true circular, cordate, discoidal to shell-shaped. Shape variability: high. **Anterior field:** slightly convex with scalloped margin. **Lateral fields:** convex and extended in dorso-ventral axis. **Posterior field:** rounded to slightly tapered end with ctenous margin. **Focus:** posterior. **Circuli:** distinct and discontinuous in the anterior and lateral fields, indistinct in the posterior field. **Radii:** primary and secondary radii are present in the anterior and lateral fields in radial orientation. **Cteni:** transforming cteni.

*Epigonus denticulatus* Dieuzeide, 1950

Family EPIGONIDAE

APP. 1, FIG. 41

**Type:** cycloid: true-cycloid. **Shape:** circular to quadrilateral: cordate or discoidal to

trapezoidal. Shape variability: moderate. **Anterior field**: flattened with smooth to waved margin. It is extended in the antero-lateral sections. **Lateral fields**: convex and extended in dorso-ventral axis, narrowing towards the posterior field. **Posterior field**: rounded to tapered end with smooth and membranous margin. **Focus**: indistinct. **Circuli**: indistinct. **Radii**: absent.

*Coryphaena equiselis* Linnaeus, 1758

Family CORYPHAENIDAE

APP. 1, FIG. 42

**Type**: cycloid: true cycloid. **Shape**: oval to quadrilateral: ovoid to rectangular. Shape variability: moderate. In area E, special thorn-like shaped scales are present. **Anterior field**: highly variable: convex to strongly pointed or flattened to concave. Smooth margin. **Lateral fields**: flattened to inclined and elongated in the antero-posterior axis. **Posterior field**: flattened to tapered end with smooth or irregular margin. **Focus**: central. **Circuli**: distinct and continuous. **Radii**: absent.

*Seriola dumerili* (Risso, 1810)

Family CARANGIDAE

APP. 1, FIG. 43

**Type**: cycloid: true cycloid. **Shape**: circular to oval: true circular to true oval. Shape variability: moderate. **Anterior field**: convex with scalloped margin. **Lateral fields**: convex, and in general, equally extended in both axes. **Posterior field**: rounded end with smooth margin. **Focus**: central. **Circuli**: distinct; discontinuous in the anterior field, continuous to discontinuous in the lateral fields, continuous in the posterior field. **Radii**: primary, secondary and tertiary radii are present in the anterior and lateral fields in radial orientation.

*Trachurus trachurus* (Linnaeus, 1758)

Family CARANGIDAE

APP. 1, FIG. 44

**Type**: cycloid: true cycloid. **Shape**: circular to intermediate: true circular or discoidal to calyx. Shape variability: high. **Anterior field**: generally flattened with waved to scalloped margin. **Lateral fields**: convex and extended in dorso-ventral axis. **Posterior field**: rounded end with smooth margin. **Focus**: central. **Circuli**: distinct; discontinuous in the anterior field, continuous in the lateral field, continuous to indistinct in the posterior field. **Radii**: primary, secondary and tertiary radii are present in the anterior field in radial orientation.

*Brama brama* (Bonnaterre, 1788)

Family BRAMIDAE

APP. 1, FIG. 45

**Type:** cycloid: spinoid. **Shape:** polygonal: pentagonal to hexagonal. Shape variability: moderate. **Anterior field:** flattened or convex with pointed apex and smooth margin. The antero-lateral corners are extended in dorso-ventral axis with a strongly pointed end resulting a bizarre thorn-like appearance of this field. **Lateral fields:** flattened to concave and extended in dorso-ventral axis. **Posterior field:** tapered end with more or less expressed spinous margin (see also discussion). **Focus:** antero-central. **Circuli:** distinct and continuous in the anterior and lateral fields, indistinct in the posterior field. **Radii:** absent. **Spines:** indistinct.

Family SPARIDAE

Scales from eight species of the family Sparidae were examined. All show a high level of similarity in their morphological characteristics. Therefore, a general description is provided of their scale characteristics including further notes on species specific features. Scale images are displayed in the APPENDIX for the following species:

*Boops boops* (Linnaeus, 1758) APP. 1, FIG. 46

*Diplodus annularis* (Linnaeus, 1758) APP. 1, FIG. 47

*Diplodus vulgaris* (Linnaeus, 1758) APP. 1, FIG. 48

*Pagellus acarne* (Risso, 1827) APP. 1, FIG. 49

*Pagellus erythrinus* (Linnaeus, 1758) APP. 1, FIG. 50

*Sarpa salpa* (Linnaeus, 1758) APP. 1, FIG. 51

*Sparus aurata* Linnaeus, 1758 APP. 1, FIG. 52

*Spondyliosoma cantharus* (Linnaeus, 1758) APP. 1, FIG. 53

#### Scale characteristics of the family Sparidae

**Type:** ctenoid: transforming cteni. **Shape:** polygonal to intermediate: pentagonal to calyx or shell-shaped. Shape variability: moderate. **Anterior field:** flattened with generally waved margin. The margin varies among species: waved in *D. annularis*, *D. vulgaris*, *P. erythrinus*, and *S. aurata*; waved to scalloped in *S. salpa*; deeply waved to dentate in *B. boops* and *P. acarne*; irregularly waved in *S. cantharus*. **Lateral fields:** convex to concave, often bulge or extend in dorso-ventral axis. The position and extent of bulging varies between species; bulging in the antero-lateral section and being concave in the mid-lateral section in *B. boops*;

bulging in the mid-lateral section in *D. annularis* and *D. vulgaris*, in the latter these fields are largely extended in dorso-ventral axis; bulging in the antero-lateral section and being concave in the postero-lateral section in *P. acarne*; bulging in the antero-lateral section without a major concavity in the postero-lateral section in *P. erythrinus*; bulging at mid-lateral section; bulging in the mid-lateral section or slightly in the antero-lateral section in *S. aurata*; bulging in the antero-lateral section with slight concavity in the postero-lateral section in *S. cantharus*. **Posterior field**: rounded to tapered end with ctenous margin. **Focus**: central in *B. boops*, *P. acarne*, *P. erythrinus*, *S. salpa*, *S. aurata*, *S. cantharus*; postero-central in *D. annularis* and *D. vulgaris*. **Circuli**: distinct and discontinuous in the anterior field, continuous to discontinuous in the lateral fields, indistinct in the posterior field. **Radii**: primary, secondary and tertiary radii are present in radial orientation. Radii usually remain within the anterior field in *D. annularis*, *D. vulgaris*, *P. erythrinus*, *S. aurata*, and partially in *S. salpa*. Radii occur both in the anterior, as well as in the lateral fields in *B. boops*, *P. acarne*, and *S. cantharus*. **Cteni**: transforming cteni.

*Spicara maena* (Linnaeus, 1758)

Family CENTRACANTHIDAE

APP. 1, FIG. 54

**Type**: ctenoid: transforming cteni. **Shape**: polygonal to intermediate: pentagonal to shell-shaped. Shape variability: moderate. **Anterior field**: flattened with waved to scalloped margin. **Lateral fields**: flattened to slightly concave and extended towards the antero-lateral section. **Posterior field**: tapered to pointed end with ctenous margin. **Focus**: central. **Circuli**: distinct and discontinuous in the anterior field, continuous to discontinuous in the lateral fields, indistinct in the posterior field. **Radii**: primary, secondary and tertiary radii are present in the anterior and lateral fields in radial orientation. **Cteni**: transforming cteni.

*Spicara smaris* (Linnaeus, 1758)

Family CENTRACANTHIDAE

APP. 1, FIG. 55

**Type**: ctenoid: transforming cteni. **Shape**: polygonal: heptagonal. Shape variability: moderate. **Anterior field**: flattened with waved to dentate margin. **Lateral fields**: concave and extended towards the antero-lateral sections. **Posterior field**: tapered to pointed end with ctenous margin. **Focus**: central. **Circuli**: distinct and discontinuous in the anterior field, continuous to discontinuous in the lateral fields, indistinct in the posterior field. **Radii**: primary, secondary and tertiary radii are present in the anterior and lateral fields in radial

orientation. *Cteni*: transforming cteni.

*Sciaena umbra* Linnaeus, 1758

Family SCIAENIDAE

APP. 1, FIG. 56

**Type**: ctenoid: transforming cteni. **Shape**: quadrilateral to intermediate: square or rectangular to calyx. Shape variability: moderate. **Anterior field**: flattened with striate margin. **Lateral fields**: flattened to slightly convex, in general, elongated in the antero-posterior axis. **Posterior field**: rounded end with ctenous margin. **Focus**: postero-central. **Circuli**: distinct; discontinuous in the anterior field, continuous in the lateral fields, continuous to discontinuous in the posterior field. **Radii**: primary, secondary and tertiary radii are present in the anterior field in parallel orientation. **Cteni**: transforming cteni.

Family MULLIDAE

Two species of the family Mullidae were investigated, i.e., *M. barbatus* and *M. surmuletus*. The scales of the two species were found to be similar in their characteristic features, thus one description is given that applies to both species. Scale images are displayed for both species.

*Mullus barbatus* Linnaeus, 1758 APP. 1, FIG. 57

*Mullus surmuletus* Linnaeus, 1758 APP. 1, FIG. 58

**Type**: ctenoid: transforming cteni. **Shape**: intermediate to oval: calyx to true oval. Shape variability: low. **Anterior field**: flattened with dentate margin. **Lateral fields**: convex and elongated in the antero-posterior axis. **Posterior field**: rounded end with ctenous margin. **Focus**: postero-central in *M. barbatus* and posterior in *M. surmuletus*. **Circuli**: distinct and discontinuous in the anterior field, continuous lateral fields, indistinct in the posterior field. **Radii**: primary and secondary radii are present in the anterior field in radial orientation. In addition, few irregular grooves can occur in the posterior field in longitudinal orientation. **Cteni**: transforming cteni.

*Chaetodon hoefleri* Steindachner, 1881

Family CHAETODONTIDAE

APP. 1, FIG. 59

**Type**: ctenoid: transforming cteni. **Shape**: circular to oval or intermediate: true circular to

ovoid or calyx. Shape variability: moderate. **Anterior field:** flattened with scalloped margin. **Lateral fields:** convex and extended in dorso-ventral axis. **Posterior field:** rounded end with ctenous margin. **Focus:** postero-central. **Circuli:** distinct and discontinuous in the anterior field, continuous to discontinuous in the lateral fields, discontinuous in the posterior field. **Radii:** primary, secondary and tertiary radii are present in the anterior and lateral fields in radial orientation. **Cteni:** transforming cteni.

*Cepola macrophthalmia* (Linnaeus, 1758)

Family CEPOLIDAE

APP. 1, FIG. 60

**Type:** cycloid: true cycloid. **Shape:** oval: ovoid. Shape variability: low. **Anterior field:** convex with scalloped margin. **Lateral fields:** flattened and elongated in antero-posterior axis. **Posterior field:** rounded end with smooth margin. **Focus:** postero-central. **Circuli:** distinct; discontinuous in the anterior and posterior fields, continuous to discontinuous in the lateral fields. **Radii:** primary, secondary and tertiary radii are present in the anterior, and partially in the lateral fields, in radial to parallel orientation.

*Chromis chromis* (Linnaeus, 1758)

Family POMACENTRIDAE

APP. 1, FIG. 61

**Type:** ctenoid: transforming cteni. **Shape:** oval or quadrilateral to intermediate: true oval or rectangular to calyx. Shape variability: moderate. **Anterior field:** flattened with scalloped margin. **Lateral fields:** differ among sampling areas: in A, B, C, D, H, and J they are rather flattened and elongated in antero-posterior axis; in E, F, G, and I they are convex and extended in dorso-ventral axis. **Posterior field:** rounded end with ctenous margin. **Focus:** postero-central. **Circuli:** distinct and discontinuous in the anterior field, continuous in the lateral fields, indistinct in the posterior field. **Radii:** primary, secondary and tertiary radii are present in the anterior field in parallel to radial orientation. **Cteni:** transforming cteni.

Family LABRIDAE

Some species of the family Labridae, analysed in this study, share a common characteristic feature, i.e., the membranous posterior field with longitudinal striae. Scales of the first three species (i.e., *Coris julis*, *Labrus viridis*, *Symphodus rostratus*) are remarkably similar, while the scale of *Thalassoma pavo* show higher similarity to the scales of *Sparisoma cretense*.

*Coris julis* (Linnaeus, 1758)

Family LABRIDAE

APP. 1, FIG. 62

**Type:** cycloid: true cycloid. **Shape:** oval to polygonal: true oval to pentagonal. Shape variability: moderate. **Anterior field:** convex with scalloped margin. **Lateral fields:** flattened and elongated in antero-posterior axis. **Posterior field:** pointed end with membranous margin. **Focus:** central. **Circuli:** distinct and discontinuous. Within the posterior field, circuli join and create distinct longitudinal striae. **Radii:** primary, secondary and tertiary radii are present in the anterior and lateral fields in radial orientation.

*Labrus viridis* Linnaeus, 1758

Family LABRIDAE

APP. 1, FIG. 63

**Type:** cycloid: true cycloid. **Shape:** oval to polygonal: true oval to pentagonal. Shape variability: moderate. **Anterior field:** flattened to slightly convex with scalloped margin. **Lateral fields:** flattened and moderately elongated in antero-posterior axis. **Posterior field:** pointed end with membranous margin and distinct longitudinal striae. **Focus:** central. **Circuli:** generally distinct and discontinuous; continuous only in a limited part of the lateral fields. Within the posterior field, circuli join and create distinct longitudinal striae. **Radii:** primary, secondary and tertiary radii are present in the anterior and lateral fields in radial orientation.

*Symphodus rostratus* (Bloch, 1791)

Family LABRIDAE

APP. 1, FIG. 64

**Type:** cycloid: true cycloid. **Shape:** oval to circular: true oval to true circular. Shape variability: moderate. **Anterior field:** flattened to slightly convex with striate to scalloped margin. **Lateral fields:** flattened to convex and moderately elongated in antero-posterior axis. **Posterior field:** rounded to tapered end with membranous margin. **Focus:** central. Instead of the presence of a clear focus, an expanded focal area is visible. Occasional grooves may be present within this area. No inner mosaic pattern, as in *Thalassoma pavo* (see below). **Circuli:** distinct and discontinuous in the anterior field, continuous to discontinuous in the lateral fields, discontinuous in the posterior field. Within the posterior field, circuli join and create distinct longitudinal striae. **Radii:** primary, secondary and tertiary radii are present in the anterior and lateral fields in radial orientation.

*Thalassoma pavo* (Linnaeus, 1758)

Family LABRIDAE

APP. 1, FIG. 65

**Type:** cycloid: true cycloid. **Shape:** oval to polygonal: true oval or oblong to hexagonal. Shape variability: moderate. **Anterior field:** flattened with conical apex and striate margin. The antero-lateral corners are slightly extended and pointed. **Lateral fields:** flattened and elongated in antero-posterior axis. **Posterior field:** rounded to tapered end with membranous margin. **Focus:** central. No clear focus point is visible. An expanded focal area is present, which is well-separated in the centre of the scale, with distinct mosaic pattern. **Circuli:** distinct and discontinuous in the anterior and lateral fields, indistinct in the posterior field. **Radii:** primary, secondary and tertiary radii are present in all four fields in radial orientation. In addition, numerous, short, vertical grooves can occur in all four fields, with predominant occurrence within the focal area; these grooves are in contact with radii.

*Sparisoma cretense* (Linnaeus, 1758)

Family SCARIDAE

APP. 1, FIG. 66

**Type:** cycloid: true cycloid. **Shape:** circular to oval: cordate to true oval. Shape variability: moderate. **Anterior field:** flattened with prominent conical apex and smooth to striate margin. The antero-lateral corners are extended and pointed. **Lateral fields:** flattened to convex, and in general, they are equally extended in both axes. **Posterior field:** rounded end with smooth margin. **Focus:** central. An expanded focal area with mosaic pattern rarely occurs (i.e., in sampling area D). **Circuli:** distinct and discontinuous in the anterior field, continuous to discontinuous in the lateral fields, indistinct in the posterior field. **Radii:** primary, secondary and tertiary radii are present in radial orientation, especially in the anterior and posterior fields. Only few radii are present in the lateral fields. In addition, few, short, vertical grooves can occur in all four fields; these grooves are in contact with radii.

*Gymnammodytes cicereus* (Raffinesque, 1810)

Family AMMODYTIDAE

APP. 1, FIG. 67-68

In *G. cicereus*, scales were available only from sampling areas D, G, H, and J. Therefore, the description refers to scales from the given areas only. **Type:** cycloid: true cycloid. **Shape:** circular: true circular to discoidal. Shape variability: moderate. **Anterior field:** flattened to convex with smooth to waved margin. **Lateral fields:** convex and slightly extended in dorso-ventral axis. **Posterior field:** rounded end with smooth margin. **Focus:** central. **Circuli:**

distinct and discontinuous. Elevated circuli are separated by longitudinal depressions (i.e., unmineralized sections). **Radii**: irregular wide grooves are present in all four fields in somewhat radial orientation.

*Trachinus draco* Linnaeus, 1758

Family TRACHINIDAE

APP. 1, FIG. 69

**Type**: cycloid: true cycloid. **Shape**: quadrilateral: square to rhomboidal. Shape variability: moderate. **Anterior field**: flattened and slightly inclined with striate margin. **Lateral fields**: flattened or inclined and moderately elongated in antero-posterior axis. **Posterior field**: rounded to tapered end with membranous margin. **Focus**: postero-central. **Circuli**: distinct; discontinuous in the anterior and posterior fields, continuous in the lateral fields. **Radii**: primary and secondary radii are present in the anterior field in parallel to radial orientation.

*Tripterygion tripteronotum* (Risso, 1810)

Family TRIPTERYGIIDAE

APP. 1, FIG. 70

**Type**: ctenoid: peripheral cteni. **Shape**: circular: true circular to discoidal. Shape variability: moderate. **Anterior field**: flattened to convex with scalloped margin. **Lateral fields**: convex and extended in dorso-ventral axis. **Posterior field**: flattened to rounded end with ctenous margin. **Focus**: postero-central. **Circuli**: distinct and discontinuous in the anterior and lateral fields, indistinct in the posterior field. **Radii**: primary and secondary radii are present in the anterior and lateral fields in parallel to radial orientation. **Cteni**: peripheral cteni.

*Gobius bucchichi* Steindachner, 1870

Family GOBIIDAE

APP. 1, FIG. 71

**Type**: ctenoid: peripheral cteni. **Shape**: circular to intermediate: true circular or discoidal to calyx. Shape variability: low. **Anterior field**: flattened to convex with striate to scalloped margin. The antero-lateral corners are extended and slightly pointed. **Lateral fields**: flattened to convex and moderately extended in dorso-ventral axis with a slightly bulging postero-lateral section. **Posterior field**: rounded end with ctenous margin. **Focus**: posterior. **Circuli**: distinct and discontinuous; continuous only in the postero-lateral section. **Radii**: primary, secondary and tertiary radii are present in the anterior and lateral fields in parallel to radial orientation. Radii often extend to the posterior field. **Cteni**: peripheral cteni.

*Gobius paganellus* Linnaeus, 1758

Family GOBIIDAE

APP. 1, FIG. 72

**Type:** ctenoid: peripheral cteni. **Shape:** circular to oval: true circular to true oval. Shape variability: low. **Anterior field:** convex with moderately scalloped margin. The antero-lateral corners are extended and slightly pointed. **Lateral fields:** flattened to convex and moderately elongated in antero-posterior axis. **Posterior field:** rounded end with ctenous margin. **Focus:** posterior. **Circuli:** distinct and discontinuous; continuous only in the postero-lateral section. **Radii:** primary and secondary radii are present in the anterior and lateral fields in parallel to radial orientation. Radii often extend to the posterior field. **Cteni:** peripheral cteni.

*Sphyraena chrysotaenia* Kluzinger, 1884

Family SPHYRAENIDAE

APP. 1, FIG. 73

**Type:** cycloid: true cycloid. **Shape:** oval to quadrilateral or intermediate: true oval to square or calyx. Shape variability: moderate. **Anterior field:** flattened with striate margin. **Lateral fields:** flattened to slightly convex and moderately elongated in antero-posterior axis. **Posterior field:** rounded end with membranous margin. **Focus:** central. **Circuli:** distinct and discontinuous in the anterior field, continuous in the lateral fields, indistinct in the posterior field. **Radii:** primary and secondary radii are present in the anterior field in radial orientation.

*Sphyraena sphyraena* (Linnaeus, 1758)

Family SPHYRAENIDAE

APP. 1, FIG. 74-75

**Type:** cycloid: spinoid. **Shape:** circular: true circular to discoidal. Shape variability: low. **Anterior field:** convex with smooth to striate margin. **Lateral fields:** convex, and in general, extended in dorso-ventral axis, except in sampling areas A, B, and J, where these fields are rather elongated in the antero-posterior axis. **Posterior field:** rounded end with spinous margin. **Focus:** central. **Circuli:** distinct and discontinuous. Circuli are highly crested and elevated creating a network of branched ridges by longitudinal interconnection. Circuli are densely interrupted by spaces perforated by unmineralized square areas. These structures align among circuli and give a loosely similar appearance to radii. **Radii:** absent. **Spines:** small number (1-4) of tiny marginal spines.

*Scomber colias* Gmelin, 1789

Family SCOMBRIDAE

APP. 1, FIG. 76

**Type:** cycloid: crenate. **Shape:** quadrilateral: square to rectangular or rhomboidal. Shape variability: high. **Anterior field:** flattened with smooth margin. **Lateral fields:** flattened or inclined and in general, extended in dorso-ventral axis, except in areas A, B, I, and J, where they are rather elongated in antero-posterior axis. **Posterior field:** rounded end with crenulated margin. **Focus:** central. **Circuli:** distinct and continuous, however, frequently become indistinct in the posterior field. **Radii:** absent.

*Tetragonurus cuvieri* Risso, 1810

Family TETRAGONURIDAE

APP. 1, FIG. 77

**Type:** cycloid: spinoid. **Shape:** quadrilateral: square to rhomboidal. Shape variability: low. **Anterior field:** flattened with smooth margin. **Lateral fields:** flattened to inclined or convex, and in general, they are slightly extended in dorso-ventral axis. **Posterior field:** rounded end with spinous margin. **Focus:** antero-central. **Circuli:** distinct and continuous in the anterior and lateral fields, indistinct in the posterior field. **Radii:** absent. **Spines:** long spines originate from the anterior field, forming longitudinal, parallel ridges that run across the entire posterior field and terminate as marginal spines.

*Capros aper* (Linnaeus, 1758)

Family CAPROIDAE

APP. 1, FIG. 78

**Type:** cycloid: spinoid. **Shape:** circular to polygonal: true circular, cordate or discoidal to pentagonal. Shape variability: high. **Anterior field:** generally convex with smooth margin. **Lateral fields:** flattened to inclined or convex, and in general, extended in the dorso-ventral axis. **Posterior field:** rounded end with smooth margin but spinous posterior field. **Focus:** postero-central. **Circuli:** distinct and continuous in the anterior and lateral fields, discontinuous to indistinct in the posterior field. **Radii:** absent. **Spines:** numerous, long and thin spines are scattered within the entire posterior field; they overreach the posterior margin. The length of the longest spine on scale C is 19% of the total scale length.

## Order PLEURONECTIFORMES

*Scophthalmus maximus* (Linnaeus, 1758)

Family PSETTODIDAE

APP. 1, FIG. 79

Typical scales are not present in adult *S. maximus*. Skin appears rather smooth with some “stony structures”. However, in early stage of development, cycloid scales can be distinguished on the ocular-side (left side) of the species.

**Type:** cycloid: true cycloid. **Shape:** oval: true oval to ovoid. Shape variability: uniform. **Anterior field:** convex with moderately scalloped margin. **Lateral fields:** slightly convex and elongated in antero-posterior axis. **Posterior field:** rounded end with smooth margin. **Focus:** central. **Circuli:** distinct and discontinuous in the anterior and posterior fields, continuous in the lateral fields. **Radii:** primary, secondary and tertiary radii occur in the anterior field in radial orientation.

*Bothus podas* (Delaroche, 1809)

Family BOTHIDAE

APP. 1, FIG. 80-81

**Type:** ctenoid: transforming cteni on ocular-side (left side); and cycloid: true cycloid on blind-side (right side). **Shape:** circular to oval: true circular to ovoid. Shape variability: low. **Anterior field:** convex with striated margin. **Lateral fields:** flattened to convex and moderately elongated in antero-posterior axis. **Posterior field:** rounded end with ctenous margin on ocular-side scales and smooth margin on blind-side scales. **Focus:** postero-central. **Circuli:** distinct and discontinuous. **Radii:** primary, secondary and tertiary radii occur in the anterior and lateral fields in radial orientation. Radii often reach the posterior field. **Cteni:** transforming cteni on the ocular-side; absent on the blind-side.

*Microchirus variegatus* (Donovan, 1808)

Family SOLEIDAE

APP. 1, FIG. 82-83

**Type:** ctenoid: transforming cteni on both sides. **Shape:** polygonal: hepta- to octagonal. Shape variability: low. **Anterior field:** flattened with scalloped margin. **Lateral fields:** flattened to concave and elongated in antero-posterior axis. The concavity of these fields is shifted slightly towards the posterior field. **Posterior field:** tapered end with ctenous margin. **Focus:** postero-central. **Circuli:** distinct; discontinuous in the anterior and posterior fields, continuous in the lateral fields. **Radii:** primary and secondary radii occur in the anterior field

in radial orientation. *Cteni*: transforming cteni on both sides.

*Monochirus hispidus* Rafinesque, 1814

Family SOLEIDAE

APP. 1, FIG. 84-85

*Type*: ctenoid: transforming cteni on both sides. *Shape*: polygonal: hepta- to octagonal. Shape variability: low. *Anterior field*: flattened with waved margin. *Lateral fields*: flattened to concave and elongated in antero-posterior axis. The concavity of these fields is shifted slightly towards the posterior field. *Posterior field*: tapered end with ctenous margin. *Focus*: postero-central. *Circuli*: distinct; discontinuous in the anterior and posterior fields, continuous in the lateral fields. *Radii*: primary and secondary radii occur in the anterior field in radial orientation. *Cteni*: transforming cteni on both sides.

*Solea solea* (Linnaeus, 1758)

Family SOLEIDAE

APP. 1, FIG. 86-87

*Type*: ctenoid: transforming cteni on both sides. *Shape*: quadrilateral: rectangular. Shape variability: uniform. *Anterior field*: flattened to concave with waved to scalloped margin. *Lateral fields*: flattened and elongated in the antero-posterior axis. *Posterior field*: rounded end with ctenous margin. *Focus*: postero-central. *Circuli*: distinct; discontinuous in the anterior and posterior fields, continuous in the lateral fields. *Radii*: primary and secondary radii occur in the anterior field in radial orientation. *Cteni*: transforming cteni on both sides.

*Symphurus nigrescens* Rafinesque, 1810

Family CYNOGLOSSIDAE

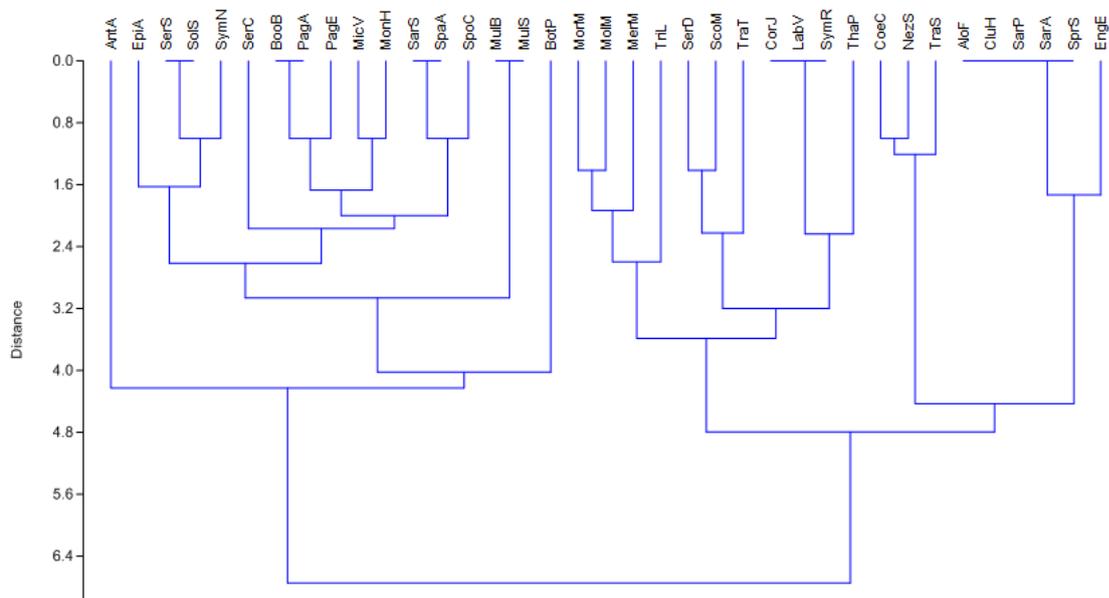
APP. 1, FIG. 88-89

*Type*: ctenoid: transforming cteni on both sides. *Shape*: quadrilateral to oval: rectangular to true oval. Shape variability: low. *Anterior field*: flattened with scalloped margin. *Lateral fields*: flattened to slightly convex and elongated in antero-posterior axis. *Posterior field*: rounded end with ctenous margin. *Focus*: postero-central. *Circuli*: distinct; discontinuous in the anterior and posterior fields, continuous in the lateral fields. *Radii*: primary and secondary radii occur in the anterior fields in radial orientation. Radii often extend to the lateral fields. *Cteni*: transforming cteni on both sides.

#### 4.1.2. Phenotypic similarities of scale characteristics among four orders of teleosts

The postulated Question 3 (“How can scale morphology be transformed into numerical data that facilitates investigation of phenotypic relations among different types of teleost scales?”) was approached by coding characteristic features of the scale morphology (TABLE 3). The coding of the scale characteristics of 37 species (TABLE 4) resulted in a data matrix (APP. 2, TABLE 1) that was utilized for further analysis as described under 3.2.1.

In the depicted dendrogram below, the scale characteristics of the 37 species cluster into two major groups. In the first cluster, scale characteristics were found to be similar among the majority of studied species that belong to the series Percomorpha of the superorder Acanthopterygii (i.e., Perciformes and Pleurinctiformes; FIG. 13). In the second cluster, similarities were found among basal teleosts, i.e., Clupeiformes and some representatives of the superorder Paracanthopterygii (i.e., Gadiformes). The scale characters of Macrouridae show stronger similarity with Clupeidae than with other representatives of Gadiformes. The remaining three families of Gadiformes (i.e., Moridae, Merlucciidae, Gadidae) exhibit similar scale characteristics and were grouped into the same subgroup (sub-cluster) as the studied species of Labridae and Scaridae, together with Carangidae and the only representative of Psettodidae.



**FIGURE 13. Dendrogram based on the between-groups-linkage method showing the phenotypic relations among the scale characteristics of the studied species**

## 4.2. Scale morphometry

### 4.2.1. Scale size and shape variability within two clupeid species

The description of the scale morphology of five clupeid species (i.e., *A. fallax*, *C. harengus*, *S. pilchardus*, *S. aurita*, *S. sprattus*) as well as the phenotypic relations among their scales (FIG. 13) showed that morphology alone cannot provide a reliable discrimination among these species. Therefore, an additional question was addressed: “Does scale morphometry allow for the differentiation among the morphologically similar scales of clupeid species?” Before approaching this question, however, morphometric parameters using relative size indices (i.e., J-indices) were calculated to determine the sampling area(s) along the fish body that vary least in their J-indices. Scales from these sampling areas are most suitable for comparative studies.

The J-indices in the two investigated clupeid species (*S. pilchardus* and *S. aurita*) demonstrated a high degree of variability. The relative scale size indices varied between species, among body areas, as well as within each area (TABLE 7 and 8).

In both species, Jsl was the least variable J-index among and within the sampling areas. In *S. pilchardus*, the variation of Jsl was the lowest ( $SD < 0.40$ ) within the sampling areas D and H, followed by areas E, G, F, and C. Higher variation of Jsl ( $SD > 0.64$ ) were found within areas J, B, I, and A (in increasing order). On the contrary, in *S. aurita*, the variation of Jsl was the lowest ( $SD < 0.10$ ) in sampling area A, followed by areas G, C, D, and B. Higher variation of Jsl ( $SD > 0.18$ ) were found within areas I, H, J, F, and E. The average variation of Jsl was smaller in *S. aurita* ( $SD = 0.5$ ) compared to *S. pilchardus* ( $SD = 0.8$ ). In *S. pilchardus*, Jsw showed lower variation ( $SD < 0.60$ ) in areas F, H, B, D, and C, followed by increasing values in areas A, E, I, G, and J. In *S. aurita*, the values of the same index were the lowest ( $SD < 0.30$ ) in areas G, A, C, and D, followed by increasing values in areas H, B, J, F, I, and E. In *S. pilchardus*, the lowest values of Jst variation ( $SD < 0.40$ ) were found, similar to Jsl, within the sampling areas D and H, followed by areas F, E, B, and A. Higher variation of Jst ( $SD > 0.65$ ) were found within areas C, I, and J. In *S. aurita*, the sampling areas with the lowest variation of Jst similarly overlap with areas where the minimum Jsl variation were found, i.e., areas A and G. Sampling areas with lower values of Jst variation ( $SD < 0.30$ ) are the areas H, C, B, and D. Higher values ( $SD > 0.30$ ) were found in areas J, I, E, and F. In both species, sampling areas C and D (i.e., below dorsal fin and above lateral line) showed lower variation in all J-indices, therefore scale sampling is recommended within this region for comparative studies.

In addition to the relative size indices, both clupeid species possess a wide repertoire of scale shapes across the entire fish body and within the sampling areas (APP. 3, TABLE 1-2). The general shape of the investigated clupeoid (i.e., cycloid: crenate) scales varied among the sampling areas from circular (i.e., true circular, cordate, discoidal) to oval (i.e., true oval, reversed ovoid). In some cases (mostly in area E), the intermediate scale shape, the irregular form was also observed. In general, *S. pilchardus* has slightly rounded circular scale shapes, whereas the scales of *S. aurita* are rather quadratic-circular in shape.

**TABLE 7. Relative size indices of the scales in *S. pilchardus***

Area	N	Minimum – Maximum			Mean ± SD		
		Jsl	Jsw	Jst	Jsl	Jsw	Jst
A	21	4.28-6.76	4.74-6.79	4.89-7.13	5.49 ± 0.79	5.88 ± 0.65	6.09 ± 0.64
B	27	4.59-6.68	4.53-6.31	4.94-6.85	5.28 ± 0.72	5.46 ± 0.48	5.75 ± 0.52
C	17	4.33-6.43	4.56-6.39	4.67-7.22	5.07 ± 0.64	5.67 ± 0.59	5.75 ± 0.66
D	21	5.41-6.52	4.77-7.10	5.30-6.63	5.79 ± 0.34	5.82 ± 0.54	6.04 ± 0.37
E	16	4.38-6.32	5.10-7.65	5.58-7.58	5.46 ± 0.50	6.57 ± 0.71	6.51 ± 0.51
F	20	5.03-6.62	5.99-6.81	5.79-7.44	5.62 ± 0.59	6.31 ± 0.22	6.49 ± 0.47
G	27	3.66-6.94	1.92-6.54	3.16-7.02	5.59 ± 0.59	5.56 ± 0.84	5.92 ± 0.64
H	28	3.69-5.01	3.13-5.65	3.46-5.04	4.15 ± 0.35	4.37 ± 0.47	4.29 ± 0.39
I	16	4.34-6.51	2.49-5.47	3.87-6.11	5.33 ± 0.78	4.45 ± 0.75	5.02 ± 0.76
J	26	3.49-5.65	2.33-5.43	3.20-6.05	4.45 ± 0.65	3.90 ± 0.86	4.45 ± 0.83
Total	219	3.49-6.94	1.92-7.65	3.16-7.58	5.18 ± 0.80	5.32 ± 1.04	5.56 ± 0.97

Note: J-indices are given in mm

**TABLE 8. Relative size indices of the scales in *S. aurita***

Area	N	Minimum – Maximum			Mean ± SD		
		Jsl	Jsw	Jst	Jsl	Jsw	Jst
A	30	2.59-2.93	2.61-3.61	2.56-3.12	2.77 ± 0.10	3.10 ± 0.24	2.82 ± 0.15
B	30	2.42-3.23	2.29-4.00	2.52-3.67	2.75 ± 0.16	3.03 ± 0.35	2.90 ± 0.27
C	30	2.77-3.28	3.19-4.01	2.82-3.73	2.96 ± 0.12	3.60 ± 0.25	3.30 ± 0.24
D	25	2.80-3.38	3.15-4.08	2.95-3.99	3.01 ± 0.13	3.65 ± 0.26	3.47 ± 0.28
E	24	2.59-4.10	3.60-6.28	3.72-5.91	3.63 ± 0.51	4.75 ± 0.87	4.76 ± 0.57
F	26	2.88-3.99	3.26-5.51	3.16-5.25	3.58 ± 0.33	3.86 ± 0.47	4.00 ± 0.57
G	26	2.84-3.24	3.07-4.05	3.10-3.76	2.99 ± 0.11	3.50 ± 0.24	3.39 ± 0.17
H	25	1.48-2.80	1.35-2.93	1.83-2.76	2.31 ± 0.31	2.22 ± 0.35	2.28 ± 0.22
I	26	3.30-4.00	2.31-4.07	2.98-5.21	3.79 ± 0.20	3.30 ± 0.50	3.77 ± 0.50
J	26	2.52-3.93	1.95-3.35	2.52-4.26	3.10 ± 0.32	2.65 ± 0.37	3.07 ± 0.33
Total	268	1.48-4.10	1.35-6.28	1.83-5.91	3.08 ± 0.50	3.36 ± 0.76	3.35 ± 0.73

Note: J-indices are given in mm

The general pattern of scale shape changes according to the anatomical location on the fish body. Scales derived from the central region of the fish body (i.e., sampling areas C, D, E, F, and G) were extended in the dorso-ventral axis, whereas scales from the upper or lower regions (areas A, B, I, and J) were rather elongated in the antero-posterior axis. This elongation was found to be most prominent in the ventral areas (I and J). Caudal peduncle scales (in area H) were characterized by being relatively shorter in the antero-posterior axis resulting a circular appearance to the scales.

#### 4.2.2. Scale shape analysis in separating two clupeid species

To investigate whether scale morphometry allows for the differentiation of the morphologically similar scales of clupeid species (Q4), multivariate statistical analyses were applied. The usefulness of scale shape indices to separate the two sympatric clupeids from each other was tested. The effect of fish size on scale shape was eliminated successfully. The size correction greatly reduced the correlation between scale shape and fish lengths ( $r^2 < 0.001$ ). Results of the discriminant analysis between *S. pilchardus* and *S. aurita* are given in TABLE 9. The overall percentage of correct classification obtained was 76%. The comparisons of shape indices using a PERMANOVA test yielded significant differences among sampling areas in both species;  $F = 15.84$ ,  $P = 0.0002$  in *S. pilchardus*, and  $F = 26.55$ ,  $P = 0.0002$  in *S. aurita*. Shape indices, however, did not show significant differences between areas A-B, A-C, B-D, B-G, D-G, and I-J in *S. pilchardus*; and between C-G, D-G, D-E, and I-J in *S. aurita* (TABLE 10). The two-way PERMANOVA test performed on combined shape indices yielded significant differences among the sampling areas of both species ( $F = 35.16$ ,  $P = 0.0002$ ) and between the two species ( $F = 17.70$ ,  $P = 0.0002$ ).

TABLE 9. Classification results of the discriminant analysis between *S. pilchardus* and *S. aurita*

	Species	Predicted Group Membership		
		<i>S. pilchardus</i>	<i>S. aurita</i>	Total
Original	<i>S. pilchardus</i>	163 (74%)	56 (26%)	219 (100%)
	<i>S. aurita</i>	56 (21%)	212 (79%)	268 (100%)
Cross-validated	<i>S. pilchardus</i>	162 (74%)	57 (26%)	219 (100%)
	<i>S. aurita</i>	59 (22%)	209 (78%)	268 (100%)

**TABLE 10. *P*-values of the PERMANOVA test showing differences among sampling areas in *S. pilchardus* and *S. aurita***

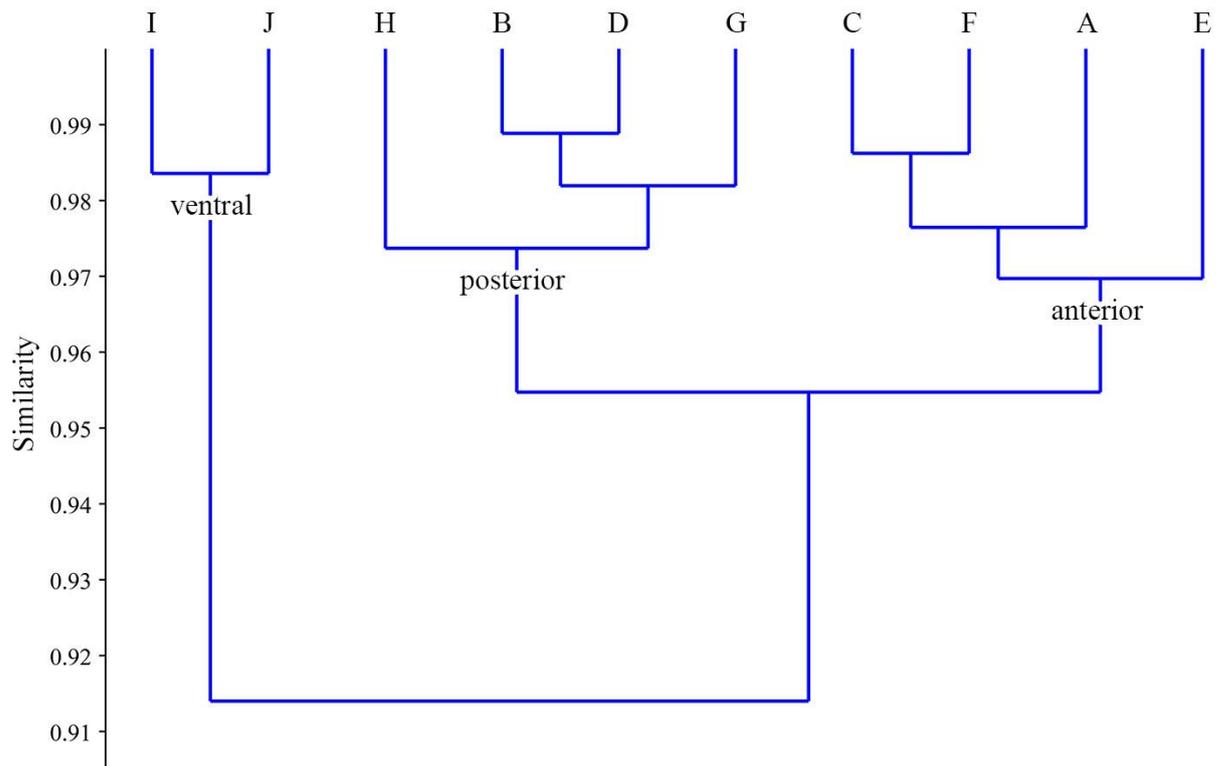
<i>S. pilchardus</i> <i>S. aurita</i>	A	B	C	D	E	F	G	H	I	J
A	-	0.2782	0.0914	0.0404*	0.0078*	0.033*	0.036*	0.0012*	0.0002*	0.0002*
B	0.0482*	-	0.0154*	0.355	0.0002*	0.002*	0.1824	0.0018*	0.0002*	0.0002*
C	0.0002*	0.0002*	-	0.0002*	0.043*	0.024*	0.0002*	0.0002*	0.0002*	0.0002*
D	0.0002*	0.0002*	0.0324*	-	0.0002*	0.0002*	0.461	0.0006*	0.0002*	0.0002*
E	0.0002*	0.0002*	0.0044*	0.055	-	0.0282*	0.0002*	0.0002*	0.0002*	0.0002*
F	0.0002*	0.0038*	0.0002*	0.0006*	0.004*	-	0.0002*	0.0002*	0.0002*	0.0002*
G	0.0002*	0.0004*	0.1224	0.1304	0.0142*	0.0048*	-	0.0058*	0.0026*	0.001*
H	0.0002*	0.0002*	0.0002*	0.0002*	0.0002*	0.0002*	0.0002*	-	0.0004*	0.0002*
I	0.0002*	0.0002*	0.0002*	0.0002*	0.0002*	0.0002*	0.0002*	0.0008*	-	0.4146
J	0.0002*	0.0002*	0.0002*	0.0002*	0.0002*	0.0002*	0.0002*	0.0056*	0.278	-

\*:  $P < 0.05$

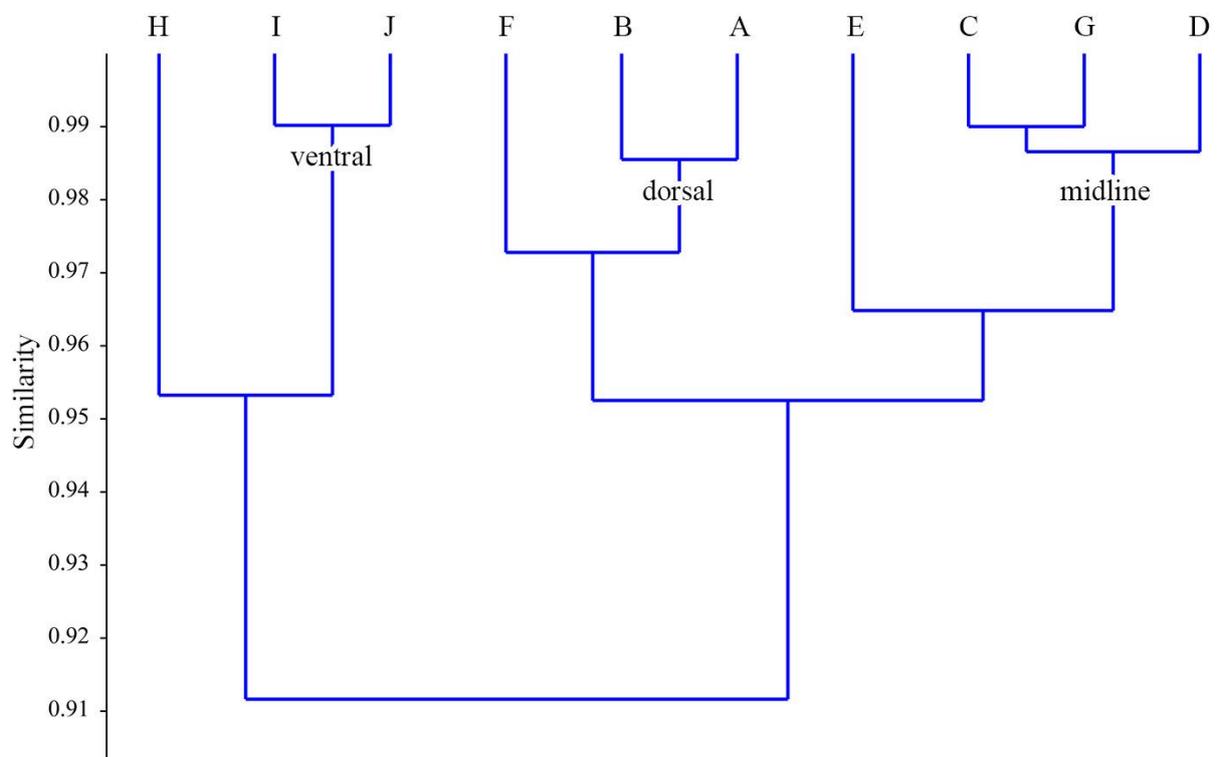
The cluster analysis outputs depicting the linkage dendrogram (Bray-Curtis' similarity) computed on scale shape indices among the sampling areas in both species are demonstrated in FIGURE 14-15.

In *S. pilchardus*, similarities in shape index values were found between sampling areas I and J (i.e., ventral part of the fish), which strongly segregated from other areas. Two other clusters of sampling areas with similarities in scale shape can be distinguished, i.e., areas E, A, F, and C (the anterior part of the fish), and areas G, D, B, and H (the posterior part of the fish). Sampling areas with similar scale shape values were found to segregate along the antero-posterior axis. The highest similarities were found between the neighbouring areas B and D (i.e., posterior, dorsal, caudal to dorsal fin and posterior, dorsal, above lateral line), as well as between C and F (i.e., anterior, dorsal, above lateral line and anterior, ventral, below lateral line (see APP. 3, FIG. 1-2).

In *S. aurita*, on the other hand, the segregation of the sampling areas according to their scale shape similarities occurs along the dorso-ventral axis (i.e., dorsal, midline, and ventral). Sampling areas with similar scale shape values were found in the ventral part of the fish (i.e., I and J), in the dorsal part in areas A and B, and in the midline in C, G, and D. Although areas H, F, and E grouped into the previous subgroups (separately), their scale shape values differ slightly from the ones found in the areas of the same cluster.



**FIGURE 14.** Linkage dendrogram showing the similarities of the scale shape indices among sampling areas in *S. pilchardus*

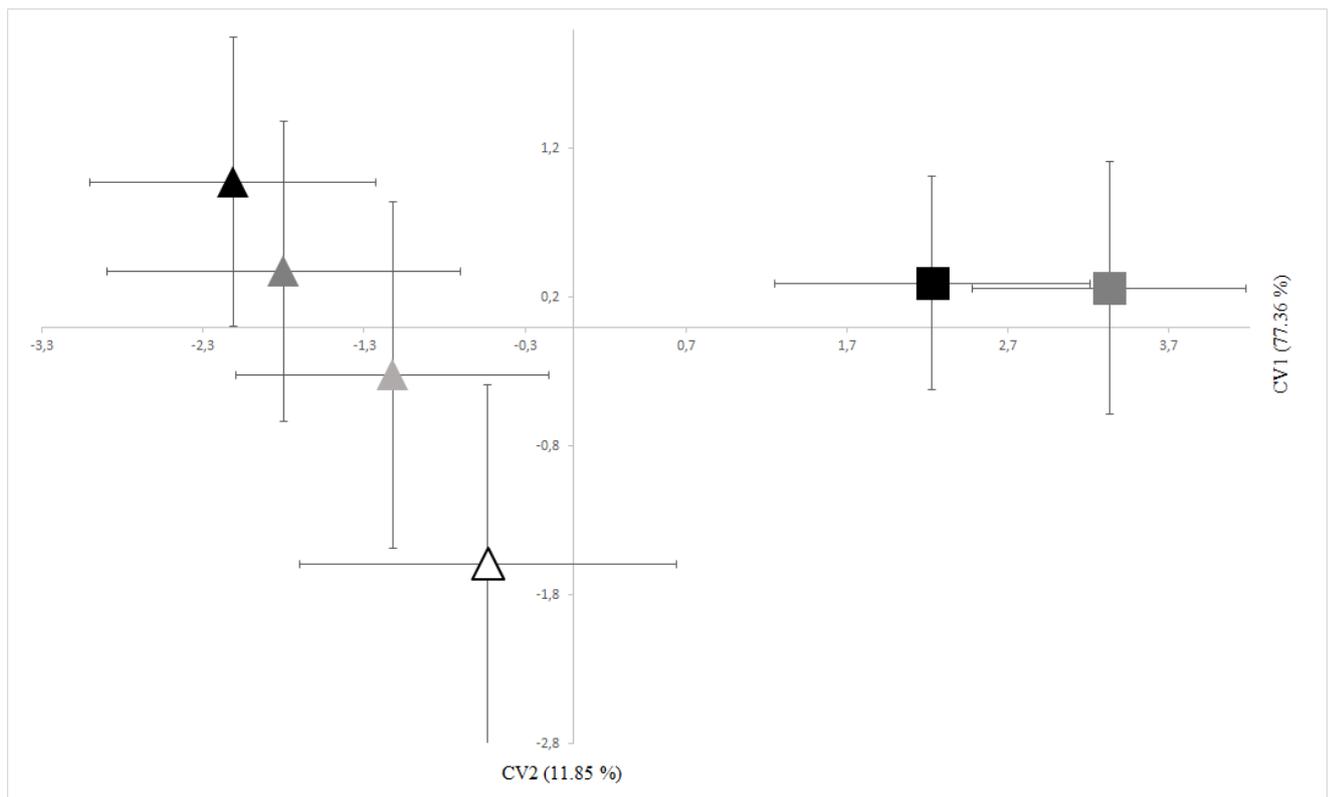


**FIGURE 15.** Linkage dendrogram showing the similarities of the scale shape indices among sampling areas in *S. aurita*

### 4.3. Scale geometric morphometry

A landmark-based geometric morphometric analysis was used to determine, whether the differences in clupeid scale shape are sufficient to enable a reliable discrimination between *S. pilchardus* and *S. aurita* as well as among their local populations.

The regression of scale shape (using Procrustes coordinates) on scale centroid size indicated notable allometry (i.e., dependence of shape on size) in all cases. The predicted percentage of the total variation in scale shape accounted for the allometric effect which was 0.87 % ( $P = 0.019$ ). This value is considered to be relatively low compared to other scale shape analyses (e.g., STASZNY *et al.*, 2013). Therefore, the use of regression residuals was justified. Scales of the two clupeid species separated significantly from each other by the DFA ( $P < 0.0001$ ) based on the scale shape. Furthermore, the mean scale shape of the two species showed no overlap in a CVA (FIG. 16).



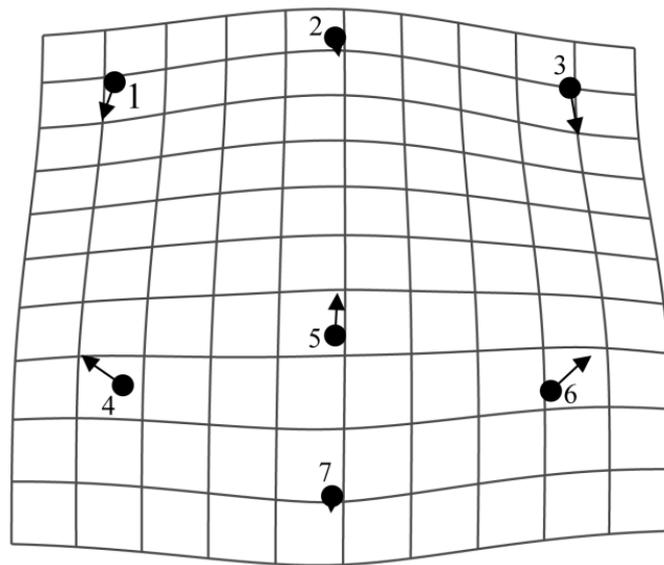
**FIGURE 16. Canonical variate analysis results comparing scale shapes of *Sardina pilchardus* and *Sardinella aurita* from different sampling sites.** Note: symbols show the group means, crosshairs show the standard deviations. Triangles indicate *S. pilchardus* populations and squares indicate *S. aurita* populations, with colour coding as follows: black for ADR; dark grey for ION; light grey for AMV; white for KAV (see FIG. 5)

The validity of the results was also confirmed by the large proportion of correct classifications with an average discrimination rate as high as 98.6 %. The two populations of *S. aurita* could also be distinguished from each other with high reliability ( $P < 0.0001$ ) and an average discrimination rate of 91 % (TABLE 11). Their mean scale shapes segregated mainly along the CV1 axis (FIG. 16). The scale shape data discriminate among local populations of *S. pilchardus* ( $P < 0.0001$ ) for all population comparisons, except for the comparisons of the northern Adriatic Sea population vs. the Inner Ionian Sea Archipelago population and the Gulf of Ambracia population vs. the Inner Ionian Sea Archipelago population ( $P = 0.001$ ). The validity of these comparisons was confirmed by high proportions of correct classifications (TABLE 11). The mean scale shape of *S. pilchardus* populations segregated on both axes in the CVA and showed no overlap. The results further indicated that the scale shape of the local population of *S. pilchardus* from the Gulf of Kavala (i.e., eastern Mediterranean Sea) is easily distinguishable from the mean scale shapes of those conspecifics sampled in the central Mediterranean Sea.

**TABLE 11. Classification results of discriminant analysis of scale shape for the two clupeid species and their local populations**

Species	Study sites	ADR	<i>S. pilchardus</i>		<i>S. aurita</i>	
			AMV	ION	KAV	AMV
<i>S. pilchardus</i>	AMV	78 %				
	ION	70 %	73 %			
	KAV	91 %	82 %	87 %		
<i>S. aurita</i>	AMV	99 %	99 %	100 %	99 %	
	KAV	98 %	97 %	98 %	99 %	91 %

Major differences in scale shape were found to exist between the two clupeids (FIG. 17). Scales of *S. aurita* have larger area with the greatest extension in the postero-lateral corners of the scale (landmark no. 4 and 6). The scales of *S. pilchardus*, on the other hand, possess a prominent apical elevation in the centre of the anterior edge (landmark no. 2), as well as in the antero-lateral corners (landmark no. 1 and 3). The latter two landmarks tend to be positioned further posteriorly to landmark no. 2 in the scales of *S. aurita*.



**FIGURE 17. Scale shape differences between *Sardina pilchardus* and *Sardinella aurita***

Note: dots represent the given position of landmarks in *S. pilchardus*, while arrows and the length of the angled lines indicate the relative deviation of landmarks of *S. aurita* from *S. pilchardus*

## 5. DISCUSSION

### 5.1. General scale morphology

#### 5.1.1. The most relevant scale characteristics that aid species identification among common Mediterranean teleosts

##### *Classification of scale types*

There is considerable variation in the literature concerning scale types (ROBERTS, 1993; KHEMIRI *et al.*, 2001), shapes and related characters (PATTERSON *et al.*, 2002; JAWAD, 2005; GHOLAMI *et al.*, 2013), and several authors feel that grouping all scales with a spiny posterior margin under the term “ctenoid” is an oversimplification (e.g., ROBERTS, 1993; KHEMIRI *et al.*, 2001). This study attempts to an improved classification of scale types. Regarding characters with an evolutionary background, instead of a purely phenological way, there is a major difference between scales made of a single bone and scales with additional ossifications (SIRE *et al.*, 2009). In contrast to other authors, this study regards all crenate and spinoid scales as sub-types of cycloid scales, and considers as ctenoid scales only those which possess separate ossifications from the main scale body. In the light of evolution, ctenoid scales likely developed only twice: in the characid family Distichodontidae and somewhere at the base or within the percomorphs (ROBERTS, 1993). Spined scales, on the other hand, certainly developed several times, as did crenate scales within the teleost lineage as variations of cycloid scales. The diversity of scale types within percomorphs, for instance, provides a good example for the changes in ontogenetic paths that result in the retention of plesiomorphic terminal character states (ROBERTS, 1993). The predominant scale type in percomorphs is the transforming ctenoid scale. However, the occurrence of cycloid, crenate, and spinoid scales within the superorder (APP. 1, TABLE 1), can be explained by “the ontogenetic modifications as well as the evolution of new characters that have probably occurred widely and independently during percomorph scale evolution” (ROBERTS, 1993).

Next to the easily definable scale types (cycloid and ctenoid), some taxa developed special types of scales making a clear designation more difficult. Clupeiform fishes form such a taxon. The characteristic morphology of their scales previously gave rise to the term “clupeoid scales” distinct from cycloid and ctenoid (TIMMS, 1905). Clupeoid scales are rather thin, relatively large, more or less circular with numerous grooves and irregular “crack” marks, and possess a crenulated posterior margin. In this study, the observed grooves in the anterior and lateral fields of crenate (clupeoid) scales were termed “transverse grooves” and

“longitudinal or irregular grooves” within the posterior field according to the orientation of the groove (see APP. 1, FIG. 2 – FIG. 7). A broad range of names can be found in the literature for the same features, e.g., “transverse grooves” by BORODIN (1924), “transverse striae (= radii)” and “longitudinal striae” by ROBERTS (1993), “fracture lines” by PATTERSON *et al.* (2002). Other unusual cycloid scales are equipped with a membranous flap on the posterior field. This feature can be seen in *Trachinus draco*, Trachinidae (APP. 1, FIG. 69), *Sphyraena chrysotaenia*, Sphyraenidae (APP. 1, FIG. 73) and most prominent in the families Labridae and Scaridae (APP. 1, FIG. 62-66). Similar membranous flaps are also known from *Albula vulpes*, *Megalops atlanticus*, *Dussumieria acuta* and *Chirocentrus dorab* (ROBERTS, 1993). According to their radii on the posterior field, Labridae and Scaridae have rather unique scales. Nevertheless, in this study, clupeid scales or membranous scales were considered as variations of cycloid scales, and no further classifying terms were introduced.

Similarly to cycloid scales, within spinoid scales unique shapes and ornamentations can occur. The most prominent features were found in *Brama brama* (APP. 1, FIG. 45), *Macrorhamphosus scolopax* (APP. 1, FIG. 30) and *Tetragonurus cuvieri* (APP. 1, FIG. 77). The spines in the latter two form smooth continuous ridges terminating in an acute spine, along the entire posterior field in *T. cuvieri*, and across the whole scale in *M. scolopax*. The scales of *B. brama* and *M. scolopax* share another characteristic feature that is the presence of a spinous projection on the anterior field in dorso-ventral orientation. ROBERTS (1993) described for *B. brama* ridges along the posterior field ending in marginal acute spines. The specimen in this study, however, showed virtually no ridges and there were no spines present. So far, specimens of *B. brama* appear to have either cycloid or spinoid scales. These differing observations cannot be satisfactorily explained in the current study and require further investigation.

Ctenoid scales were divided into three sub-types based on ROBERTS (1993): peripheral, transforming and whole cteni. The latter calls for cteni growing in several rows without any transformation of the cteni in the older rows. Among the studied scales, this condition was found only in members of the family Mugilidae. It may represent a precursor of the transforming cteni type, but further clarification of this issue is required.

### ***Classification of scale shapes***

Scales have very diverse outlines with considerable intra- and interspecific variation. There is no obvious way of grouping these, and, therefore, there is no standard classification of scale shapes (e.g., PATTERSON *et al.*, 2002; JAWAD, 2005; MATONDO *et al.*, 2010; GANZON

*et al.*, 2012; GHOLAMI *et al.*, 2013). In this study, the scales were sorted by major geometric shape categories with several subcategories in the attempt to provide a generally usable scale shape classification system.

Although a clear classification of scale shapes appears to be difficult in many cases, this is one of the most important characters in taxon identification. In some cases, a first glance of the scale shape provides useful identification clues: e.g., *Atherina* species have a unique octogonal scale shape (APP. 1, FIG. 24).

### ***Scale ornamentations***

Some features of the observed scales do not fit into a simple classification scheme, especially the ones related to circuli. Instead of merging superficially similar structures, the observed ornamentations were described separately in the results section. In *Molva molva* (APP. 1, FIG. 20) elevated brick-like structures are present. Comparable structures were reported for other Gadidae species by PATTERSON *et al.* (2002) who referred to them as “intermarkings”. The authors described intermarkings as “perpendicular or irregular lines/structures between the circuli that can align between circuli to give a loosely similar appearance to radii”. In cases where surface ornamentations are arranged in an orderly pattern, it may become difficult to decide, whether “intermarkings” or the ornamentations of circuli are indeed present, or whether they rather constitute highly elevated circuli that are regularly divided by radii as in *Trisopterus luscus* (APP. 1, FIG. 21). The comparative study of gadiform scales by KHEMIRI *et al.* (2001) revealed that the scale surface ornamentation, represented by circuli, are variably developed but always present in this family. “Whenever mineralization occurs only at the level of the circuli, the space between two adjacent circuli remains unmineralized” (KHEMIRI *et al.*, 2001). In this case, circuli can be recognized clearly as individual concentric lines on the scale surface. However, when the mineralization happens also in the space between the circuli, the surface becomes ornamented with a network of branched ridges, as in *Sphyaena sphyraena* (APP. 1, FIG. 75). Similarly, in *Maurolicus muelleri* surface ornamentations occur in variable shape creating linear depressions (APP. 1, FIG. 11A) or uneven ridges (APP. 1, FIG. 11B) in radial orientation.

Radii are characterized by the absence of superficial mineralized layers (KHEMIRI *et al.*, 2001). As a result, they appear as even grooves often in radial orientation on the surface interrupting circuli. Radii are most common on the anterior field, but they can also be distributed over the entire scale surface, as in *T. luscus* and in *Bothus podas* (APP. 1, FIG. 21 and FIG. 80-81) where scales have highly elevated circuli that are densely carved by radii.

The same appears to be true for *Gymnammodytes cicerelus* (APP. 1, FIG. 67-68), but with considerably widened radii becoming more irregular in the posterior field.

### ***Scales from different body areas***

Even though this study focused on regular body scales, thus excluding lateral line scales, ventral scutes, scales on fin bases or cheeks etc., there is still notable variability within a single specimen. In few species, different types of scales are present on a single specimen, e.g., *Epinephelus aeneus* (APP. 1, FIG. 37) and *Eutrigla gurnardus* (APP. 1, FIG. 33) possess both cycloid and ctenoid scales. Only in few cases, such as in flatfishes, the scales from the sampled body areas A to J appear almost identical. For most species, scale size and shape varies significantly along the body. In general, scales found in sampling areas A, B, H, and most of the scales in I and J are smaller in size compared to scales from areas C, D, E, F, and G. Most of the time, scales found in areas E and F were the largest in size related to the standard length of the fish, whereas scales in area H were the smallest in size, and scales in area C and D appeared to be most consistent in size.

A general pattern of scale shape variation occurs along the fish body. Scales in areas A, B, I, and J are rather elongated along the antero-posterior axis, whereas they extend in dorso-ventral axis in areas C, D, F, and G. Scales in area H are either square-shaped, circular or slightly elongated, and they are more compressed than scales derived from other areas. In area E, directly behind the operculum, scale shape differs in most cases from the “typical” shape for a given species. The least variability in scale shape was again found in areas C or D (i.e., below the dorsal fin and above the lateral line). Therefore, for morphometric analyses, e.g., when comparing species or populations, scales from this region are likely to provide the most reliable results. In morphometric studies, scales from a region comparable or identical to sampling areas C or D are already commonly used (e.g., RICHARDS & ESTEVES, 1997; POULET *et al.*, 2005; IBÁÑEZ *et al.*, 2007; STASZNY *et al.*, 2012; GHOLAMI *et al.*, 2013). Shape, relative size and/or characteristics of scales may change during ontogeny (JACOT, 1920; SIRE, 1986; SIRE & ARNULF, 2000), which is why morphometric studies have to take into account also the developmental stage before comparing taxa or groups with each other.

Changes in scale shape along the antero-posterior and dorso-ventral axes of the body appear to be related to the curvature of the fish (IBÁÑEZ *et al.*, 2009). For instance, in the posterior region of the fish body (sampling area H), surface area decreases and curvature increases. Scales found in this area are therefore more compressed along the dorso-ventral axis (equivalent to an elongation along the antero-posterior axis), so that scale rows fit into

the smaller surface area. On the other hand, scales derived from the mid-section of the fish body (i.e., especially from sampling areas F and G – ventral areas below the lateral line) are expanded along their dorso-ventral axis. The curvature of the fish body and thus the pattern of variation in scale shape along the body may be related functionally to the swimming mode (IBÁÑEZ *et al.*, 2009). In general, the more primitive fishes tend to have hydrodynamic adaptations to create lift, whereas the more advanced forms appear to evolve static or passive means to achieve neutral buoyancy (NYBAKKEN, 2001). Clupeiforms, for instance, are representatives of basal actinopterygians and demonstrate a subcarangiform swimming mode, based on the extended classification scheme by LINDSEY (1978). Although there are notable differences in the phylogenetic relationships between clupeid species and *Mugil* species, the pattern of scale shape variation along the body is similar to the pattern found by IBÁÑEZ *et al.* (2009) in *Mugil* species and *Dicentrarchus labrax*. This observation further supports IBÁÑEZ's findings that the pattern of scale shape variation may depend on the swimming mode. Furthermore, the variety of ecological niches occupied by teleost species is reflected in the diversity of specialized adaptations of their body shape (SUMICH & MORRISSEY, 2004). Therefore, the scale shape variation along the fish body is more likely related to the functional adaptation to a habitat and/or swimming mode rather than mirroring the phylogenetic relationships of certain taxa.

#### ***Use of the scale atlas for species identification***

The results presented show an amazing variety of scale types, shapes and ornamentations. Morphological features of relevance are mostly the shape and the pattern on the surface of a scale. A single scale often bears sufficient information to assign it to a taxon, often at species level. The scales of the family Macrouridae (APP. 1, FIG. 14-16), for example, are easily recognizable, while at the same time, the number and shape of the spines differ among the species thus allowing a clear species differentiation. Even for additional macrourid species described by KHEMIRI *et al.* (2001), spine shape and pattern also appears to be species-specific. In other taxa, species identification may be much more difficult, e.g. in *Spicara* species (APP. 1, FIG. 54-55) or in gobies (APP. 1, FIG. 71-72). Especially of the latter group, only two of many species were studied here. In such cases, a simple identification by consulting this atlas does not appear feasible. If the respective information is available, however, morphometric analysis may still allow species identification (e.g., JAWAD, 2005). To provide a better orientation between closely related species, the relative scale sizes (J-indices) and the focal index (Fi) are represented in APPENDIX 1, TABLE 1, to aid species differentiation.

Although perhaps immensely useful, there are at least three notable shortcomings of the morphological descriptions: (1) the selection of usually the most common species, (2) the disregard of intra-specific scale variability, and (3) the lack of ontogenetic information of scale growth in the different species. A study taking all species and their ontogenetic stages into account, however, hardly appeared feasible and future investigations are necessary to supplement the information of this study. Nevertheless, the scale atlas presented herein will provide a helpful orientation for the identification of fish scales from the Mediterranean region.

### **5.1.2. Phylogenetic information inferred from scales or how scale morphology can be utilized in systematic studies of teleosts**

The potential of studying scales in an evolutionary setting becomes visible already by the species selection presented herein. The distribution of grooves on the scales of the clupeiform fishes, for example, appears to allow for some phylogenetic grouping. In some species, such as *Alosa fallax* (APP. 1, FIG. 3) and *Clupea harengus* (APP. 1, FIG. 4), grooves run quite regular in dorso-ventral orientation, and are usually restricted to the anterior and lateral fields. This pattern is best visible in the larger body scales. In *Sardina pilchardus* (APP. 1, FIG. 5), *Sardinella aurita* (APP. 1, FIG. 6) and *Sprattus sprattus* (APP. 1, FIG. 7), however, grooves are usually interrupted in the middle and – especially in the latter species – less regularly distributed. In *Engraulis encrasicolus* (APP. 1, FIG. 2), the grooves are more irregular, running radially, and transverse grooves are present in the posterior field as well. Whether these different types of groove groupings indicate the evolutionary history and phylogenetic relationships needs to be shown by future studies. Nonetheless, it appears likely that the type of grooves in clupeiform fishes bears valuable information about their phylogenetic relationships.

It may not be surprising that closely related species have similar scales (PATTERSON *et al.*, 2002; JAWAD, 2005; YOKOGAWA & WATANABE, 2011), but life history, habitat characteristics and other factors may have also a strong influence on the scale morphology of a fish species (JOHAL & DUA, 1994, 1995; DAPAR *et al.*, 2012; GANZON *et al.*, 2012; IBÁÑEZ *et al.*, 2012). Since the effect of the environment might be manifested in scale morphology, scales of species that share similar or identical living conditions may look alike, for instance scales of *Thalassoma pavo* (Labridae, APP. 1, FIG. 65) and *Sparisoma cretense* (Scaridae, APP. 1, FIG. 66). Scales of *T. pavo* resemble more the scales of *S. cretense* than those of other labrid

species investigated in this study (i.e., *Coris julis* FIG. 62, *Labrus viridis* FIG. 63, and *Symphodus rostratus* FIG. 64). Labrid and scarid scales are true cycloid with membranous posterior fields. In contrast to the above mentioned three labrid species, the scales of *T. pavo* and *S. cretense* have a rather smooth posterior margin, radii in all fields and web-like ornamentation of radii in the centre of the scale. Although belonging to different families, both species share similar ecological habitats. Both species are found mostly on rocky shores, whereas the other three labrid species prefer different habitats close to seagrass meadows and/or among mixtures of sandy and rocky ground (QUIGNARD & PRAS, 1986). Whether or not the similarity of scale shape or characteristic features and habitat are causally related, warrants further investigation.

The results of this study show that much information provided by teleost scales is not yet fully understood in its biological context. The incorporation of scale characteristics into a data matrix to investigate phenotypic relationships among teleost scales is promising. A clear segregation occurred in scale characters between basal teleost lineages and more advanced taxa (FIG. 13). However, analyses merely based on phenotypic relations unlikely can lead to conclusions in a phylogenetic context. The combination of scale morphology of adult specimens with the ontogeny of the representative characters might gain more information on the placement of several groups within the teleostean lineage.

Further comparative analyses might focus on specific fish groups whose position in the tree of actionpterygians is still under debate, for instance, the positioning of the orders Salmoniformes and Esociformes. Molecular studies (CAMPBELL *et al.*, 2013; RONDEAU *et al.*, 2014) repeatedly placed the pike-like fishes (Esociformes) as a sister group to the Salmoniformes. However, Salmoniformes are likely to be placed somewhere within the paraphyletic group of “basal euteleosts”. Multidisciplinary studies are definitely needed for both marine and freshwater taxa to gain a better understanding of the phylogenetic relationships of fishes, as well as the development of scale types and shapes.

## **5.2. Scale size and shape variation in two clupeid species: Can scale morphometry differentiate between morphologically similar scales?**

In contrast to general morphological descriptions, relative scale size (J-indices) and scale shape indices (Si), which to date have received little attention in the field of fish taxonomy, can be reliable tools to distinguish fish species. Furthermore, the variation of J-indices within and among body areas in the studied clupeids showed that scales derived from certain body

areas possess lower plasticity than others. Due to the lower variation of J-indices, scales derived from sampling areas C and D (below the dorsal fin and above the lateral line) serve a better purpose for comparative studies. This confirms the applicability of the “key sampling area” generally used in scale morphometric studies (e.g., IBÁÑEZ *et al.*, 2007; STASZNY *et al.*, 2012; GHOLAMI *et al.*, 2013). Variations in relative scale size have been successfully used for the discrimination between several species of the genus *Aphanius* (Cyprinodontidae) by ESMAELI *et al.* (2012) and GHOLAMI *et al.* (2013). Their studies already opined that J-indices are suitable for separation of some fish species of the same genus. However, variation of J-indices between closely related or sympatric species and populations did not result in high overall classification success.

On the basis of the morphometric shape analysis presented here, it is inferred that the scale shape of clupeids can provide a reliable discrimination between sympatric species. To identify sympatric species correctly via scale analysis is useful for fishery science as well as for other fields of ecology. In dietary studies of marine mammals, for instance, ecologists mostly use indirect methods to identify prey items (BOWEN & SINIFF, 1999), resulting in various severe limitations. Under certain circumstances, the identification of scales lost by prey represents a useful approach (BRÄGER *et al.*, in press). Although gross morphological analysis of fish scales allows for the identification of prey genera and even species, most of the time, reliable differentiation between closely related species can be problematic. Some clupeid genera, particularly *Sardina* and *Sardinops*, possess considerable similarity and plasticity in their scale shape and characteristics (PATTERSON *et al.*, 2002). The same applies to *Sardina* and *Sardinella* species. Scales of *S. pilchardus* and *S. aurita* show strong similarities in their morphology. Furthermore, when scales are derived from predation events on mixed-species fish schools (and of mixed body areas), correct identification is problematic (BRÄGER *et al.*, in press). In the present study, scales of *S. pilchardus* and *S. aurita* were successfully separated by discriminant analysis based on the standardized scale shape indices. Although there are some body areas that show higher similarities in their scale shape within the same species than others, the multivariate analysis on combined shape indices yielded significant differences among the body areas of each of the two species and between the two species as well. Therefore, the application of morphometric analysis using scale shape indices is likely to help overcome the limitations of morphological analysis, thus aid a more reliable identification of prey species.

### **5.3. Differentiation between two sympatric clupeid species and among their local populations using the landmark-based geometric morphometric approach**

The third part of this study shows that geometric morphometric analysis of scales provides a powerful tool to discriminate between *S. pilchardus* and *S. aurita*, as well as among their local populations. In contrast to comparative analyses of scales merely based on morphology or traditional morphometry, this method is capable of detecting differences in scale geometrics that are unattainable with the former approaches. The large number of examined scale samples that can be easily screened using geometric morphometric analysis further amplifies the reliability of the results. Therefore, the findings indicate a solution to other lepidological studies where a more reliable discrimination between clupeids and their populations is needed (e.g., PATTERSON *et al.*, 2002; DRAGO *et al.*, 2009). Furthermore, the ability to achieve differentiation between the two sympatric clupeid species as well as among their local populations via scale shape offers a rapid and cost-effective approach towards a better understanding of their population connectivity.

According to the results, the scale characteristics of local populations of *S. pilchardus* group together (i.e., as a species). Nonetheless, the mean values of scale shape from the four study sites can be readily differentiated from each other. Only the scales of *S. pilchardus* from the Adriatic Sea and adjacent waters (i.e., central Mediterranean Sea) show greater similarity in shape than the ones derived from the eastern Mediterranean Sea, and the segregation of the population from Gulf of Kavala based on scale shape is even more obvious. Populations of *S. pilchardus* in close geographic proximity were expected to show modest genetic differentiation (GONZALEZ & ZARDOYA, 2007). Earlier DNA surveys using allozyme, mitochondrial and microsatellite data, however, did not find any evidence for *S. pilchardus* populations from the Adriatic and the adjacent Ionian Sea being genetically different from each other (e.g., TINTI *et al.*, 2002; RUGGERI *et al.*, 2013). Their distributions may be rather restricted effectively by environmental factors such as changes in the sea surface temperature or productivity or by hydrographic boundaries (OLIVAR *et al.*, 2001; SOMARAKIS *et al.*, 2006).

It should be noted, however, that environmental factors can affect fish morphology and scale shape as well (IBÁÑEZ *et al.*, 2007). Therefore, it might be possible that slight morphological variation of scale shapes among neighbouring populations detected in this study may have been caused by hydrographical and ecological factors such as water depth and productivity. For example, the clupeids sampled in the Adriatic and Ionian Sea inhabit deep, oligotrophic waters (mostly > 50 m in depth), whereas the Gulf of Ambracia group is

linked to shallow, eutrophic waters (mostly < 50 m in depth). The existence of morphological variation between *S. pilchardus* populations has been previously documented by ALEGRÍA-HERNÁNDEZ *et al.* (1986), who suggested the presence of two distinct stocks within the Adriatic basin based on morphological and meristic parameters. In the coastal waters between South Carolina and Florida, morphological variation has been detected in a genetically homogenous population of *S. aurita* (KINSEY *et al.*, 1994). The study concluded that the single, panmictic population was structured in regional allometric patterns that diminished as the fish grew older. Furthermore, ecological factors such as the proximity to embayments appeared to influence some of the observed morphological variation. Nonetheless, it is now commonly accepted that morphological variation in scales has both environmental and genetic components, but stable differences in shape among groups of fish may reveal different growth rates, mortality rates, or reproductive rates that are relevant for the definition of populations (CADRIN *et al.*, 2005).

Ecological factors thus may have led to a morphological differentiation between the Adriatic and Ionian or Ionian and Ambracian populations of *S. pilchardus* mediated by phenotypic plasticity as in the case of *S. aurita* in Florida. Congruently, the more recent application of molecular and morphometric methods did also not detect significant differences between Adriatic *S. pilchardus* and those from the adjacent Ionian Sea (TINTI *et al.*, 2002; RUGGERI *et al.*, 2013). Between Ionian and Aegean *S. pilchardus* populations, however, genetic heterogeneity was detected previously suggesting reduced panmixia (SPANAKIS *et al.*, 1989), which is well supported by the findings of this study. Similarly, a fragmented genetic structure in *S. pilchardus* populations inhabiting the western Mediterranean Sea had been revealed previously (RAMON & CASTRO, 1997). The existence of well-defined geographic and hydrographic boundaries such as the Greek peninsula and its related hydrographic conditions have been cited previously to explain the population structure of this pelagic fish inhabiting Greek waters (TINTI *et al.*, 2002). The differences in scale shape between the Adriatic-Ionian *S. pilchardus* groups and the group from the geographically more distant area in the eastern Mediterranean Sea may confirm recent population structuring and a pattern of isolation by distance as suggested by GONZALEZ & ZARDOYA (2007). From a conservation and fishery management perspective, these conclusions provide valuable demographic information to be taken into consideration when managing the exploitation of these commercially important fish populations.

## SUMMARY

Fish scales possess valuable information on many aspects of the bearers' biology which can be equally useful in systematics and ecology. However, a suitable approach of retrieving this information is essential.

The primary aim of this study to establish a system that facilitates scale identification in the common Mediterranean teleost species has been successfully achieved with the creation of a scale atlas. The great diversity of scale structures presented with the seemingly endless variations on each of the morphological features render this atlas a relevant reference catalogue for species identification. The novel classification system of scale morphology, introduced in this study, not only provides an orientation among the vast diversity of scales, but also aids the specific identification. Furthermore, the well-defined categories provide an opportunity for integration of scale descriptions and for transformation of scale characteristics for numerical analysis. Among the characteristic features, scale type and the shape of the scale, as well as the distribution of grooves or ridges on the scale's surface are most useful to determine a taxon. Type, shape and ornamentation of single scales allow the designation to certain taxa; in many cases to species level. In some cases, however, scale features show a high degree of plasticity and vary significantly within a single specimen depending on the body area sampled. Therefore, the representation of scales from several body areas is important when producing a scale catalogue for species identification.

In a phylogenetic context, the classification of scale types found in this study differs from traditional views by assigning crenate and spinoid scales as subtypes of cycloid instead of ctenoid scales. Evidence from previous ontogenetic studies of scale development and juvenile structures retained in adult scales had suggested already that crenate, spinoid and ctenoid scales each develop from a generalized cycloid state. Ctenoid spines in teleosts were already shown to be derived structures rather than plesiomorphic characters, which further supports their separation from other forms of spined scales (i.e., crenate and spinoid). The classification of the scales of 80 Mediterranean teleosts revealed some phylogenetic relationships among the major teleost groups. The phenotypic relationships among scale characteristics showed that ctenoid scales with transforming cteni clustered among representatives of the orders Perciformes and Pleuronectiformes. In contrast, the rather basal forms of scale types, e.g., crenate scales of the Clupeidae and spinoid scales found among the Macrouridae, segregated in their morphological characteristics from the scales of phylogenetically advanced taxa.

Although the morphological analysis is the primary step towards successful identification, in some cases scale morphology alone does not allow a reliable species determination. Therefore, the second aim of this study was to apply additional methodological approaches to enable a successful differentiation in a model system of morphologically similar scales of sympatric clupeid species. On the basis of the morphometric shape analysis, the scales of European sardine *Sardina pilchardus* and round sardinella *Sardinella aurita* from the eastern Mediterranean Sea were successfully separated by discriminant analysis based on the standardized scale shape indices. Although there are body areas with higher degrees of similarity in scale shape within the same species, the multivariate analysis on combined shape indices yielded significant differences between the two species. The application of morphometric analysis using scale shape indices helps to overcome the limitations of morphological analysis, thus aiding more reliable species identification. Furthermore, the morphometric analysis revealed size and shape differences of scales along the fish body. Changes in scale size and shape along the body appear to be related to the curvature and the swimming mode in different taxa. Consequently, the similarity among different species in patterns of scale shape variation across the fish body might become the object of further analyses to shed light on the differences in morphology and phylogenetic relationships.

Beyond species determination, the separation of the local populations of *S. pilchardus* and *S. aurita* based on scale shape differences was attempted. Landmark-based geometric morphometric analysis was applied to reveal differences in scale shape between the two clupeids as well as among the local populations of each species. The findings indicated that scale shape of *S. pilchardus* and *S. aurita* from the central and eastern Mediterranean Sea form separate morphometric groups, thus supporting previous genetic studies that suggested a genetic differentiation due to isolation by distance. The geometric morphometric method allows rapid differentiation between two clupeid species providing an insight into the segregation of their populations.

In conclusion, fish scale analysis has proven to be a useful tool in species identification and many other research fields. The utilization of the established classification system of scale morphology has the potential to unify scale descriptions among studies which could not only integrate scale identification but would also facilitate the exploitation of the phylogenetic information stored in scale morphology.

## LITERATURE CITED

- ALEGRÍA-HERNÁNDEZ, V., JARDAS, I. & SINOVCIC, G. (1986): Observations on the differences between sardine, *Sardina pilchardus* (Walb.) subpopulations from the eastern Adriatic. In: *FAO Fisheries Report No. 345*. FAO, Rome, Italy, pp. 137-160.
- ANDERSON, M.J. (2001): A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26: 32-46.
- AGASSIZ, L. (1833-1843): *Recherches sur les poissons fossiles*. Vol. 1.-5. Imprimerie de Petitpierre, Neuchâtel.
- BACHA, M., JEMAA, S., HAMITOCHE, A., RABHI, K. & AMARA, R. (2014): Population structure of the European anchovy, *Engraulis encrasicolus*, in the SW Mediterranean Sea, and the Atlantic Ocean: evidence from otolith shape analysis. *ICES Journal of Marine Science*, 71: 2429-2435.
- BASU, A., MUSTAFIZ, S., ISLAM, M.R., BJORNDALEN, N., RAHAMAN, M.S. & CHAALAL, O. (2006): A comprehensive approach for modeling sorption of lead and cobalt ions through fish scales as an adsorbent. *Chemical Engineering Communications*, 193: 580-605.
- BATTS, B.S. (1964): Lepidology of the adult pleuronectiform fishes of Puget Sound, Washington. *Copeia*, 1964: 666-672.
- BAUDELLOT, E. (1873): *Recherches sur la structure et le développement des écailles des poissons osseux*. Vol. 2. Archives de Zoologie Expérimentale et Générale, pp. 87-244.
- BORODIN, N. (1924): Age of shad (*Alosa sapidissima* Wilson) as determined by the scales. *Transactions of the American Fisheries Society*, 54: 178-184.
- BOWEN, W.D. & SINIFF, D.B. (1999): Distribution, population biology, and feeding ecology of marine mammals. In: REYNOLDS, J.E. & ROMMEL, S.A. (Eds.), *Biology of marine mammals*. Smithsonian Institution Press, Washington, WA, pp. 451-455.
- BRÄGER, ZS., GONZALVO, J., AGAZZI, S. & BEARZI, G. (in press): Identification of bottlenose dolphin (*Tursiops truncatus*) prey using fish scale analysis. *Aquatic Mammals*
- BRÄGER, ZS. & MORITZ, T. (in press): A scale atlas for common Mediterranean teleost fishes. *Vertebrate Zoology*
- BROWN, C.S. (1992): *Archeology of Mississippi*. University Press of Mississippi, Oxford, p. 87.
- CADRIN, S.X., FRIEDLAND, K.D. & WALDMAN, J.R. (2005): *Stock identification methods: applications in fishery science*. Elsevier Academic Press, Amsterdam, p. 719.

- CAMPBELL, M.A., LÓPEZ, J.A., SADO, T. & MIYA, M. (2013): Pike and salmon as sister taxa: detailed intraclade resolution and divergence time estimation of Esociformes + Salmoniformes based on whole mitochondrial genome sequences. *Gene*, 530: 57-65.
- CASOTTI, R., LANDOLFI, A., BRUNET, C., D'ORTENZIO, F., MANGONI, O., RIBERA D'ALCALÀ, M. & DENIS, M. (2003): Composition and dynamics of the phytoplankton of the Ionian Sea (eastern Mediterranean). *Journal of Geophysical Research: Oceans (1978–2012)*, p. 108.
- CASSELMAN, J.M., COLLINS, J.J., CROSSMAN, E.J., IHSEN, P.E. & SPANGER, G.R. (1981): Lake whitefish (*Coregonus clupeaformis*) stocks of the Ontario waters of Lake Huron. *Canadian Journal of Fisheries and Aquatic Sciences*, 38: 1772–1789.
- CASSELMAN, J.M. (1987): Determination of age and growth. In: WEATHERLEY, A.H. & GILL, H.S. (Eds.), *The biology of fish growth*. Academic Press, London, pp. 209-242.
- CASTEEL, R.W. (1972): A key, based on scales, to the families of native California freshwater fishes. *Proceedings of the California Academy of Sciences*, 7: 75-86.
- CASTEEL, R.W. (1973): The scales of the native freshwater fish families of Washington. *Northwest Science*, 47: 230-238.
- CASTEEL, R.W. (1974): On the remains of fish scales from archaeological sites. *American Antiquity*, 39: 557-581.
- CHU, Y.T. (1935): Comparative studies on the scales and on the pharyngeals and their teeth in Chinese cyprinids, with particular reference to taxonomy and evolution. *Biological Bulletin of St. John's University*, 2: 1-225.
- CHUGUNOVA, N.I. (1959): *Age and growth studies in fish: a systematic guide for ichthyologists*. Israel Program for Scientific Translations. Izdatel'stvo Akademii Nauk SSSR, Moskva, p. 132.
- COBURN, M.M. & GAGLIONE, J.I. (1992): A comparative study of percoid scales (Teleostei: Perciformes). *Copeia*, 4: 986-1001.
- COCKERELL, T.D.A. (1910): The scales of the African characinid fishes. *Smithsonian Miscellaneous Collections*, 56: 1-10.
- COCKERELL, T.D.A. (1913): Observations on fish scales. *Bulletin of the United States Bureau of Fisheries*, 32: 119-174.
- COCKERELL, T.D.A. (1915): Scales of Panama fishes. *Proceedings of the Biological Society of Washington*, 28: 151-160.
- COCKERELL, T.D.A. (1919): Some American Cretaceous fish scales. *United States Geological Survey, Professional Paper*, 120: 765-206.

- COOPER, E.L. (1951): Validation of the use of scales of brook trout, *Salvelinus fontinalis*, for age determination. *Copeia*, 1951: 141-148.
- COTTRELL, P.E., TRITES, A.W. & MILLER, E.H. (1996): Assessing the use of hard parts in faeces to identify harbour seal prey: results of captive-feeding trials. *Canadian Journal of Zoology*, 74: 875-880.
- DAPAR, M.L.G., TORRES, M.A.J., FABRICANTE, P.K. & DEMAYO, C.G. (2012): Scale morphology of the Indian goatfish, *Parupeneus indicus* (Shaw, 1803) (Perciformes: Mullidae). *Advances in Environmental Biology*, 6: 1426-1432.
- DAVID, L.R. (1944): Use of fossil fish scales in micropaleontology. *Carnegie Institution of Washington, Geophysical Laboratory, Publication No. 551*, pp. 25-43.
- DAVIDSON, T.A., SAYER, C.D., PERROW, M.R. & TOMLINSON, M.L. (2003): Representation of fish communities by scale sub-fossils in shallow lakes: implications for inferring percid-cyprinid shifts. *Journal of Paleolimnology*, 30: 441-449.
- DÍAZ-OCHOA, J.A., LANGE, C.B., PANTOJA, S., DE LANGE, G.J., GUTIÉRREZ, D., MUÑOZ, P. & SALAMANCA, M. (2009): Fish scales in sediments from off Callao, central Peru. *Deep Sea Research Part II: Topical Studies in Oceanography*, 56: 1124-1135.
- DRAGO, T., FERREIRA-BARTRINA, V., SANTOS, A.M.P. & PINHEIRO, J. (2009): The use of fish remains in sediments for the reconstruction of paleoproductivity. In: *IOP Conference Series: Earth and Environmental Science 5*, IOP Publishing, p. 012012.
- ELLIOTT, N.G., HASKARD, K. & KOSLOW, J.A. (1995): Morphometric analysis orange roughy (*Hoplostethus atlanticus*) off the continental slope of southern Australia. *Journal of Fish Biology*, 46: 202-220.
- ELMER, K.R., KUSCHE, H., LEHTONEN, T.K. & MEYER, A. (2010): Local variation and parallel evolution: morphological and genetic diversity across a species complex of neotropical crater lake cichlid fishes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365: 1763–1782.
- ESCHMEYER, W.N. (2014): Catalog of fishes: genera, species, references. *California Academy of Sciences, Institute for Biodiversity Science and Sustainability*. Available at: <http://research.calacademy.org/research/ichthyology/catalog/fishcatmain.asp> (last accessed 28 October 2015).
- ESMAEILI, H.R. (2001): *Biology of an exotic fish, silver carp, Hypophthalmichthys molitrix, from Gobindsagar reservoir, India*. PhD thesis. Punjab University, India.
- ESMAEILI, H.R. & GHOLAMI, Z. (2011): Scanning electron microscopy of the scale morphology in cyprinid fish, *Rutilus frisii kutum* Kamenskii, 1901 (Actinopterygii:

- Cyprinidae). *Iranian Journal of Fisheries Sciences*, 10: 155-166.
- ESMAEILI, H.R., ANSARI, T.H. & TEIMORY, A. (2007): Scale structure of a cyprinid fish, *Capoeta damascina* (Valenciennes in Cuvier and Valenciennes, 1842) using scanning electron microscope (SEM). *Iranian Journal of Science & Technology, Trans. A*, 31: 255-262.
- ESMAEILI, H.R., TEIMORI, A., GHOLAMI, Z., ZAREI, N. & REICHENBACHER, B. (2012): Re-validation and re-description of an endemic and endangered species, *Aphanius pluristriatus* (Jenkins, 1910) (Teleostei, Cyprinodontidae), from southern Iran. *Zootaxa*, 3208: 58–67.
- EWINS, P.J., WESELOH, D.V., GROOM, J.H., DOBOS, R.Z. & MINEAU, P. (1994): The diet of herring gulls (*Larus argentatus*) during winter and early spring on the lower Great Lakes. *Hydrobiologia*, 279/280: 39-55.
- FERENTINOS, G., PAPTAEODOROU, G., GERAGA, M., IATROU, M., FAKIRIS, E., CHRISTODOULOU, D., DIMITRIOU, E. & KOUTSIKOPOULOS, C. (2010): Fjord water circulation patterns and dysoxic/anoxic conditions in a Mediterranean semi-enclosed embayment in the Amvrakikos Gulf, Greece. *Estuarine, Coastal and Shelf Science*, 88: 473-481.
- FIGIS (2004): A world overview of species of interest to fisheries. *Sardina pilchardus*, Species Identification and Data Programme. FIGIS Species Fact Sheets. *FAO-FIGIS*.
- FORD, J.K.B. & ELLIS, G.M. (2006): Selective foraging by fish-eating killer whales *Orcinus orca* in British Columbia. *Marine Ecology Progress Series*, 316: 185-199.
- GANZON, M.A.M., TORRES, M.A.J., GOROSPE, J.J. & DEMAYO, C.G. (2012): Variations in scale morphology between sexes of the spotted barb, *Puntius binotatus* (Valenciennes, 1842) (Actinopterygii: Cyprinidae). *2<sup>nd</sup> International Conference on Environment and BioScience*, IACSIT Press, Singapore, pp. 80-84.
- GARDUÑO-PAZ, M.V., DEMETRIOU, M. & ADAMS, C.E. (2010): Variation in scale shape among alternative sympatric phenotypes of Arctic charr *Salvelinus alpinus* from two lakes in Scotland. *Journal of Fish Biology*, 76: 1491-1497.
- GHOLAMI, Z., TEIMORI, A., ESMAEILI, H.R., SCHULZ-MIRBACH, T. & REICHENBACHER, B. (2013): Scale surface microstructure and scale size in the tooth-carp genus *Aphanius* (Teleostei, Cyprinodontidae) from endorheic basins in Southwest Iran. *Zootaxa*, 3619: 467-490.
- GONZALEZ, E.G. & ZARDOYA, R. (2007): Relative role of life-history traits and historical factors in shaping genetic population structure of sardines (*Sardina pilchardus*). *BMC*

*Evolutionary Biology*, 7: 197.

- GUNN, J.S., CLEAR, N.P., CARTER, T.I., REES, A.J., STANLEY, C.A., FARLEY, J.H. & KALISH, J.M. (2008): Age and growth in southern bluefin tuna, *Thunnus maccoyii* (Castelnau): Direct estimation from otoliths, scales and vertebrae. *Fisheries Research*, 92: 207-220.
- HAMMER, Ø., HARPER, D.A.T. & RYAN, P.D. (2001): PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4: 1-9.
- HARABAWY, A.S.A., MEKKAWY, I.A.A. & ALKALADI, A. (2012): Identification of three fish species of genus *Plectorhynchus* from the Red Sea by their scale characteristics. *Life Science Journal*, 9: 4472-4485.
- HARDY, B.L. & MONCEL, M.-H. (2011): Neanderthal use of fish, mammals, birds, starchy plants and wood 125-250,000 years ago. *PLoS ONE*, 6: e23768.
- HELFMAN, G.S., COLLETTE, B.B., FACEY, D.E. & BOWEN, B.W. (2009): *The diversity of fishes*. 2<sup>nd</sup> edition. Wiley-Blackwell, Hong Kong, pp. 36-38.
- HOLMGREN-URBA, D. & BAUMGARTNER, T.R. (1993): A 250-year history of pelagic fish abundances from the anaerobic sediments of the central Gulf of California. *California Cooperative Oceanic Fisheries Investigations*, 34: 60-68.
- HUGHES, D.R. (1980): Preparation of fish scales for SEM. *Micron*, 11: 423-424.
- HUGHES, D.R. (1981): Development and organization of the posterior field of ctenoid scales in the Platycephalidae. *Copeia*, 1981: 596-606.
- HUYSEUNE, A. & SIRE, J.-Y. (1998): Evolution of patterns and processes in teeth and tooth-related tissues in non-mammalian vertebrates. *European Journal of Oral Sciences*, 106: 437-481.
- IBÁÑEZ, A.L., COWX, I.G. & O'HIGGINS, P. (2007): Geometric morphometric analysis of fish scales for identifying genera, species, and local populations within Mugilidae. *Canadian Journal of Fisheries and Aquatic Sciences*, 64: 1091-1100.
- IBÁÑEZ, A.L., COWX, I.G. & O'HIGGINS, P. (2009): Variation in elasmoid fish scale patterns is informative with regard to taxon and swimming mode. *Zoological Journal of the Linnean Society*, 155: 834-844.
- IBÁÑEZ, A.L., ESPINO-BARR, E. & GALLARDO-CABELLO, M. (2012): Population connectivity among geographic variants within the Lutjanidae (Pisces) of the Mexican Pacific coast through fish scale shape recognition. *Scientia Marina*, 76: 667-675.
- JACOT, A.P. (1920): Age, growth and scale characters of the mullets, *Mugil cephalus* and *Mugil curema*. *Transactions of the American Microscopical Society*, 39: 199-229.
- JARVIS, R.S., KLODOWSKI, H.F. & SHELDON, S.P. (1978): New method of quantifying scale

- shape and an application to stock identification in Walleye (*Stizostedion vitreum vitreum*). *Transactions of the American Fisheries Society*, 107: 528–534.
- JAWAD, L.A. (2005): Comparative scale morphology and squamation patterns in triplefins (Pisces: Teleostei: Perciformes: Tripterygiidae). *Tuhinga*, 16:137-167.
- JAWAD, L.A. & AL-JUFALI, S.M. (2007): Scale morphology of greater lizardfish *Saurida tumbil* (Bloch, 1795) (Pisces: Synodontidae). *Journal of Fish Biology*, 70: 1185-1212.
- JOHAL, M.S. & TANDON, K.K. (1992): Age and growth of carp *Catla catla* (Hamilton, 1822) from northern India. *Fisheries Research*, 14: 83-90.
- JOHAL, M.S. & DUA, A. (1994): SEM study of the scales of freshwater snakehead, *Chana punctatus* (Bloch) upon exposure to endosulfan. *Bulletin of Environmental Contamination and Toxicology*, 52: 718-721.
- JOHAL, M.S. & DUA, A. (1995): Elemental, lepidological and toxicological studies in *Channa punctatus* (Bloch) upon exposure to an organochlorine pesticide. *Bulletin of Environmental Contamination and Toxicology*, 55: 916-921.
- JOHNSON, G.D. (1984): Percoidei: development and relationships. In: MOSER, H.G., RICHARDS, W.J., COHEN, D.M., FAHAY, M.P., KENDALL, A.W. & JR. RICHARDSON, S.L. (Eds.), *Ontogeny and systematics of fishes*. Special Publication No. 1. American Society of Ichthyologists and Herpetologists, Lawrence, pp. 464-498.
- KARACHLE, P.K. & STERGIOU, K.I. (2014): Feeding and ecomorphology of three clupeoids in the North Aegean Sea. *Mediterranean Marine Science*, 15: 9-26.
- KAUR, N. & DUA, A. (2004): Species specificity as evidenced by scanning electron microscopy of fish scales. *Current Science*, 87: 692-696.
- KHEMIRI, S., MEUNIER, F.J., LAURIN, M. & ZYLBERBERG, L. (2001): Morphology and structure of the scales in the Gadiformes (Actinopterygii: Teleostei: Paracanthopterygii) and a comparison to the elasmoid scales of other Teleostei. *Cahiers de Biologie Marine*, 42: 345-362.
- KINSEY, S.T., ORSOY, T., BERT, T.M. & MAHMOUDI, B. (1994): Population structure of the Spanish sardine *Sardinella aurita*: natural morphological variation in a genetically homogeneous population. *Marine Biology*, 118: 309-317.
- KLINGENBERG, C.P. (2011): MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources*, 11: 353-357.
- KOUNTOURA, K. & ZACHARIAS, I. (2011): Temporal and spatial distribution of hypoxic/seasonal anoxic zone in Amvrakikos Gulf, Western Greece. *Estuarine, Coastal and Shelf Science*, 94: 123-128.

- LAGLER, K.F. (1947): Lepidological studies 1. Scale characters of the families of Great Lake fishes. *Transactions of the American Microscopical Society*, 66: 149-171.
- LAKE, J.L., RYBA, S.A., SERBST, J.R. & LIBBY, A.D. (2006): Mercury in fish scales as an assessment method for predicting muscle tissue mercury concentrations in largemouth bass. *Archives of environmental contamination and toxicology*, 50: 539-544.
- DE LAMATER, E.D. & COURTENAY, W.R. (1973): Variations in structure of the lateral-line canal on scales of Teleostean fishes. *Zeitschrift für Morphologie der Tiere*, 75: 259-266.
- LINDSEY, C.C. (1978): Form, function and locomotory habits in fish. In: HOAR, D. & RANDALL, D.J. (Eds.), *Fish physiology*. Vol. VII. Academic Press, New York, pp. 1-100.
- LIPPITSCH, E. (1990): Scale morphology and squamation patterns in cichlids (Teleostei, Perciformes). A comparative study. *Journal of Fish Biology*, 37: 265-291.
- LIU, W.T., ZHANG, Y., LI, G.Y., MIAO, Y.Q. & WU, X.H. (2008): Structure and composition of teleost scales from snakehead *Channa argus* (Cantor) (Perciformes: Channidae). *Journal of Fish Biology*, 72: 1055-1067.
- LLEONART, J., SALAT, J. & TORRES, G.J. (2000): Removing allometric effects of body size in morphological analysis. *Journal of Theoretical Biology*, 205: 85-93.
- MATONDO, D.-A.P., TORRES, M.A.J., TABUGO, S.R.M. & DEMAYO, C.G. (2010): Describing variations in scales between sexes of the yellowstriped goatfish, *Upeneus vittatus* (Forsk., 1775) (Perciformes: Mullidae). *Egyptian Academic Journal of Biological Sciences*, 2: 37-50.
- MAUCLINE, J. & GORDON, J.D.M. (1984): Occurrence of stones, sediment and fish scales in stomach contents of demersal fish of the Rockall Trough. *Journal of Fish Biology*, 24: 357-362.
- MCCULLY, H.H. (1961): *The comparative anatomy of the scales of serranid fishes*. Unpublished Ph.D. Thesis, Stanford University.
- MOSHER, K.H. (1969): Identification of Pacific salmon and steelhead trout by scale characteristics. *U.S. Fish and Wildlife Service Circular*, 317: 1-17.
- MUGIYA, Y., HAKOMORI, T. & HATSUTORI, K. (1991): Trace metal incorporation into otoliths and scales in the goldfish, *Carassius auratus*. *Comparative Biochemistry and Physiology Part C*, 99: 327-331.
- NELSON, N.C. (1918): Chronology in Florida. *American Museum of Natural History, Anthropological Papers*, 22: 75-103.
- NELSON, J.N. (2006): *Fishes of the world*. 4<sup>th</sup> edition. John Wiley & Sons, New Jersey.
- NYBAKKEN, J.W. (2001): *Marine Biology, an ecological approach*. 5<sup>th</sup> edition. Benjamin

- Cummings, San Francisco, pp. 98-102.
- OLIVAR, M.P., SALAT, J. & PALOMERA, I. (2001): Comparative study of spatial distribution patterns of the early stages of anchovy and pilchard in the NW Mediterranean Sea. *Marine Ecology Progress Series*, 217: 111-120.
- OTTAWAY, E.M. (1978): Rhythmic growth activity in fish scales. *Journal of Fish Biology*, 12: 615-623.
- PATTERSON, R.T., WRIGHT, C., CHANG, A.S., TAYLOR, L.A., LYONS, P.D., DALLIMORE, A. & KUMAR, A. (2002): Atlas of common squamatological (fish scale) material in coastal British Columbia and an assessment of the utility of various scale types in paleofisheries reconstruction. *Palaeontologia Electronica*, 4: 1-88.
- PATTERSON, R.T., PROKOPH, A., KUMAR, A., CHANG, A.S. & ROE, H.M. (2005): Late Holocene variability in pelagic fish scales and dinoflagellate cysts along the west coast of Vancouver Island, NE Pacific Ocean. *Marine Micropaleontology*, 55: 183-204.
- PAXTON, J.R. & ESCHMEYER W.N. (1995): *Encyclopedia of Fishes*. 2<sup>nd</sup> edition. Academic Press, San Diego, p. 240.
- PENNINGTON, W. & FROST, W.E. (1961): Fish vertebrae and scales in a sediment core from Esthwaite Water (English Lake District). *Hydrobiologia*, 17: 183-190.
- PITTA, P., KARAKASSIS, I., TSAPAKIS, M. & ZIVANOVIC, S. (1998): Natural vs. mariculture induced variability in nutrients and plankton in the eastern Mediterranean. *Hydrobiologia*, 391: 179-192.
- DE PONTUAL, H. & PROUZET, P. (1987): Atlantic salmon, *Salmo salar* L., stock discrimination by scale shape analysis. *Aquaculture and Fisheries Management*, 18: 277–289.
- POULET, N., REYJOL, Y., COLLIER, H. & LEK, S. (2005): Does fish scale morphology allow the identification of populations at local scale? A case study for rostrum dace *Leuciscus leuciscus burdigalensis* in River Viaur (SW France). *Aquatic Sciences*, 67: 122-127.
- QUIGNARD, J.-P. & PRAS, A. (1986): Labridae. – Scaridae. In: WHITEHEAD, P.J.P., BAUCHOT, M.-L., HUREAU, J.-C., NIELSEN, J. & TORTONESE, E. (Eds.), *Fishes of the North-eastern Atlantic and the Mediterranean*. Vol. 2. United Nations Educational, Scientific and Cultural Organization (UNESCO), Paris, pp. 919-944.
- RAHAMAN, M.S., BASU, A. & ISLAM, M.R. (2008): The removal of As(III) and As(V) from aqueous solutions by waste materials. *Bioresource Technology*, 99: 2815-2823.
- RAMON, M.M. & CASTRO, J.A. (1997): Genetic variation in natural stocks of *Sardina pilchardus* (sardines) from the western Mediterranean Sea. *Heredity*, 78: 520-528.

- REIF, W.-E. & RICHTER, M. (2001): Revisiting the lepidomorph and odontode regulation theories of dermo-skeletal morphogenesis. *Neues Jahrbuch für Geologie und Paläontologie*, 219: 285-304.
- RICHARDS, R.A. & ESTEVES, C. (1997): Use of scale morphology for discriminating wild stocks of Atlantic striped bass. *Transactions of the American Fisheries Society*, 126: 919-925.
- ROBERTS, C.D. (1993): Comparative morphology of spined scales and their phylogenetic significance in the Teleostei. *Bulletin of Marine Science*, 52: 60-113.
- ROHLF, F.J. (2015a): TpsUtil, file utility program, version 1.60. Department of Ecology and Evolution, State University of New York at Stony Brook. Available at: <http://life.bio.sunysb.edu/morph/> (last accessed 30 March 2015).
- ROHLF, F.J. (2015b): TpsDig, digitize landmarks and outlines, version 2.17. Department of Ecology and Evolution, State University of New York at Stony Brook. Available at: <http://life.bio.sunysb.edu/morph/soft-dataacq.html> (last accessed 30 March 2015).
- RONDEAU, E.B., MINKLEY, D.R., LEONG, J.S., MESSMER, A.M., JANTZEN, J.R., VON SCHALBURG, K.R., LEMON, C., BIRD, N.H. & KOOP, B.F. (2014): The Genome and Linkage Map of the Northern Pike (*Esox lucius*): Conserved Synteny Revealed between the Salmonid Sister Group and the Neoteleostei. *PLoS One*, 9: e102089.
- RUGGERI, P., SPLENDIANI, A., BONANOMI, S., ARNERI, E., CINGOLANI, N., SANTOJANNI, A., COLELLA, S., DONATO, F., GIOVANNOTTI, M. & CAPUTO BARUCCHI, V. (2013): Searching for a stock structure in *Sardina pilchardus* from the Adriatic and Ionian seas using a microsatellite DNA-based approach. *Scientia Marina*, 77: 565-574.
- SIRE, J.-Y. (1986): Ontogenic development of surface ornamentation in the scales of *Hemichromis bimaculatus* (Cichlidae). *Journal of Fish Biology*, 28: 713-724.
- SIRE, J.-Y. (1990): From ganoid to elasmoid scales in the actinopterygian fishes. *Netherlands Journal of Zoology*, 40: 75-92.
- SIRE, J.-Y. & GÉRAUDIE, J. (1983): Fine structure of the developing scale in the cichlid *Hemichromis bimaculatus* (Pisces, Teleostei, Perciformes). *Acta Zoologica (Stockholm)*, 64: 1-8.
- SIRE, J.-Y. & ARNULF, I. (2000): Structure and development of the ctenial spines on the scales of a teleost fish, the cichlid *Cichlasoma nigrofasciatum*. *Acta Zoologica (Stockholm)*, 81: 139-158.
- SIRE, J.-Y. & HUYSEUNE, A. (2003): Formation of dermal skeletal and dental tissues in fish: a comparative and evolutionary approach. *Biological Reviews*, 78: 219–249.

- SIRE, J.-Y. & AKIMENKO, M.A. (2004): Scale development in fish: a review, with description of sonic hedgehog (shh) expression in the zebrafish (*Danio rerio*). *International journal of developmental biology*, 48: 233-247.
- SIRE, J.-Y., ALLIZARD, F., BABIAR, O., BOURGUIGNON, J. & QUILHAC, A. (1997): Scale development in zebrafish (*Danio rerio*). *Journal of Anatomy*, 190: 545-561.
- SIRE, J.-Y., DONOGHUE, P.C.J. & VICKARYOUS, M.K. (2009): Origin and evolution of the integumentary skeleton in non-tetrapod vertebrates. *Journal of Anatomy*, 214: 409-440.
- SOMARAKIS, S., GANIAS, K., SIAPATIS, A., KOUTSIKOPOULOS, C., MACHIAS, A. & PAPACONSTANTINO, C. (2006): Spawning habitat and daily egg production of sardine (*Sardina pilchardus*) in the eastern Mediterranean. *Fisheries Oceanography*, 15: 281-292.
- SOUTAR, A. & ISAACS, J.D. (1969): History of fish populations inferred from fish scales in anaerobic sediments off California. *California Cooperative Oceanic Fisheries Investigations*, 13: 63-70.
- SOUTAR, A. & ISAACS, J.D. (1974): Abundance of pelagic fish during the 19<sup>th</sup> and 20<sup>th</sup> centuries as recorded in anaerobic sediments off California. *Fishery Bulletin*, 72: 257-273.
- SPANAKIS, E., TSIMENIDES, N. & ZOUROS, E. (1989): Genetic differences between populations of sardine, *Sardina pilchardus*, and anchovy, *Engraulis encrasicolus*, in the Aegean and Ionian seas. *Journal of Fish Biology*, 35: 417-437.
- STASZNY, Á., FERINCZ, A., WEIPERTH, A., HAVAS, E., URBANYI, B. & PAULOVITS, G. (2012): Scale-morphometry study to discriminate Gibel carp (*Carassius gibelio*) populations in the Balaton-Catchment (Hungary). *Acta Zoologica Academiae Scientiarum Hungaricae*, 58: 19-27.
- STASZNY, Á., HAVAS, E., KOVÁCS, R., URBÁNYI, B., PAULOVITS, G., BENCSIK, D., FERINCZ, Á., MÜLLER, T., SPECZIÁR, A., BAKOS, K. & CSENKI, Z. (2013): Impact of environmental and genetic factors on the scale shape of zebrafish, *Danio rerio* (Hamilton 1822): a geometric morphometric study. *Acta Biologica Hungarica*, 64: 462-475.
- SUMICH, J.L. & MORRISSEY, J.F. (2004): *Introduction to the biology of marine life*. 8<sup>th</sup> edition. Jones and Bartlett, Sudbury, p. 166.
- TANDON, K.K. & JOHAL, M.S. (1996): *Age and growth in Indian freshwater fishes*. Narendra Publishing House, New Delhi, p. 232.
- TAYLOR, H.F. (1916): The structure and growth of the scales of the squeteague and the pigfish as indicative of life history. *U.S. Bureau of Fisheries, Bulletin*, No. 34, pp. 285-330.
- THORPE, R.S. (1975): Quantitative handling of characters useful in snake systematics with

- particular reference to intraspecific variation in the Ringed Snake *Natrix natrix* (L.). *Biological Journal of the Linnean Society*, 7: 27-43.
- TIMMS, H.W.M. (1905): The development, structure, and morphology of the scales in some teleostean fish. *Quarterly Journal of Microscopical Science*, 49: 39-69.
- TINTI, F., DI NUNNO, C., GUARNIERO, I., TALENTI, M., TOMMASINI, S., FABBRI, E. & PICCINETTI, C. (2002): Mitochondrial DNA sequence variation suggests the lack of genetic heterogeneity in the Adriatic and Ionian stocks of *Sardina pilchardus*. *Marine Biotechnology*, 4: 163-172.
- TSAGARAKIS, K., COLL, M., GIANNOULAKI, M., SOMARAKIS, S., PAPACONSTANTINO, C. & MACHIAS, A. (2010): Food-web traits of the North Aegean Sea ecosystem (Eastern Mediterranean) and comparison with other Mediterranean ecosystems. *Estuarine, Coastal and Shelf Science*, 88: 233-248.
- TSIKLIRAS, A.C. (2014): Sympatric clupeoid fish larvae in the eastern Mediterranean: coexistence or avoidance? *Advances in Ecology*, 2014: 274180.
- TSIKLIRAS, A.C. & ANTONOPOULOU, E. (2006): Reproductive biology of round sardinella (*Sardinella aurita*) in north-eastern Mediterranean. *Scientia Marina*, 70: 281-290.
- TSIKLIRAS, A.C. & KOUTRAKIS, E.T. (2013): Growth and reproduction of European sardine, *Sardina pilchardus* (Pisces: Clupeidae), in northeastern Mediterranean. *Cahiers de Biologie Marine*, 54: 365-374.
- TSIKLIRAS, A.C., KOUTRAKIS, E.T. & STERGIU, K.I. (2005): Age and growth of round sardinella (*Sardinella aurita*) in the northeastern Mediterranean. *Scientia Marina*, 69: 231-240.
- TSIKLIRAS, A.C., KOUTRAKIS, E.T., SYLAIOS, G.K. & KALLIANIOTIS, A.A. (2009): Summer distribution of fish larvae in northern Aegean Sea. *Journal of the Marine Biological Association of the United Kingdom*, 89: 1137-1146.
- TUSET, V.M., LOZANO, I.J., GONZALEZ, J.A., PERTUSA, J.F. & GARCIA-DIAZ, M.M. (2003): Shape indices to identify regional differences in otolith morphology of comber *Serranus cabrilla* (L. 1758). *Journal of Applied Ichthyology*, 19: 88-93.
- VALDÉS, J., ORTLIEB, L., GUTIERREZ, D., MARINOVIC, L., VARGAS, G. & SIFEDDINE, A. (2008): 250 years of sardine and anchovy scale deposition record in Mejillones Bay, northern Chile. *Progress in Oceanography*, 79: 198-207.
- VALLENTYNE, J.R. (1960): On fish remains in lacustrine sediments. *American Journal of Science*, 258-A: 344-349.
- VAN OOSTEN, J. (1957): The skin and scales. In: BROWN, M.E. (ed.) *The physiology of fishes*.

Academic Press Publishers, New York.

- WALKER, W.M. (1936): The Troyville mound, Catahoula Parish, Louisiana. *Bulletin of the Bureau of American Ethnology*, 113:1-73.
- WILLIAMSON, W.C. (1851): Investigations into the structure and development of the scales and bones of fishes. *Philosophical Transactions of the Royal Society of London*, 141: 643-702.
- WHITEHEAD, P.J.P. (1985): *FAO species catalogue Vol. 7. Clupeoid fishes of the world (suborder Clupeioidi). An annotated and illustrated catalogue of the herrings, sardines, pilchards, sprats, anchovies and wolfherrings. Part 1 Chirocentridae, Clupeidae and Pristigasteridae.* Rome: FAO Fisheries Synopsis 125, pp. 1-303.  
<http://www.fao.org/docrep/009/ac482e/ac482e00.htm>
- YOKOGAWA, K. & WATANABE, K. (2011): Squamatological differences between two closely related flatfish species of the genus *Pleuronichthys* (Pleuronectidae, Pleuronectiformes), with a proposal of unambiguous distinctive characters. *Ichthyological Research*, 58: 24-32.
- ZELDITCH, M.L., SWIDERSKI, D.L., SHEETS, H.D. & FINK, W.L. (2004): *Geometric morphometrics for biologists: a primer.* Amsterdam: Elsevier Academic Press.

## LIST OF PUBLICATIONS

### PUBLICATIONS BASED ON THE TOPIC OF THE DISSERTATION

BRÄGER, ZS., GONZALVO, J., AGAZZI, S. & BEARZI, G. (2016): Identification of bottlenose dolphin (*Tursiops truncatus*) prey using fish scale analysis. *Aquatic Mammals*, 42: 63-73.  
Doi: 10.1578/AM.42.1.2016.63

**IF: 1.015**

BRÄGER, ZS., MORITZ, T., TSIKLIRAS, A.C., GONZALVO, J., RADULOVIĆ, M. & STASZNY, Á. (2016): Scale morphometry allows discrimination of European sardine *Sardina pilchardus* and round sardinella *Sardinella aurita* and among their local populations. *Journal of Fish Biology*, 88:1273-1281. Doi:10.1111/jfb.12907

**IF: 1.658**

BRÄGER, ZS. & MORITZ, T. (in press): A scale atlas for common Mediterranean teleost fishes. *Vertebrate Zoology*

**IF: 0.593**

**Cumulative impact factor: 3.266**

## OTHER PUBLICATIONS

**PERESZLÉNYI, ZS.** (2013): *A tengerbiológia alapjai*. (Eng.: Basics of marine biology). Oktatói jegyzet. Kék Bolygó Egyesület, Budapest, Hungary, pp. 1-231.

**BRÄGER, ZS., NÉMETH, SZ. & REGÖS, J.** (2014): *Bevezetés a tengerek ökológiájába*. (Eng.: Introduction to marine ecology). E-Book. ISBN: 978-963-642-639-2. University of Pécs, TTK, Pécs, Hungary, pp. 1-96. <http://www.okologia.pte.hu/Files/BevTenOkol.pdf>

**BRÄGER, S., KOPCSÁNYI, T. & BRÄGER, ZS.** (2014): First sightings of living Cuvier's beaked whales (*Ziphius cavirostris*) in Albanian waters. *Marine Biodiversity*, 44: 553-557. Doi: 10.1007/s12526-014-0232-5.

## CONFERENCE PRESENTATIONS

**PERESZLÉNYI, ZS., BEARZI, G., AGAZZI, S. & GONZALVO, J.** (2009): Feeding behaviour of common bottlenose dolphins (*Tursiops truncatus*) in the Amvrakikos Gulf, Greece. *5<sup>th</sup> Annual Meeting of the Ecology and Behaviour*, Lyon, France, 6-10 April 2009.

**BRÄGER, S. & PERESZLÉNYI, ZS.** (2013): Marine Mammal Research in a nutshell. *2<sup>nd</sup> Interdisciplinary Doctoral Conference*, Pécs, Hungary, 15-17 May 2013.

**BRÄGER, ZS.** (2014): Feeding ecology and dietary studies of Cetaceans. *Annual Meeting of the Natural History Society of Jamaica*, Kingston, Jamaica, 5 June 2014.

**BRÄGER, ZS. & MORITZ, T.** (2014): Fish Scale Atlas of the Mediterranean Sea. *11. Tagung der Gesellschaft für Ichthyologie*, Stralsund, Germany, 25-28 September 2014.

**MERTZEN, M., MORITZ, T. & BRÄGER, ZS.** (2015): Morphologie und evolutionäre Geschichte der Heringsschuppen. *12. Tagung der Gesellschaft für Ichthyologie*, Berlin, Germany, 05-11 November 2015.

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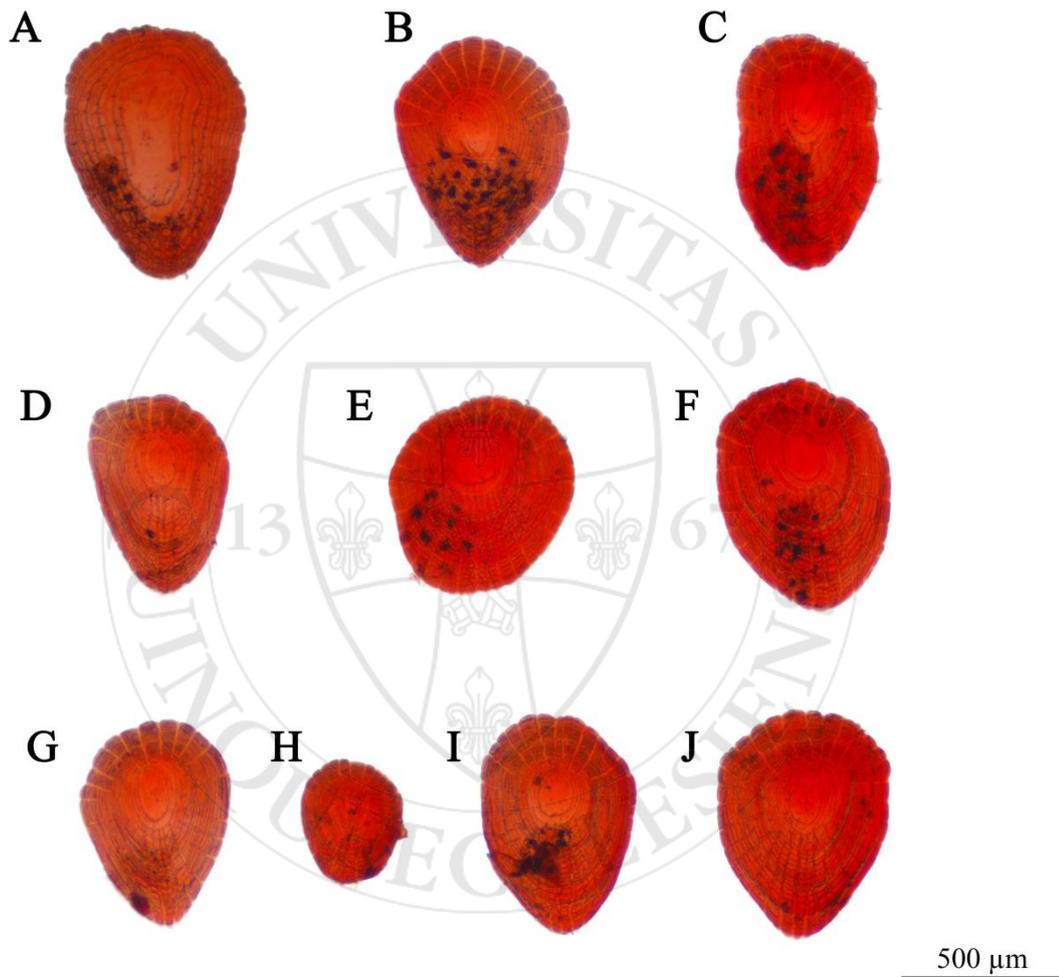
Last but not least, I want to thank my parents, family members, and all of my friends for their love and support they have given me during the last few years.

My sincerest gratitude goes to my husband, Dr. Stefan Bräger, whose love, support, and understanding helped to put my mind at ease during those long and frequent absences from home. Without you, this dream, as many others, could not have become reality.

I dedicate this thesis to my grandfather, Július Pereszlényi sen., who always had confidence in me, but sadly did not get to see the completion of this dissertation.

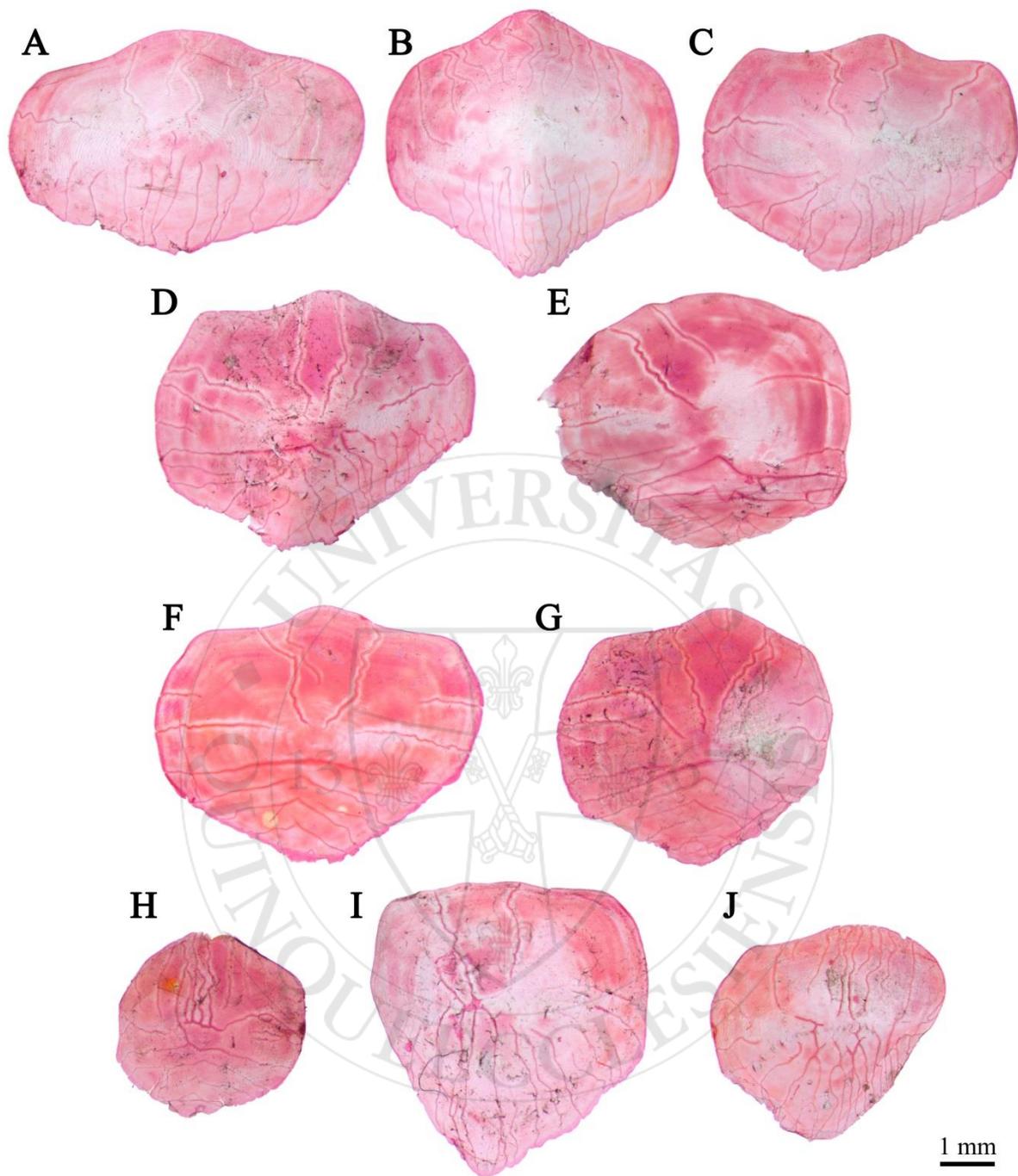
## APPENDICES

### APPENDIX 1. Scales of 80 teleosts from the Mediterranean region

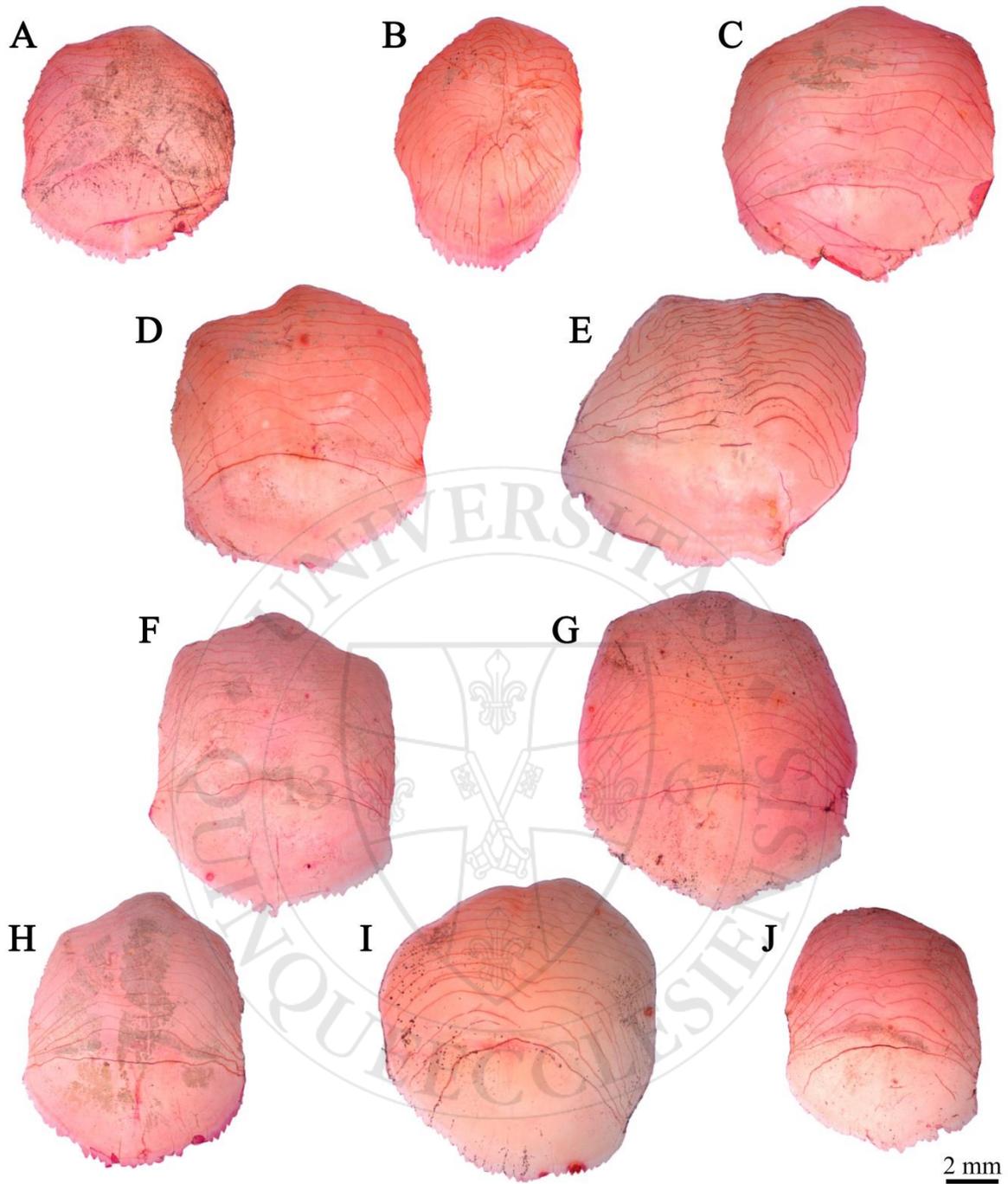


**FIGURE 1.** *Notacanthus bonaparte*

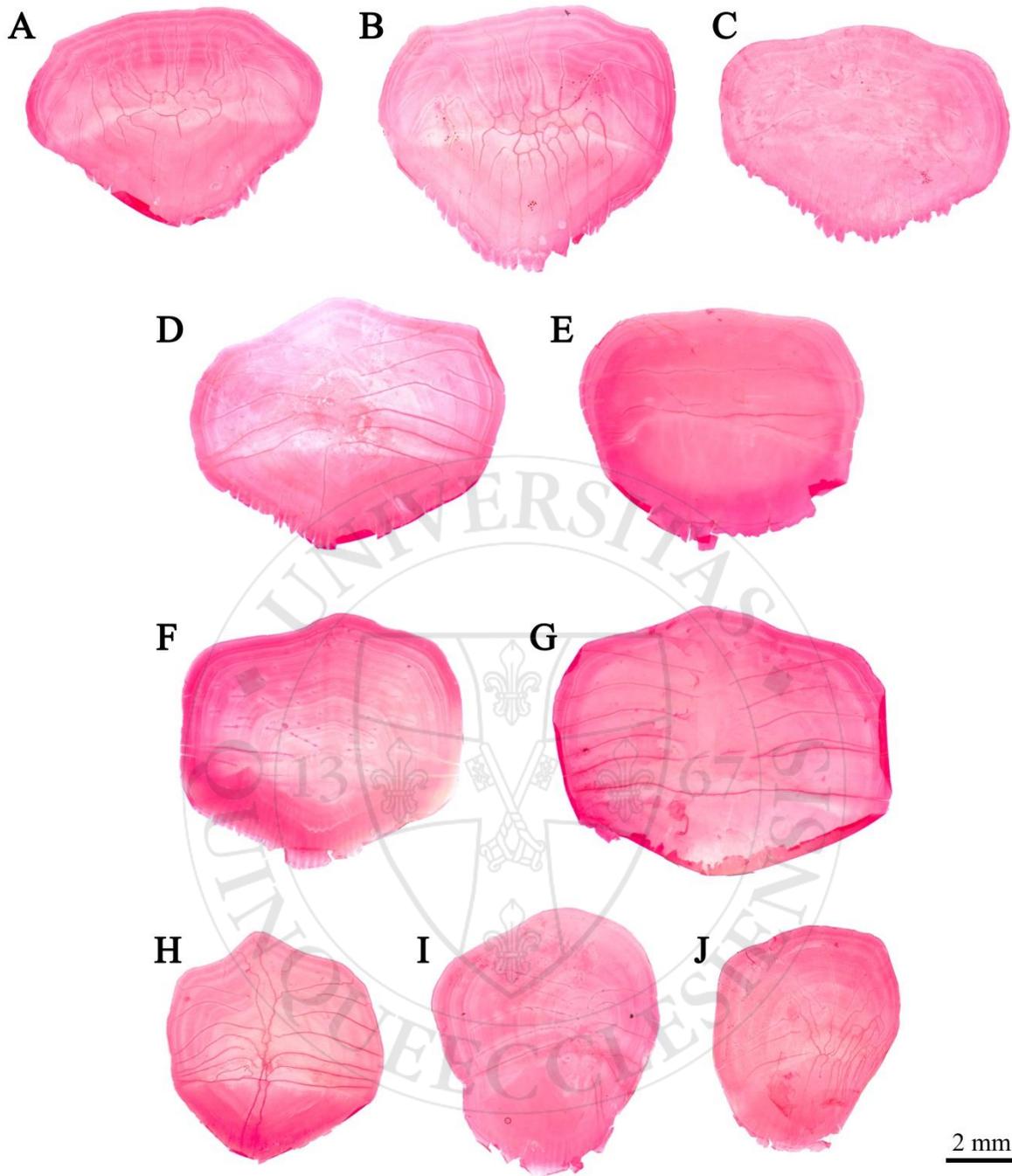
190 mm TL, Costa Brava, Spain, DMM IE/4609



**FIGURE 2.** *Engraulis encrasicolus*  
108 mm SL, Mali Lošinj, Croatia, DMM IE/9008

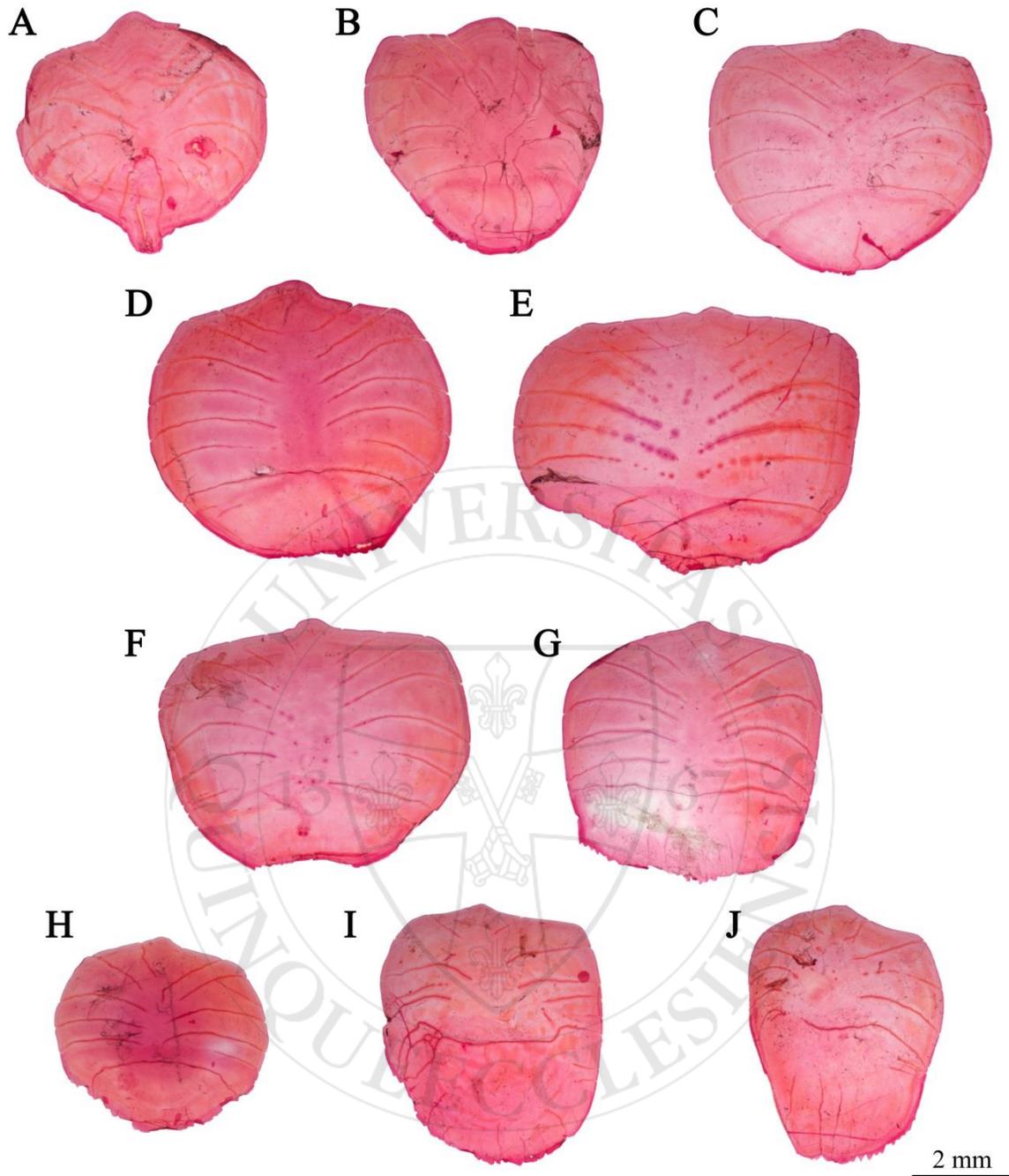


**FIGURE 3.** *Alosa fallax*  
330 mm SL, Baltic Sea, Germany, DMM IE/4361



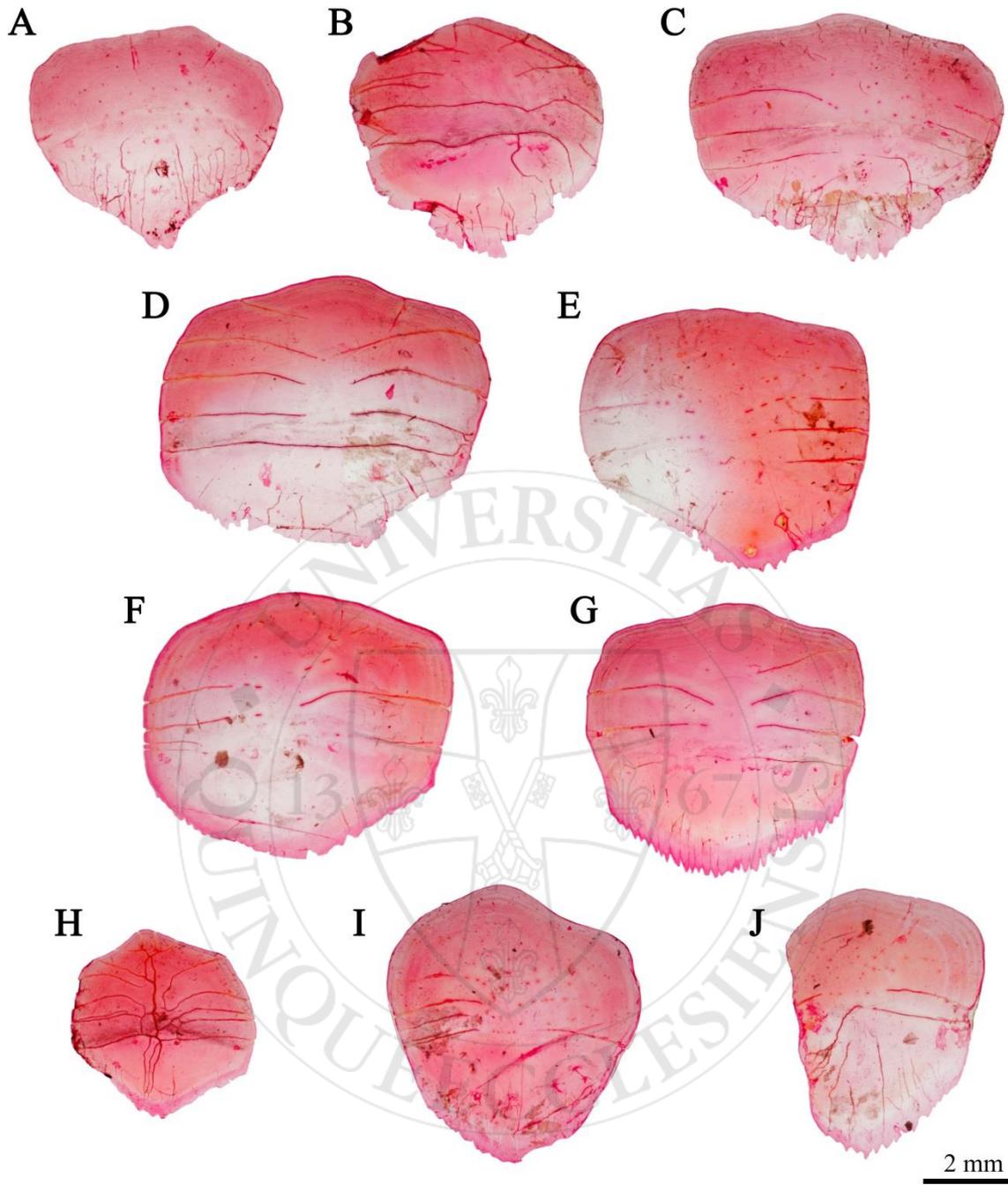
**FIGURE 4.** *Clupea harengus*

210 mm SL, Costa Brava, Spain, DMM IE/4611

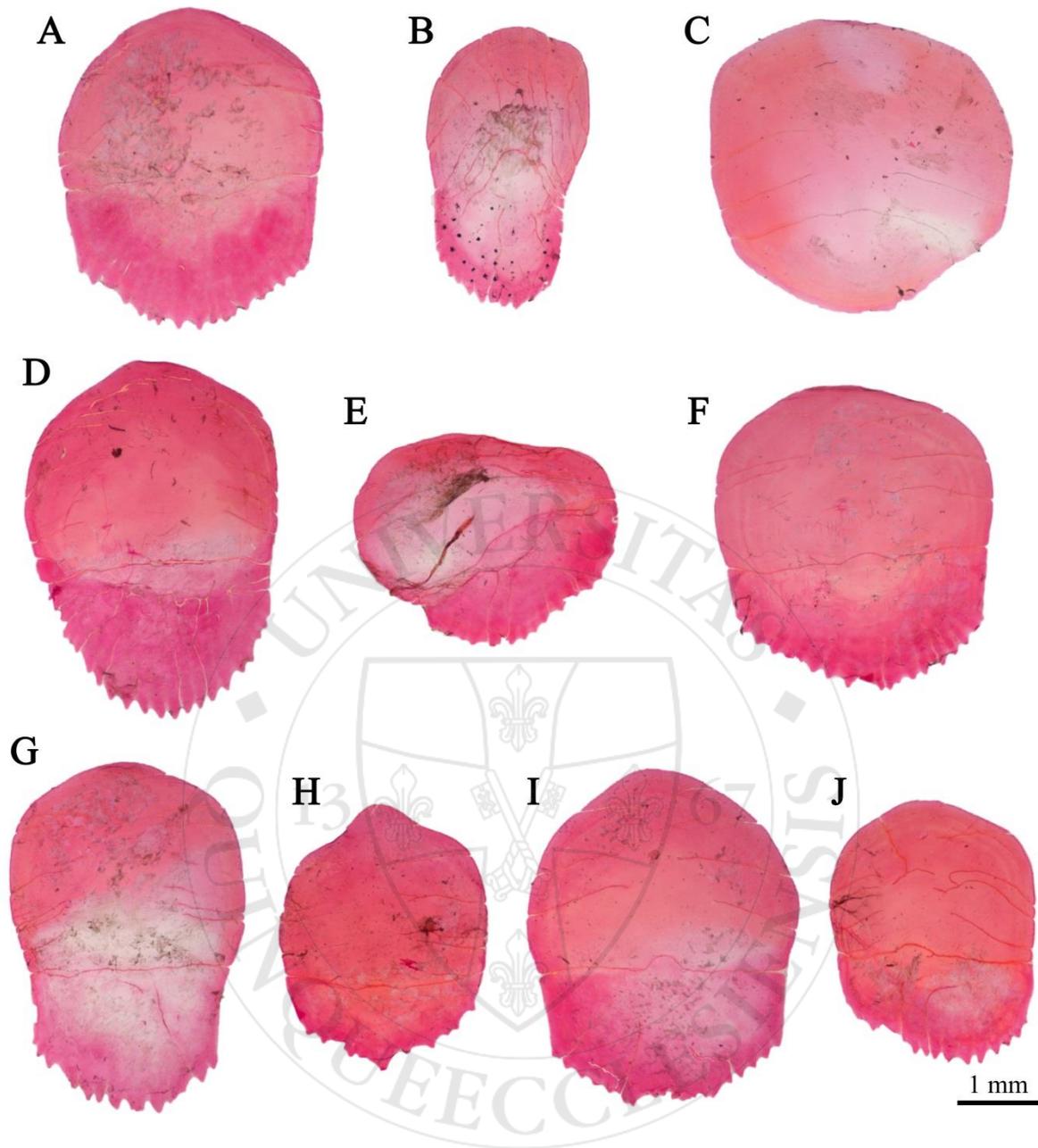


**FIGURE 5.** *Sardina pilchardus*

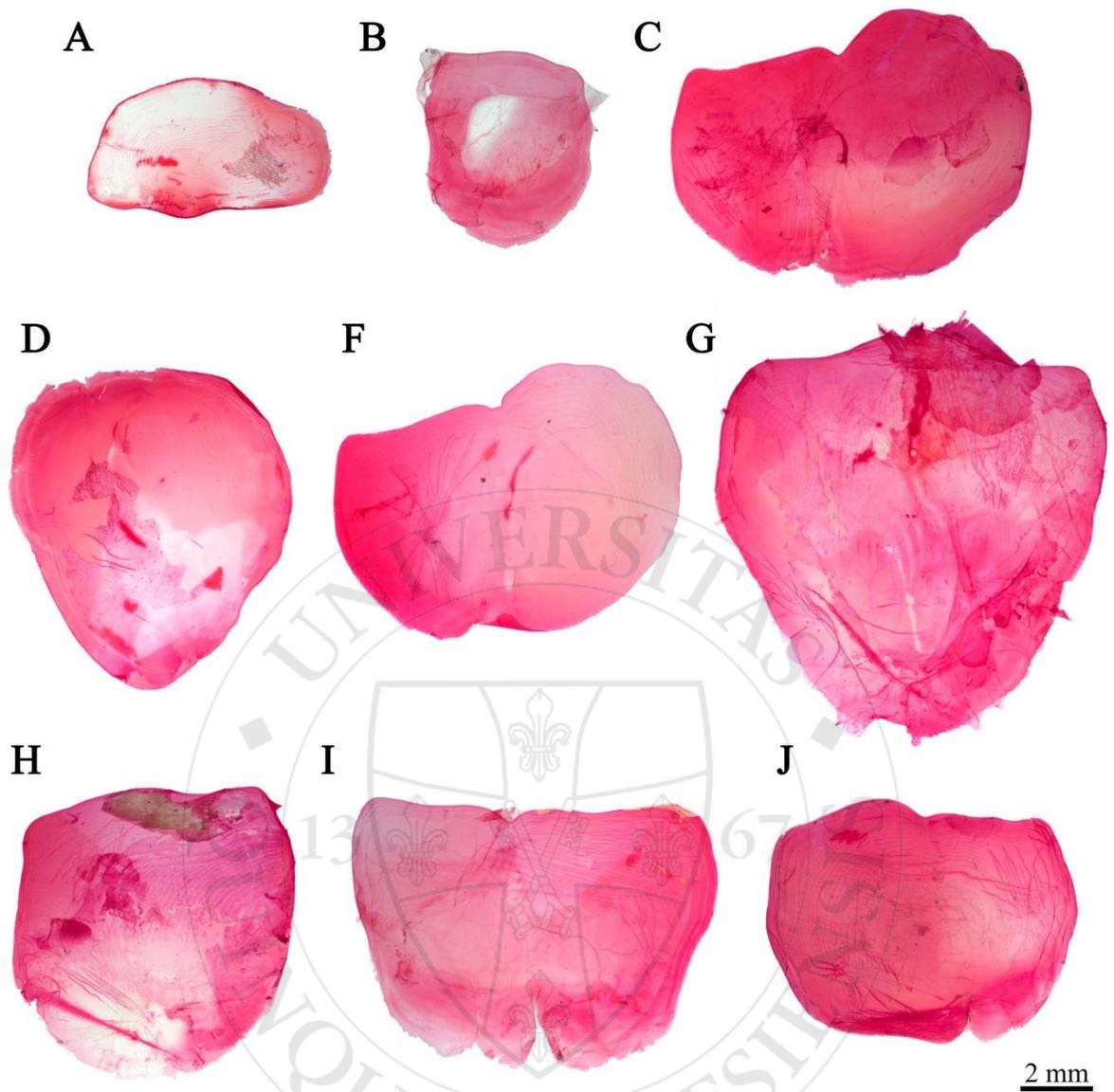
115 mm SL, Mali Lošinj, Croatia, DMM IE/9009



**FIGURE 6.** *Sardinella aurita*  
132 mm SL, Costa Brava, Spain, DM IE/5843



**FIGURE 7.** *Sprattus sprattus*  
108 mm SL, North Sea, DM IE/6726

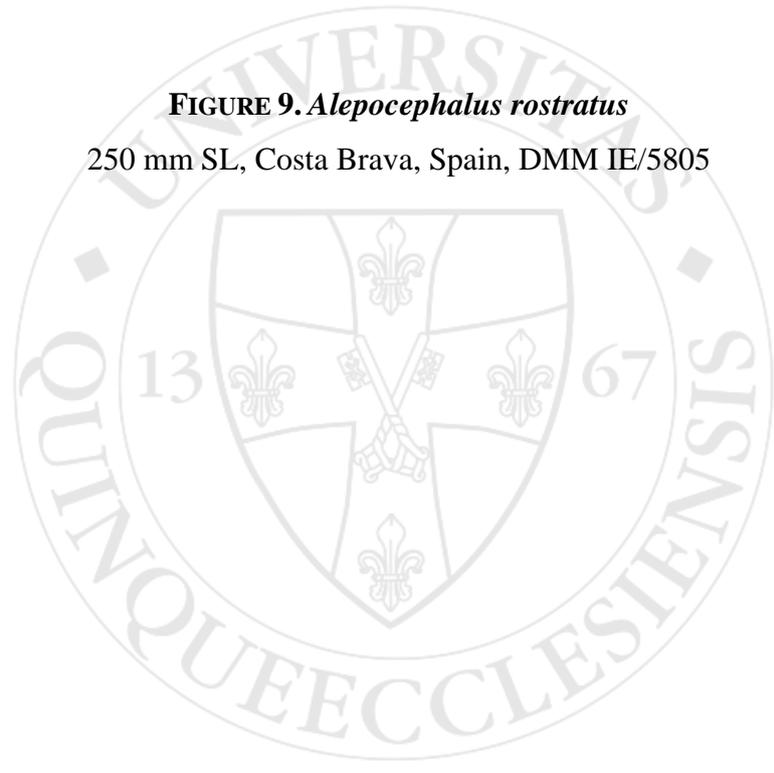


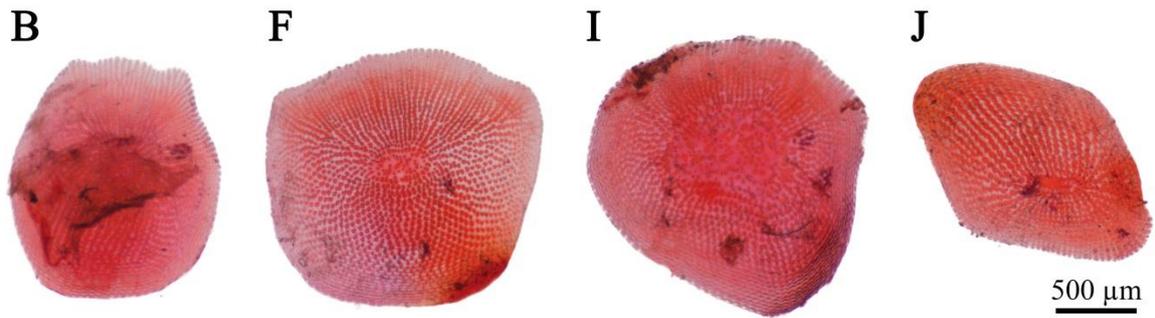
**FIGURE 8.** *Argentina sphyraena*

185 mm SL, Costa Brava, Spain, DMM IE/5003



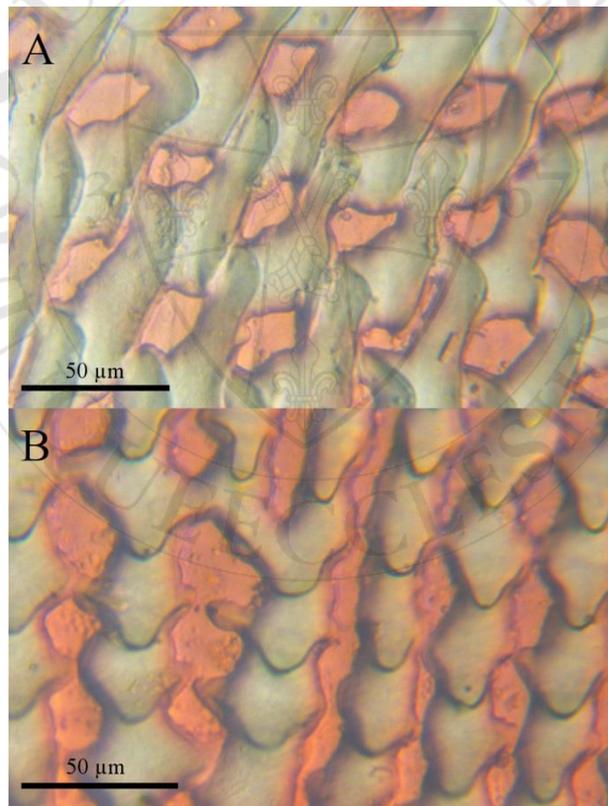
**FIGURE 9.** *Alepocephalus rostratus*  
250 mm SL, Costa Brava, Spain, DMM IE/5805





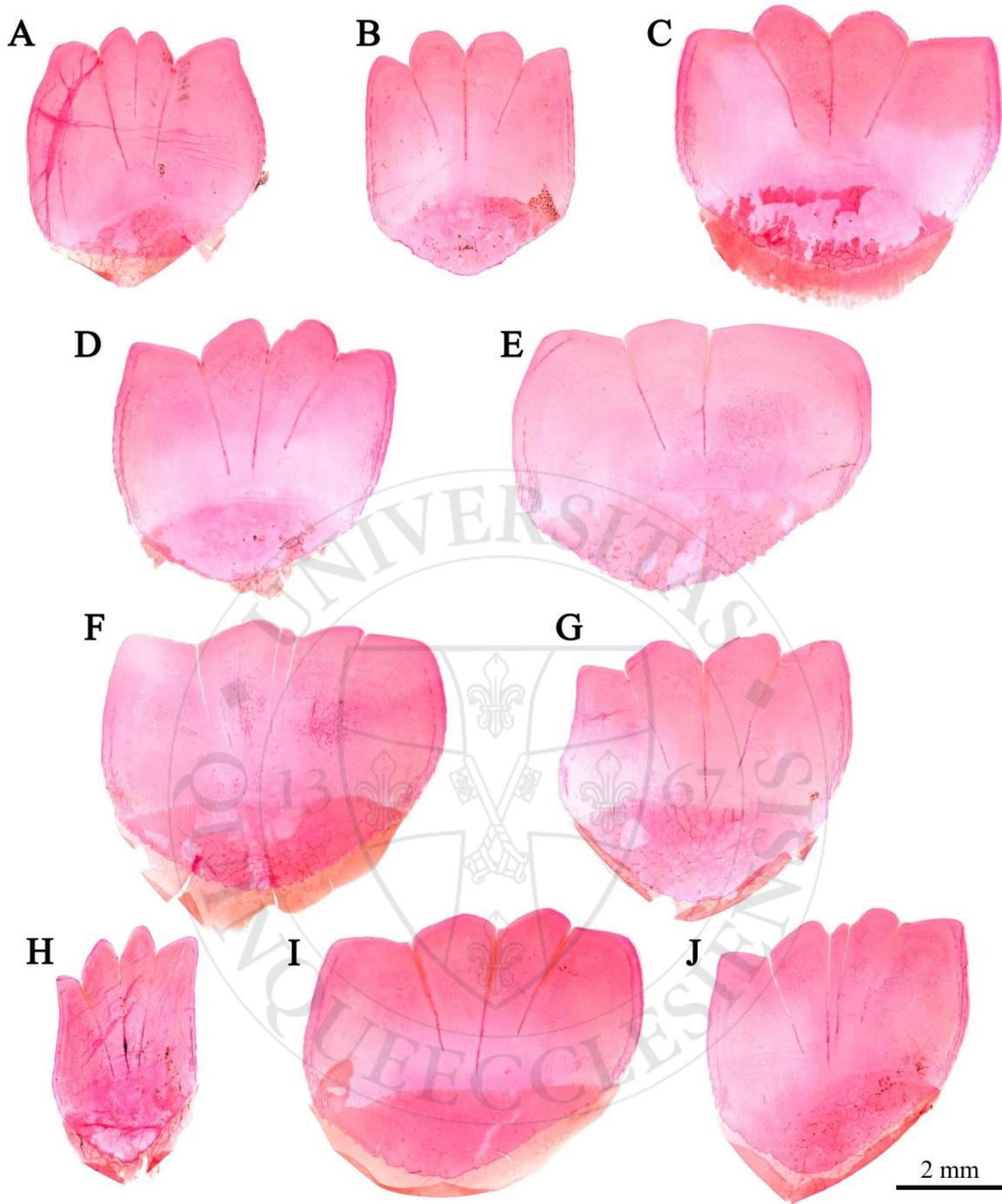
**FIGURE 10.** *Maurolicus muelleri*

4 specimens, 42-51 mm SL, Costa Brava, Spain, DMM IE/5048



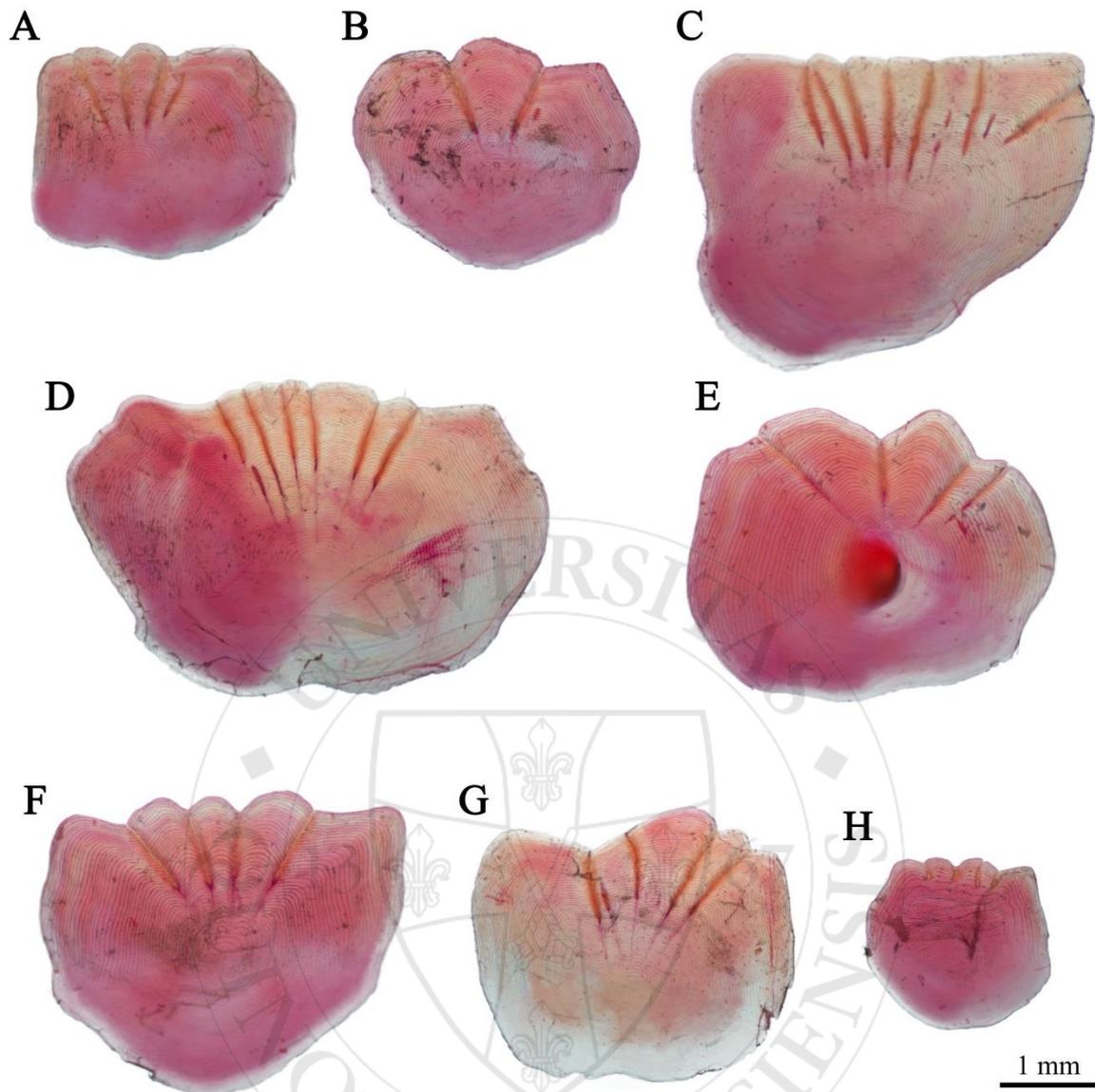
**FIGURE 11.** Surface ornamentations of the scale of *M. muelleri* in sampling area F

Note: A: individual scale ornamentations; B: scale ornamentations forming ridges

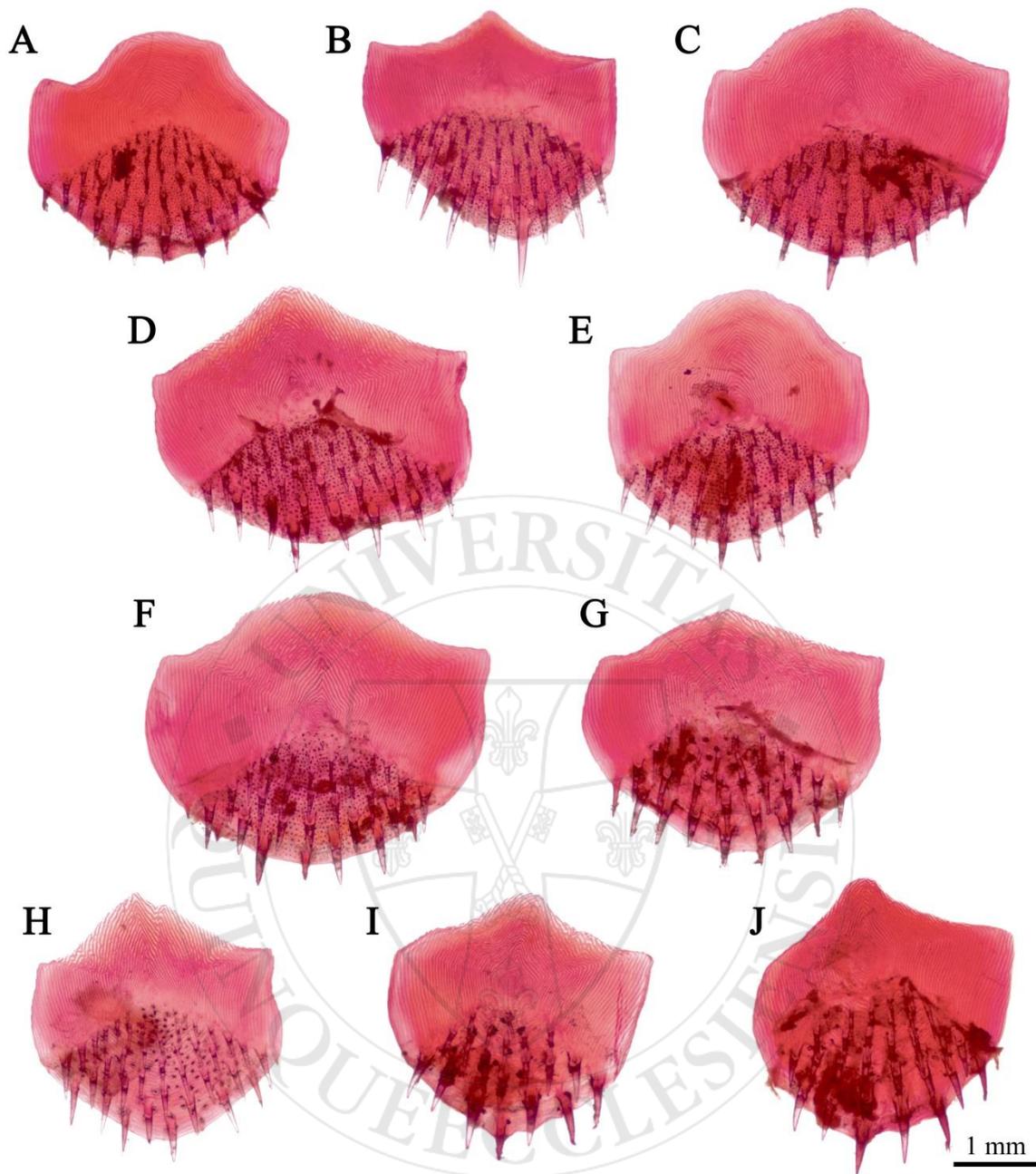


**FIGURE 12.** *Synodus saurus*

164 mm SL, Costa Brava, Spain, DMM IE/5026



**FIGURE 13.** *Myctophum punctatum*  
68 mm SL, Costa Brava, Spain, DMM IE/5114



**FIGURE 14.** *Coelorhynchus caelorhynchus*  
219 mm SL, Costa Brava, Spain, DMM IE/5899

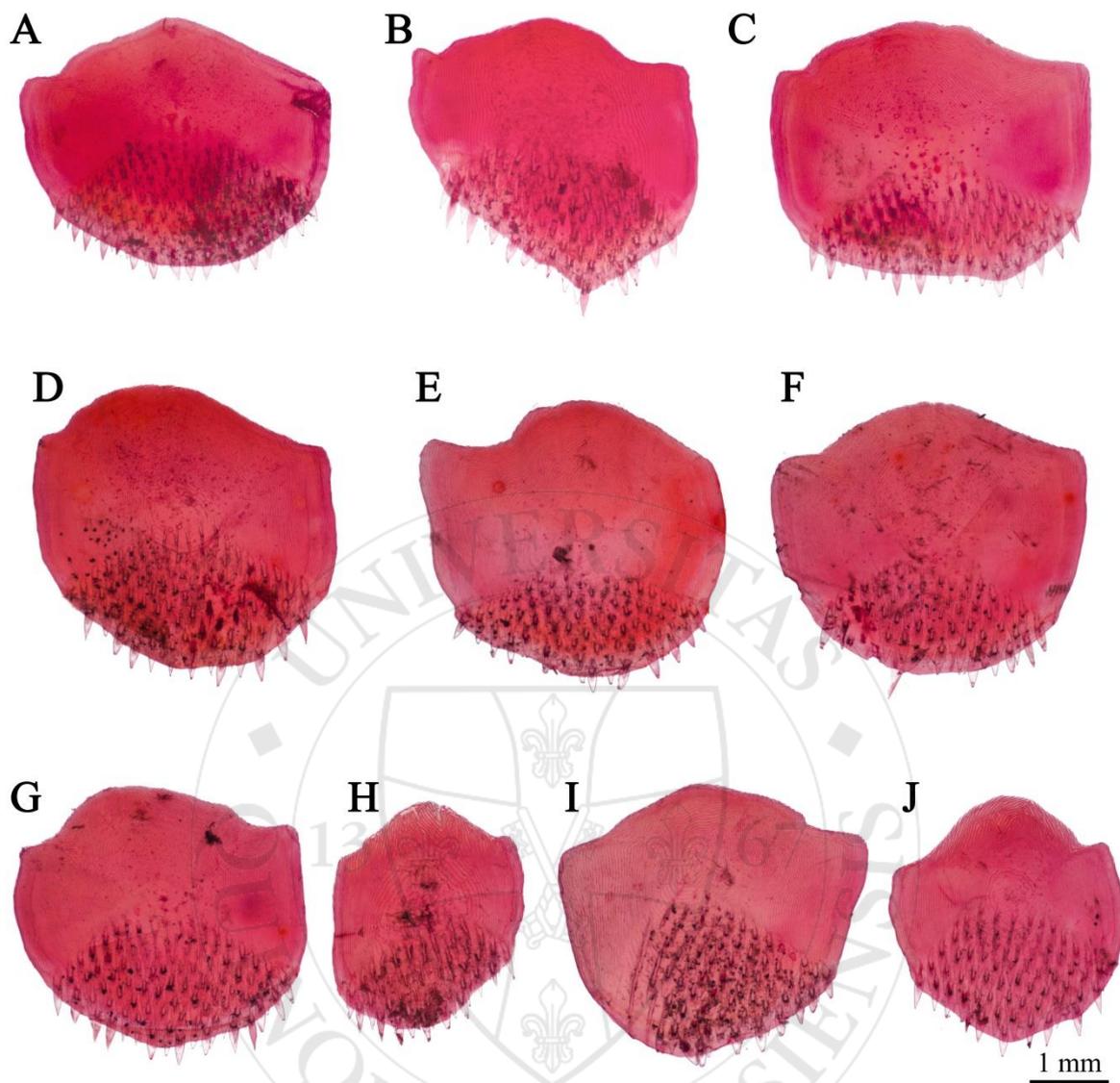
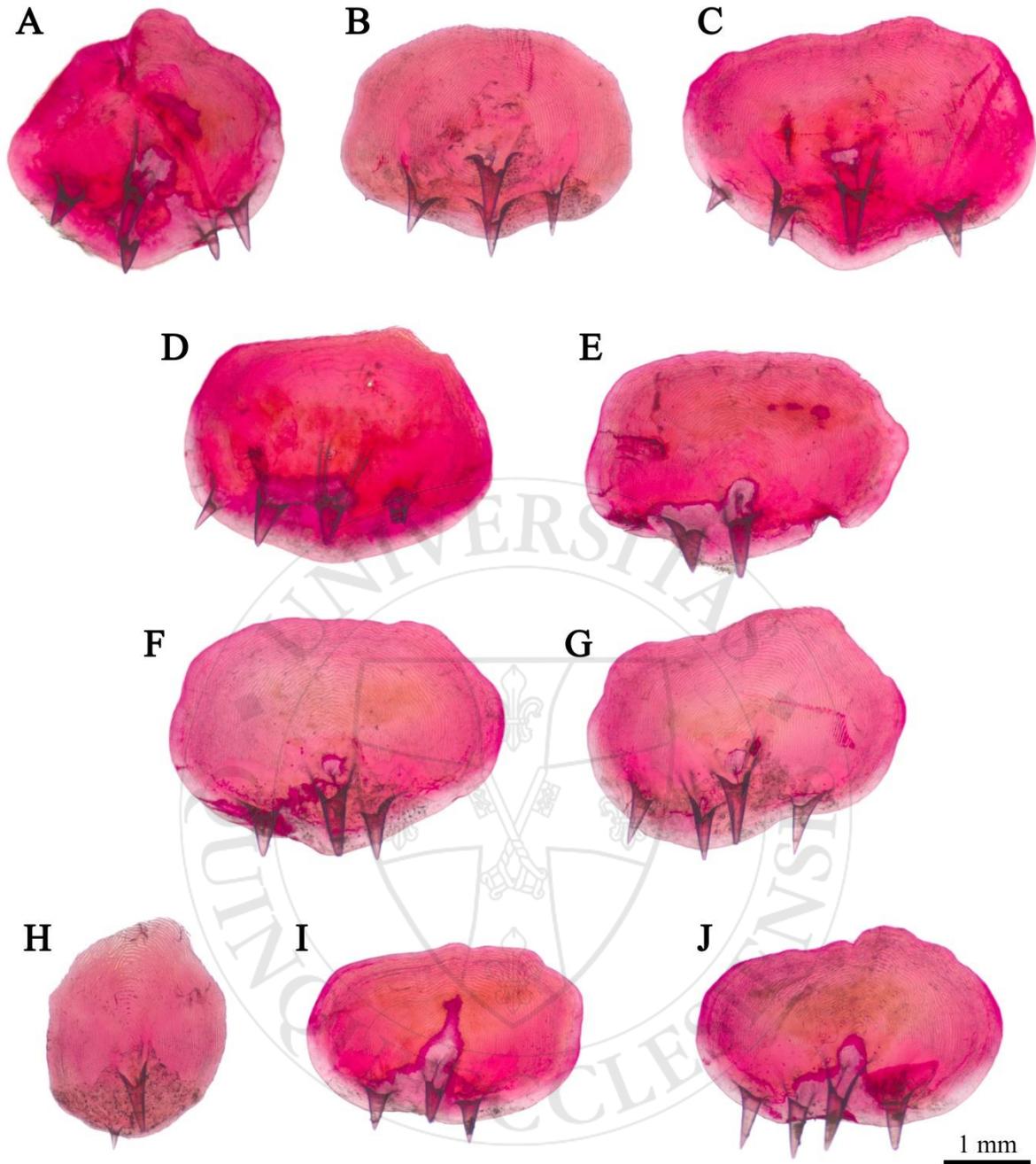
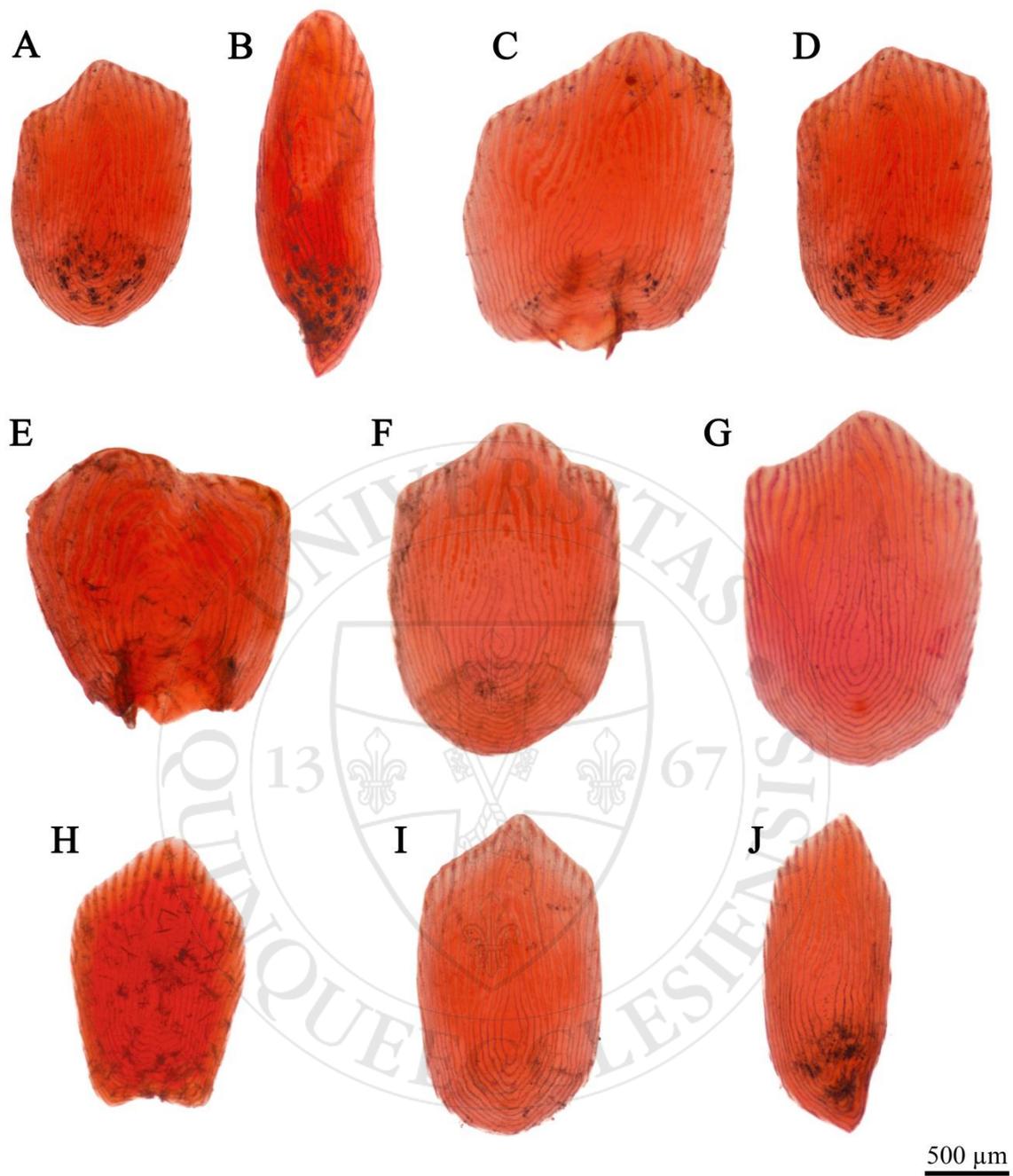


FIGURE 15. *Nezumia sclerorhynchus*

232 mm TL, Costa Brava, Spain, DMM IE/5902

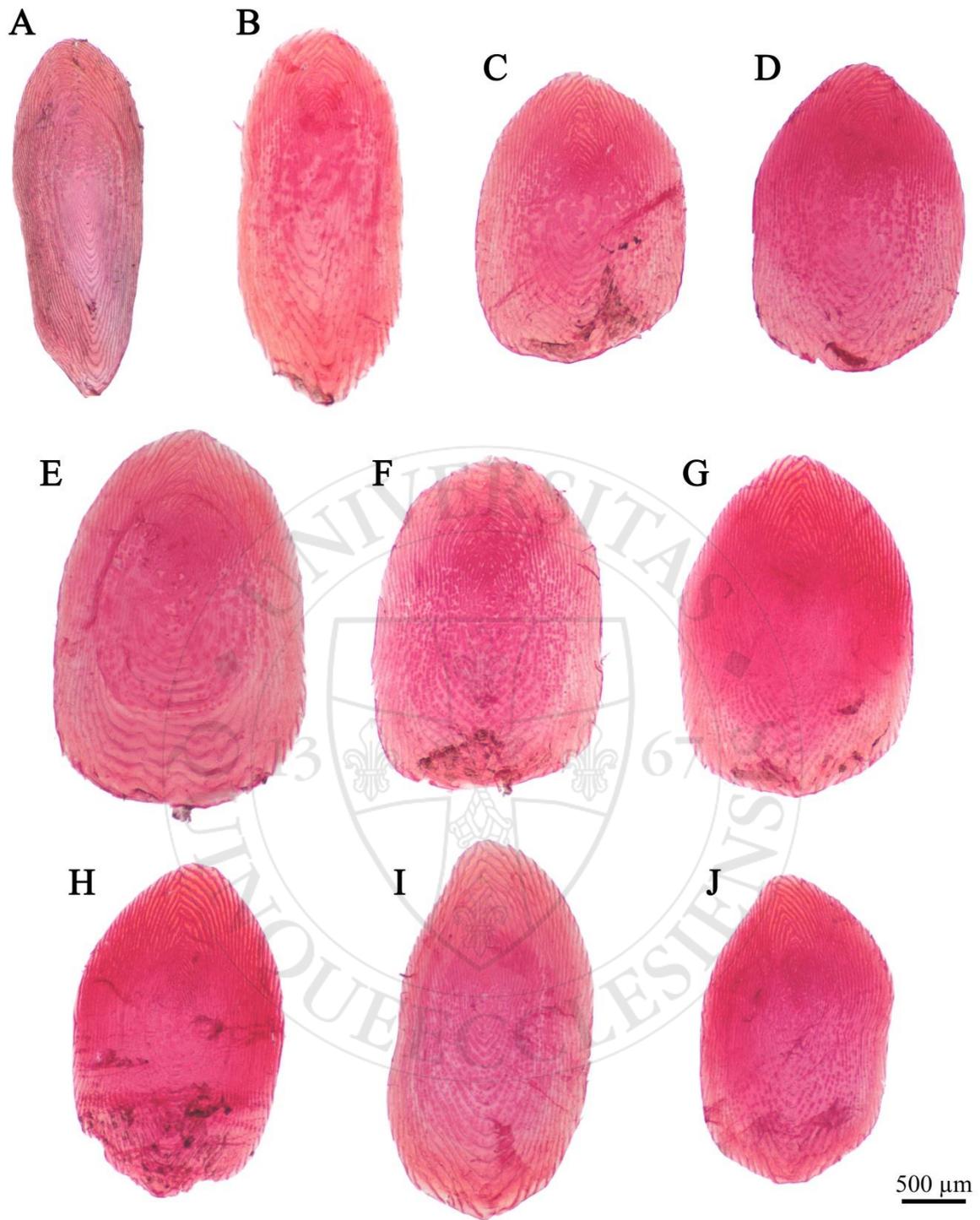


**FIGURE 16.** *Trachyrincus scabrus*  
300 mm SL, Costa Brava, Spain, DMM IE/5103

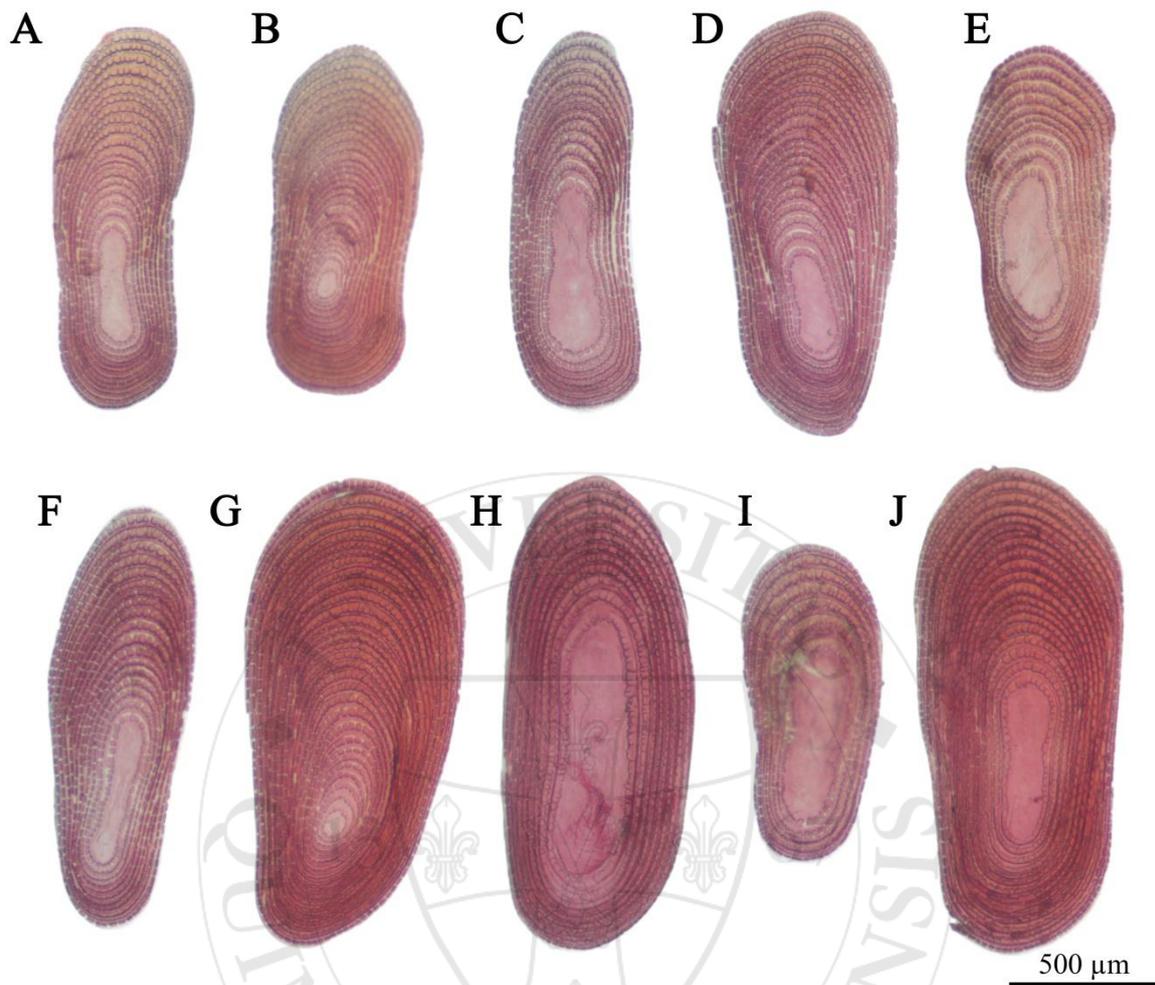


**FIGURE 17.** *Mora moro*

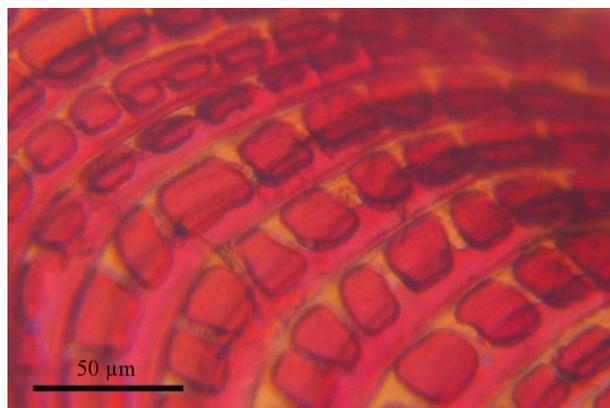
3 specimens, 86-99 mm SL, Costa Brava, Spain, DMM IE/5900



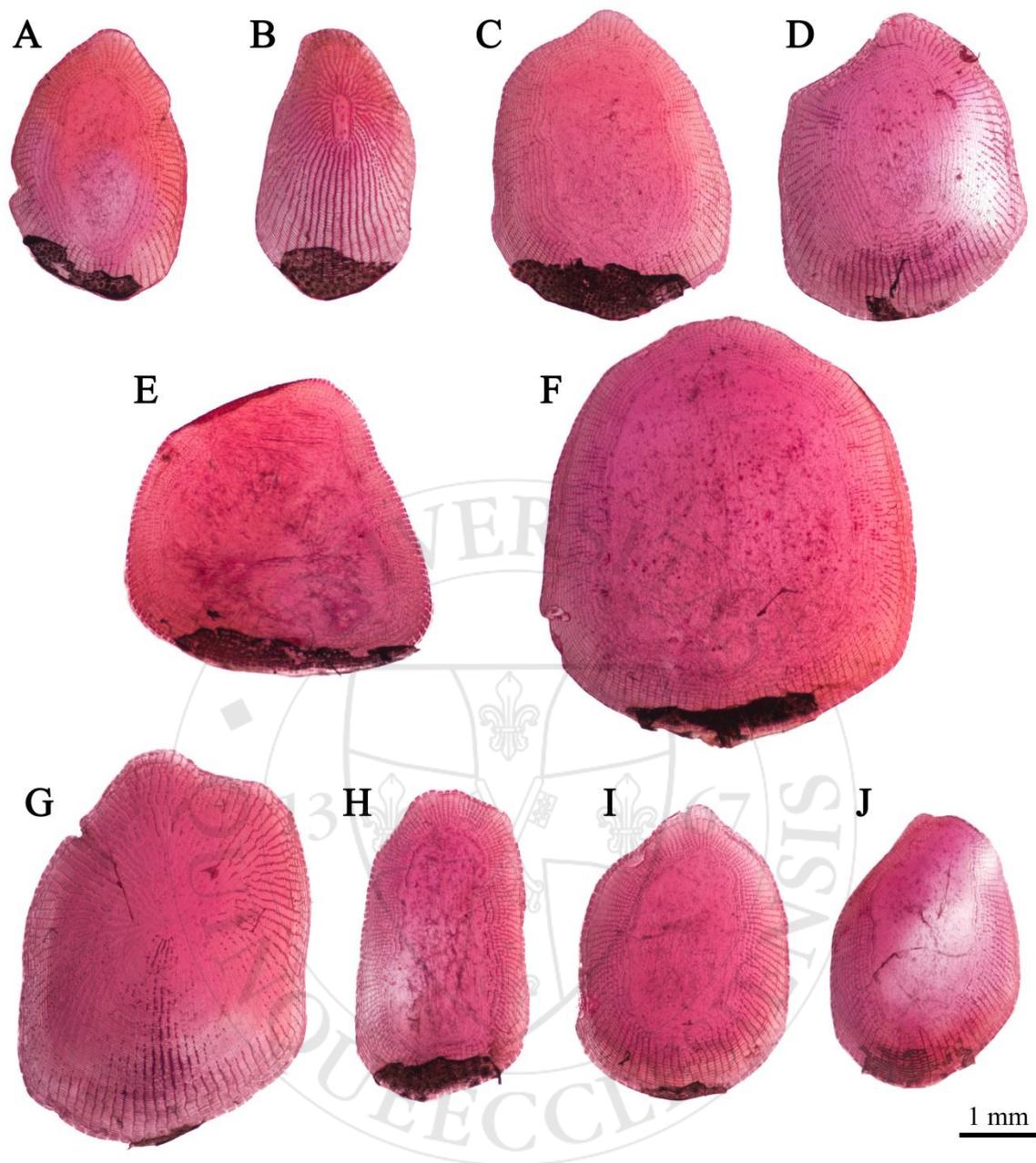
**FIGURE 18.** *Merluccius merluccius*  
323 mm SL, Mali Lošinj, Croatia, DMM IE/9010



**FIGURE 19. *Molva molva***  
340 mm SL, North Atlantic, Norway, DMM IE/5642

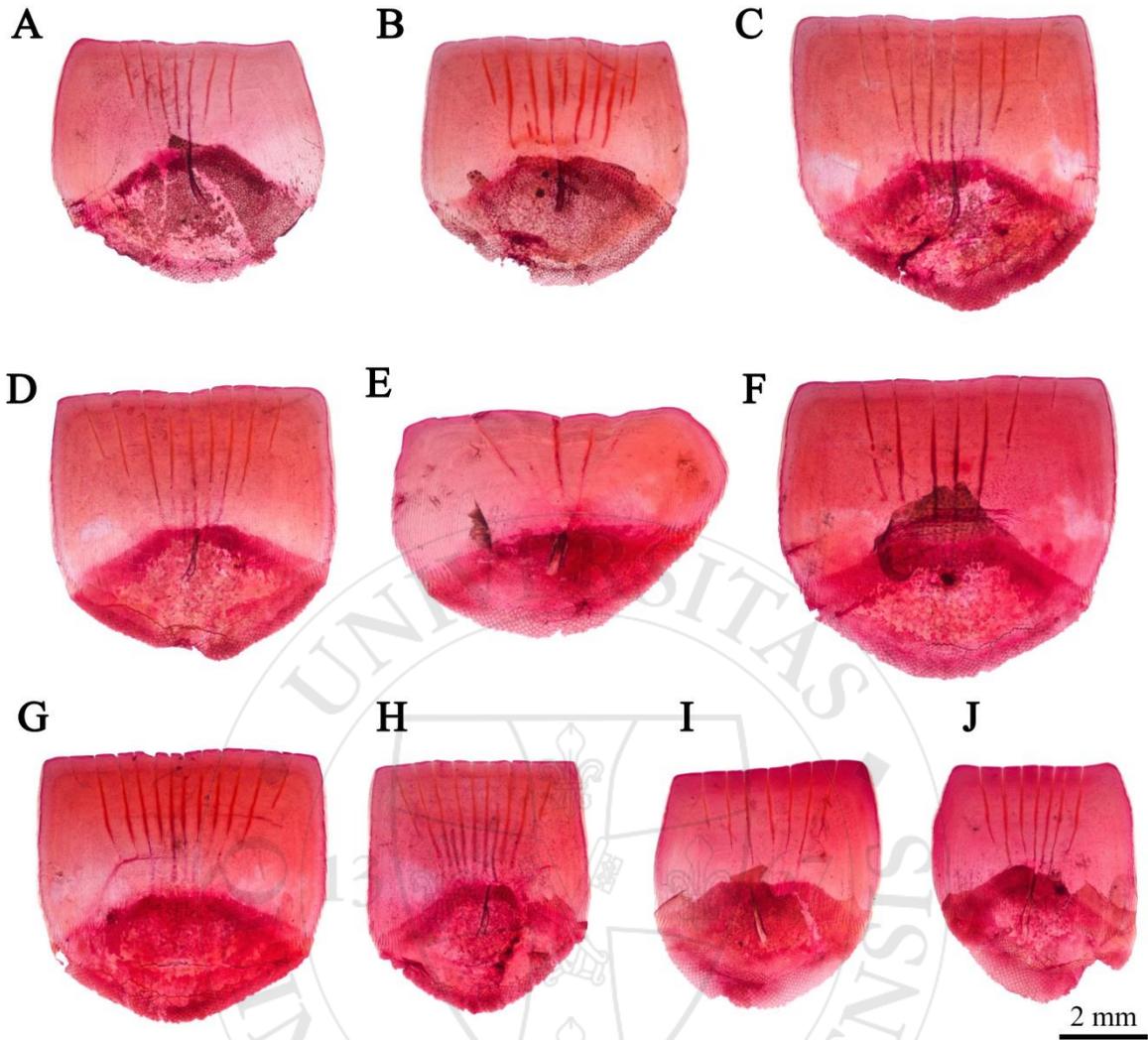


**FIGURE 20. Surface ornamentations of the scale of *M. molva* in sampling area C**

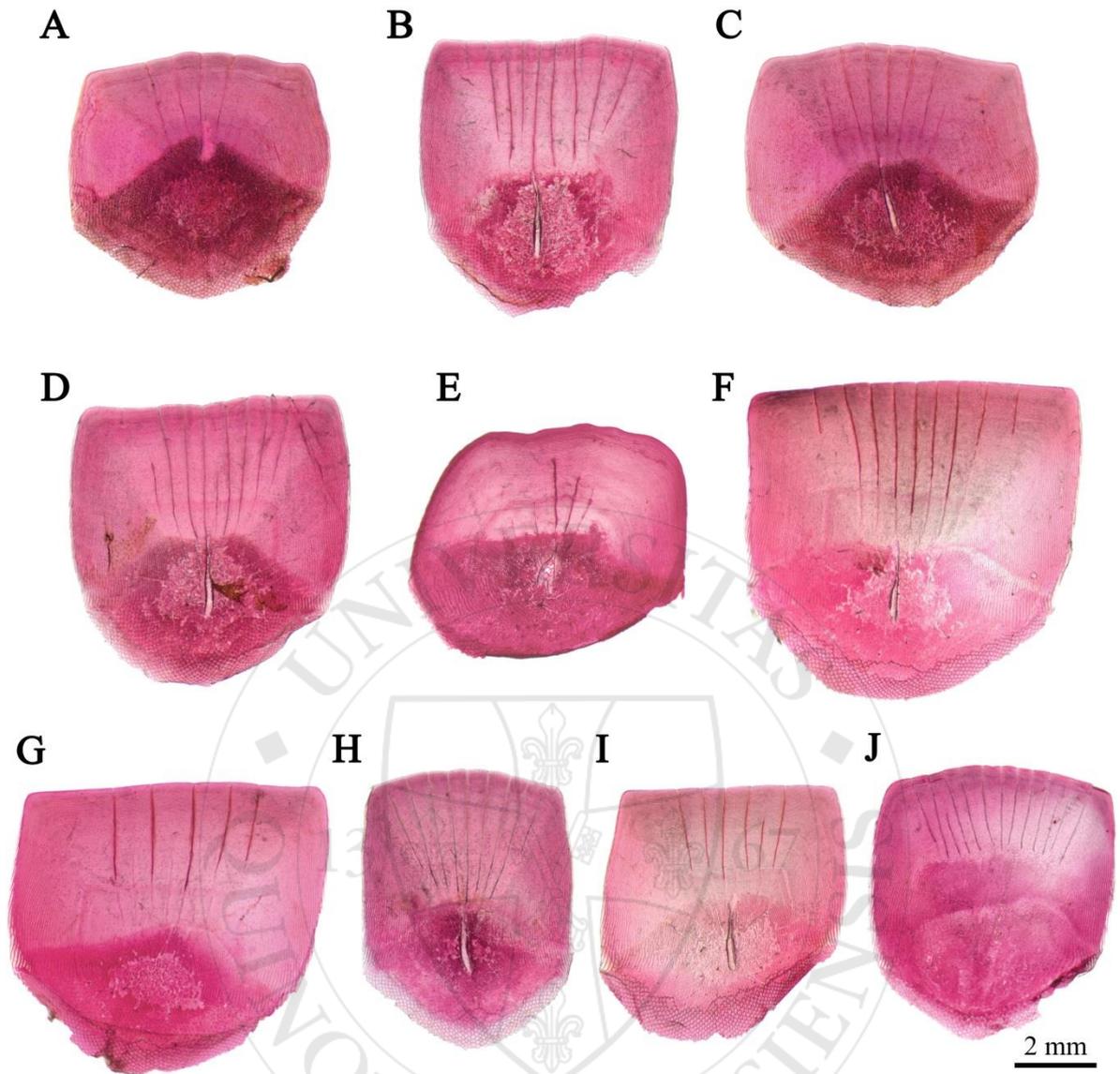


**FIGURE 21.** *Trisopterus luscus*

278 mm SL, North Atlantic, United Kingdom, DMM IE/9011

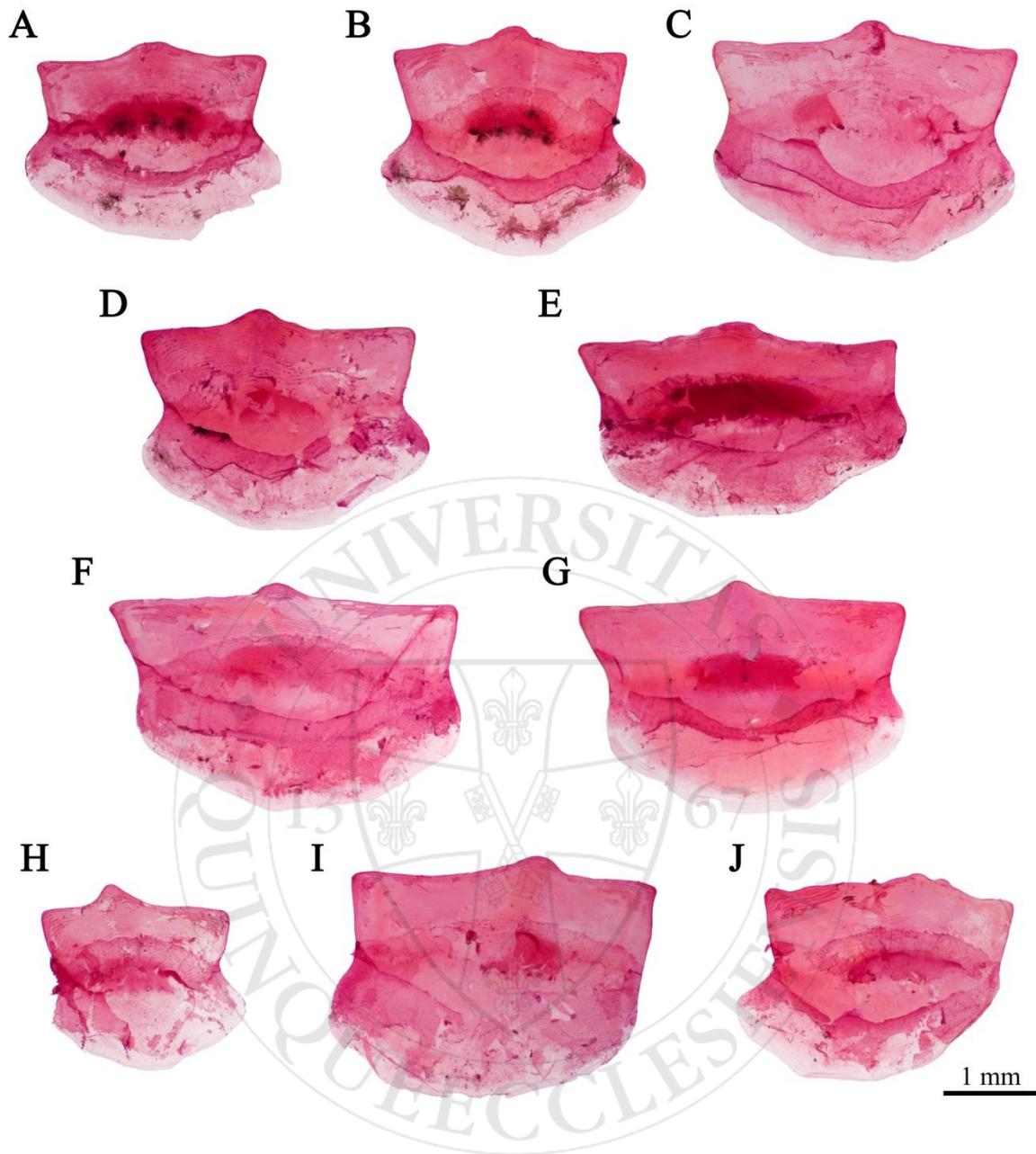


**FIGURE 22.** *Chelon labrosus*  
181 mm SL, Elba, Italy, DMM IE/6188



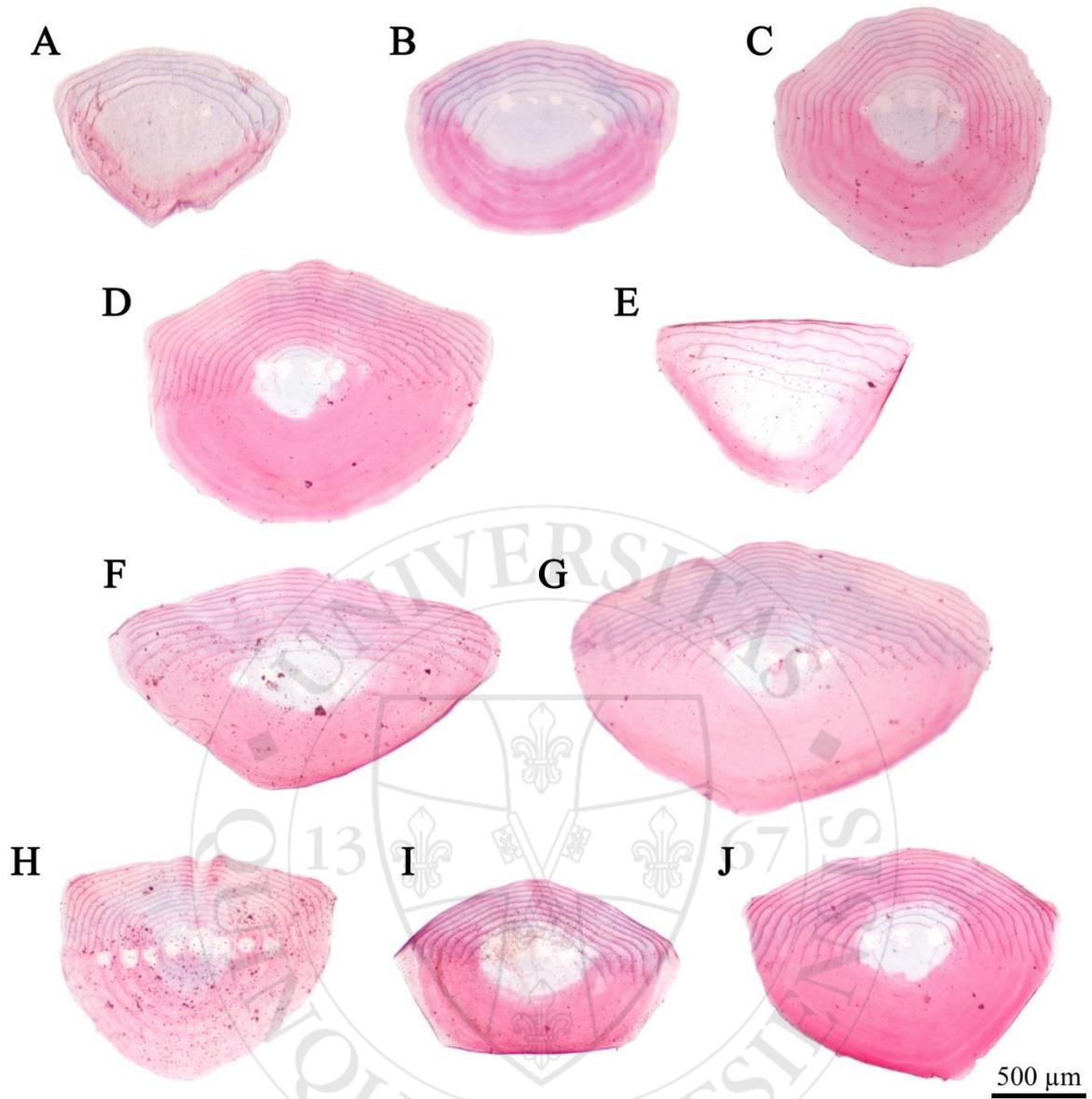
**FIGURE 23.** *Liza aurata*

207 mm SL, Gulf of Ambracia, Greece, DMM IE/9012



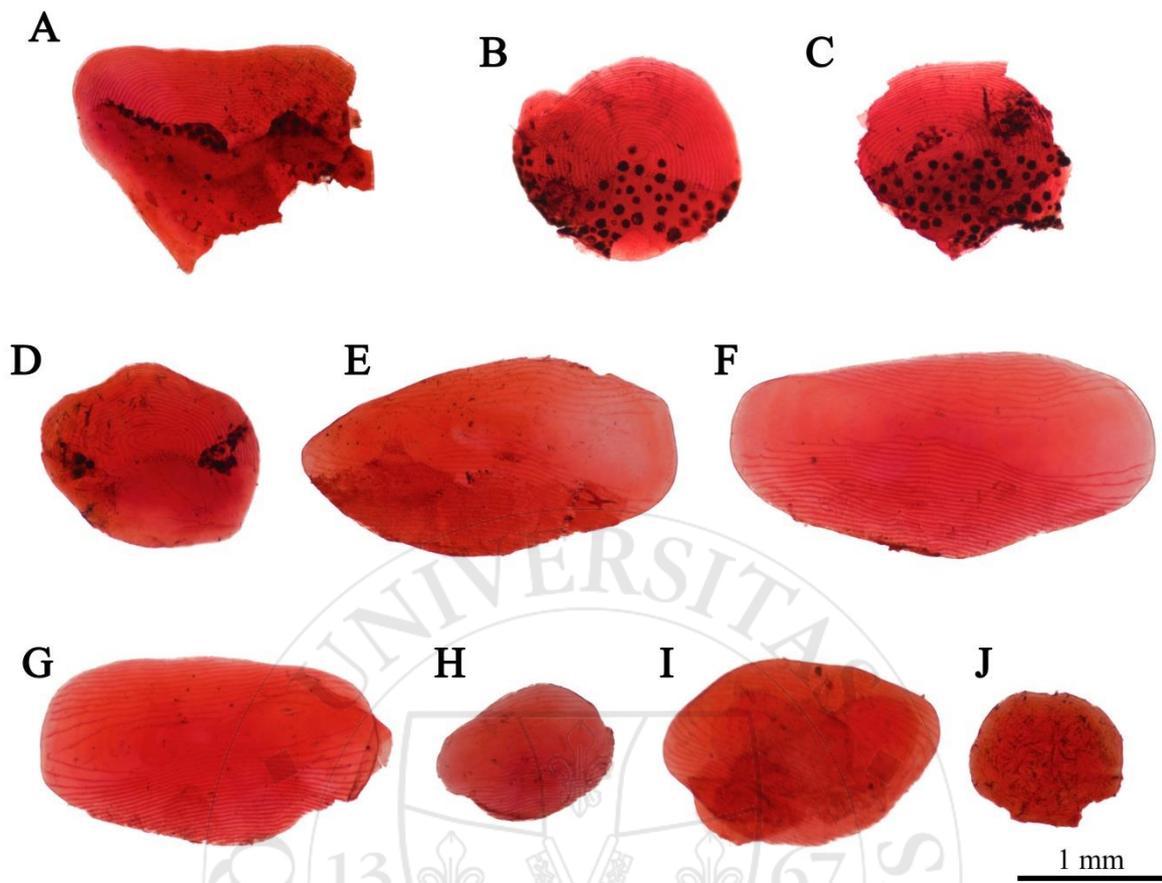
**FIGURE 24.** *Atherina hepsetus*

101 mm SL, Costa Brava, Spain, DMM IE/5080

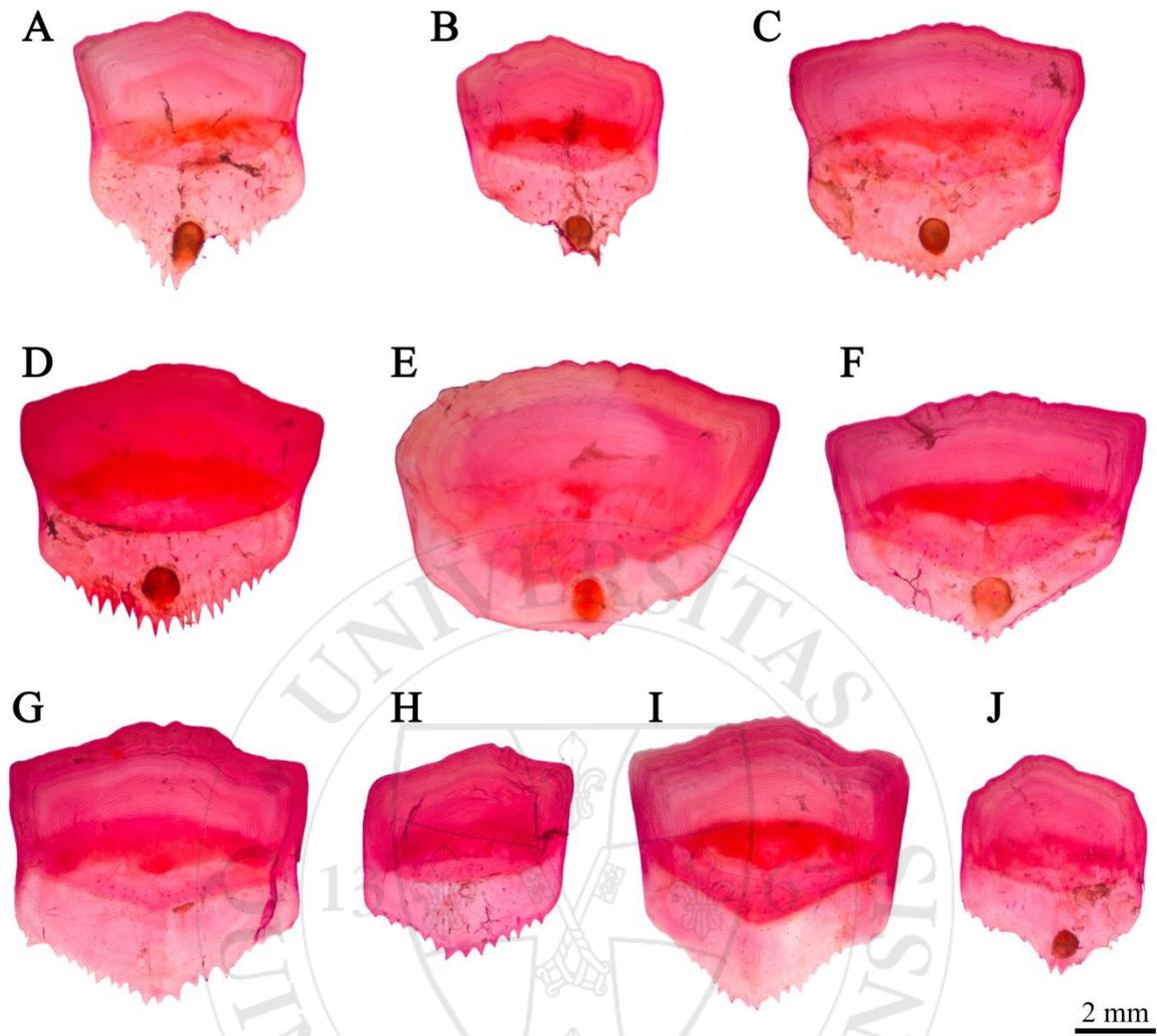


**FIGURE 25.** *Atherinomorus lacunosus*

72 mm SL, Turkey, DMM IE/9013

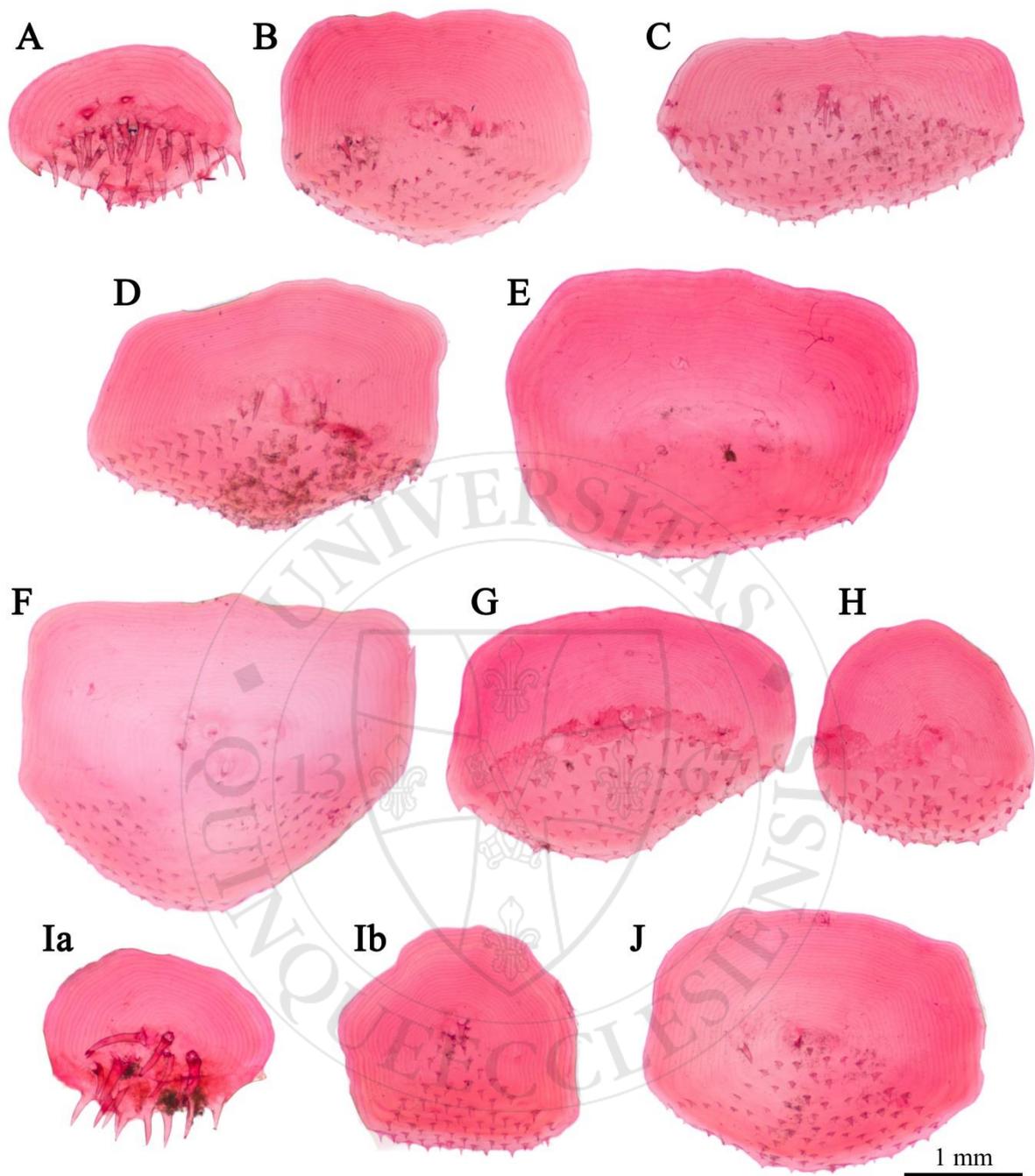


**FIGURE 26.** *Belone belone*  
222 mm SL, Elba, Italy, DMM IE/6175



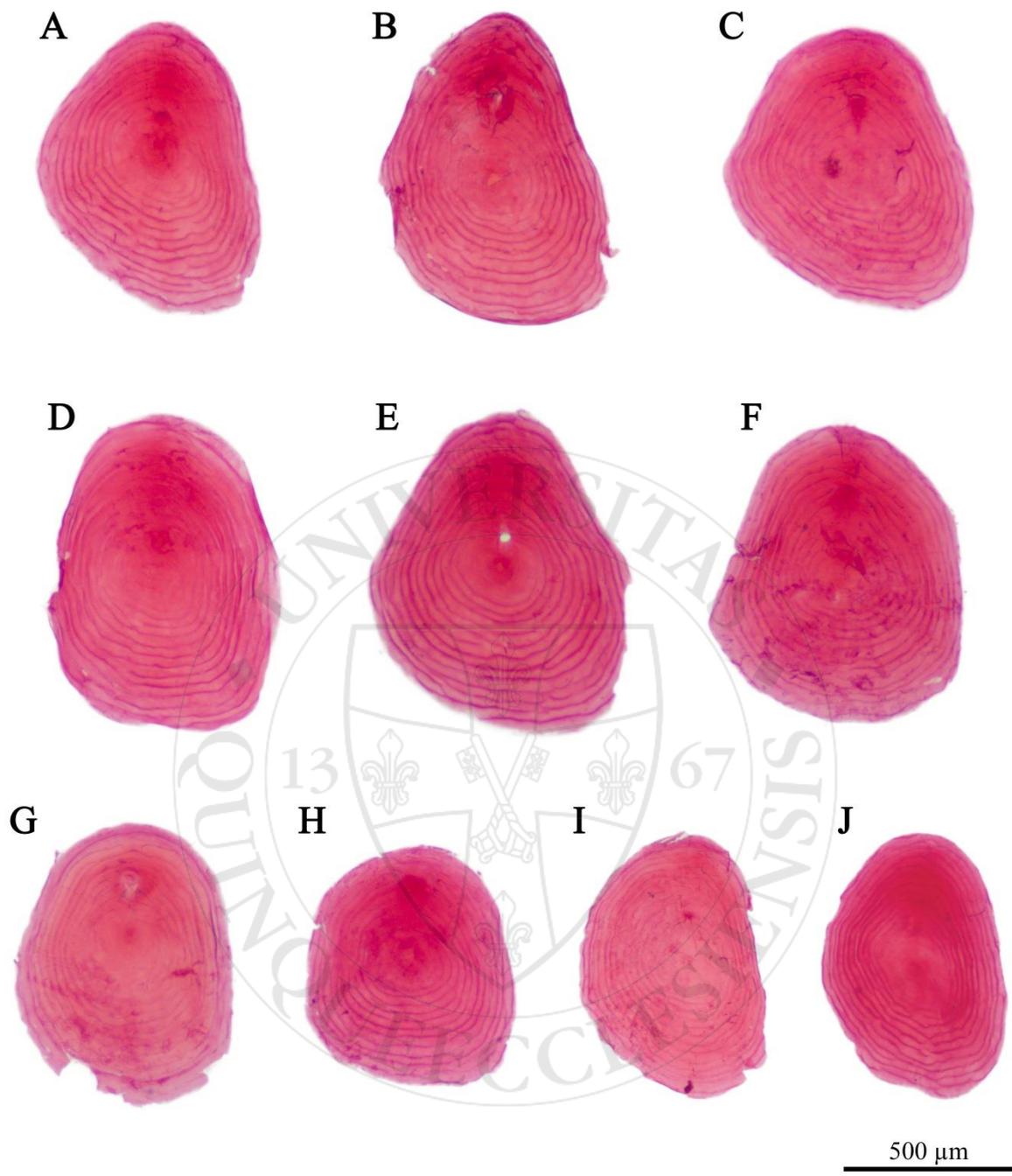
**FIGURE 27.** *Beryx decadactylus*

313 mm SL, Central Atlantic, Azores, DMM IE/6718

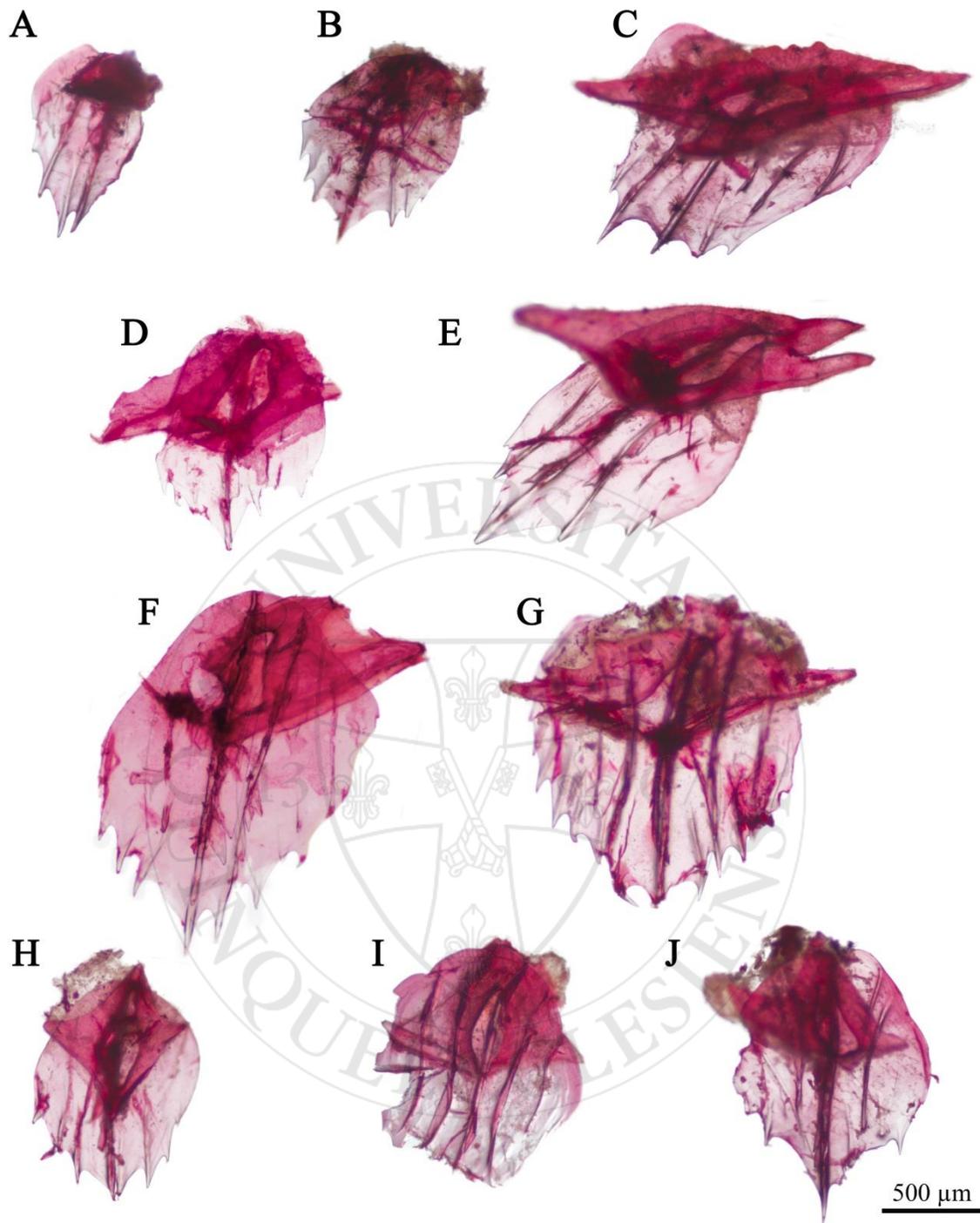


**FIGURE 28.** *Hoplostethus mediterraneus*

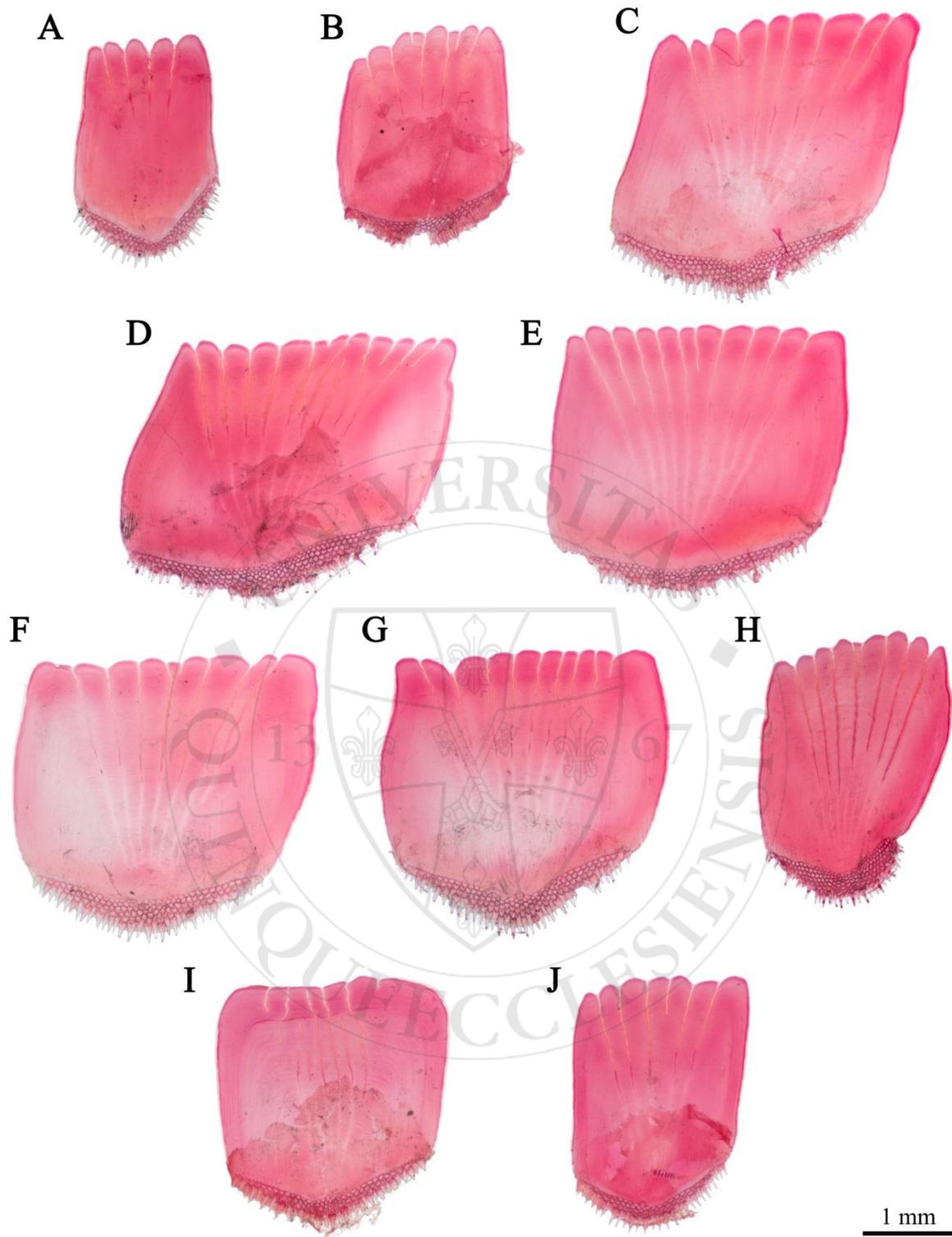
109 mm SL, Costa Brava, Spain, DMM IE/5896



**FIGURE 29.** *Zeus faber*  
82 mm SL, Costa Brava, Spain, DMM IE/5039

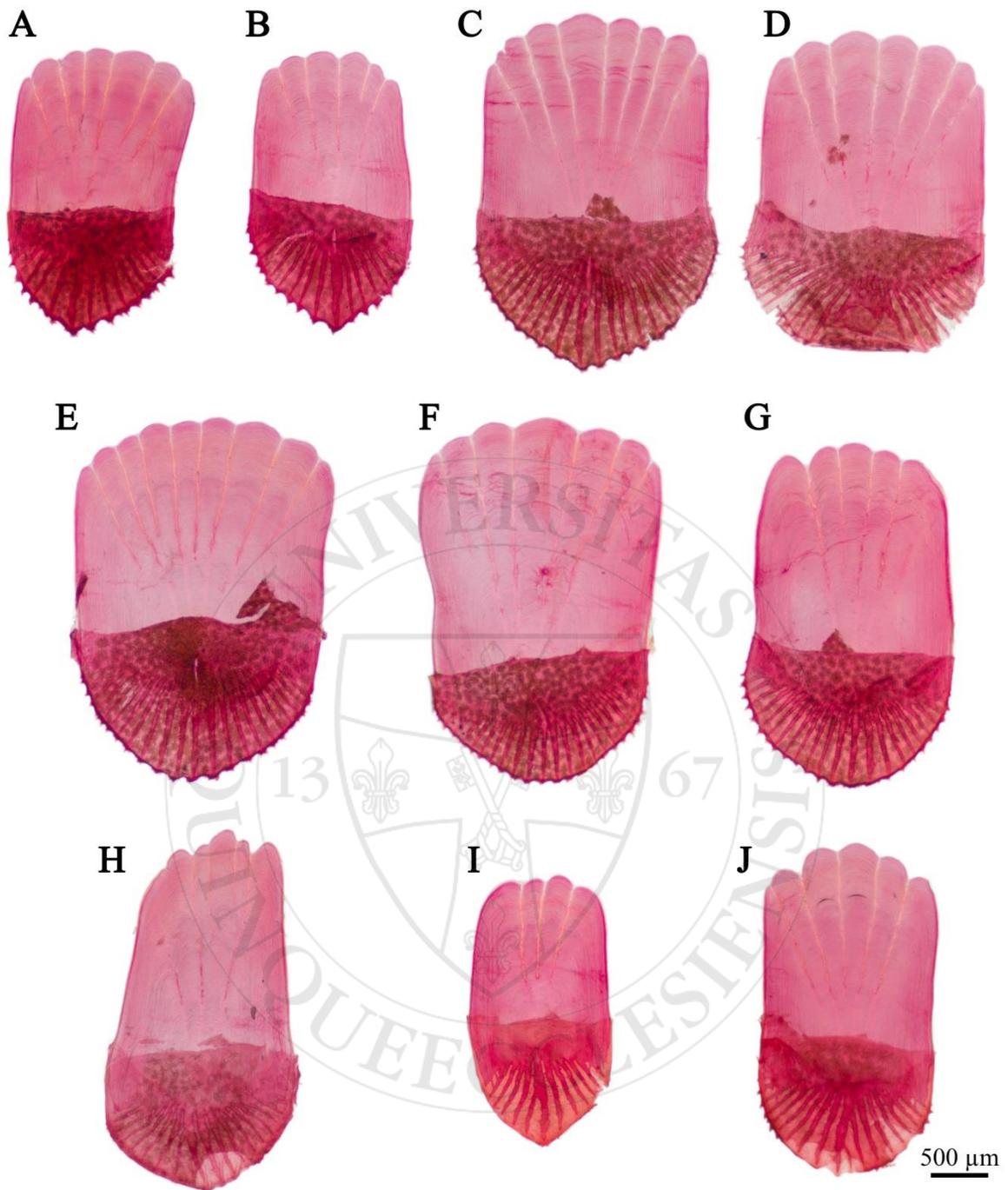


**FIGURE 30.** *Macrorhamphosus scolopax*  
114 mm SL, Central Atlantic, Azores, DMM IE/6520



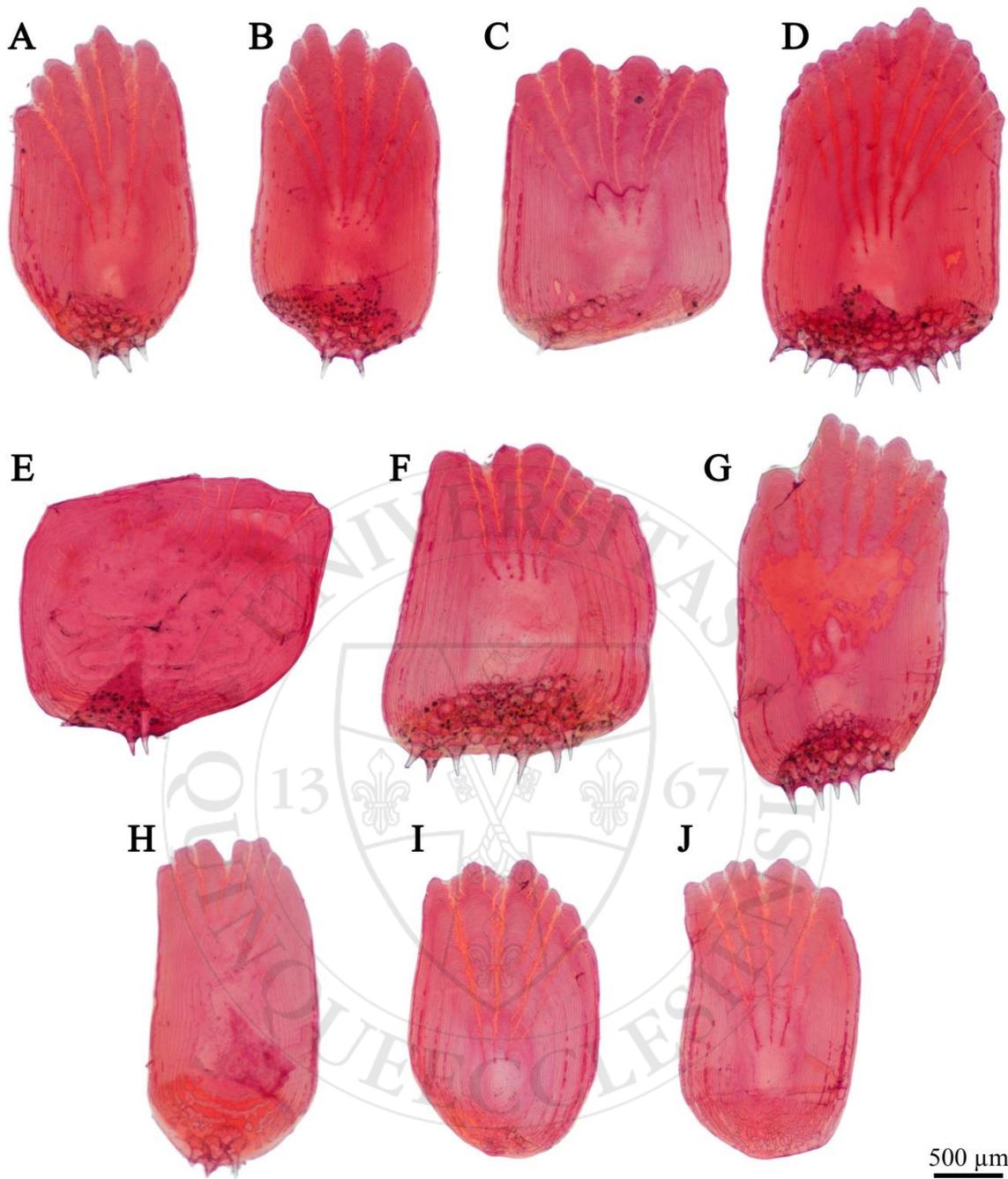
**FIGURE 31.** *Helicolenus dactylopterus*

131 mm SL, Costa Brava, Spain, DMM IE/5818



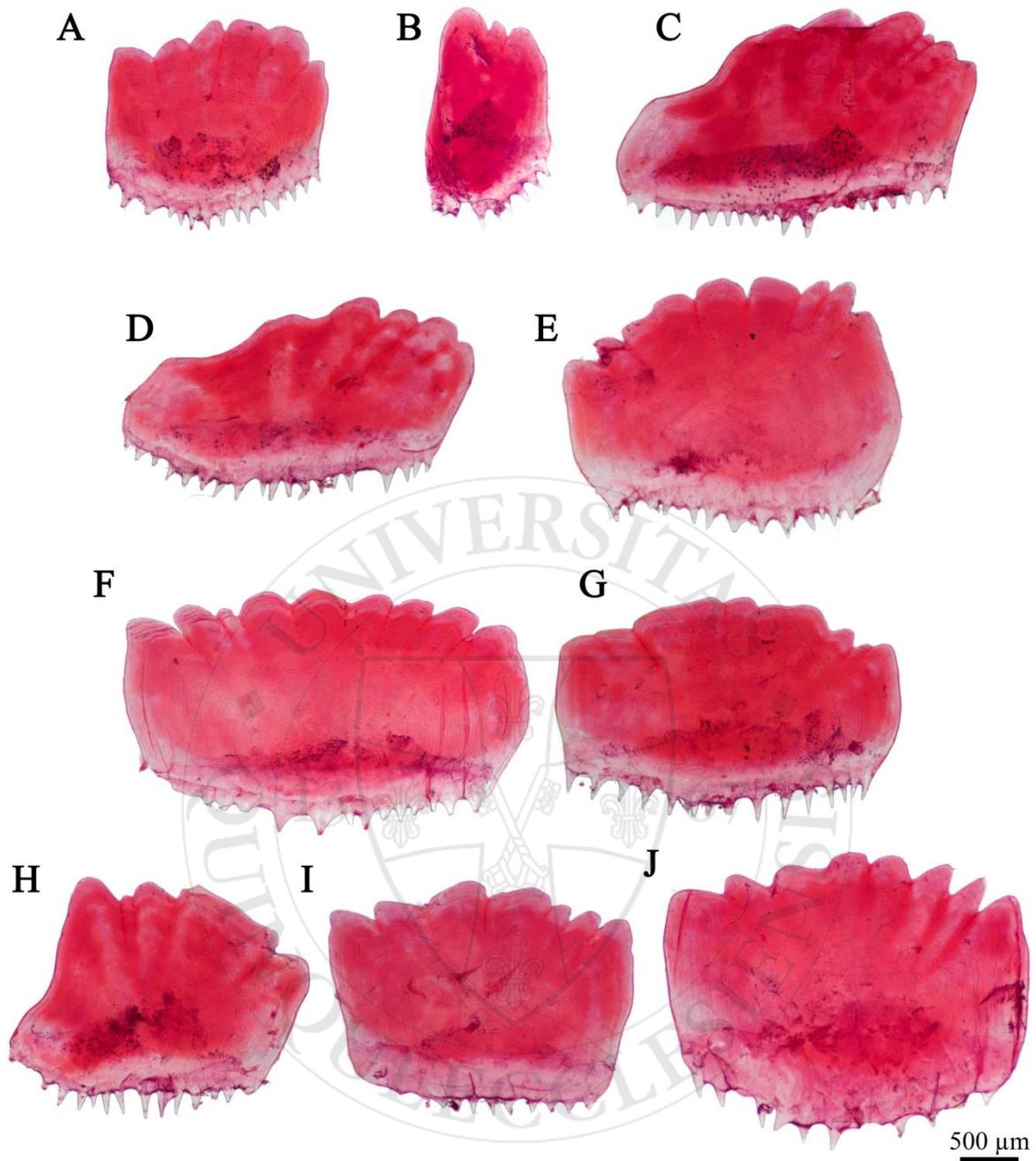
**FIGURE 32.** *Scorpaena porcus*

114 mm SL, Costa Brava, Spain, DMM IE/4571

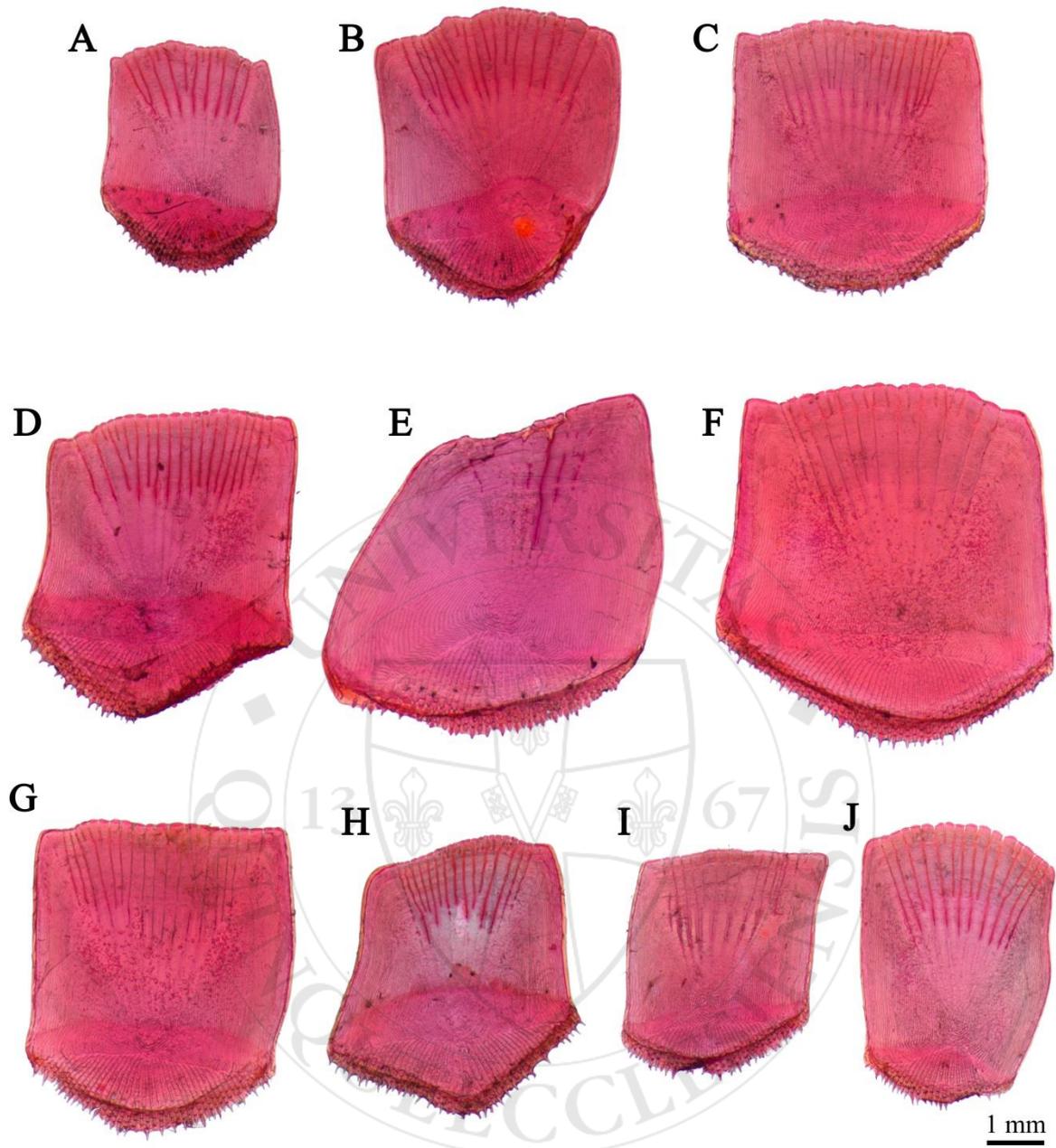


**FIGURE 33.** *Eutrigla gurnardus*

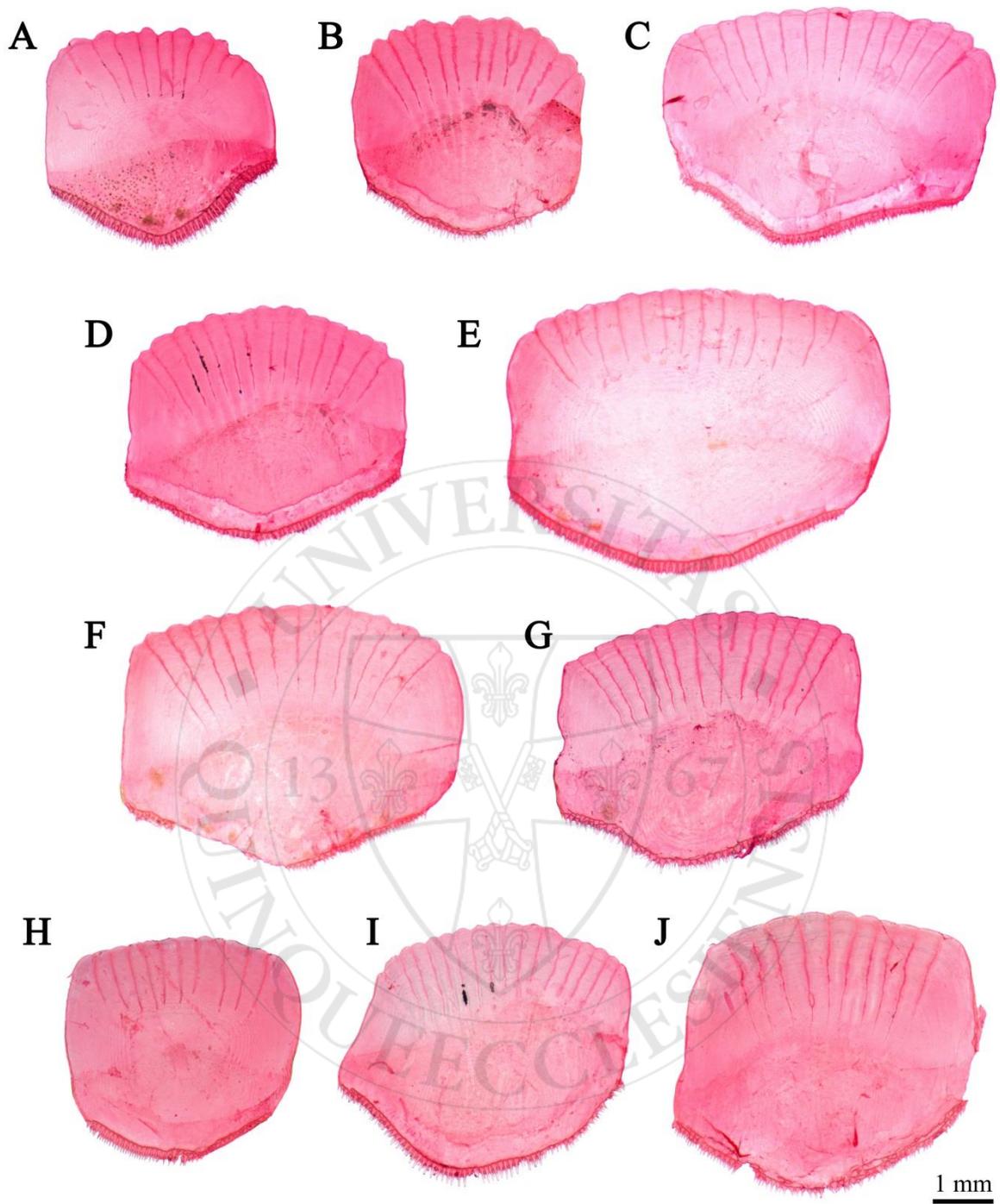
220 mm SL, Costa Brava, Spain, DMM IE/5089



**FIGURE 34.** *Lepidotrigla cavillone*  
87 mm SL, Costa Brava, Spain, DMM IE/4993

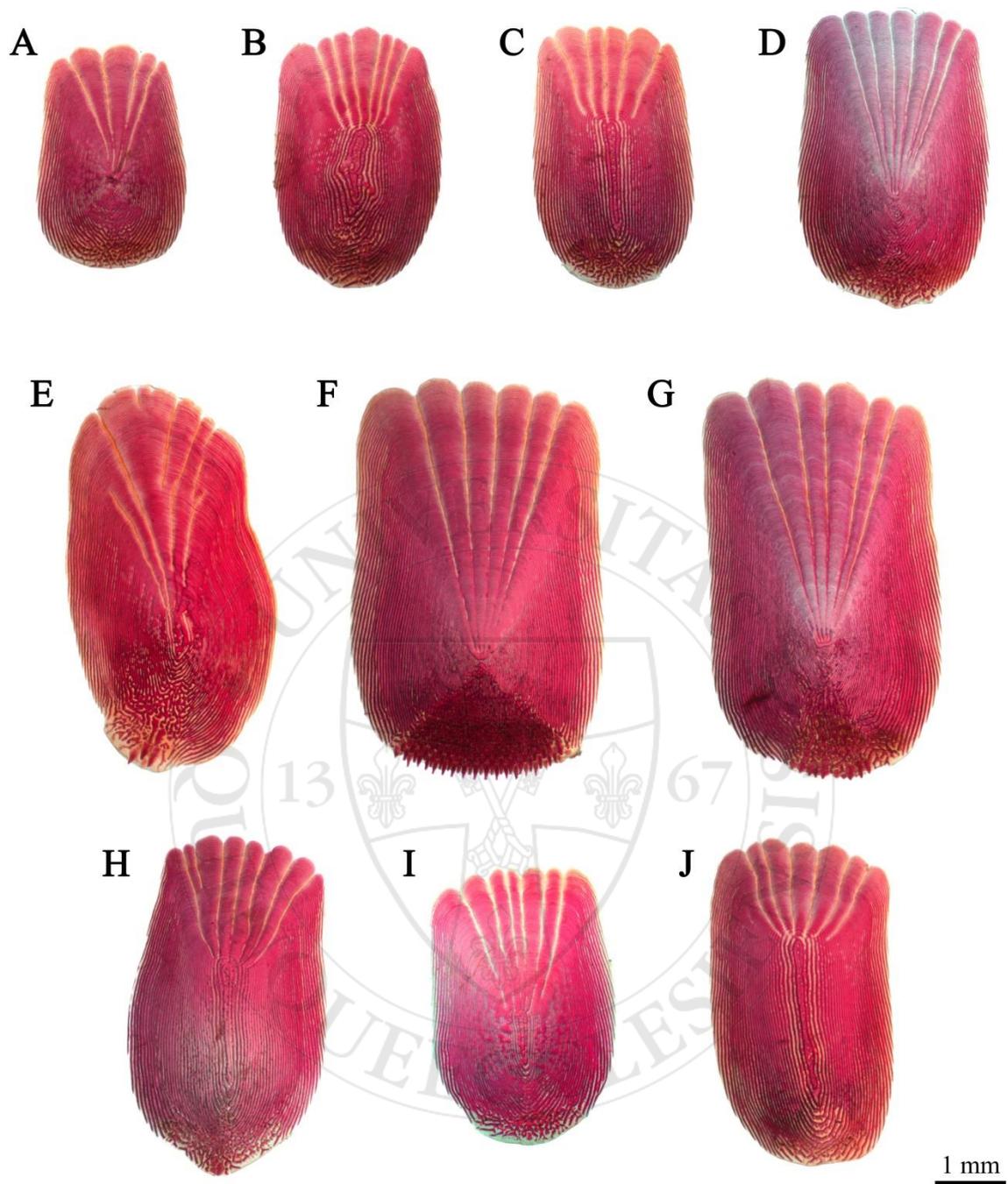


**FIGURE 35.** *Dicentrarchus labrax*  
264 mm SL, Mali Lošinj, Croatia, DMM IE/9014



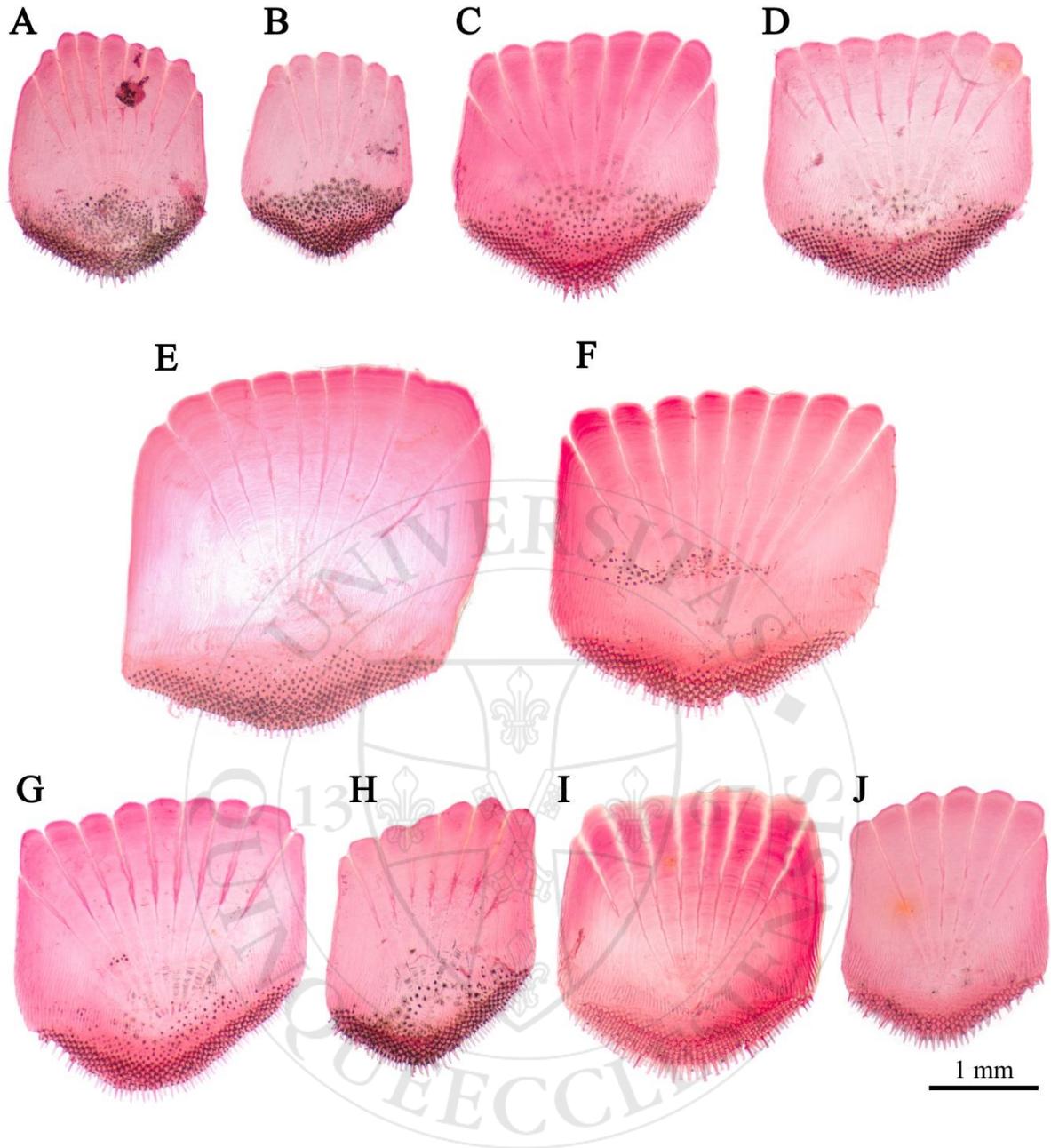
**FIGURE 36.** *Anthias anthias*

134 mm SL, Costa Brava, Spain, DMM IE/4594



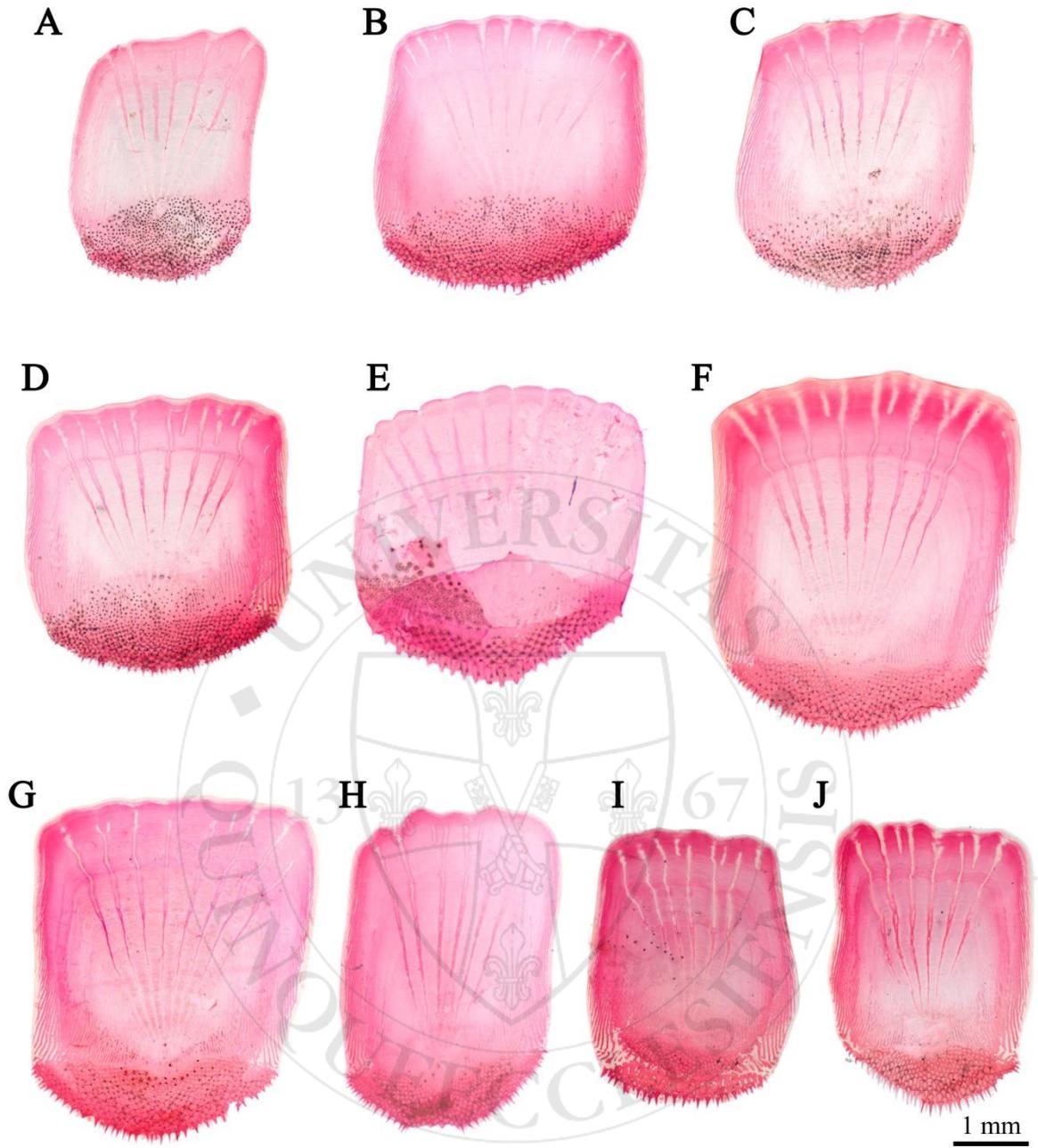
**FIGURE 37.** *Epinephelus aeneus*

264 mm SL, Central East Atlantic, Morocco, DMM IE/1233



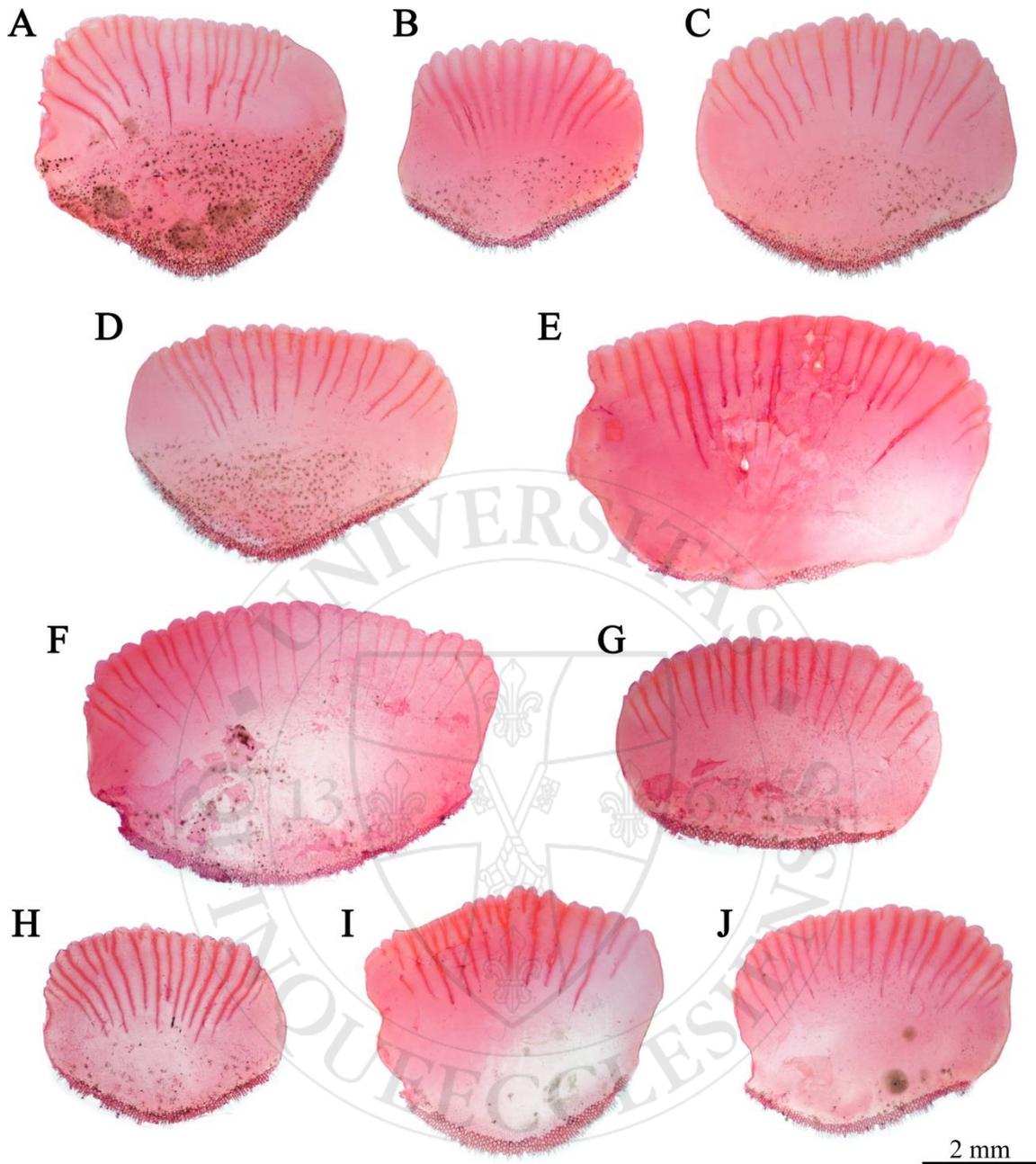
**FIGURE 38.** *Serranus cabrilla*

137 mm SL, aquarium specimen, Spain, DMM IE/5942



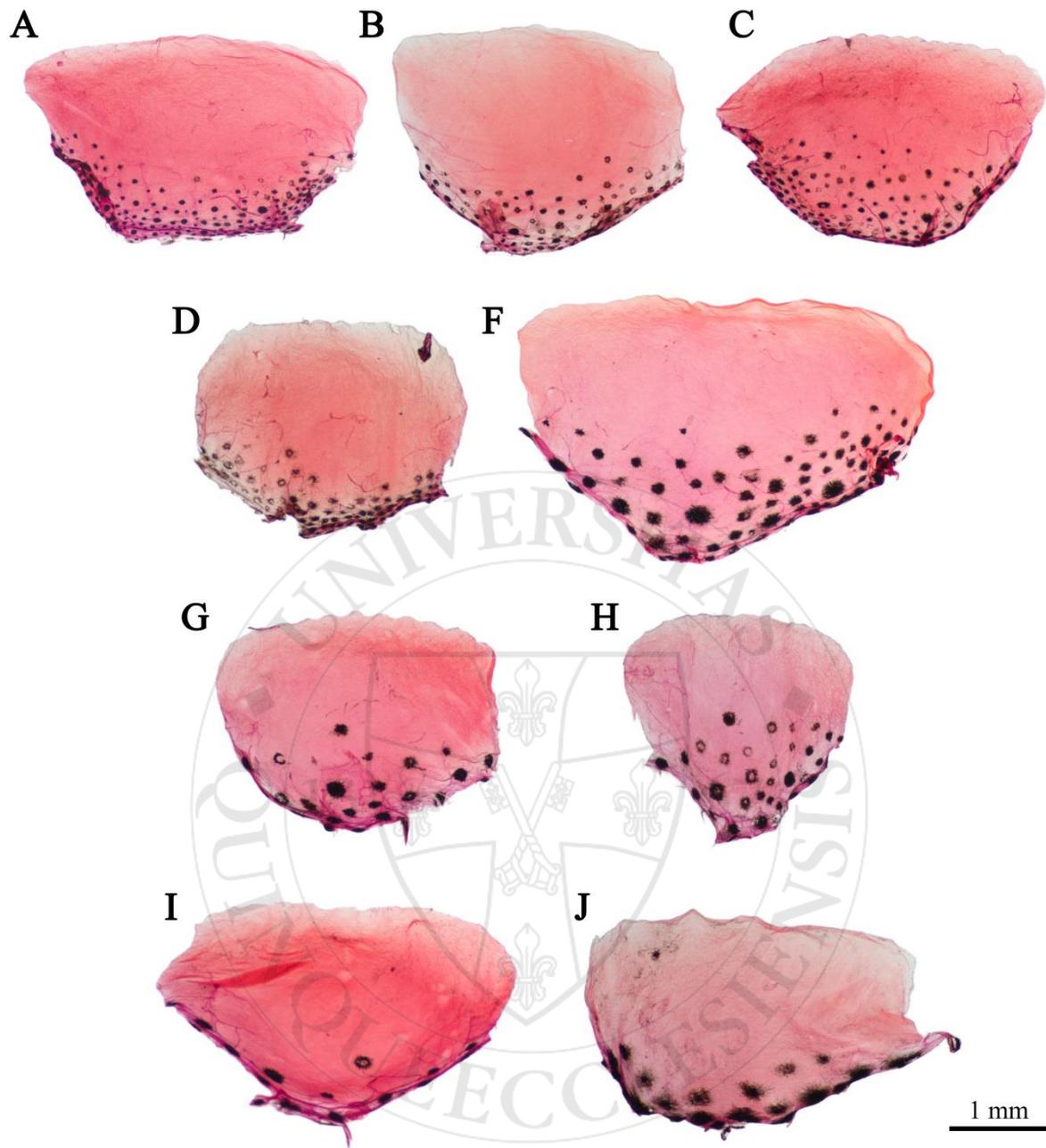
**FIGURE 39.** *Serranus scriba*

183 mm SL, Costa Brava, Spain, DMM IE/5024



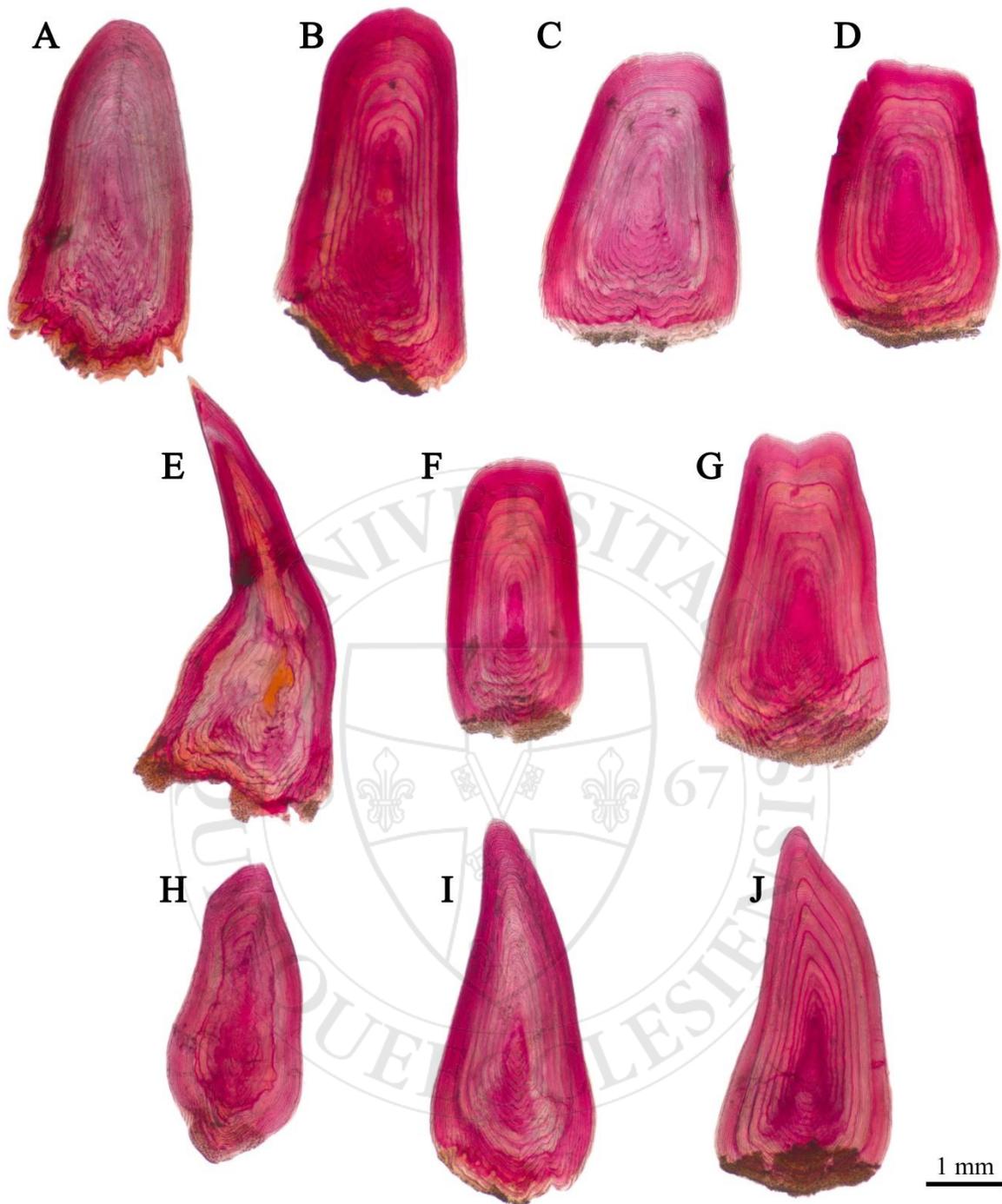
**FIGURE 40.** *Apogon imberbis*

89 mm SL, aquarium specimen, DMM IE/5593

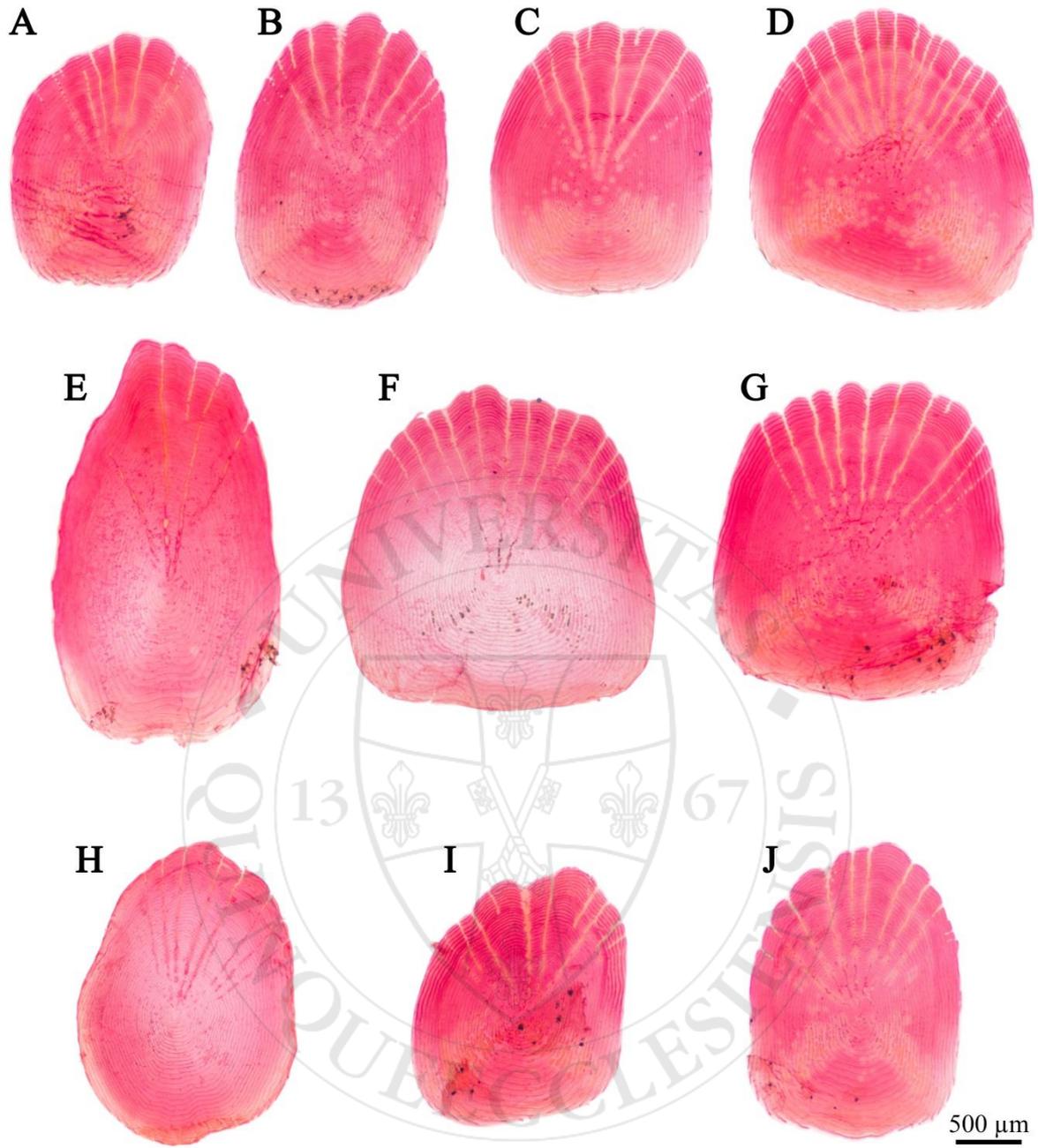


**FIGURE 41.** *Epigonus denticulatus*

117 mm SL, Costa Brava, Spain, DMM IE/4642

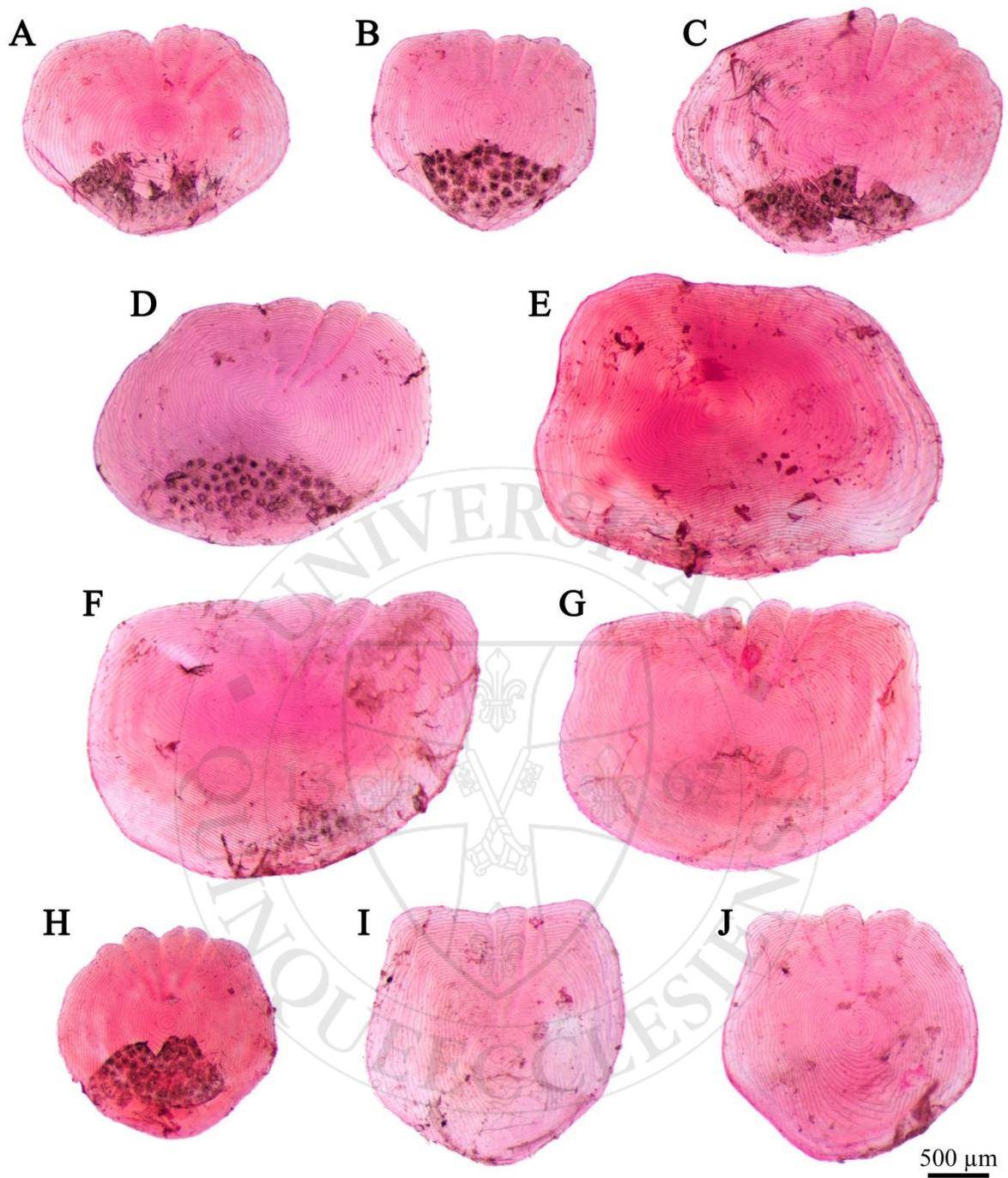


**FIGURE 42.** *Coryphaena equiselis*  
434 mm SL, Algarve, Portugal, DMM IE/5566

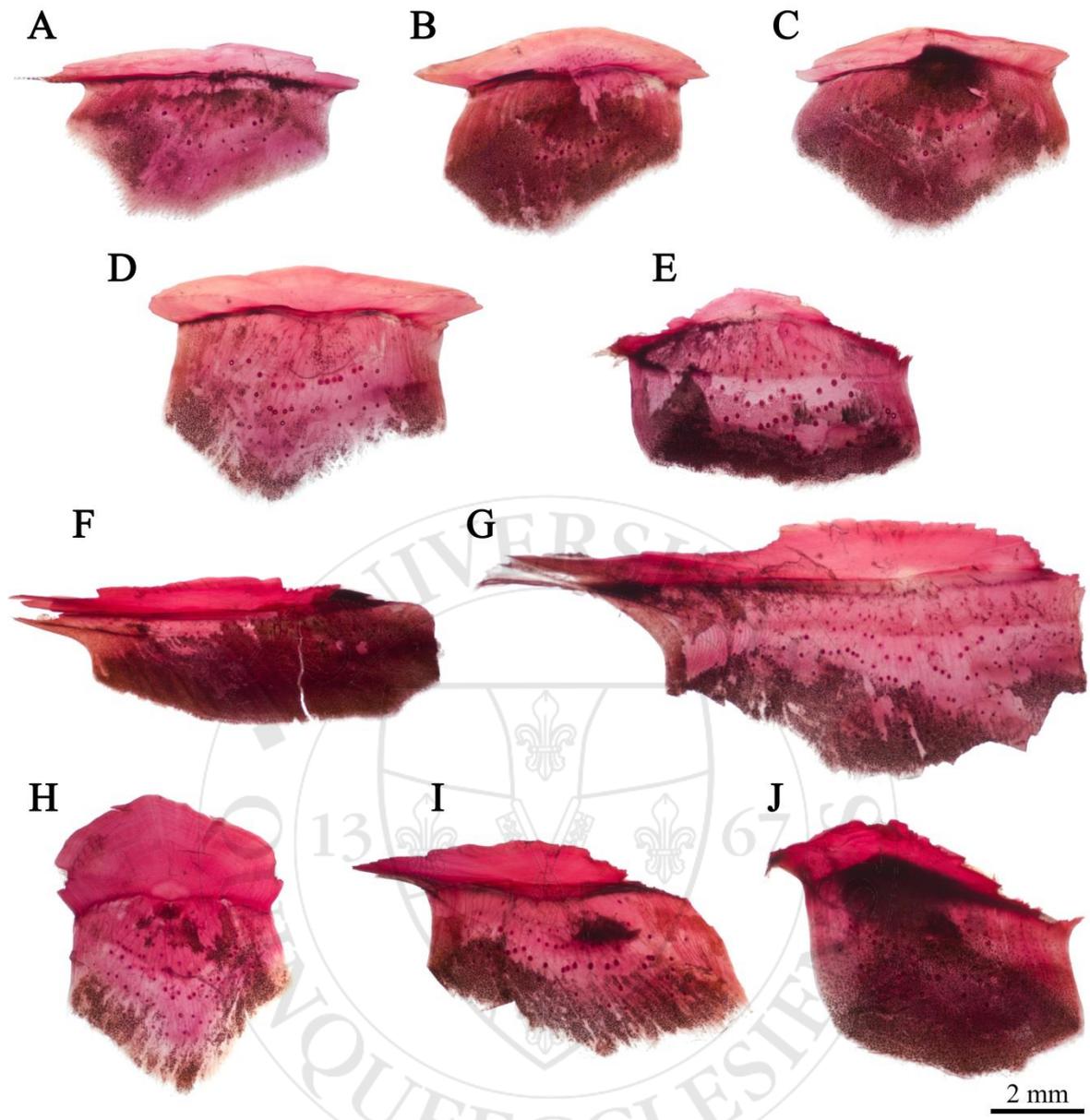


**FIGURE 43.** *Seriola dumerili*

233 mm SL, Costa Brava, Spain, DMM IE/5000

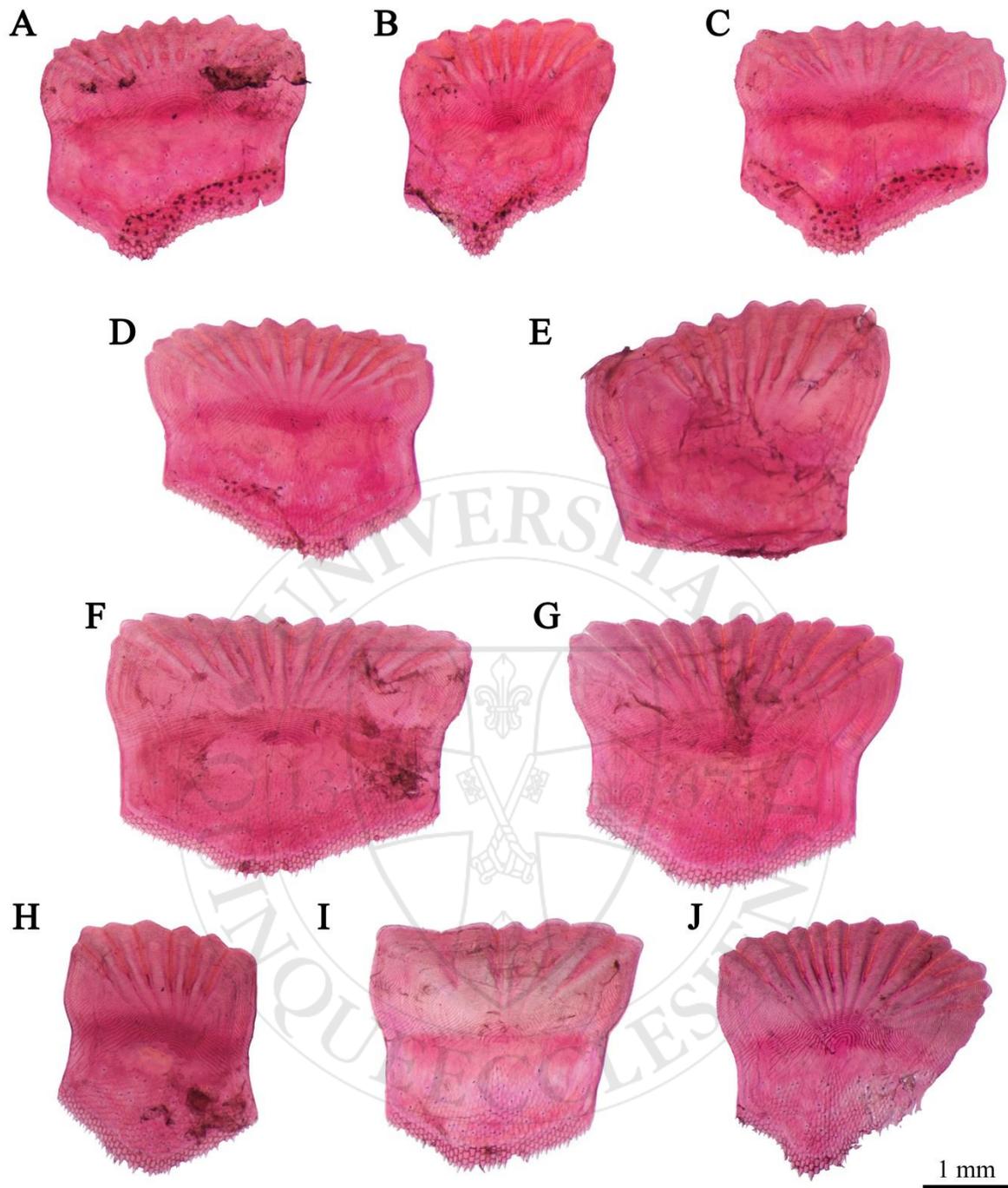


**FIGURE 44.** *Trachurus trachurus*  
199 mm SL, Mali Lošinj, Croatia, DMM IE/9015



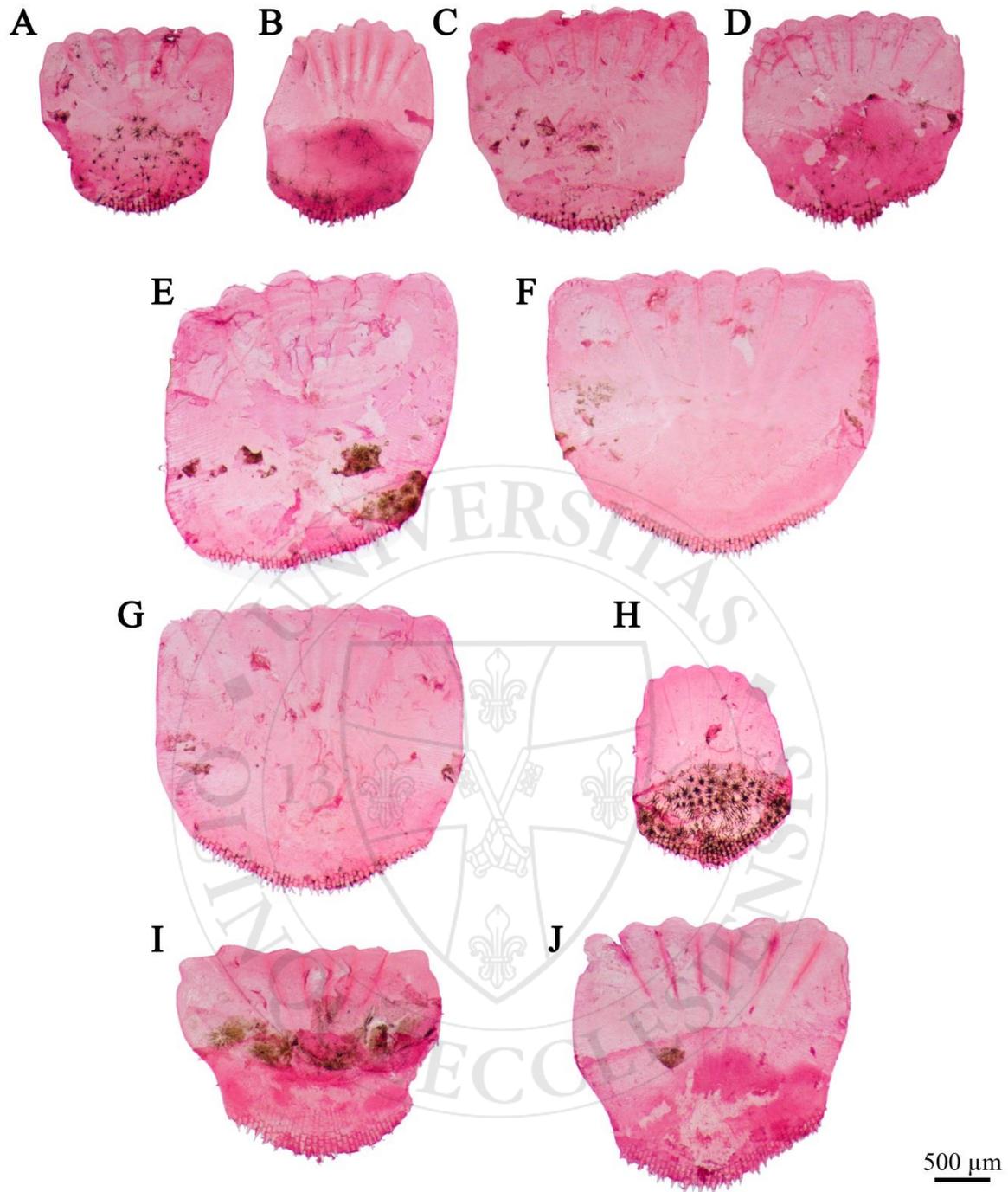
**FIGURE 45. *Brama brama***

325 mm SL, Central East Atlantic, Morocco, DMM IE/0451

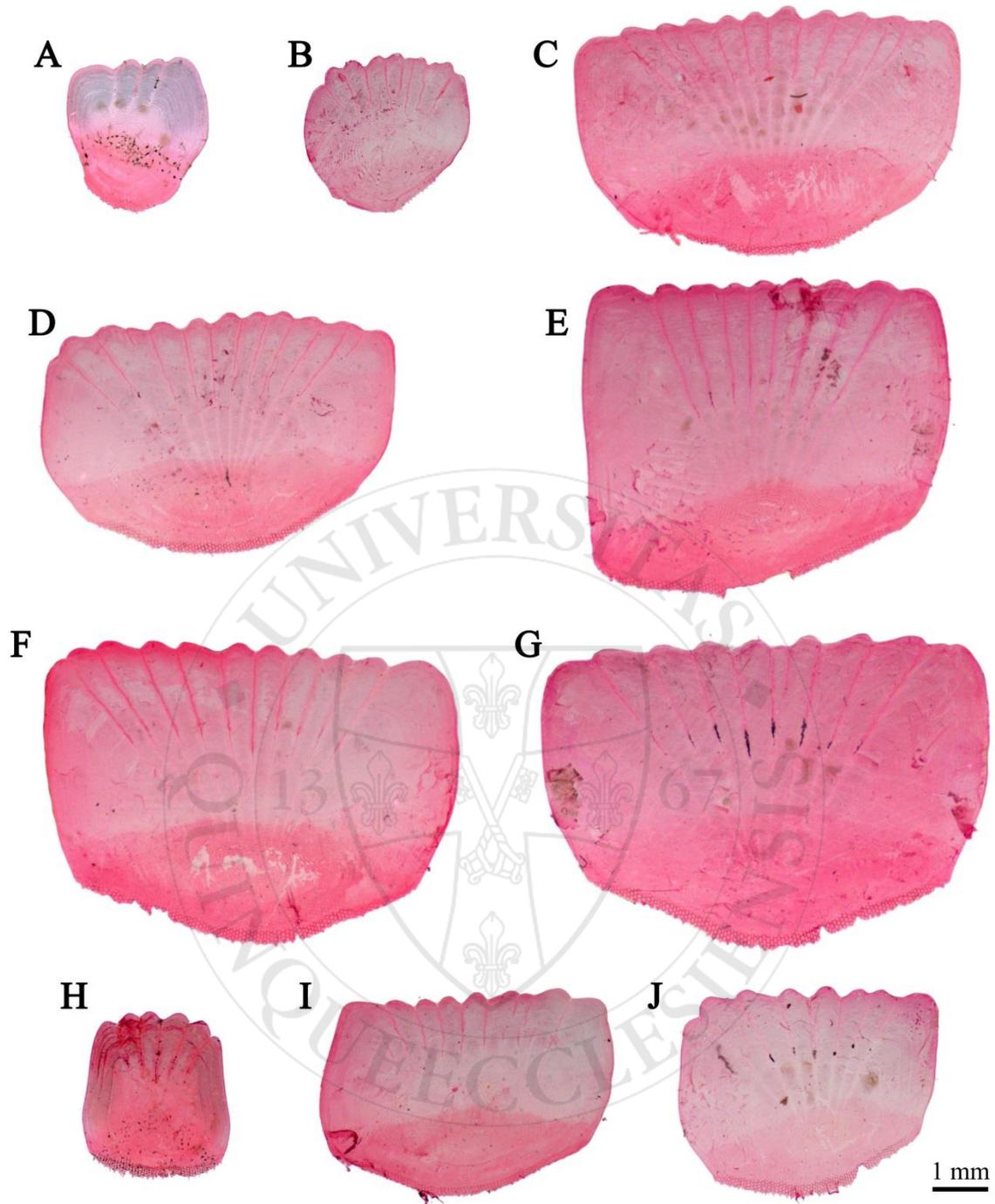


**FIGURE 46.** *Boops boops*

128 mm SL, Gulf of Ambracia, Greece, DMM IE/9016

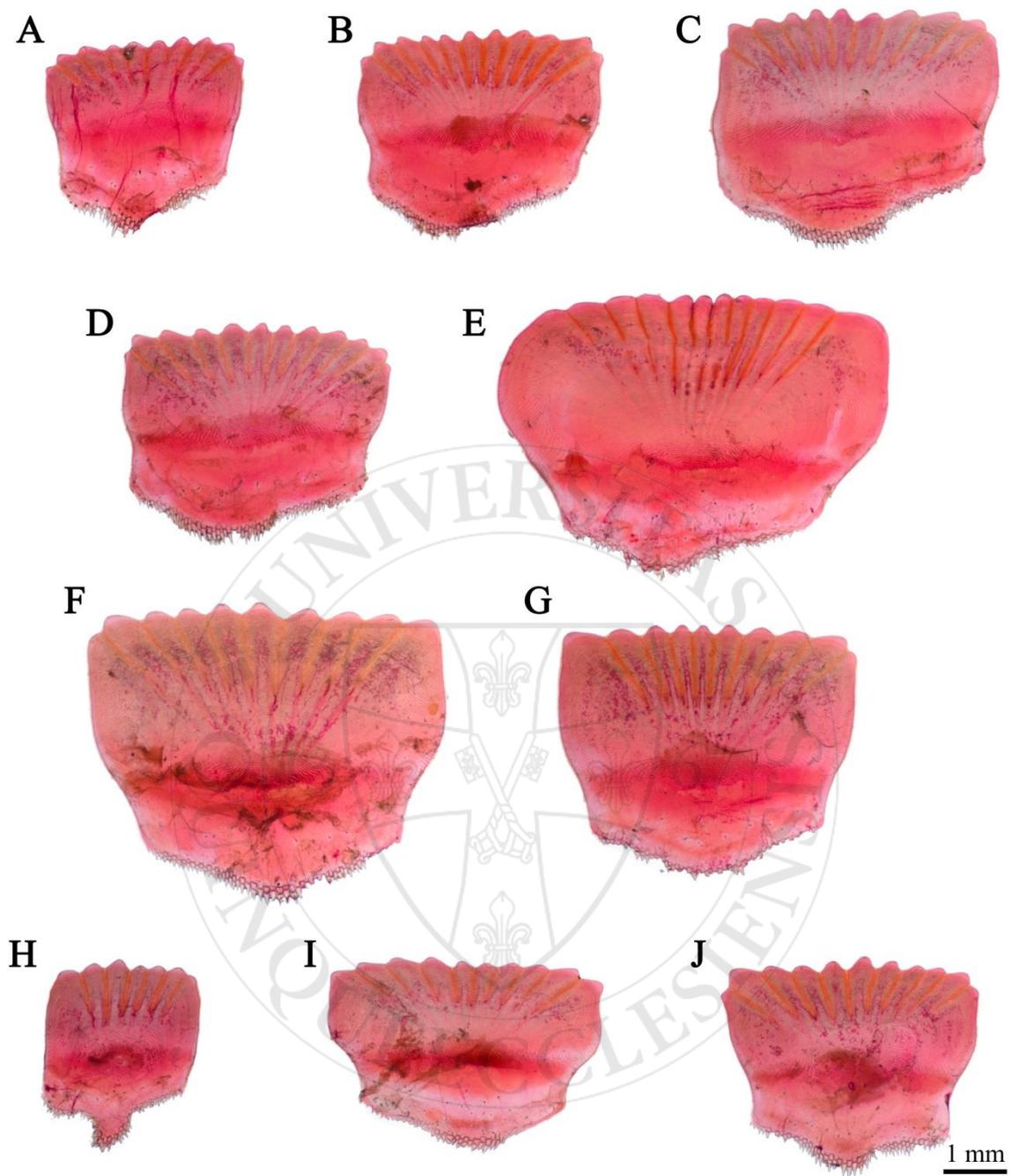


**FIGURE 47.** *Diplodus annularis*  
73 mm SL, Costa Brava, Spain, DMM IE/5033



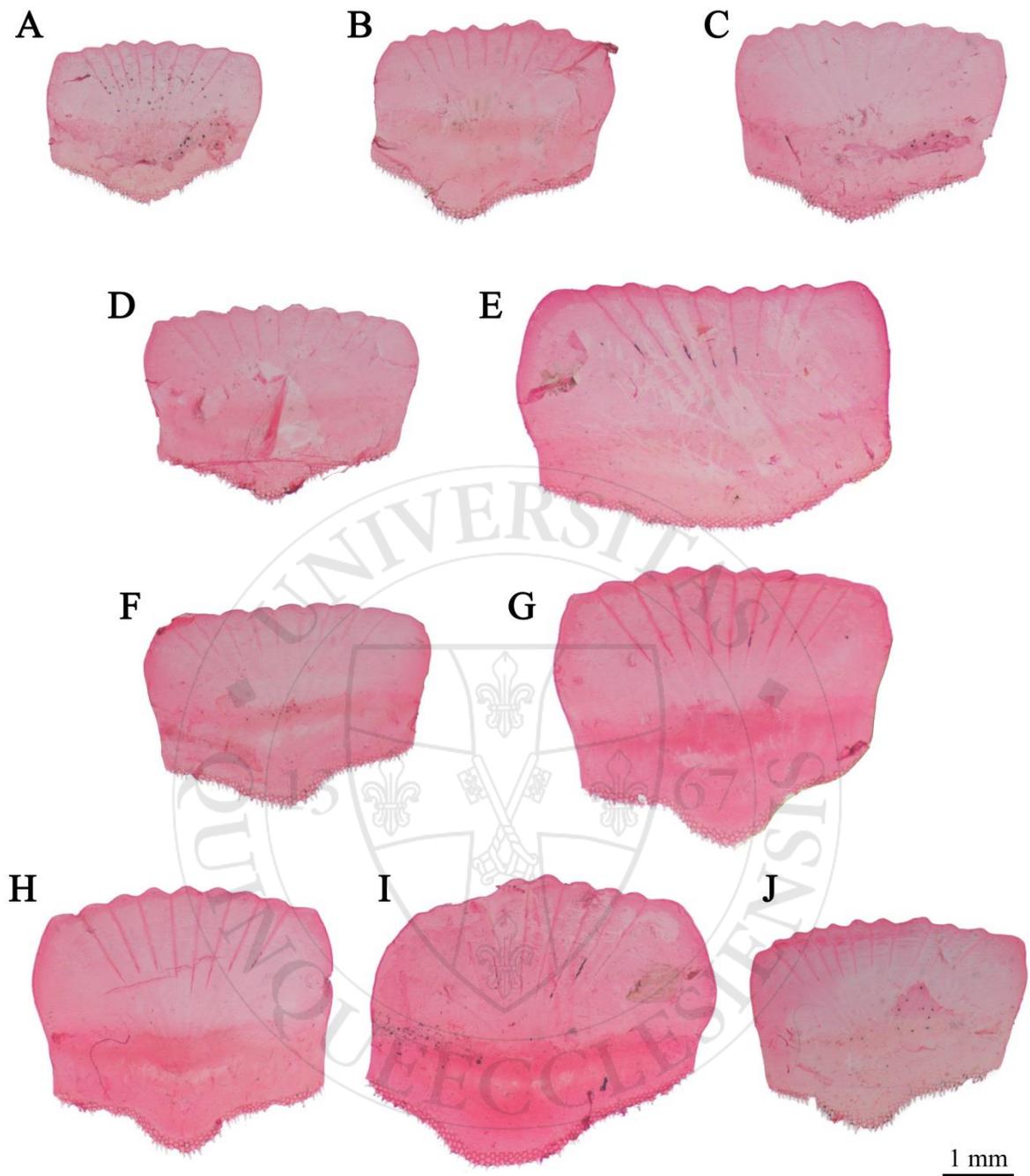
**FIGURE 48.** *Diplodus vulgaris*

128 mm SL, Costa Brava, Spain, DMM IE/5030



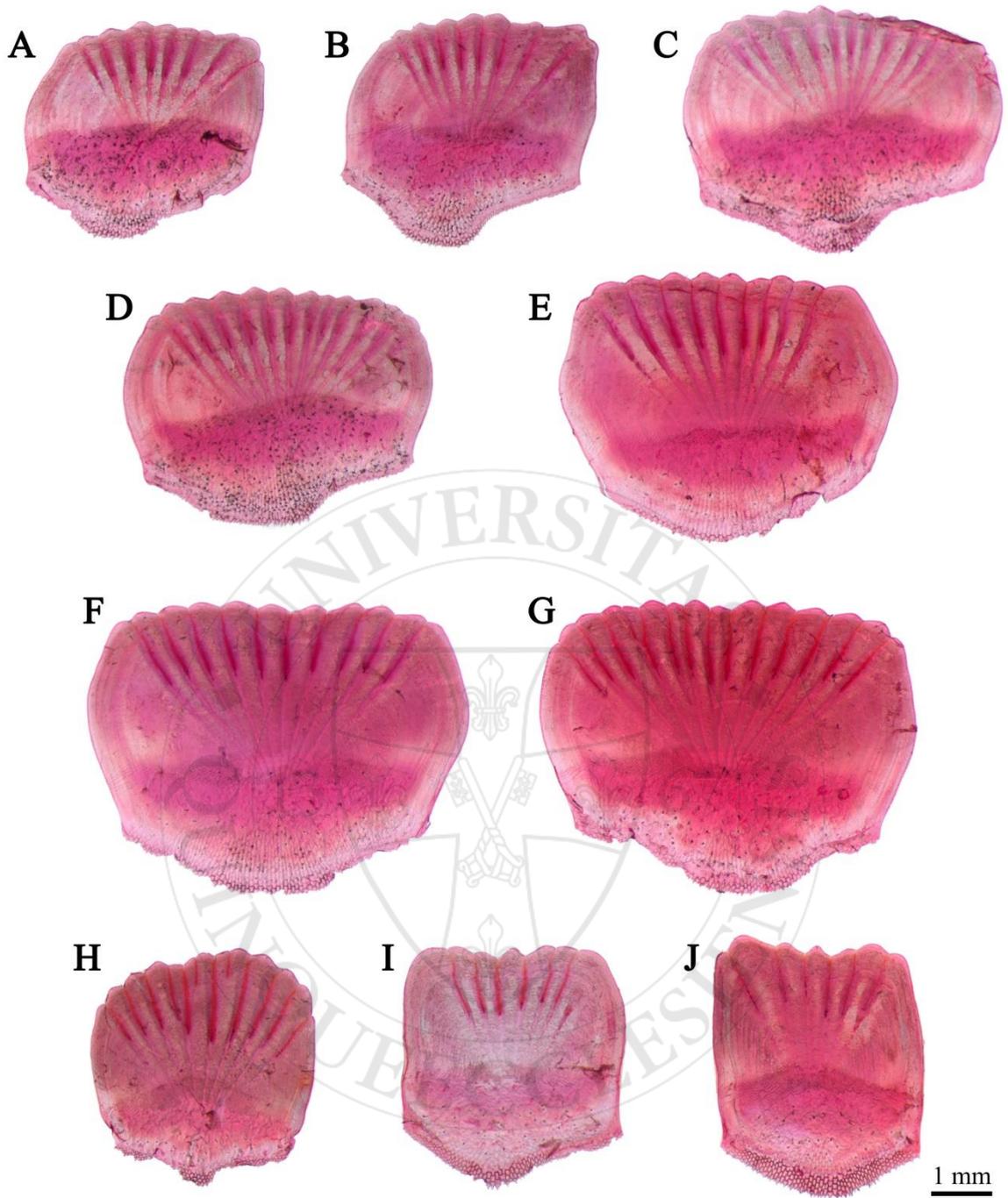
**FIGURE 49.** *Pagellus acarne*

153 mm SL, Cote Vermeille, France, DMM IE/6095



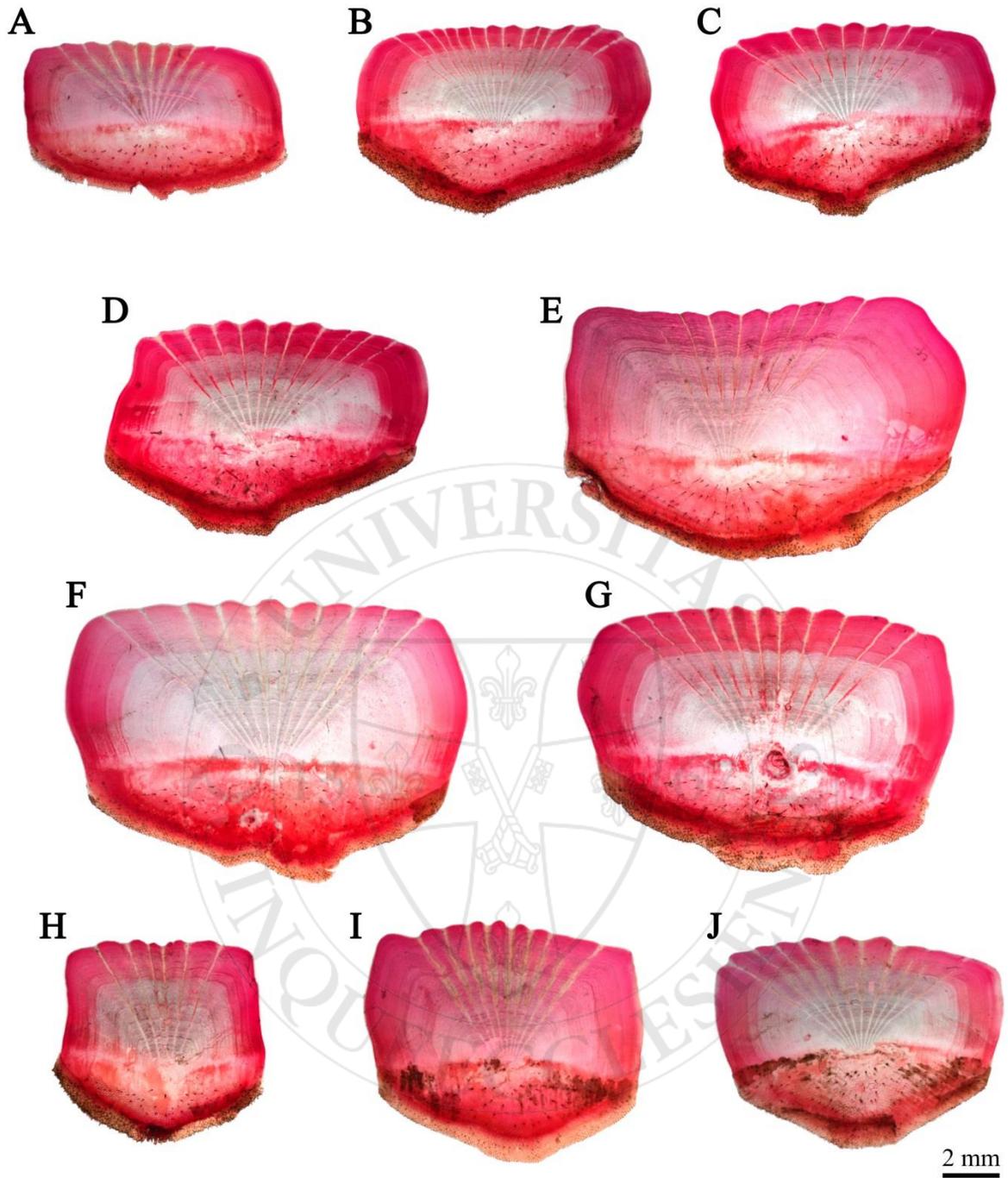
**FIGURE 50.** *Pagellus erythrinus*

130 mm SL, Costa Brava, Spain, DMM IE/5064



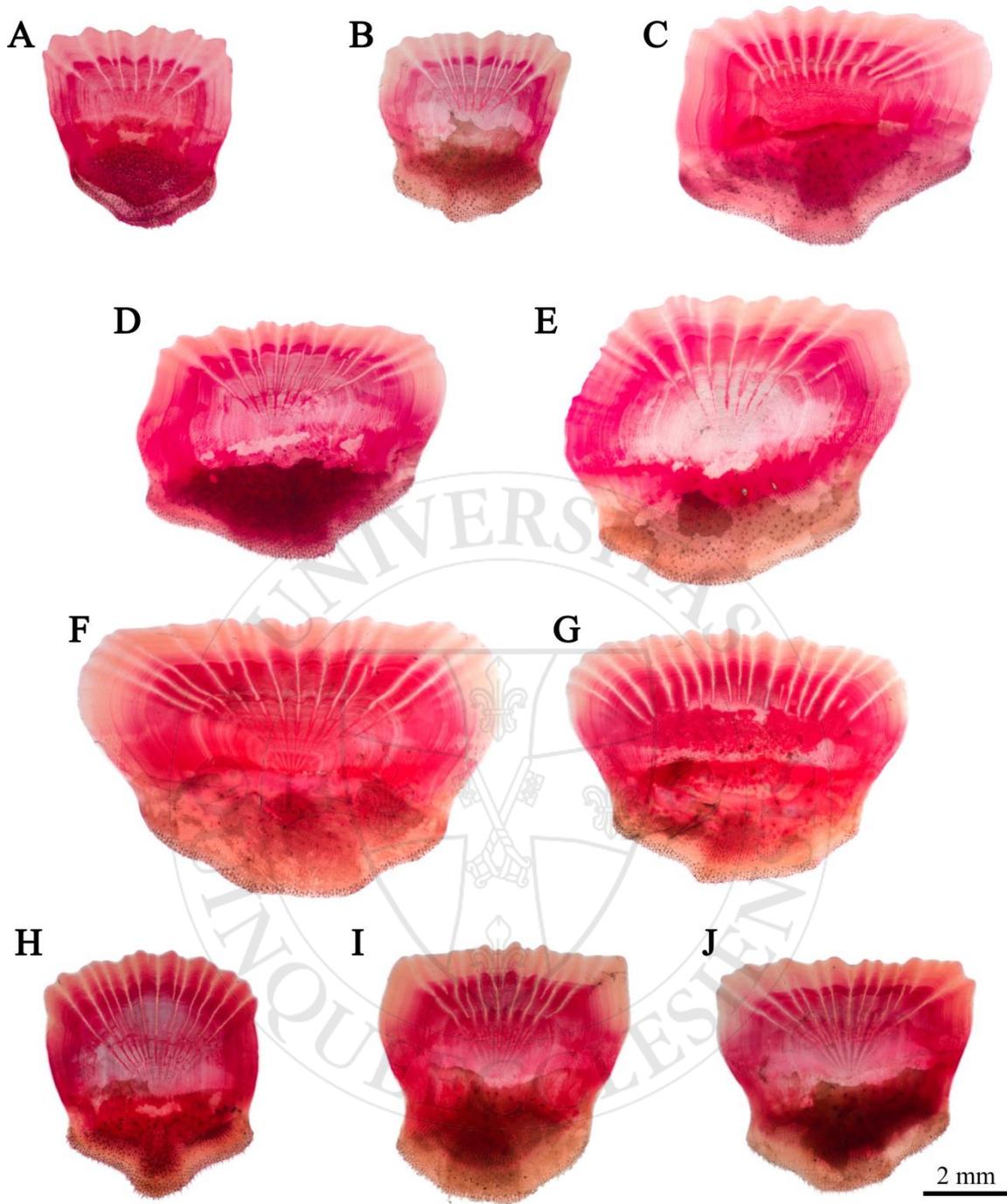
**FIGURE 51.** *Sarpa salpa*

174 mm SL, Mali Lošinj, Croatia, DMM IE/9017

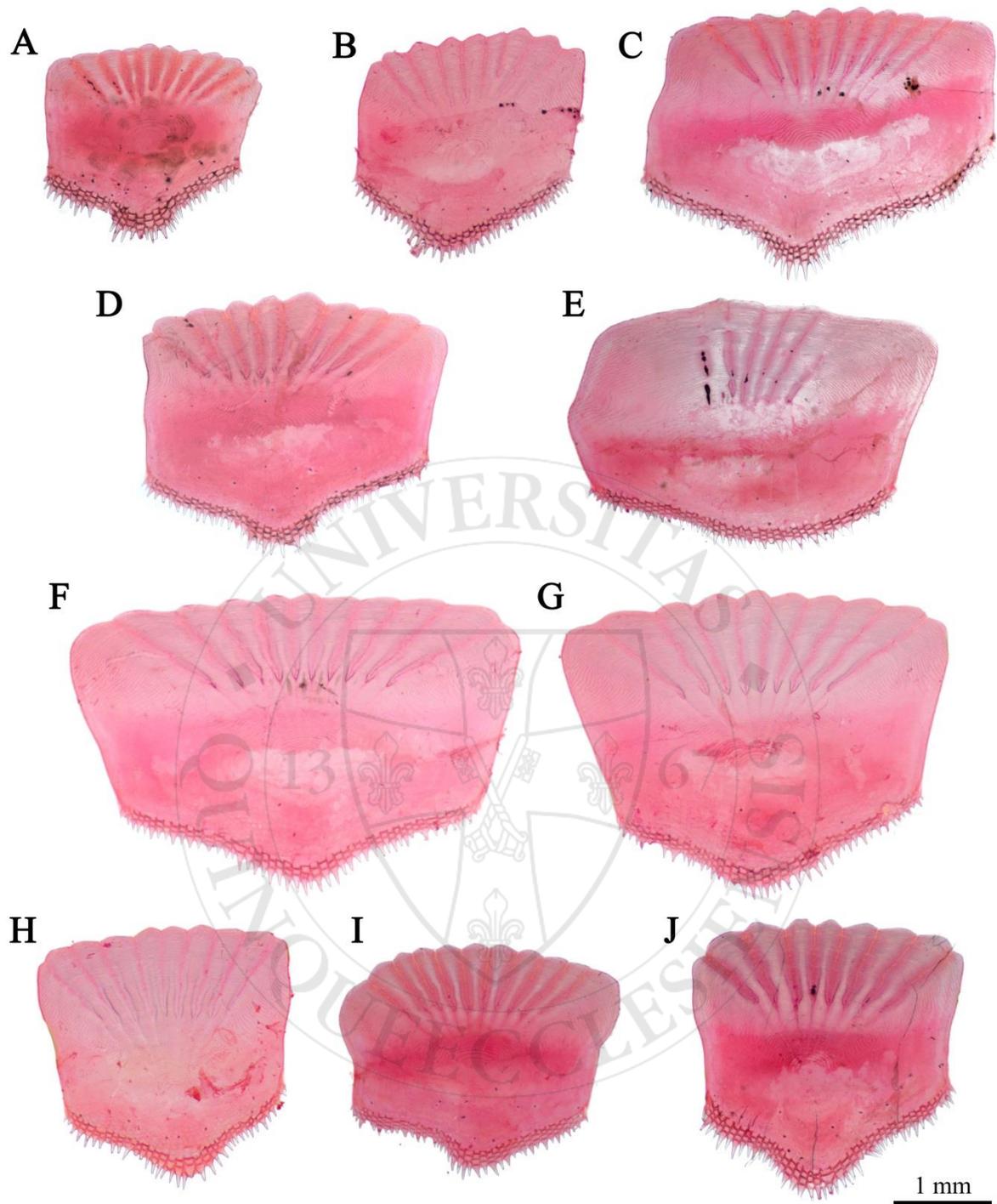


**FIGURE 52.** *Sparus aurata*

318 mm SL, aquarium specimen, DMM IE/9023

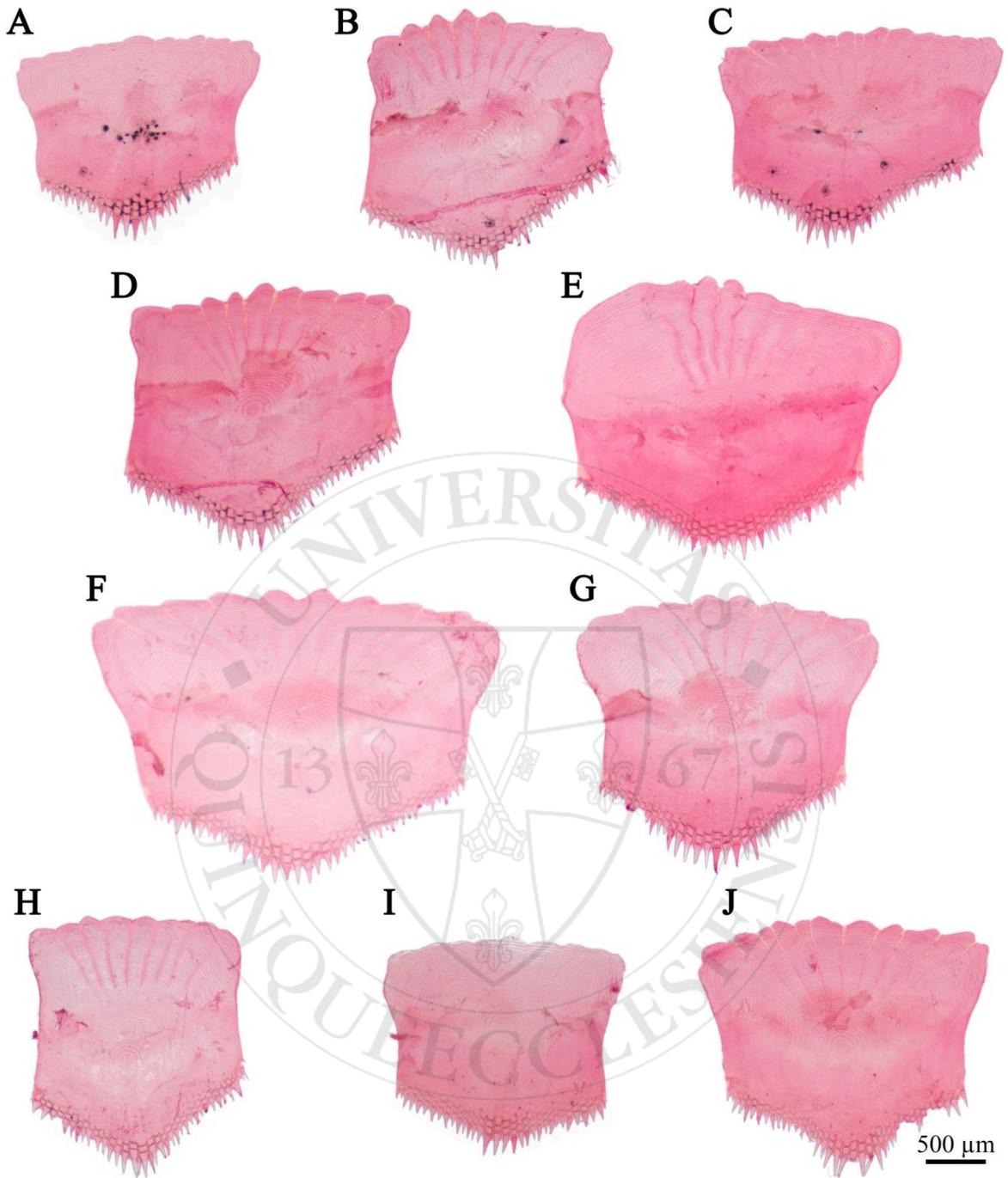


**FIGURE 53.** *Spondyliosoma cantharus*  
264 mm SL, aquarium specimen, DMM IE/9024



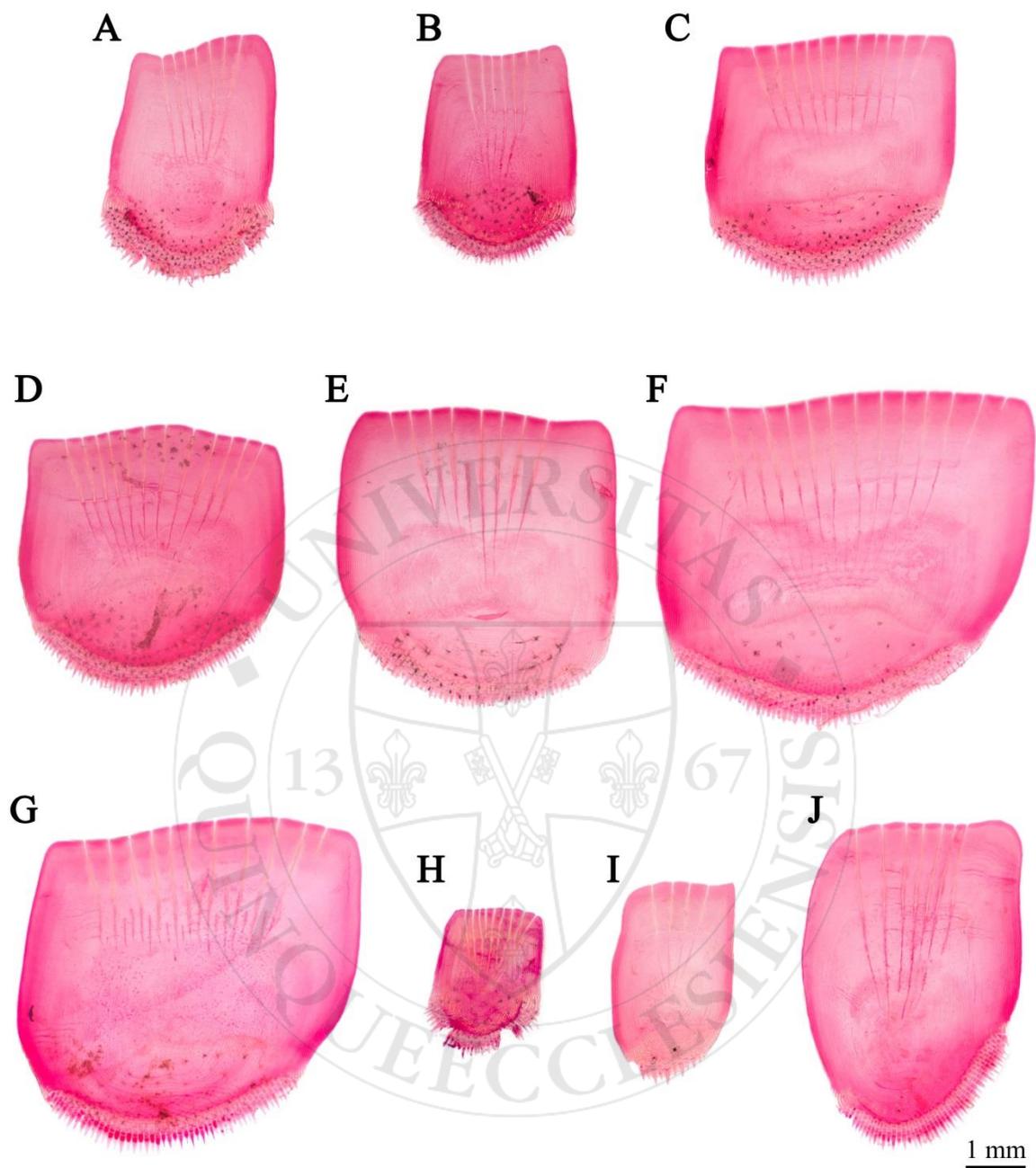
**FIGURE 54.** *Spicara maena*

123 mm SL, Costa Brava, Spain, DMM IE/5056



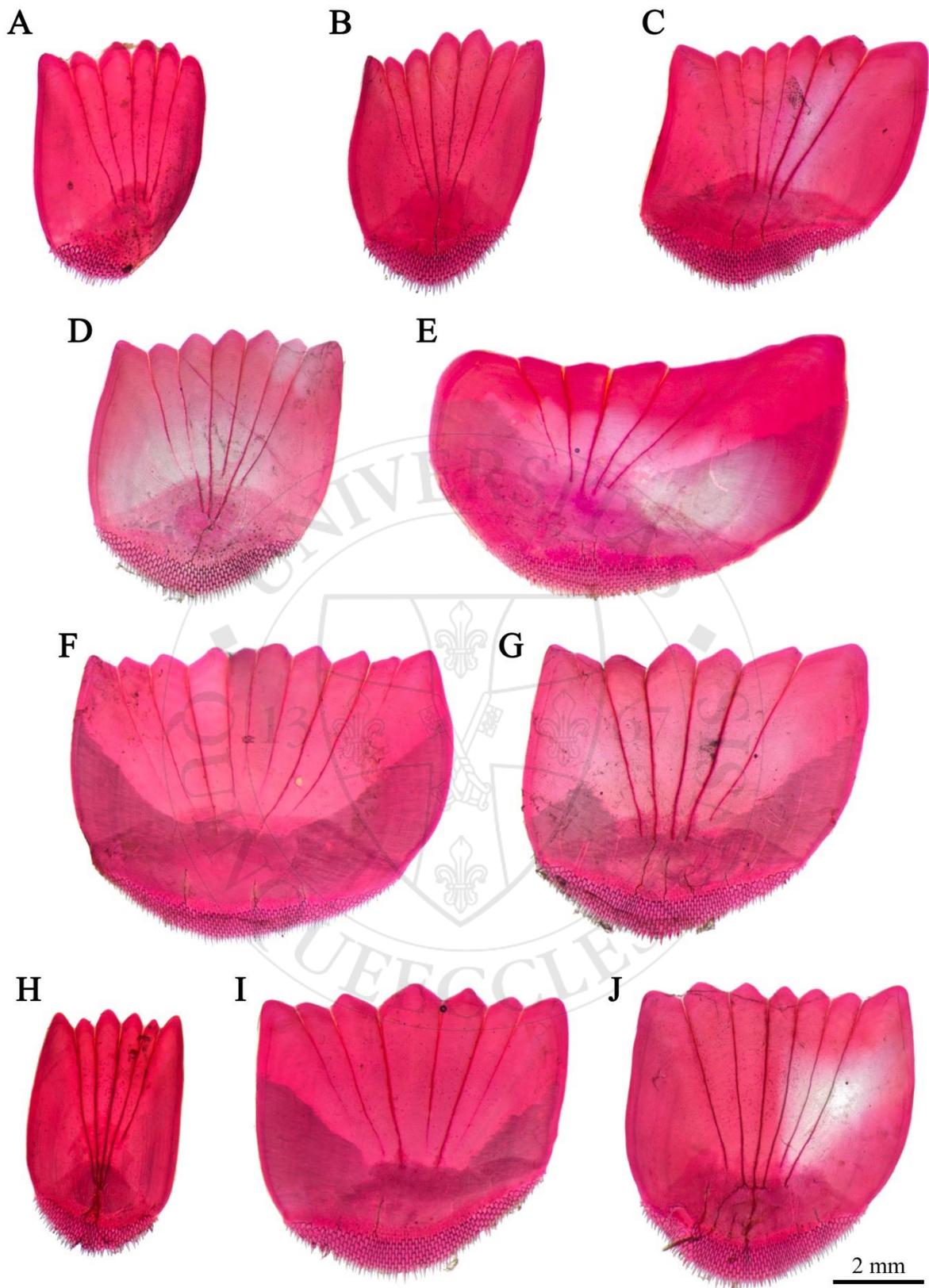
**FIGURE 55.** *Spicara smaris*

113 mm SL, Costa Brava, Spain, DMM IE/5058



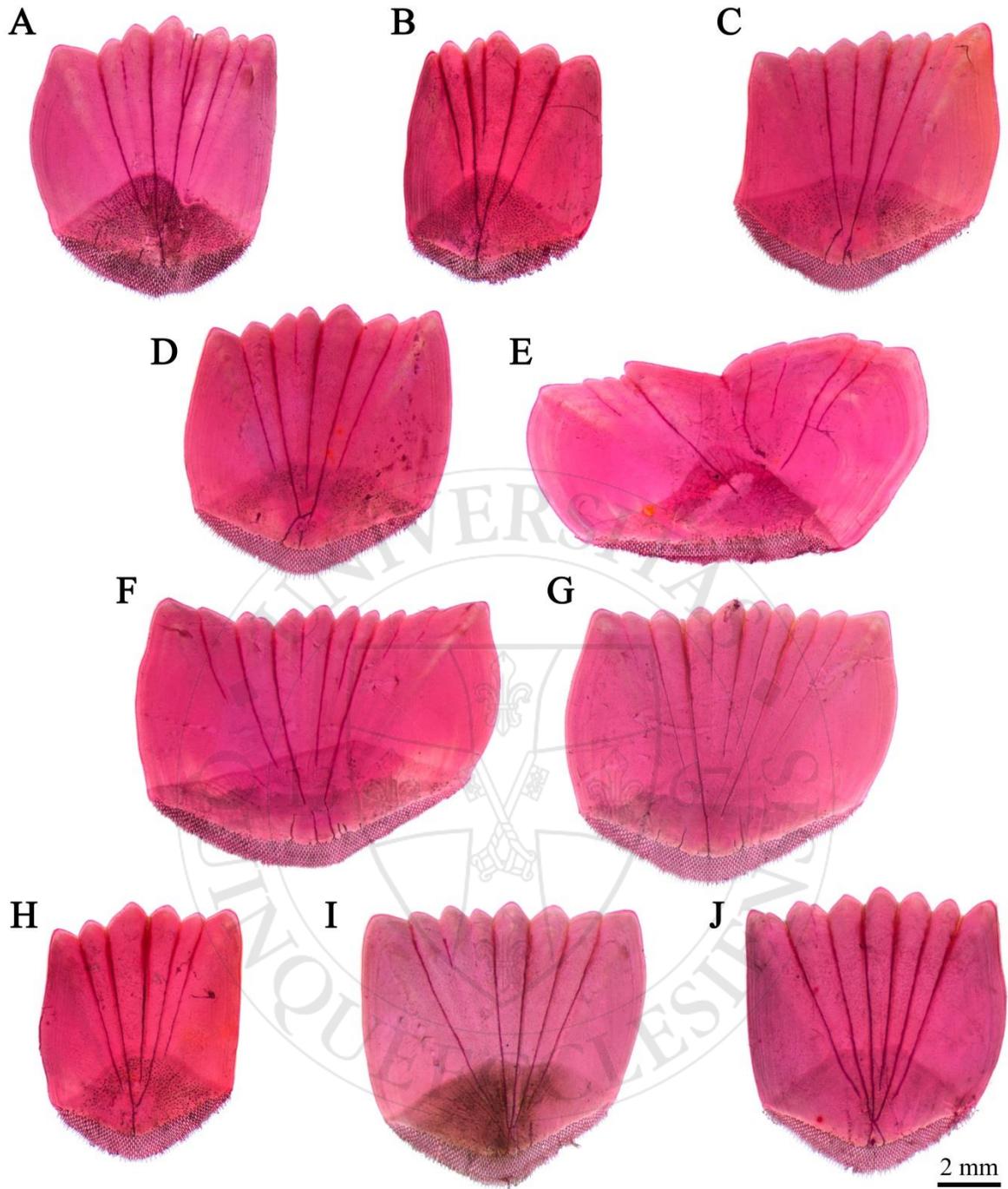
**FIGURE 56. *Sciema umbra***

179 mm SL, Costa Brava, Spain, DMM IE/4588



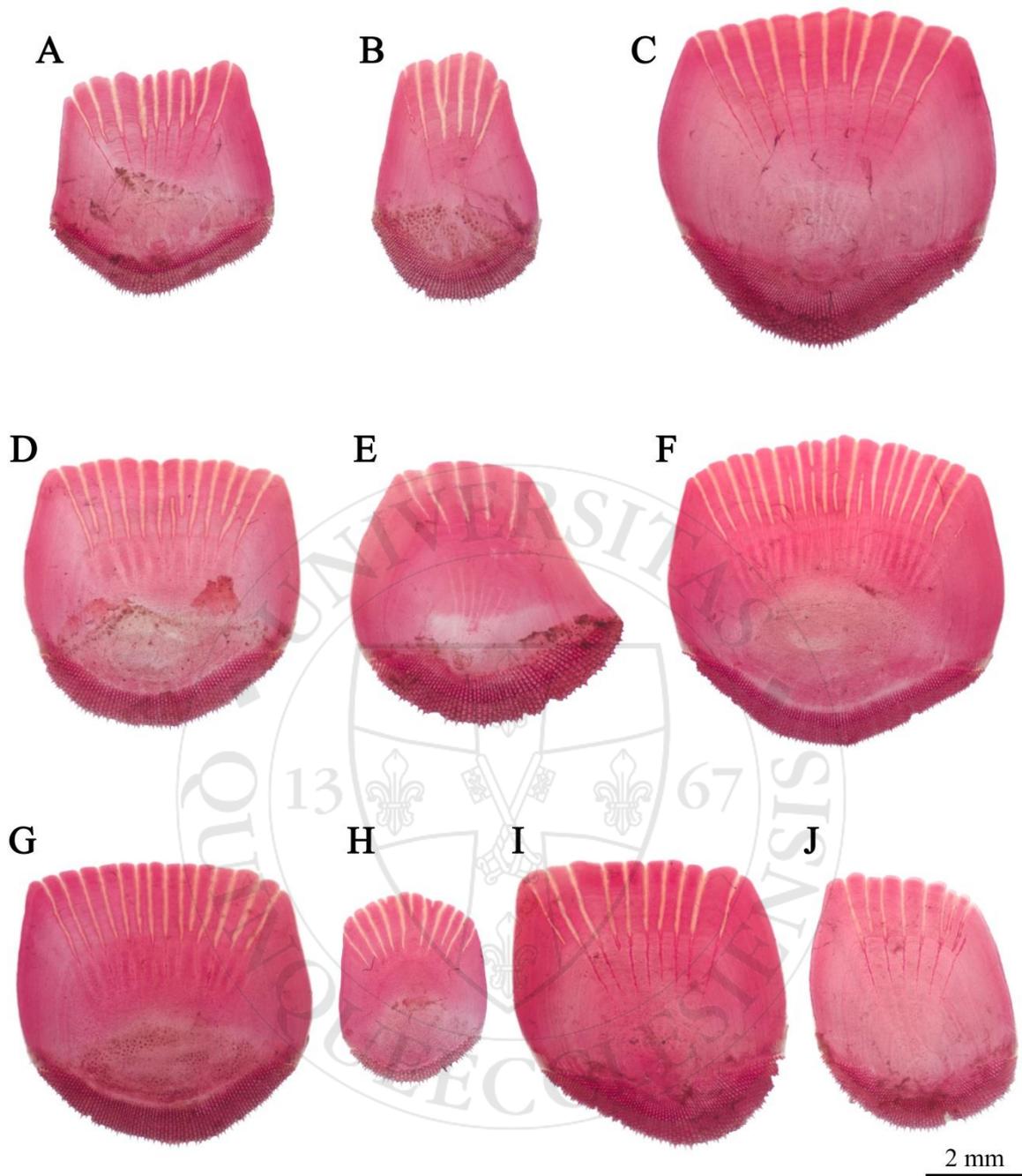
**FIGURE 57.** *Mullus barbatus*

114 mm SL, Gulf of Ambracia, Greece, DMM IE/9018



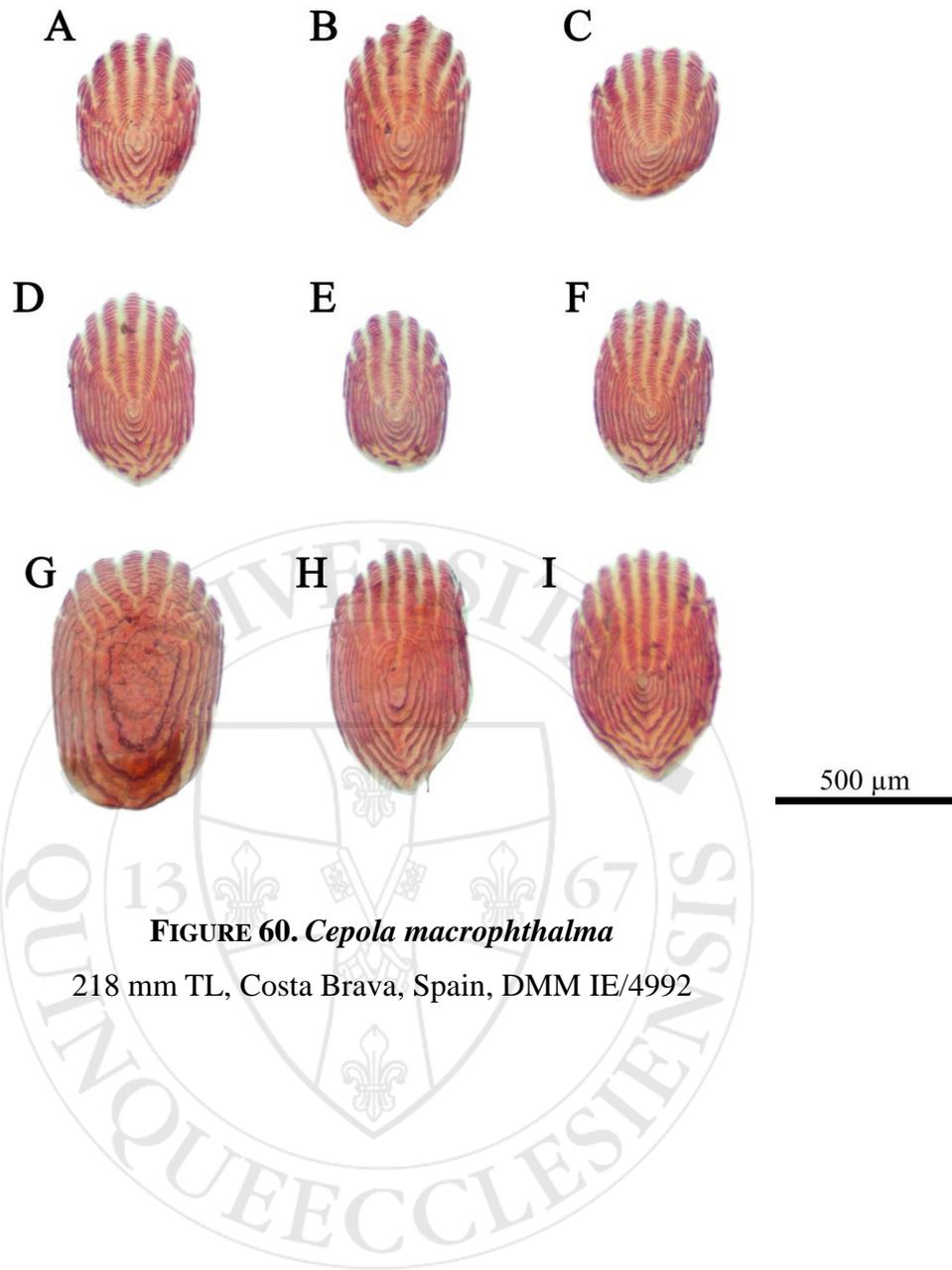
**FIGURE 58.** *Mullus surmuletus*

164 mm SL, Mali Lošinj, Croatia, DMM IE/9019



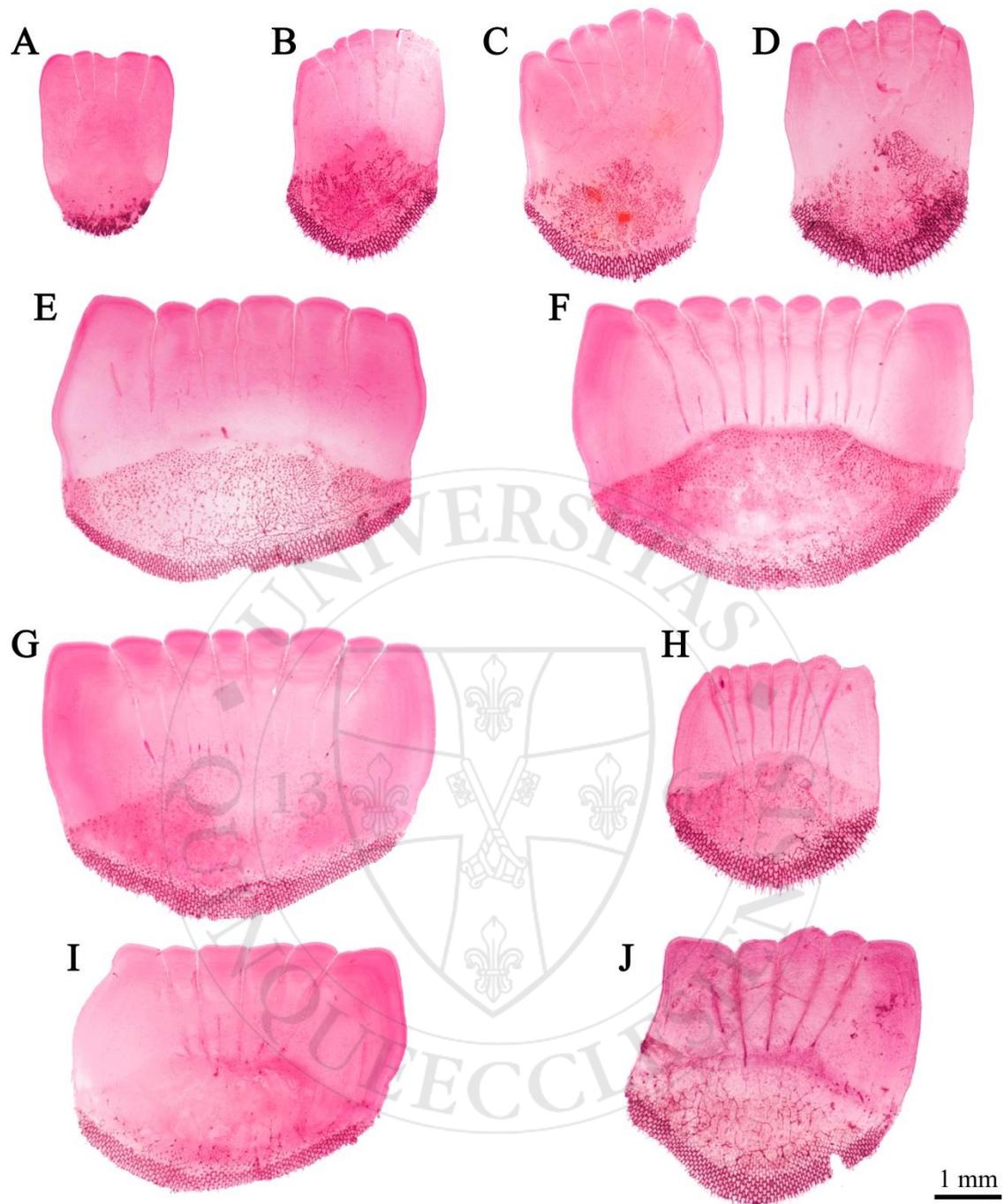
**FIGURE 59.** *Chaetodon hoefleri*

132 mm SL, Central East Atlantic, Morocco, DMM IE/2267



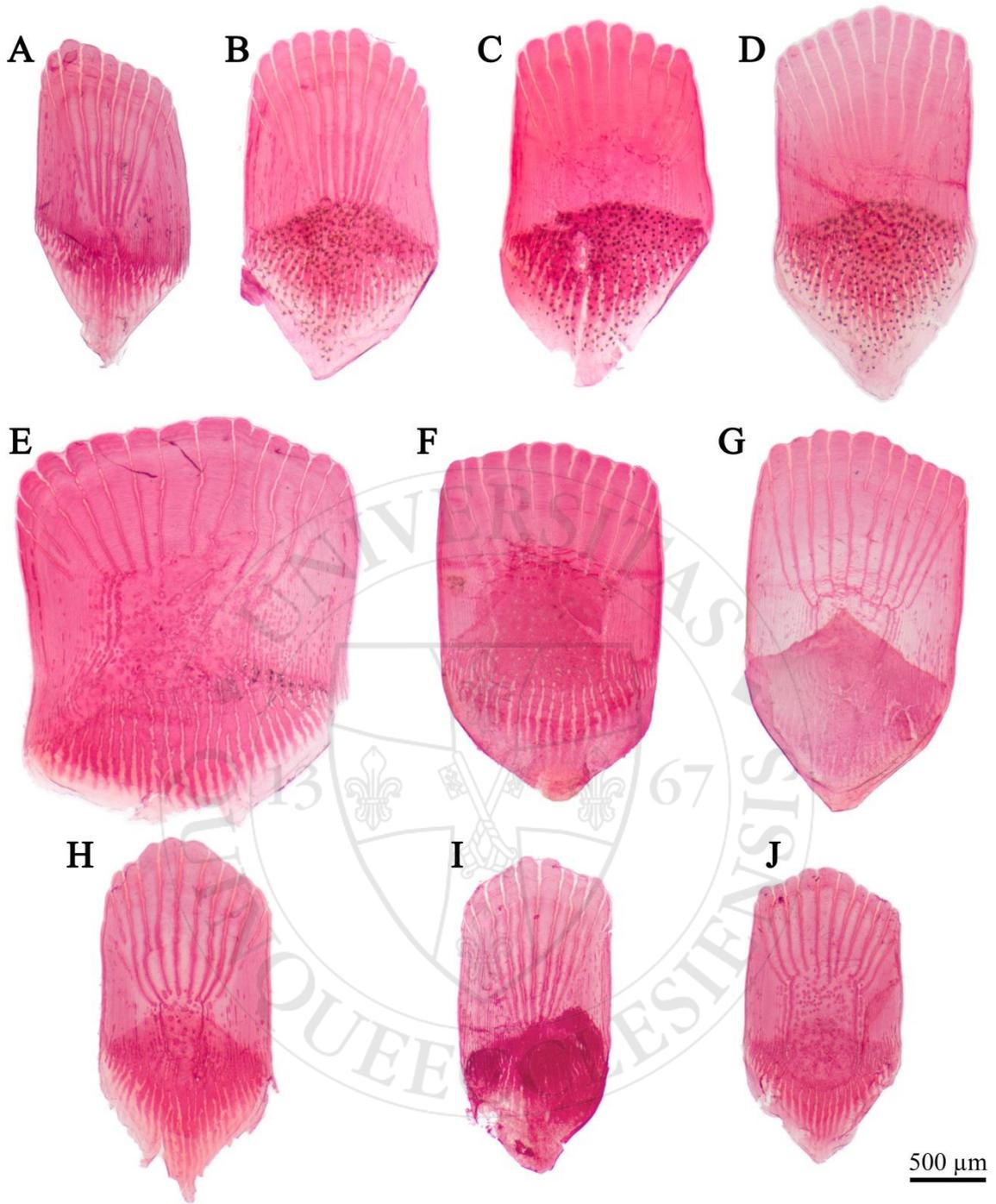
**FIGURE 60.** *Cepola macrophthalma*

218 mm TL, Costa Brava, Spain, DMM IE/4992

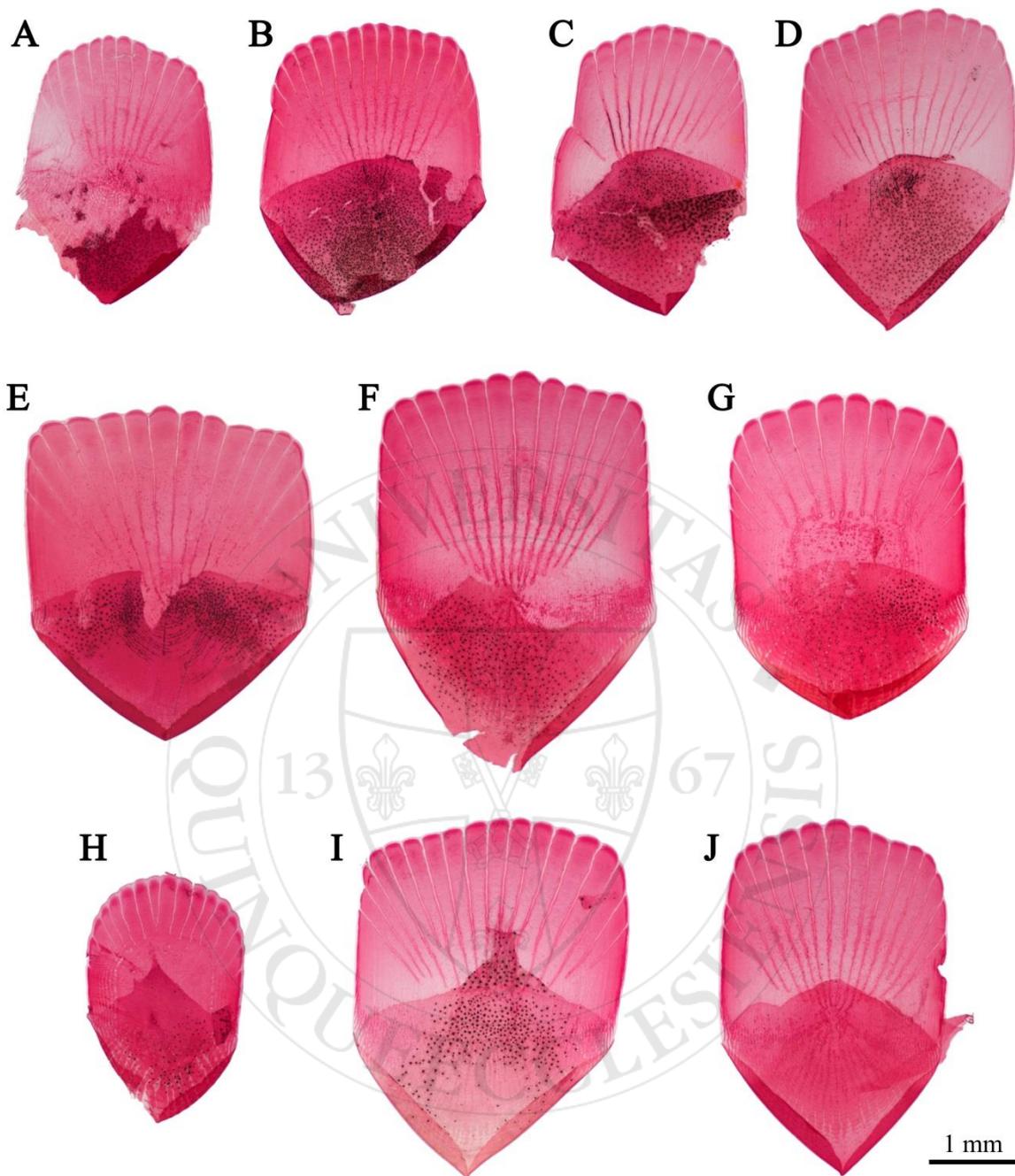


**FIGURE 61.** *Chromis chromis*

68 mm SL, aquarium specimen, Spain, DMM IE/5943

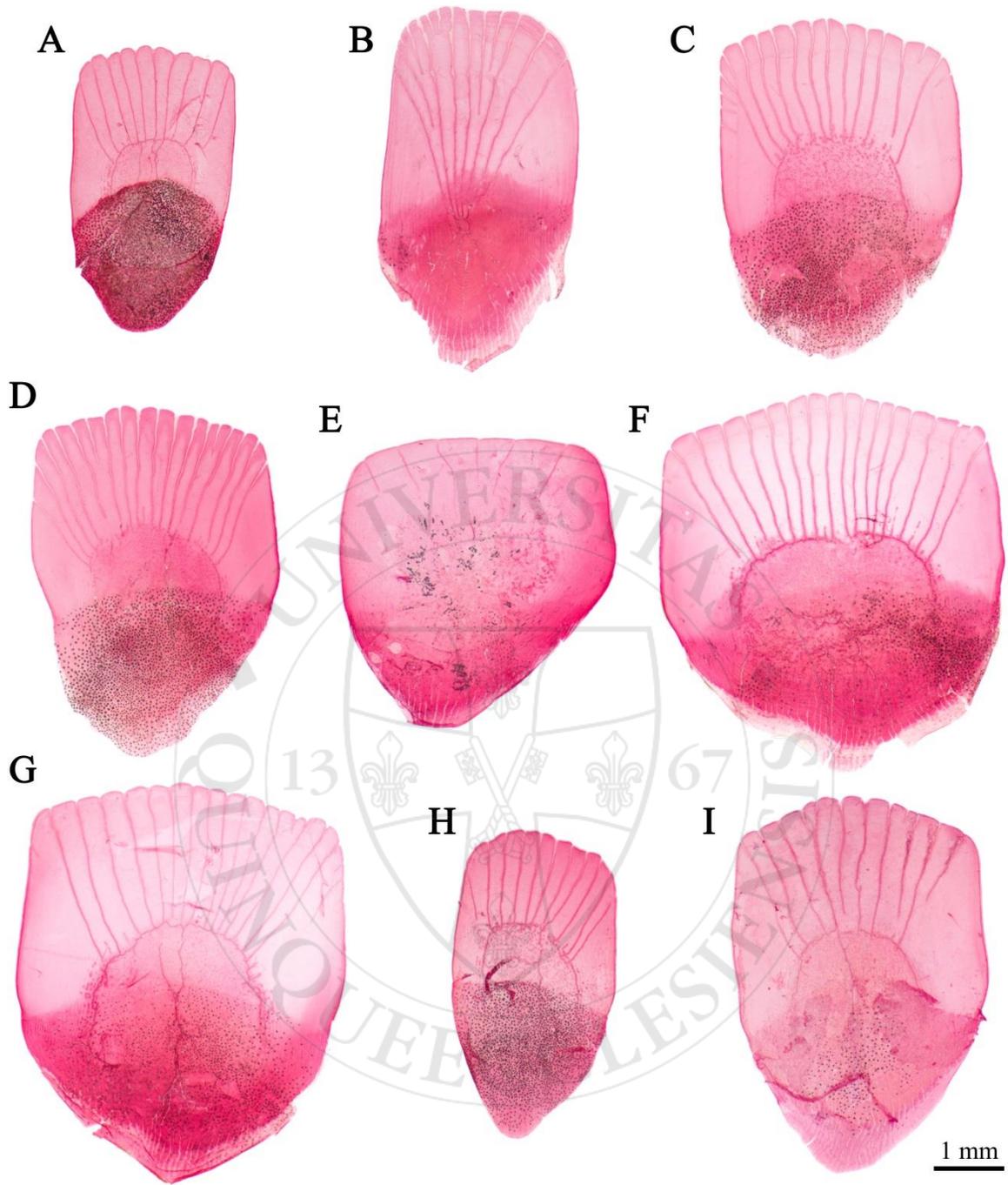


**FIGURE 62.** *Coris julis*  
96 mm SL, Costa Brava, Spain, DMM IE/5020



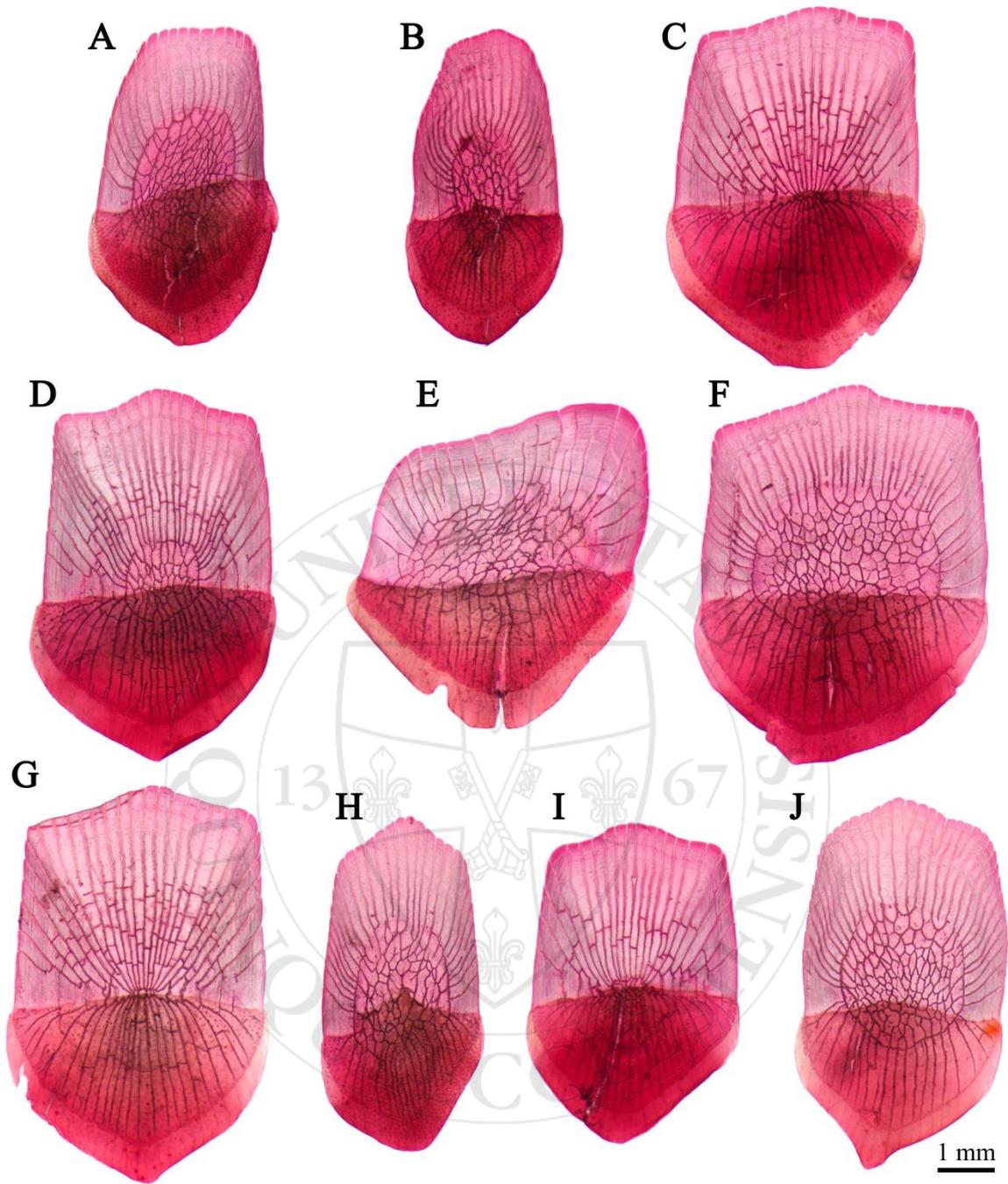
**FIGURE 63.** *Labrus viridis*

106 mm SL, Costa Brava, Spain, DMM IE/5886



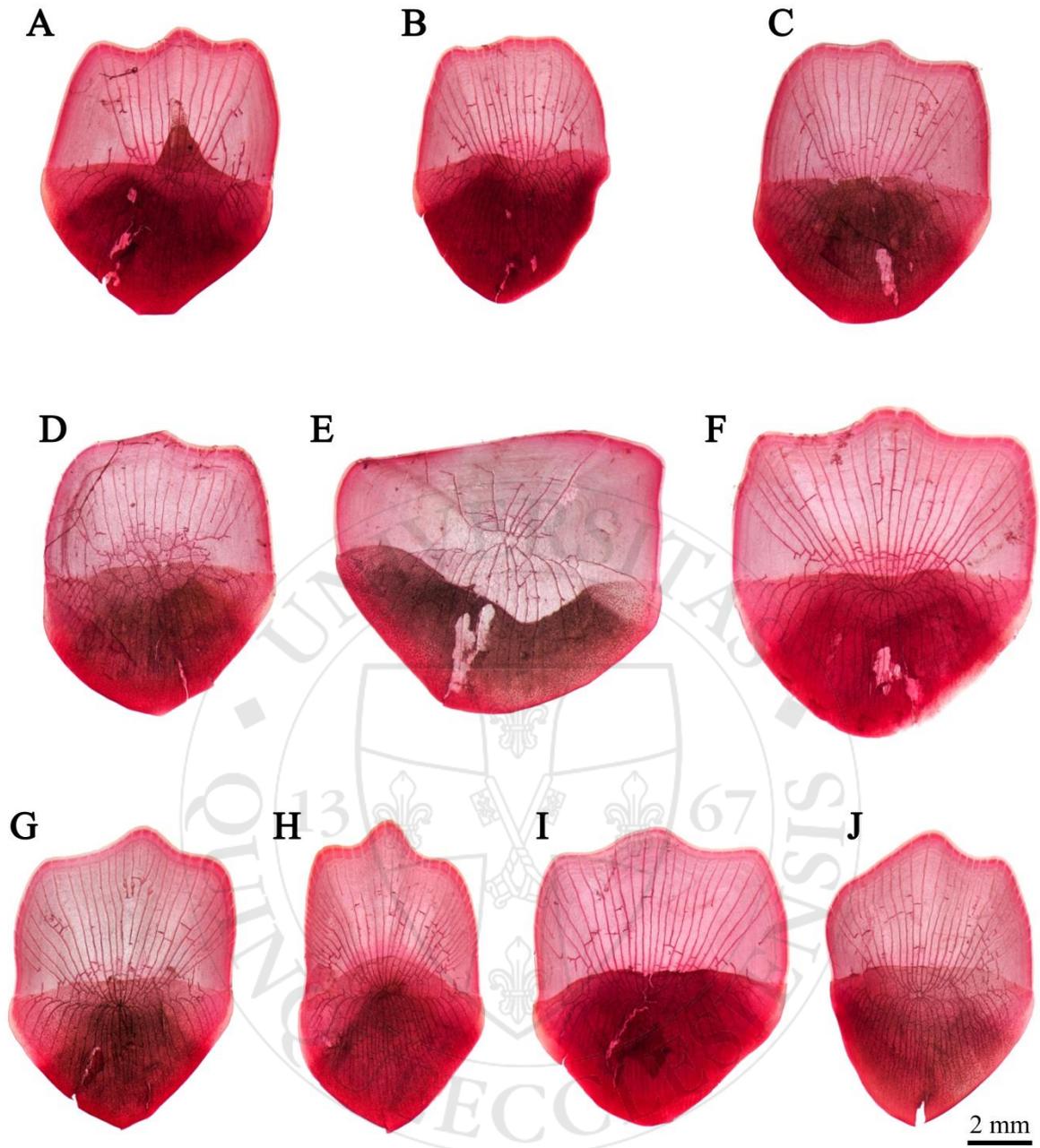
**FIGURE 64.** *Symphodus rostratus*

84 mm SL, aquarium specimen, Spain, DMM IE/5945



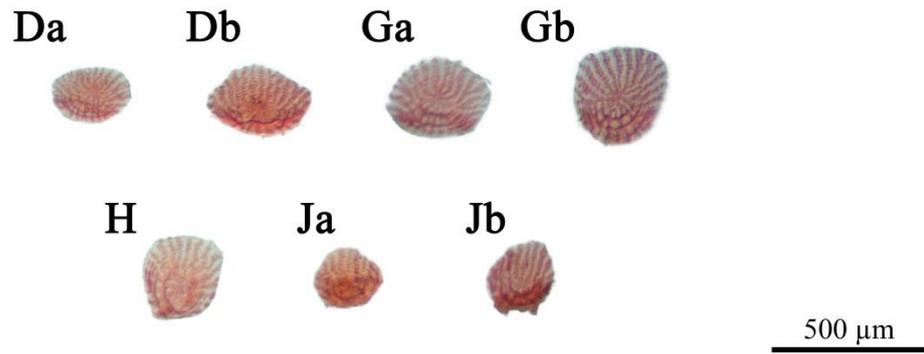
**FIGURE 65.** *Thalassoma pavo*

95 mm SL, Central Atlantic, Azores, DMM IE/6113

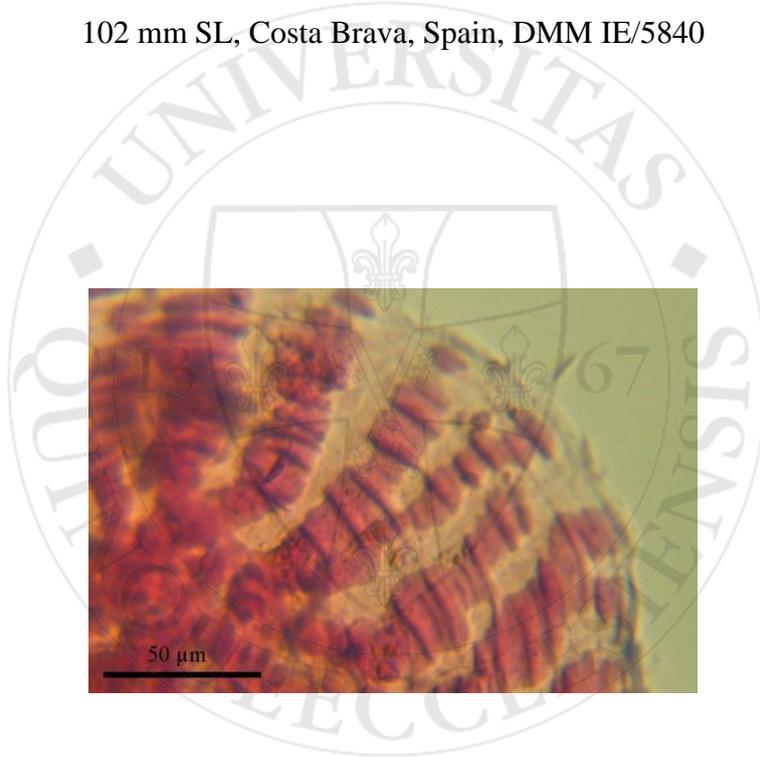


**FIGURE 66.** *Sparisoma cretense*

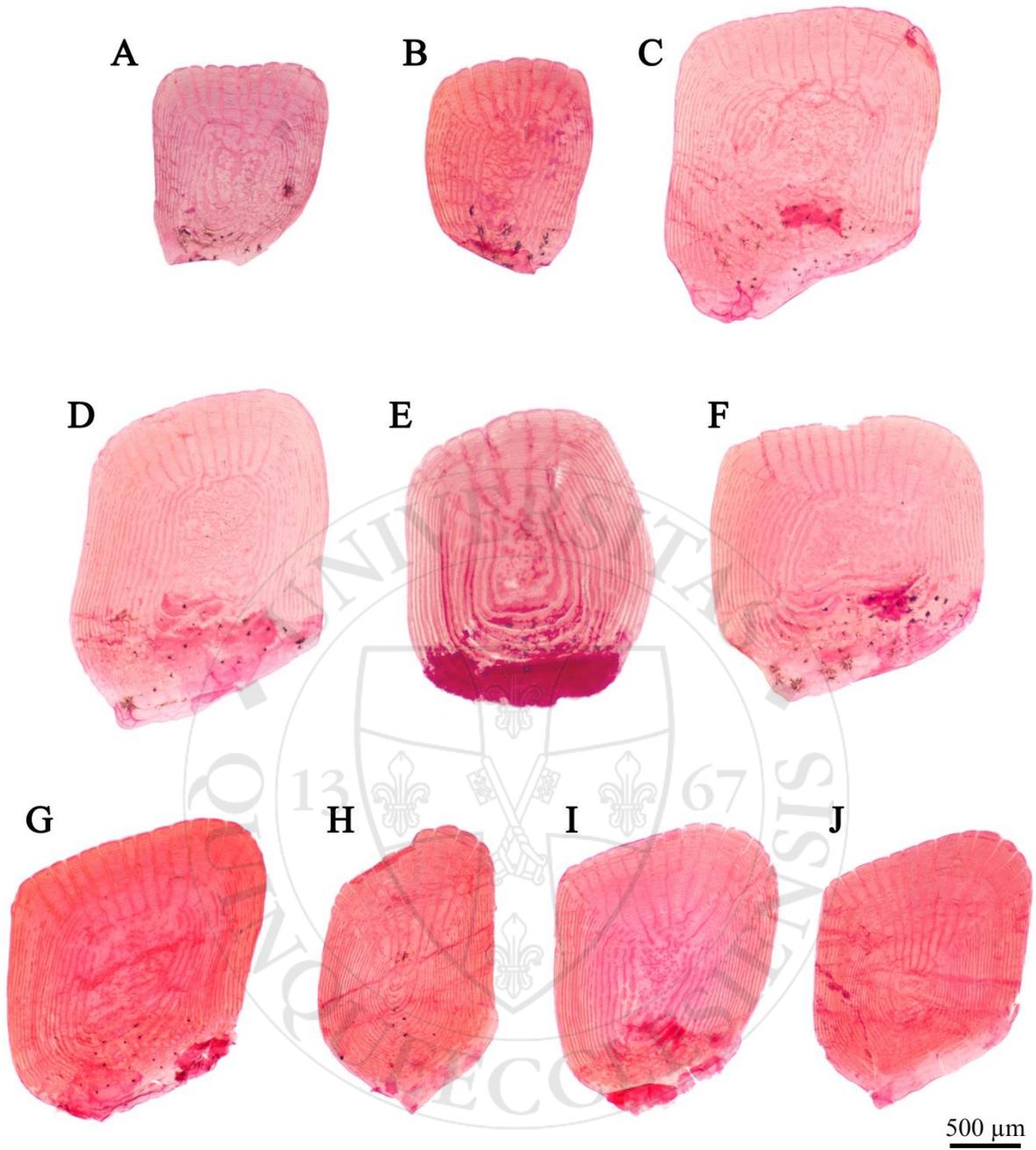
110 mm SL, Central Atlantic, Azores, DMM IE/6115



**FIGURE 67.** *Gymnammodytes cicereus*  
102 mm SL, Costa Brava, Spain, DMM IE/5840

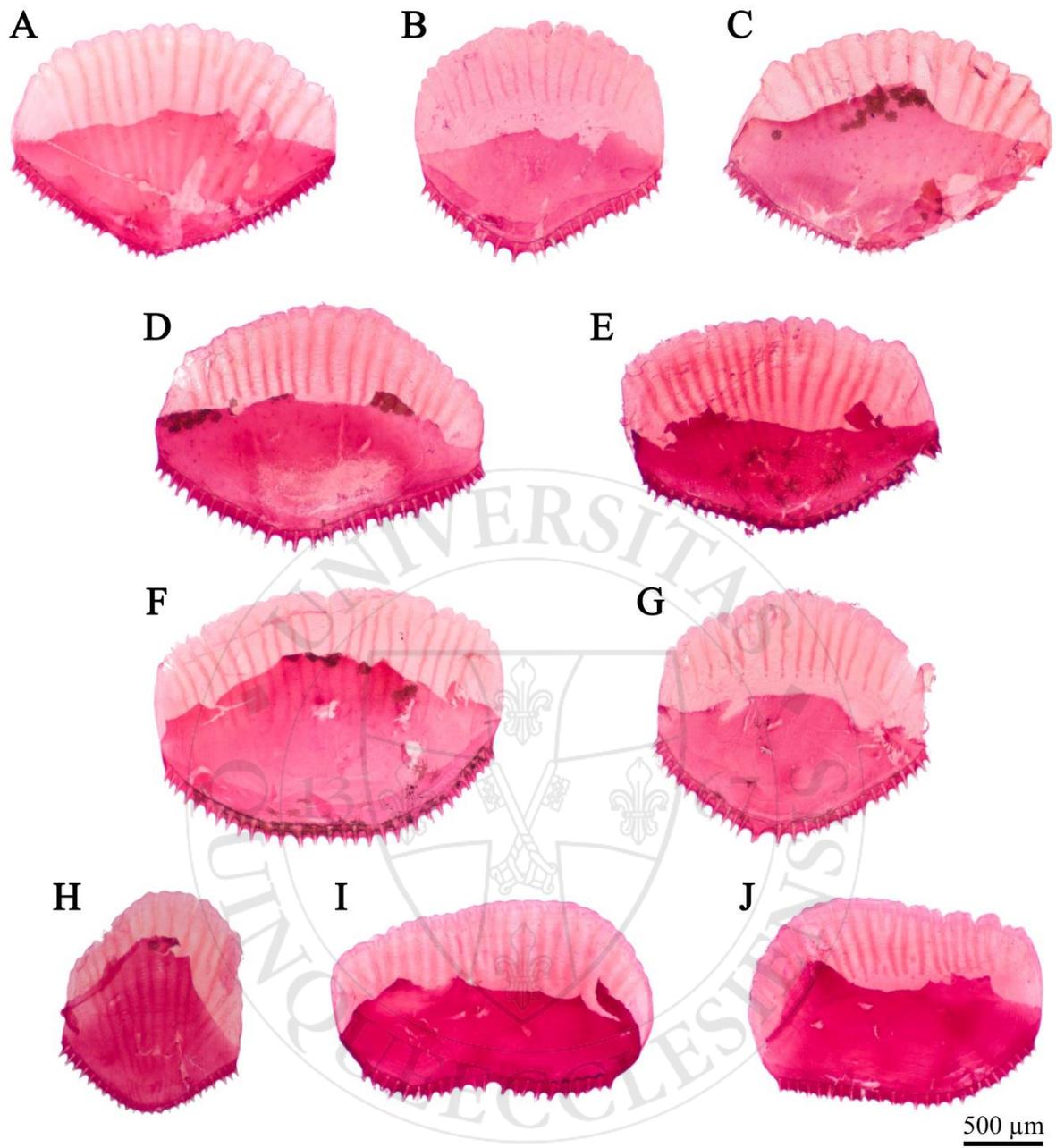


**FIGURE 68.** Scale surface of *G. cicereus* in sampling area D

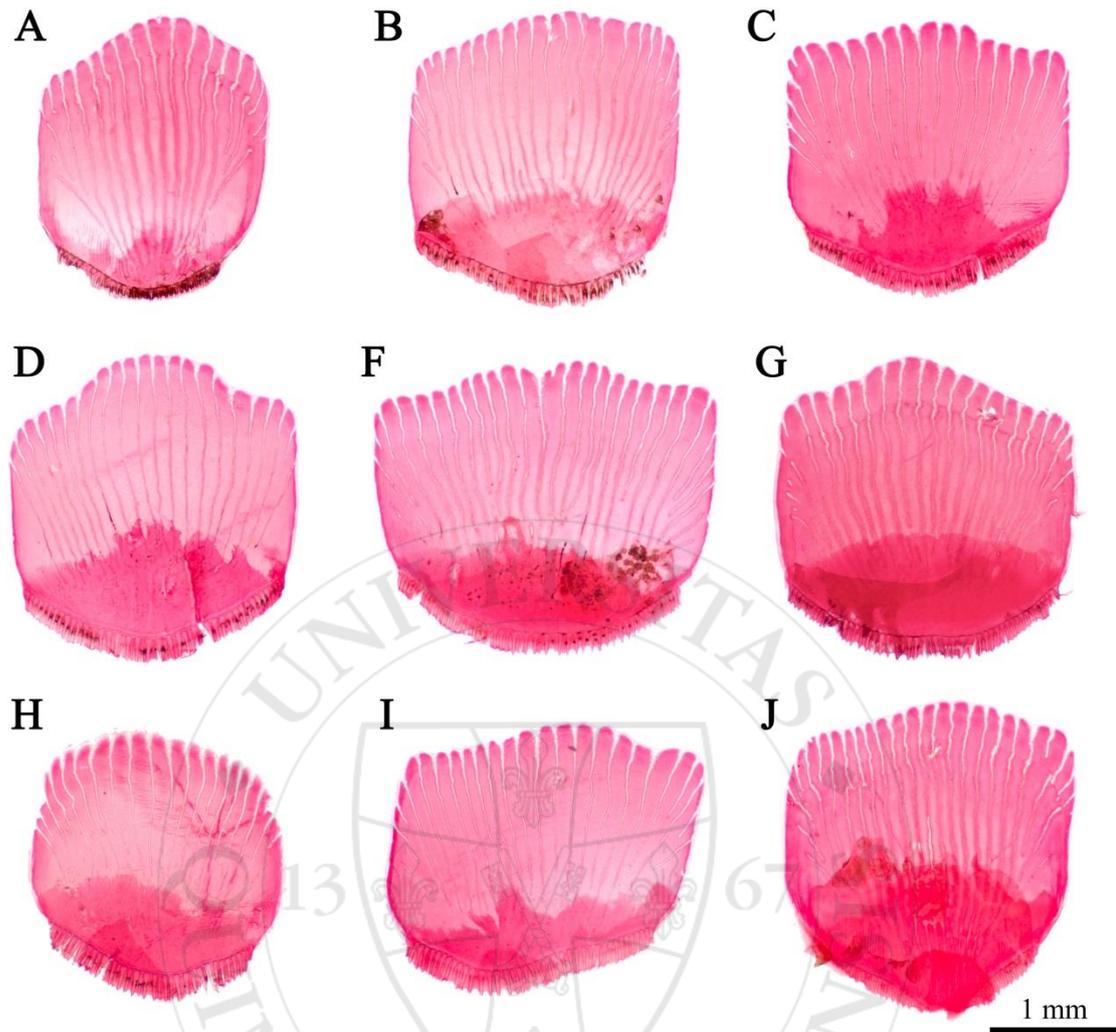


**FIGURE 69.** *Trachinus draco*

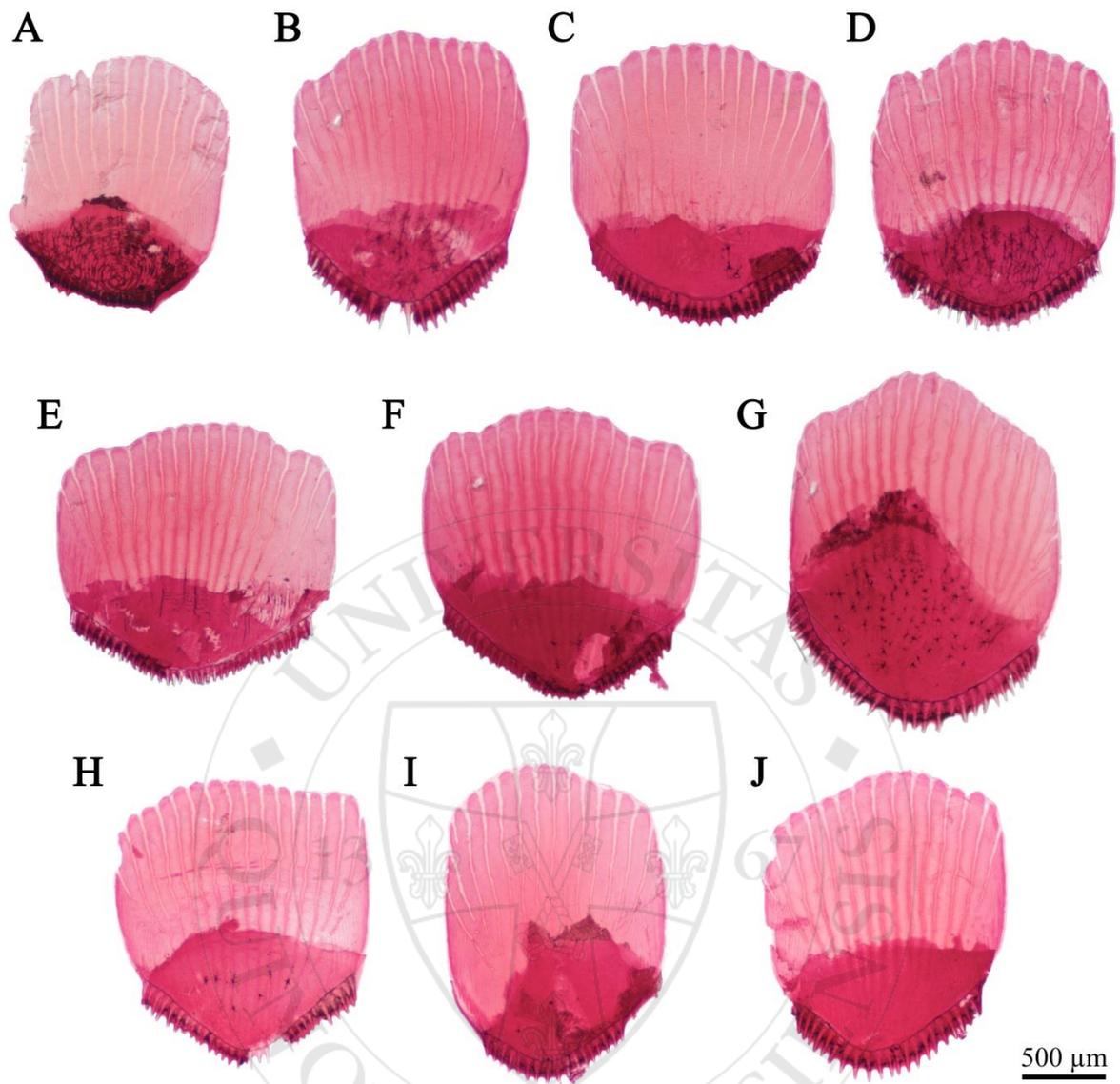
179 mm SL, Wismar Bay, Germany, DMM IE/4561



**FIGURE 70.** *Tripterygion tripteronotum*  
53 mm SL, Costa Brava, Spain, DMM IE/4570

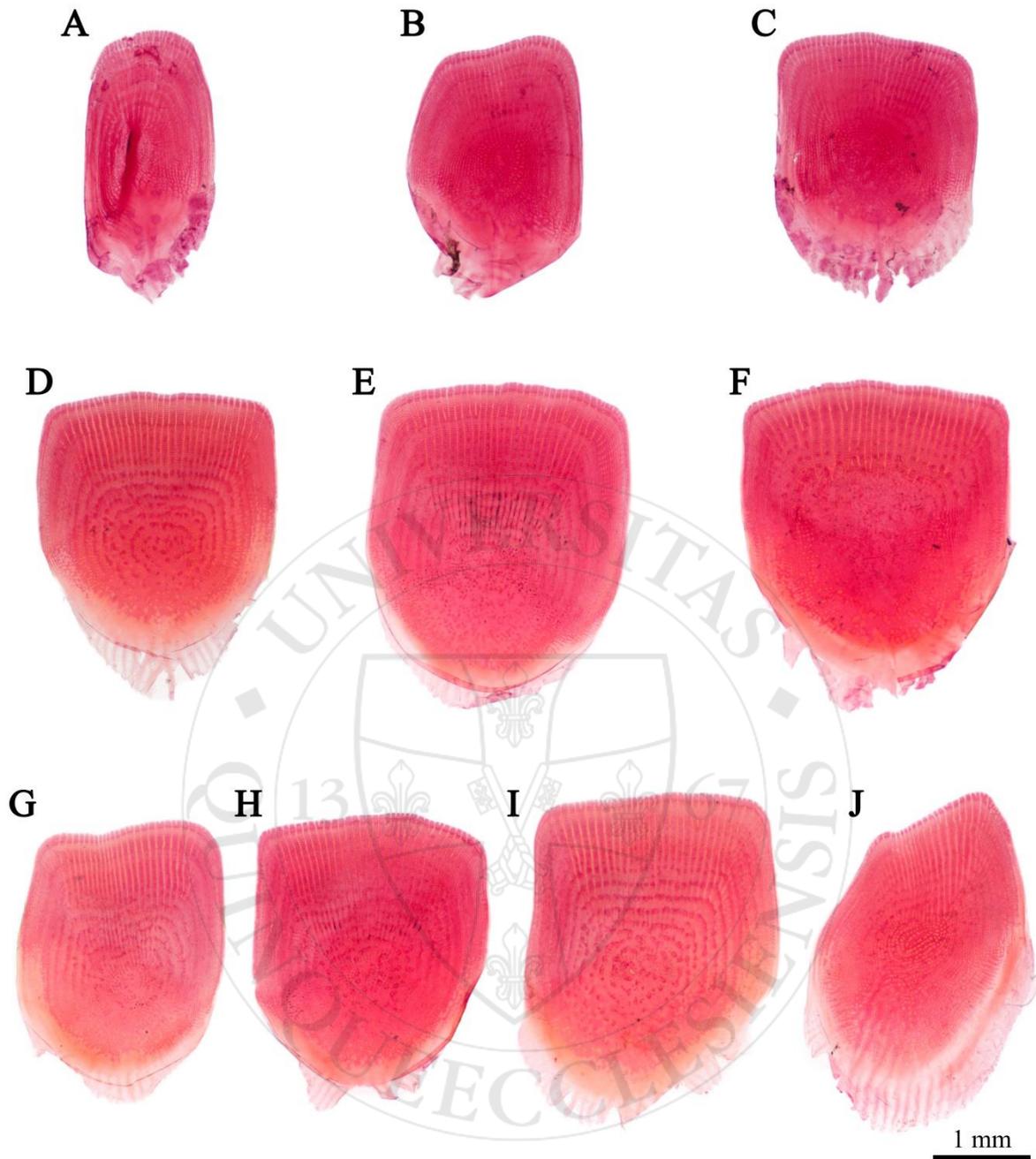


**FIGURE 71.** *Gobius bucchichi*  
81 mm SL, Costa Brava, Spain, DMM IE/5861

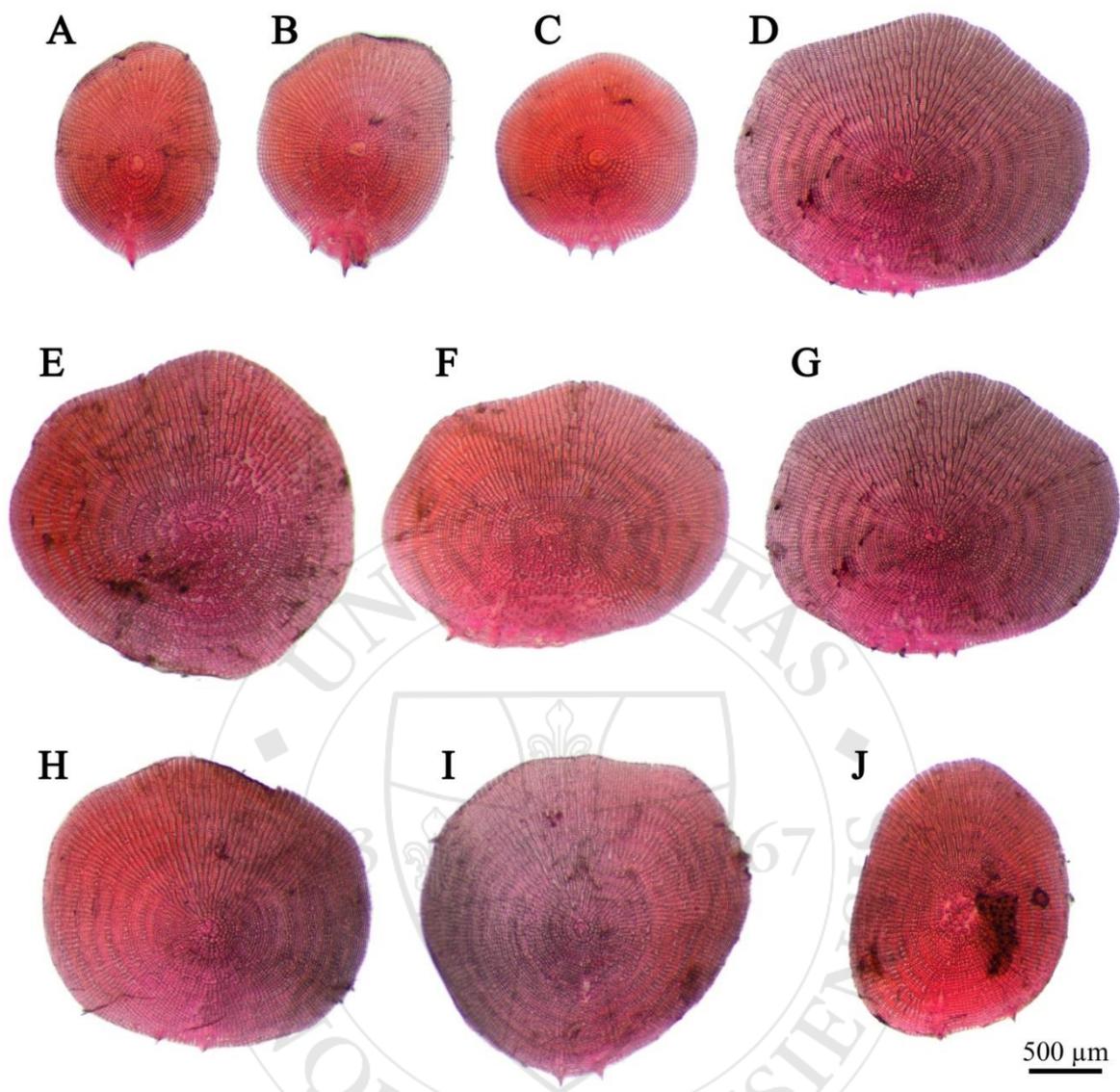


**FIGURE 72.** *Gobius paganellus*

61 mm SL, Costa Brava, Spain, DMM IE/5870

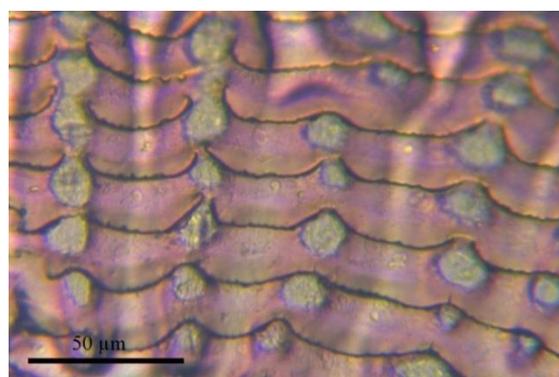


**FIGURE 73.** *Sphyraena chrysotaenia*  
194 mm SL, Cilician Sea, Turkey, DMM IE/3864

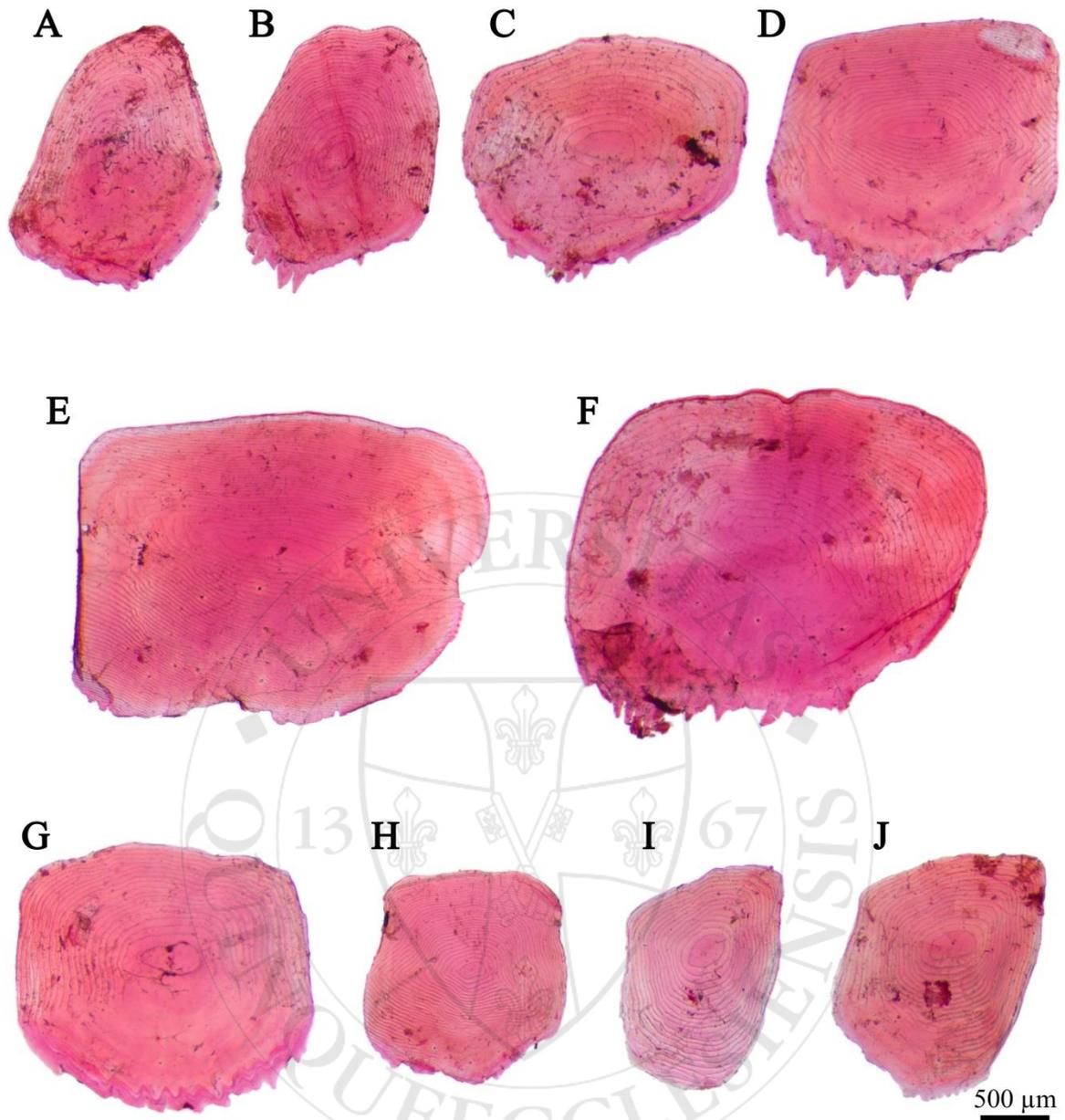


**FIGURE 74.** *Sphyaena sphyraena*

268 mm SL, Gulf of Ambracia, Greece, DMM IE/9020



**FIGURE 75.** Surface ornamentations of the scale of *S. sphyraena* in sampling area H



**FIGURE 76.** *Scomber colias*

215 mm SL, Mali Lošinj, Croatia, DMM IE/9021

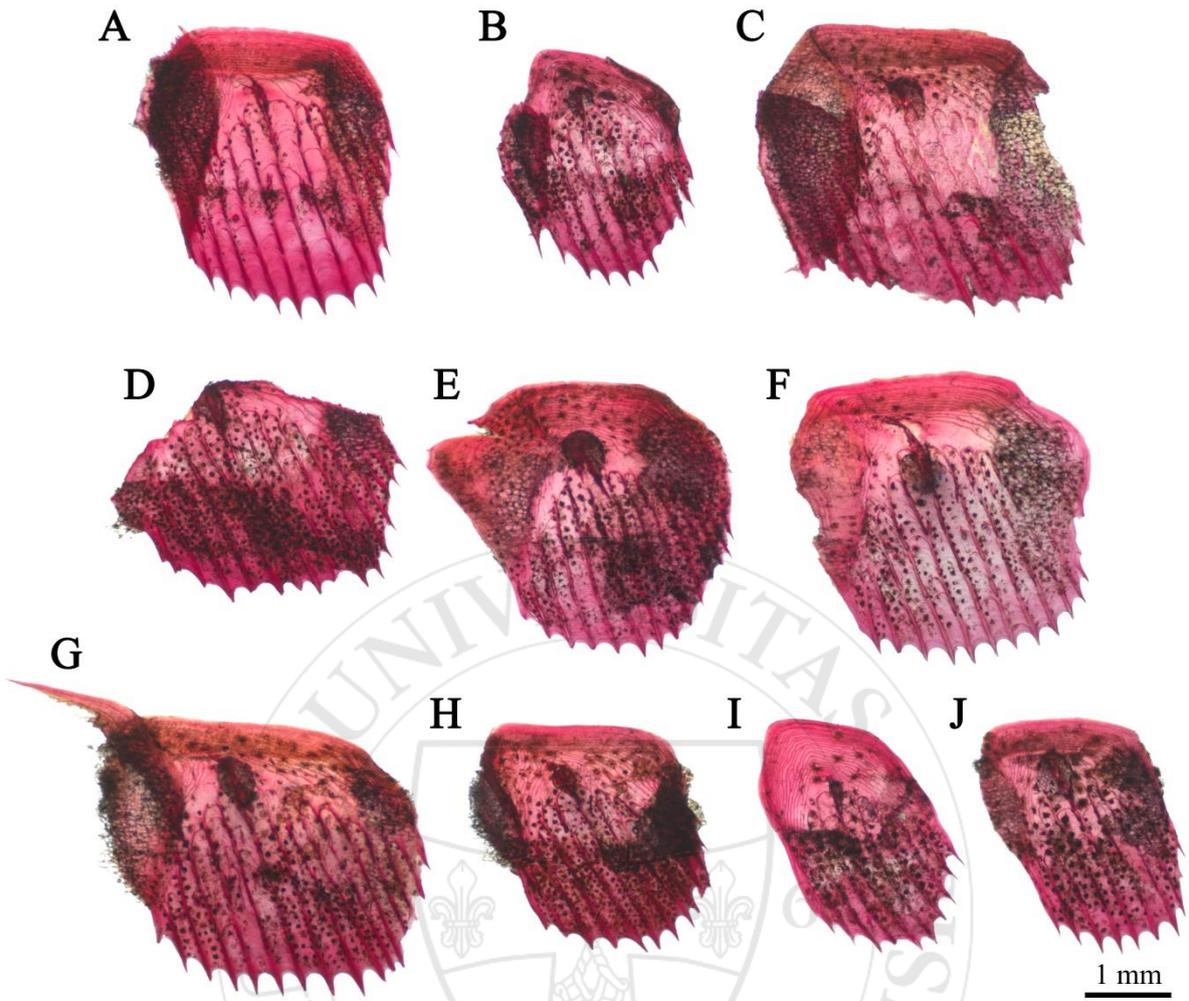
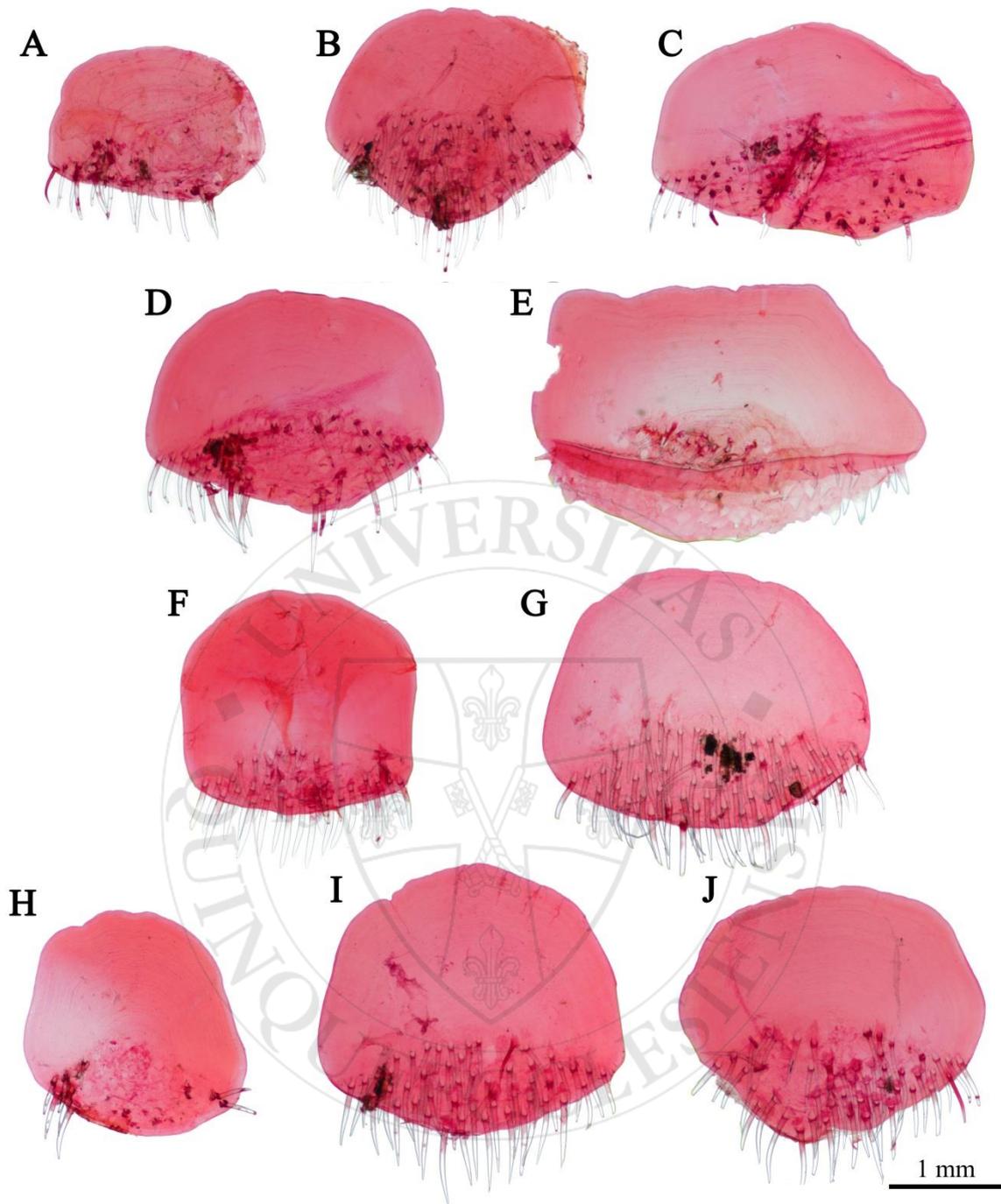
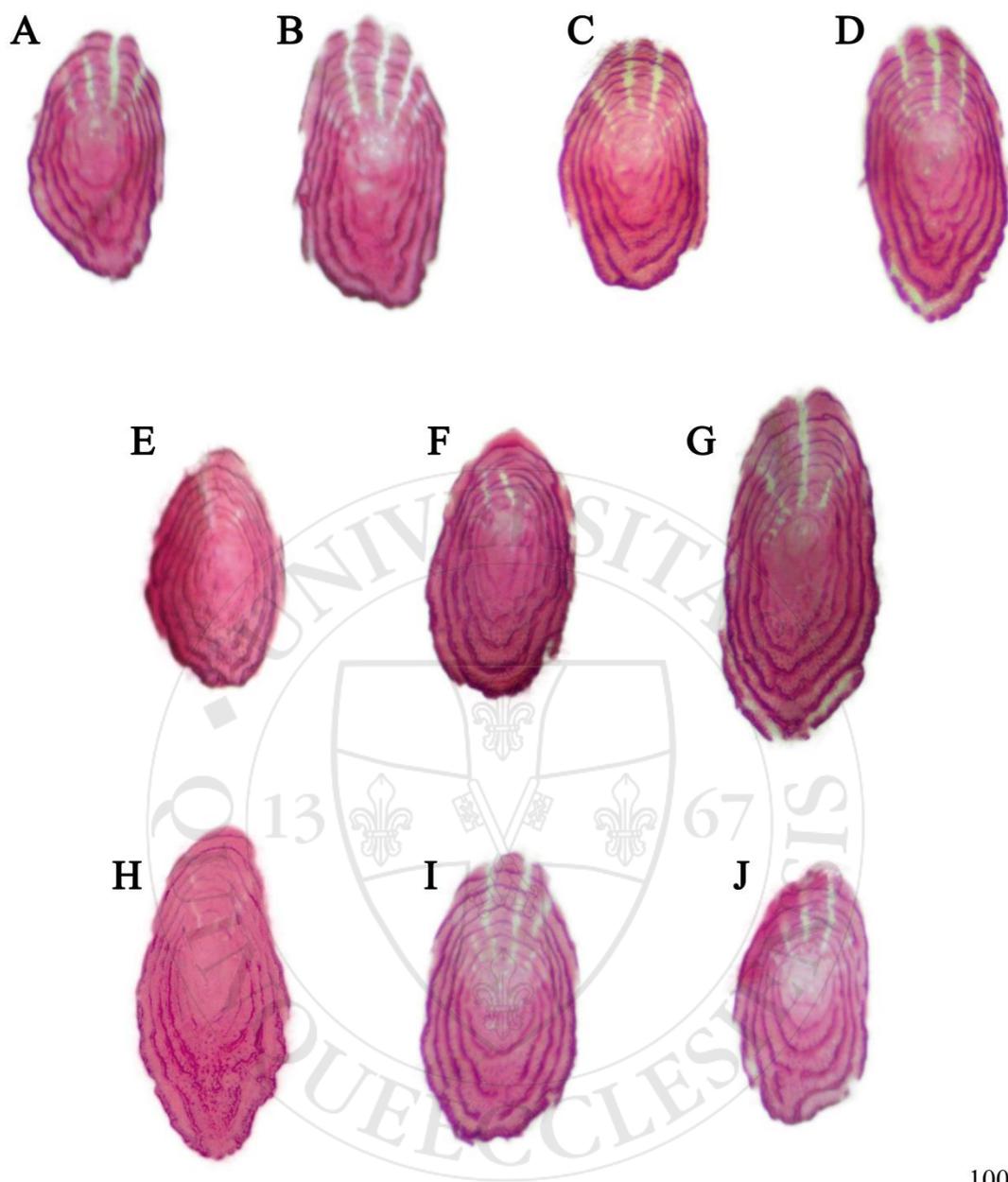


FIGURE 77. *Tetragonurus cuvieri*

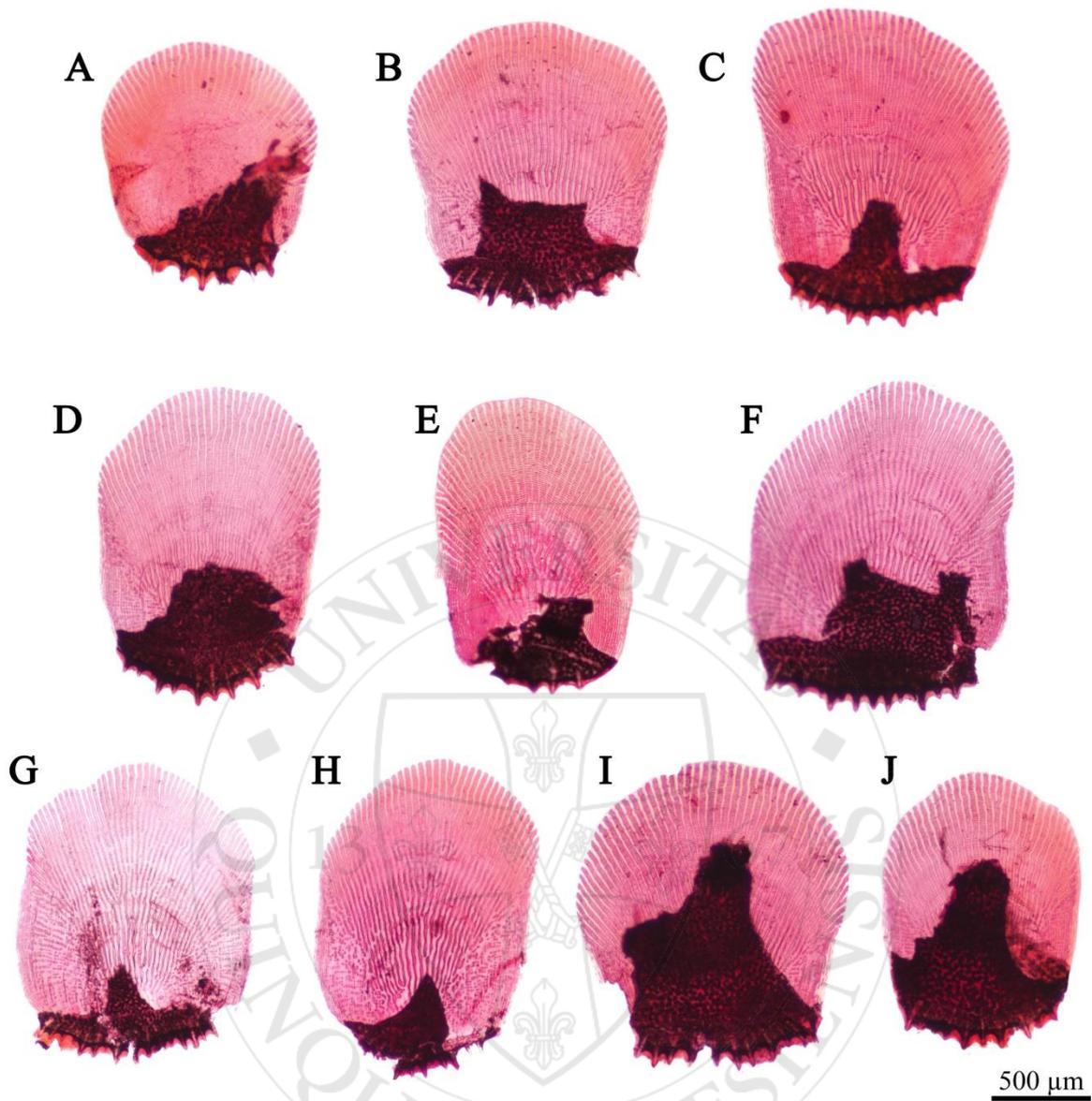
298 mm SL, South East Atlantic, Namibia, DMM IE/3469



**FIGURE 78.** *Capros aper*  
81 mm SL, Costa Brava, Spain, DMM IE/5038

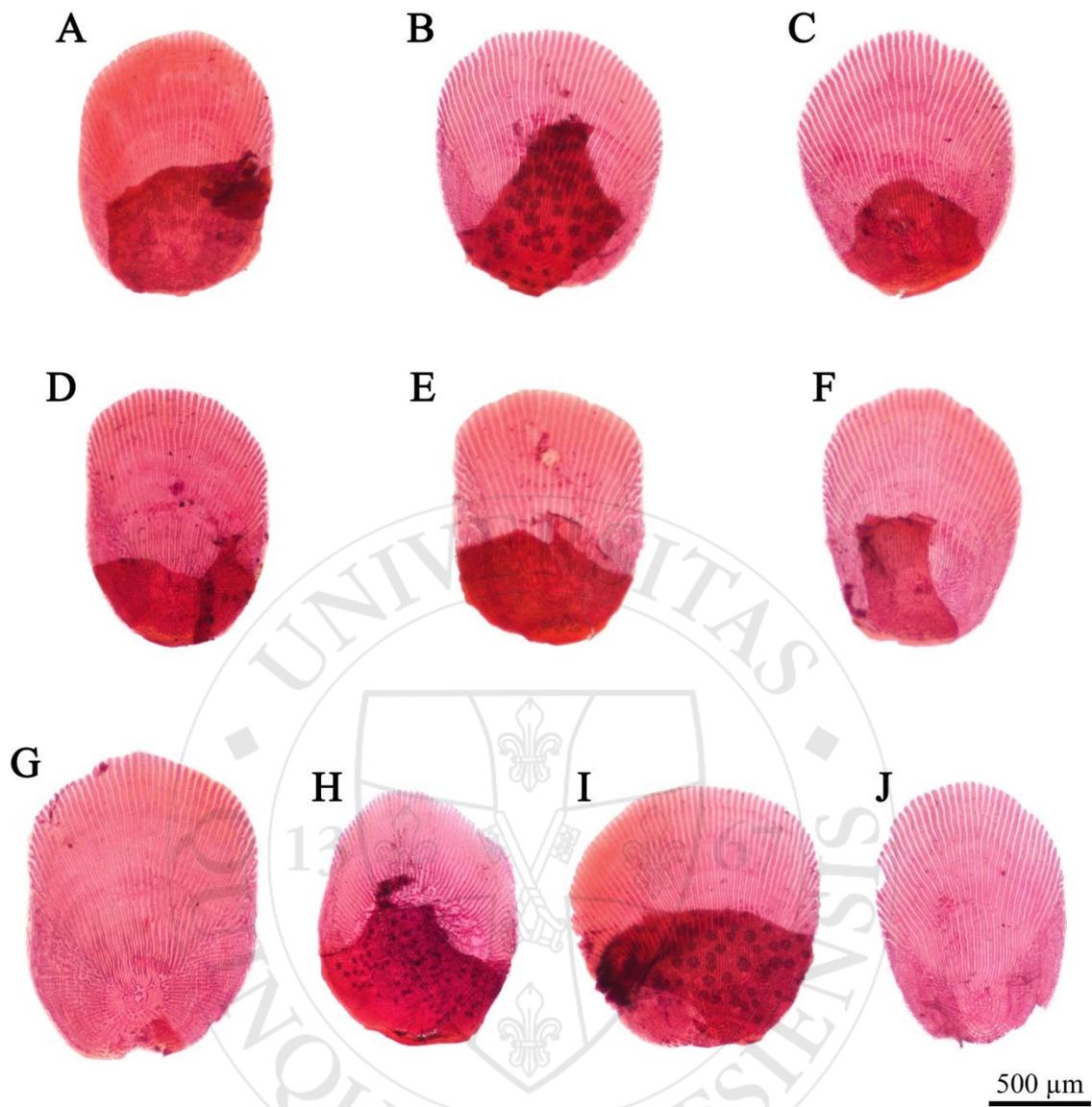


**FIGURE 79.** *Scophthalmus maximus*  
ocular-side (left side); 45 mm SL, Baltic Sea, Germany, DMM IE/9022



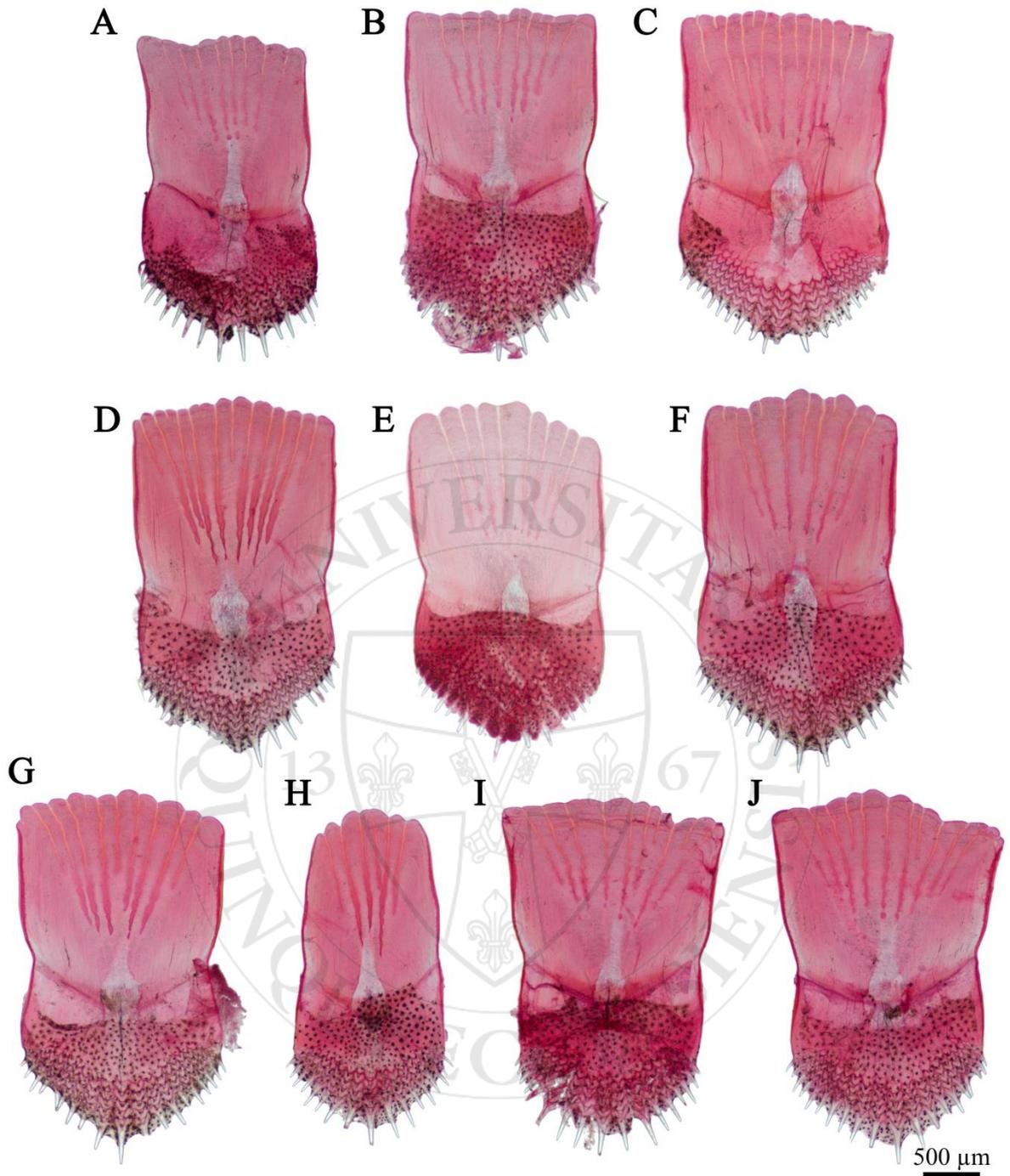
**FIGURE 80.** *Bothus podas*

ocular-side (left side); 132 mm SL, Central Atlantic, Azores, DMM IE/6627



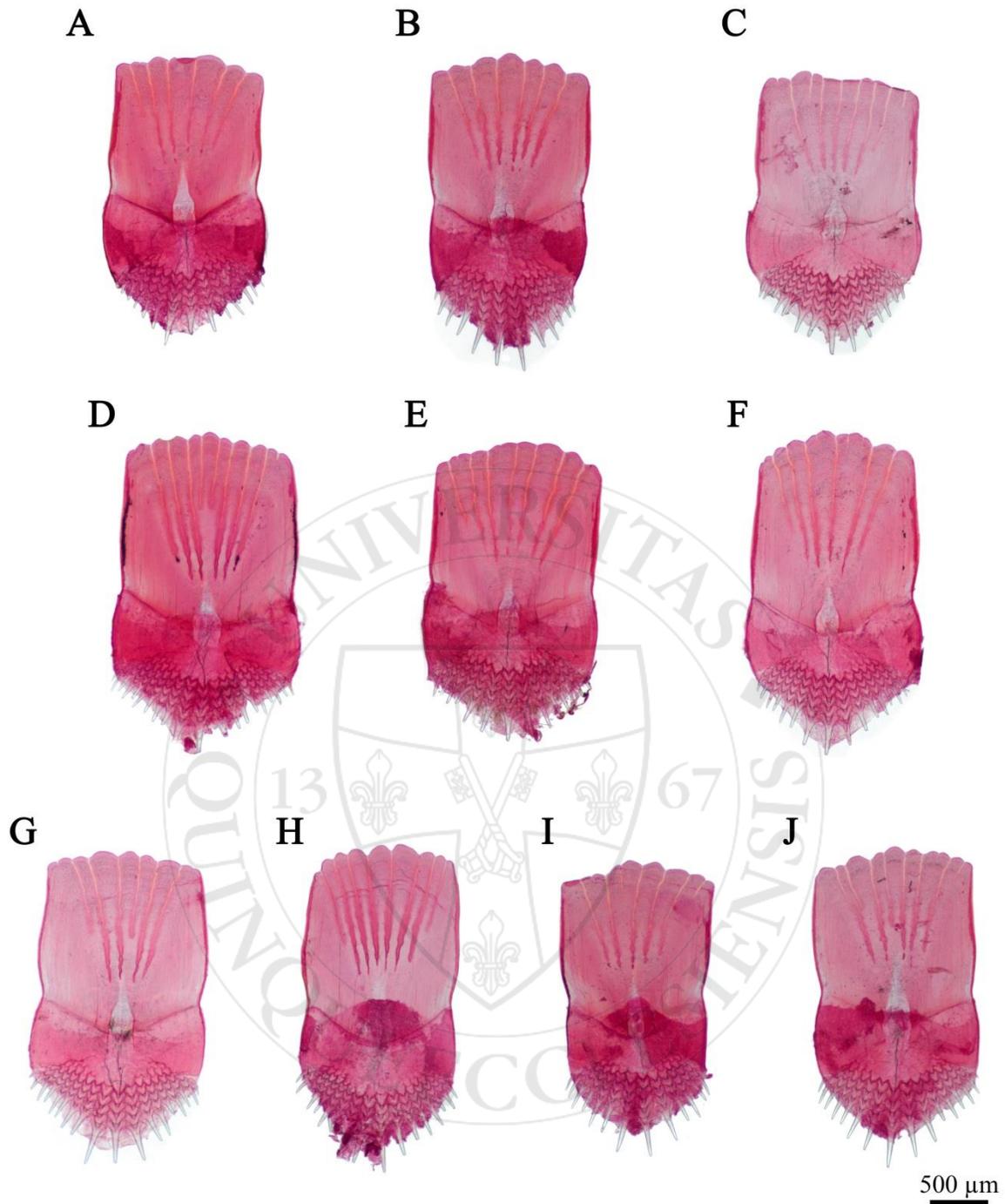
**FIGURE 81.** *Bothus podas*

blind-side (right side); 132 mm SL, Central Atlantic, Azores, DMM IE/6627

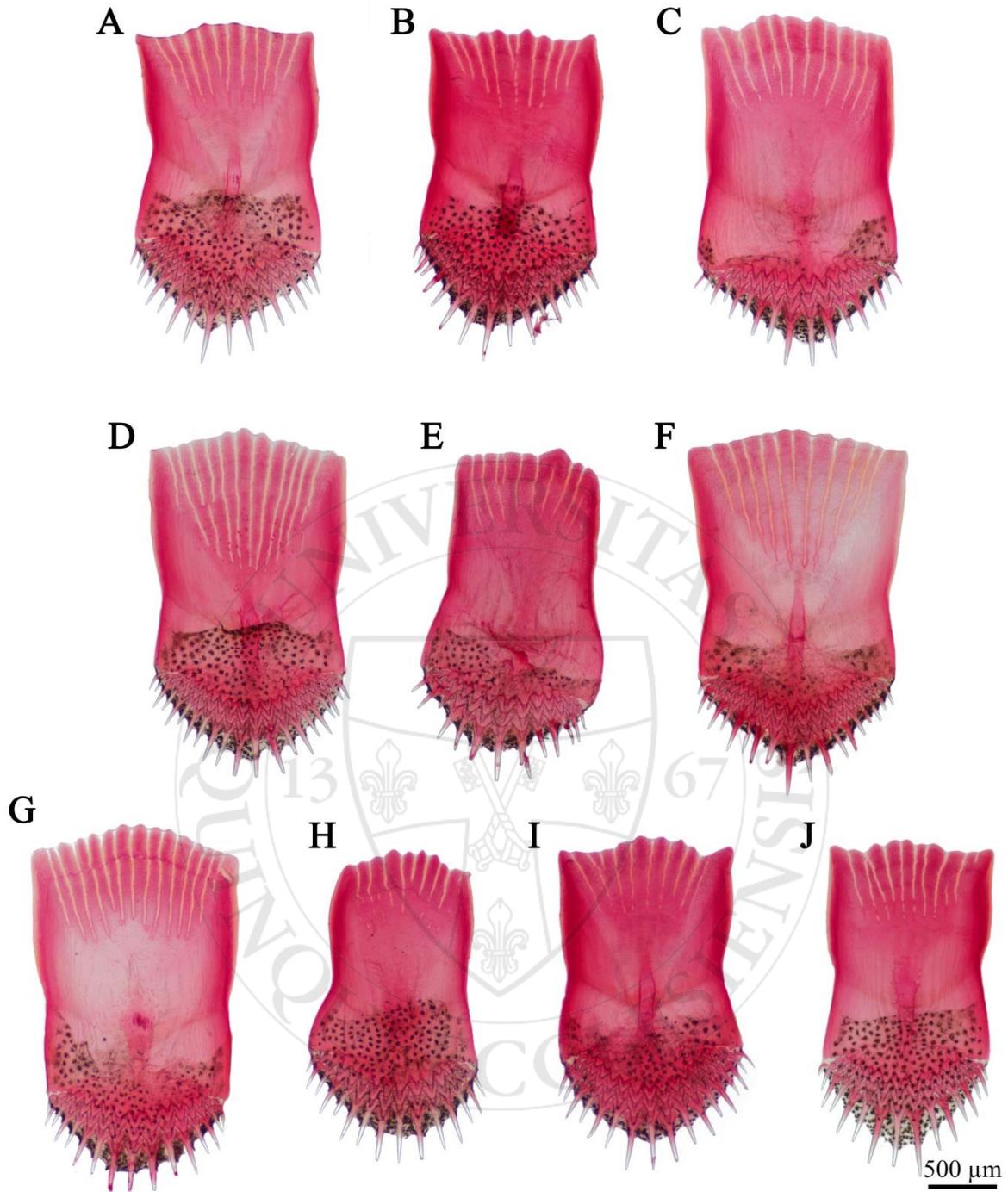


**FIGURE 82.** *Microchirus variegatus*

ocular-side (right side); 129 mm SL, Costa Brava, Spain, DMM IE/5101

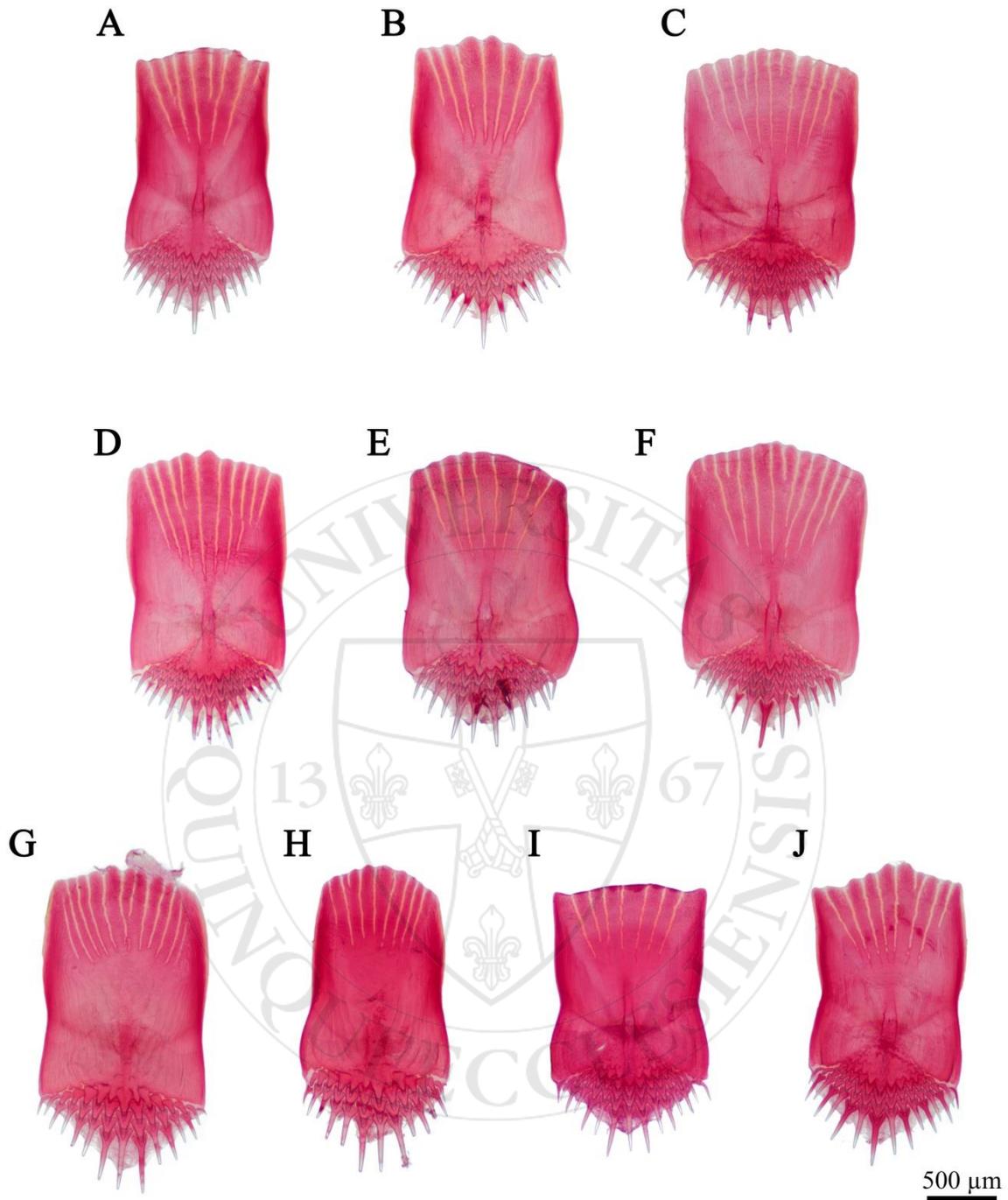


**FIGURE 83.** *Microchirus variegatus*  
blind-side (left side); 129 mm SL, Costa Brava, Spain, DMM IE/5101



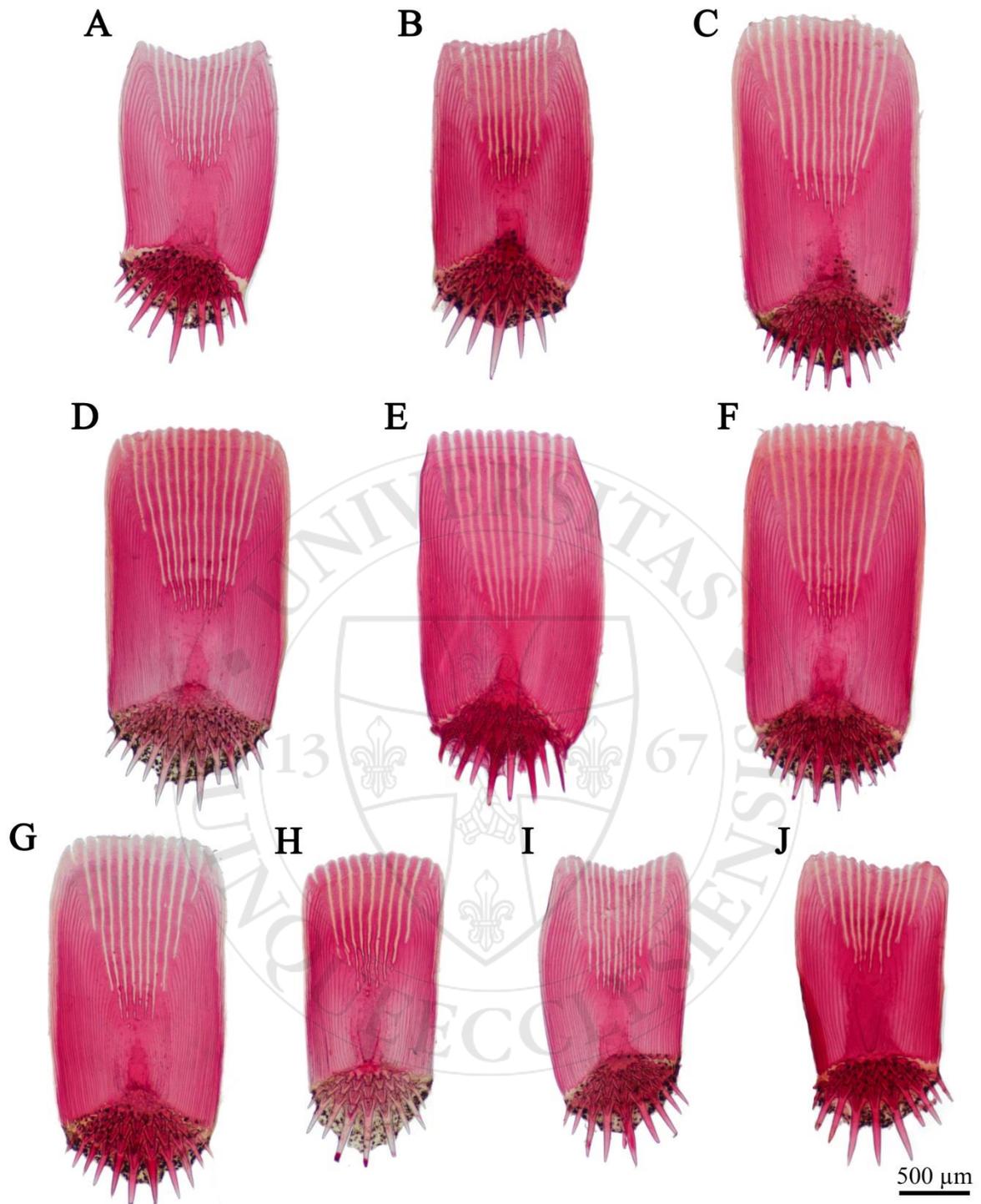
**FIGURE 84. *Monochirus hispidus***

ocular-side (right side); 104 mm SL, Costa Brava, Spain, DMM IE/4600



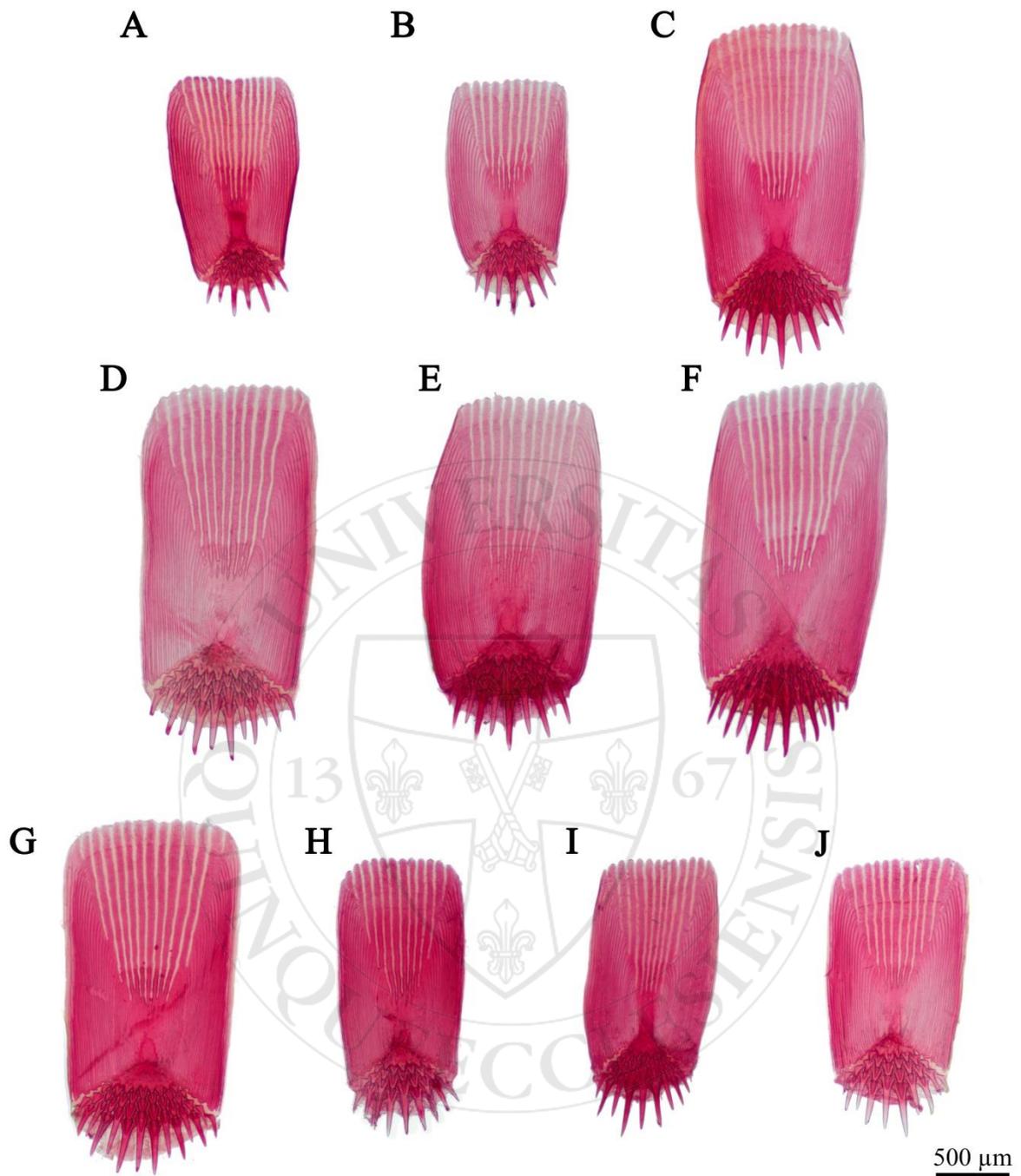
**FIGURE 85. *Monochirus hispidus***

blind-side (left side); 104 mm SL, Costa Brava, Spain, DMM IE/4600



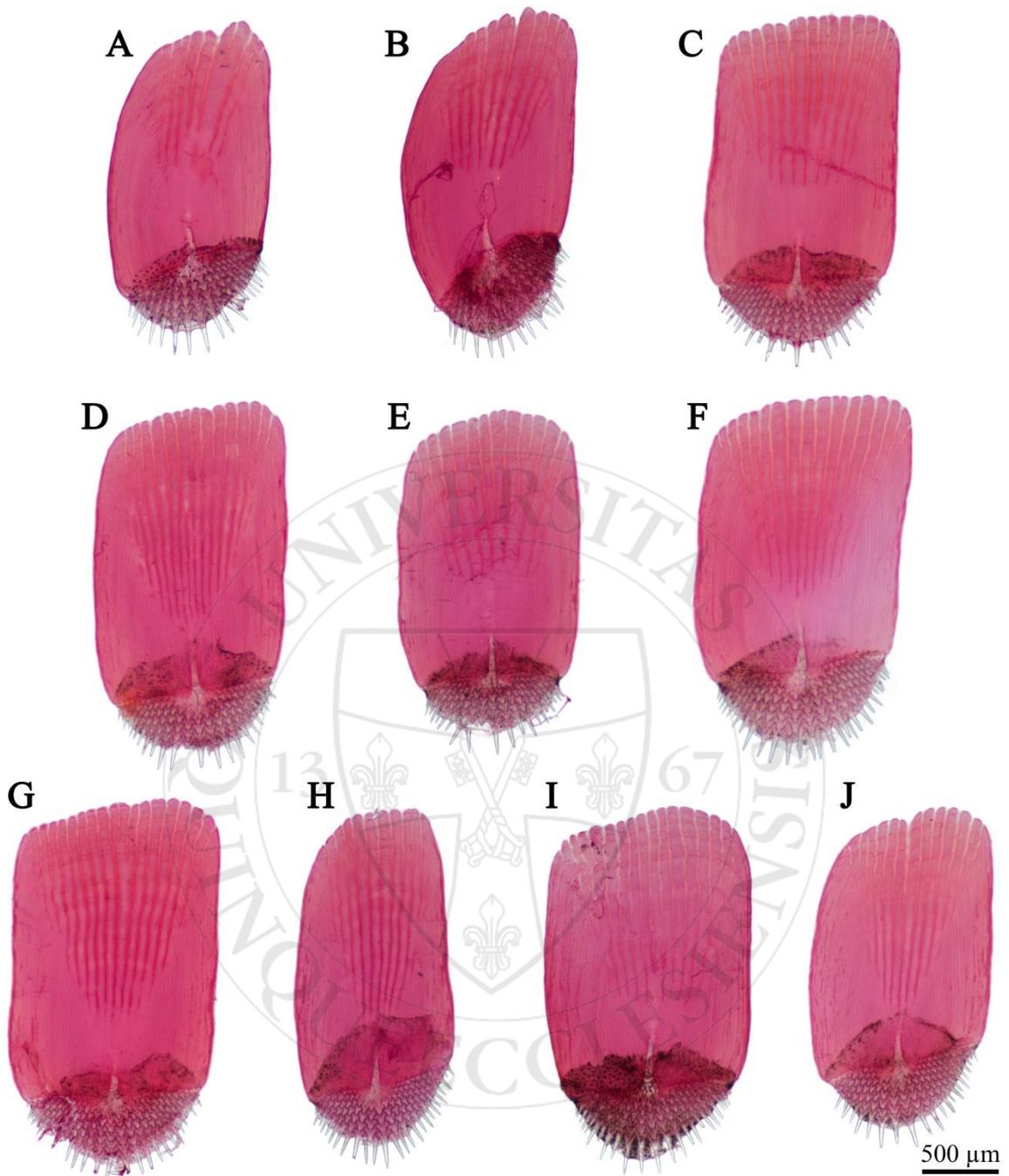
**FIGURE 86.** *Solea solea*

ocular-side (right side); 187 mm SL, Elbe estuary, Germany, DMM IE/4782



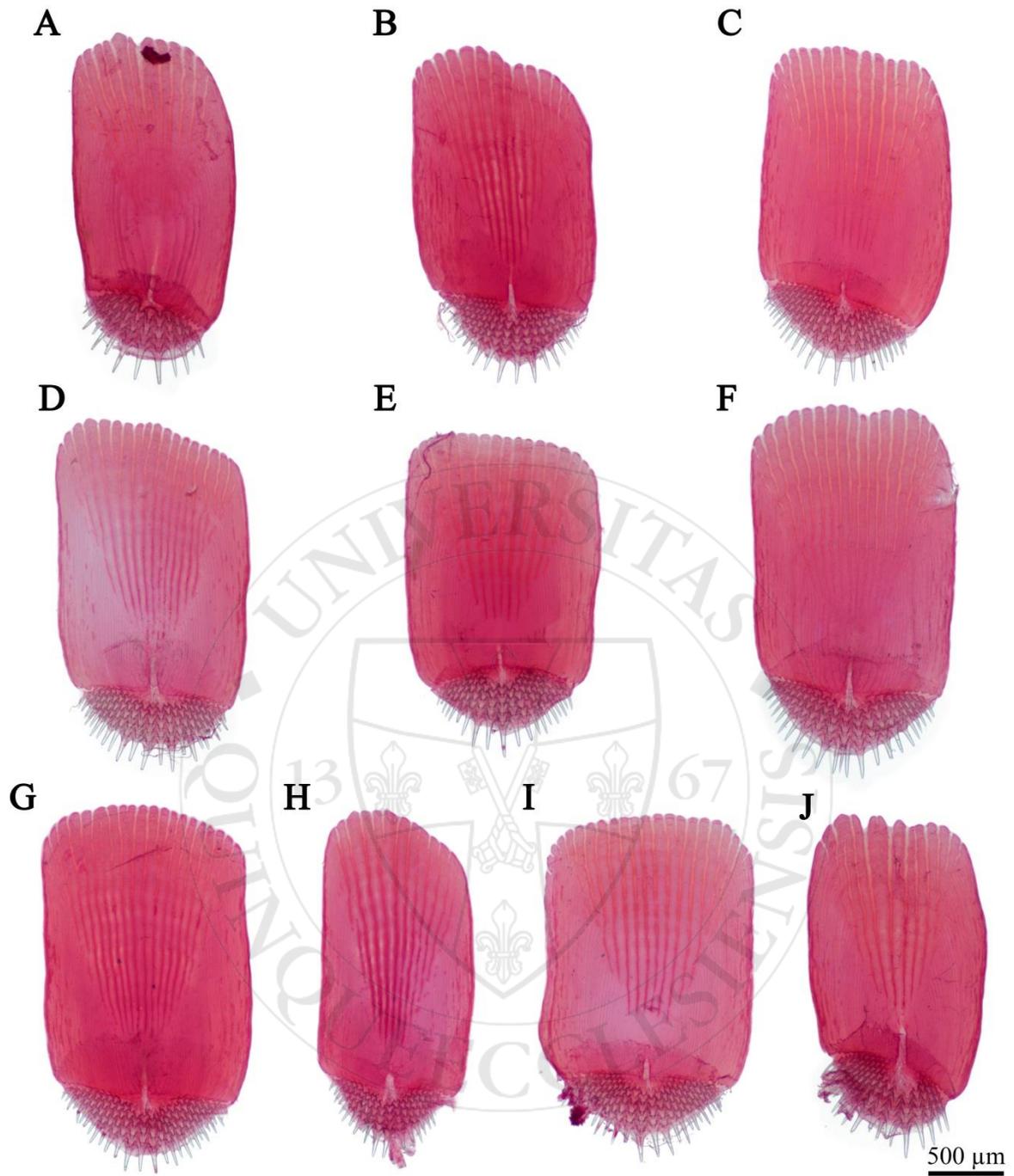
**FIGURE 87.** *Solea solea*

blind-side (left side); 187 mm SL, Elbe estuary, Germany, DMM IE/4782



**FIGURE 88.** *Symphurus nigrescens*

ocular-side (left side); 104 mm SL, Costa Brava, Spain, DMM IE/5100



**FIGURE 89.** *Symphurus nigrescens*

blind-side (right side); 104 mm SL, Costa Brava, Spain, DMM IE/5100

**TABLE 1. List of fish species and their scale types with exemplary size indices of scales derived from sampling area C (i.e., anterior, dorsal, above lateral line)**

Scale type abbreviations based on ROBERTS (1993): Cy – true cycloid scale; Cr – crenate scale; Sp – spinoid scale; Pe – ctenoid scale with peripheral cteni; Tr – ctenoid scale with transforming cteni; Wh – ctenoid scale with whole cteni. The relative scale sizes (J-indices) for the scale length (Jsl), scale width (Jsw) and scale transverse diameter (Jst) were calculated following ESMAEILI (2001):  $Jsl/Jsw/Jst = \text{length (width or transverse diameter) of scale (in mm)} / \text{fish standard length (in mm)} \times 100$  (see point 3.2.2.2). Focal index (Fi) is given as the distance (in mm) from the outermost edge of the anterior field to the focus / the distance (in mm) from the outermost edge of the anterior field to the outermost edge of the posterior field.

Teleostei	Scale type	Jsl	Jsw	Jst	Fi
<b>ALBULIFORMES</b>					
Notacanthidae					
<i>Notacanthus bonaparte</i>	Cy	0.39	0.23	0.34	0.36
<b>CLUPEIFORMES</b>					
Engraulidae					
<i>Engraulis encrasicolus</i>	Cr	4.11	5.44	5.30	0.50
Clupeidae					
<i>Alosa fallax</i>	Cr	3.20	3.14	3.44	0.67
<i>Clupea harengus</i>	Cr	2.93	3.87	3.79	0.56
<i>Sardina pilchardus</i>	Cr	4.34	4.94	4.86	0.66
<i>Sardinella aurita</i>	Cr	3.78	4.76	4.60	0.61
<i>Sprattus sprattus</i>	Cr	3.37	3.45	3.66	0.62
<b>ARGENTINIFORMES</b>					
Argentinidae					
<i>Argentina sphyraena</i>	Cy	4.18	5.40	5.06	0.73
Alepocephalidae					
<i>Alepocephalus rostratus</i>	Cy	3.44	2.06	3.39	0.02
<b>STOMIIFORMES</b>					
Sternoptychidae					
<i>Maurollicus muelleri</i>	Cy	3.49	3.72	3.97	0.44
<b>AULOPIIFORMES</b>					
Synodontidae					
<i>Synodus saurus</i>	Cy	3.49	3.75	3.97	0.64
<b>MYCTOPHIFORMES</b>					
Myctophidae					
<i>Myctophum punctatum</i>	Cy	4.90	6.15	6.84	0.58
<b>GADIFORMES</b>					
Macrouridae					
<i>Coelorinchus caelorhinchus</i>	Sp	1.37	1.67	1.67	0.40
<i>Nezumia sclerorhynchus</i>	Sp	1.43	1.75	1.81	0.60
<i>Trachyrincus scabrus</i>	Sp	0.98	1.36	1.29	0.48
Moridae					
<i>Mora moro</i>	Cy	2.16	1.79	2.24	0.70
Merlucciidae					
<i>Merluccius merluccius</i>	Cy	0.71	0.51	0.66	0.47
Gadidae					
<i>Molva molva</i>	Cy	0.37	0.13	0.33	0.59
<i>Trisopterus luscus</i>	Cy	1.47	1.13	1.36	0.30
<b>MUGILIFORMES</b>					
Mugilidae					
<i>Chelon labrosus</i>	Wh	3.71	3.85	4.48	0.72
<i>Liza aurata</i>	Wh	2.83	3.13	3.30	0.70
<b>ATHERINIFORMES</b>					
Atherinidae					
<i>Atherina hepsetus</i>	Cy	2.57	3.22	3.39	0.49
<i>Atherinomorus lacunosus</i>	Cy	1.93	2.06	2.04	0.44

<b>BELONIFORMES</b>						
Belonidae						
	<i>Belone belone</i>	Cy	0.62	0.67	0.67	0.47
<b>BERYCIFORMES</b>						
Berycidae						
	<i>Beryx decadactylus</i>	Sp	2.07	2.37	2.42	0.44
Trachichthyidae						
	<i>Hoplostethus mediterraneus</i>	Sp	1.54	2.89	2.65	0.47
<b>ZEIFORMES</b>						
Zeidae						
	<i>Zeus faber</i>	Cy	1.03	0.87	0.98	0.44
<b>GASTEROSTEIFORMES</b>						
Macroramphosidae						
	<i>Macrorhamphosus scolopax</i>	Sp	1.04	1.73	1.39	0.33
<b>SCORPAENIFORMES</b>						
Sebastidae						
	<i>Helicolenus dactylopterus</i>	Tr	2.43	2.63	3.18	0.82
Scorpaenidae						
	<i>Scorpaena porcus</i>	Sp	2.75	1.87	2.43	0.63
Triglidae						
	<i>Eutrigla gurnardus</i>	Cy/Tr	1.01	0.78	1.10	0.75
	<i>Lepidotrigla cavillone</i>	Sp	2.13	3.55	3.65	0.70
<b>PERCIFORMES</b>						
Moronidae						
	<i>Dicentrarchus labrax</i>	Tr	1.85	1.74	2.13	0.64
Serranidae						
	<i>Anthias anthias</i>	Pe	2.94	4.10	4.05	0.88
	<i>Epinephelus aeneus</i>	Cy/Tr	1.43	0.87	1.40	0.57
	<i>Serranus cabrilla</i>	Tr	1.80	1.79	2.03	0.77
	<i>Serranus scriba</i>	Tr	2.03	1.75	2.26	0.72
Apogonidae						
	<i>Apogon imberbis</i>	Tr	5.08	6.24	5.92	0.88
Epigonidae						
	<i>Epigonus denticulatus</i>	Cy	1.88	2.88	2.43	n/a*
Coryphaenidae						
	<i>Coryphaena equiselis</i>	Cy	0.90	0.61	0.91	0.45
Carangidae						
	<i>Seriola dumerili</i>	Cy	0.91	0.73	0.92	0.60
	<i>Trachurus trachurus</i>	Cy	1.04	1.38	1.22	0.59
Bramidae						
	<i>Brama brama</i>	Sp	1.40	1.85	1.86	0.23
Sparidae						
	<i>Boops boops</i>	Tr	2.13	2.45	2.59	0.45
	<i>Diplodus annularis</i>	Tr	2.85	3.08	3.37	0.62
	<i>Diplodus vulgaris</i>	Tr	3.51	5.40	5.29	0.80
	<i>Pagellus acarne</i>	Tr	2.39	2.93	3.16	0.57
	<i>Pagellus erythrinus</i>	Tr	2.22	2.96	3.09	0.60
	<i>Sarpa salpa</i>	Tr	2.34	3.01	3.06	0.46
	<i>Sparus aurata</i>	Tr	2.13	3.12	3.09	0.55
	<i>Spondylisoma cantharus</i>	Tr	2.10	2.77	2.95	0.52
Centracanthidae						
	<i>Spicara maena</i>	Tr	2.14	2.93	3.15	0.58
	<i>Spicara smaris</i>	Tr	1.53	2.08	2.20	0.49
Sciaenidae						
	<i>Sciaena umbra</i>	Tr	2.33	2.36	2.86	0.63
Mullidae						
	<i>Mullus barbatus</i>	Tr	4.86	5.60	6.45	0.73
	<i>Mullus surmuletus</i>	Tr	5.17	5.11	6.00	0.81
Chaetodontidae						
	<i>Chaetodon hoefleri</i>	Tr	5.31	5.24	5.51	0.76

Cepolidae						
<i>Cepola macrophthalmia</i>	Cy	0.20	0.17	0.21	0.72	
Pomacentridae						
<i>Chromis chromis</i>	Tr	6.13	4.53	5.70	0.73	
Labridae						
<i>Coris julis</i>	Cy	2.55	1.48	2.13	0.52	
<i>Labrus viridis</i>	Cy	3.19	2.14	2.96	0.58	
<i>Symphodus rostratus</i>	Cy	5.87	4.17	5.63	0.51	
<i>Thalassoma pavo</i>	Cy	6.66	4.72	6.37	0.54	
Scaridae						
<i>Sparisoma cretense</i>	Cy	8.02	6.65	7.74	0.54	
Ammodytidae						
<i>Gymnamodytes cicerelus</i>	Cy	0.26	0.34	0.31	0.50	
Trachinidae						
<i>Trachinus draco</i>	Cy	1.27	1.10	1.44	0.63	
Tripterygiidae						
<i>Tripterygion tripteronotum</i>	Pe	2.70	3.90	3.42	0.67	
Gobiidae						
<i>Gobius bucchichi</i>	Pe	2.72	2.77	3.13	0.90	
<i>Gobius paganellus</i>	Pe	2.81	2.61	2.94	0.87	
Sphyraenidae						
<i>Sphyraena chrysotaenia</i>	Cy	1.44	1.08	1.42	0.44	
<i>Sphyraena sphyraena</i>	Sp	0.52	0.52	0.53	0.54	
Scombridae						
<i>Scomber colias</i>	Cr	0.85	0.97	1.00	0.51	
Tetragonuridae						
<i>Tetragonurus cuvieri</i>	Sp	1.15	1.29	1.43	0.28	
Caproidae						
<i>Capros aper</i>	Sp	2.40	3.54	3.30	0.70	
PLEURONECTIFORMES						
Psettodidae						
<i>Scophthalmus maximus</i>	Cy**/-	1.00/-	0.59/-	0.91/-	0.47/-	
Bothidae						
<i>Bothus podas</i>	Tr/Cy	1.22/1.00	0.98/0.81	1.24/0.93	0.77/0.79	
Soleidae						
<i>Microchirus variegatus</i>	Tr/Tr	2.27/1.82	1.46/1.18	2.19/1.66	0.65/0.61	
<i>Monochirus hispidus</i>	Tr/Tr	2.33/1.93	1.40/1.22	2.15/1.74	0.62/0.63	
<i>Solea solea</i>	Tr/Tr	1.36/1.17	0.72/0.62	1.29/1.10	0.73/0.70	
Cynoglossidae						
<i>Symphurus nigrescens</i>	Tr/Tr	2.10/2.04	1.24/1.24	1.97/1.91	0.77/0.74	

Note: in some species, more than one scale type can occur, indicated with “/” mark.

In Pleuronectiformes, information is provided on scales derived from both sides of the specimens (i.e., ocular/blind side).

\* n/a: not applicable. \*\* Scales of *Scophthalmus maximus* were sampled at early stage of development.

## APPENDIX 2.

**TABLE 1. Data matrix of informative coded characters of scale morphology**

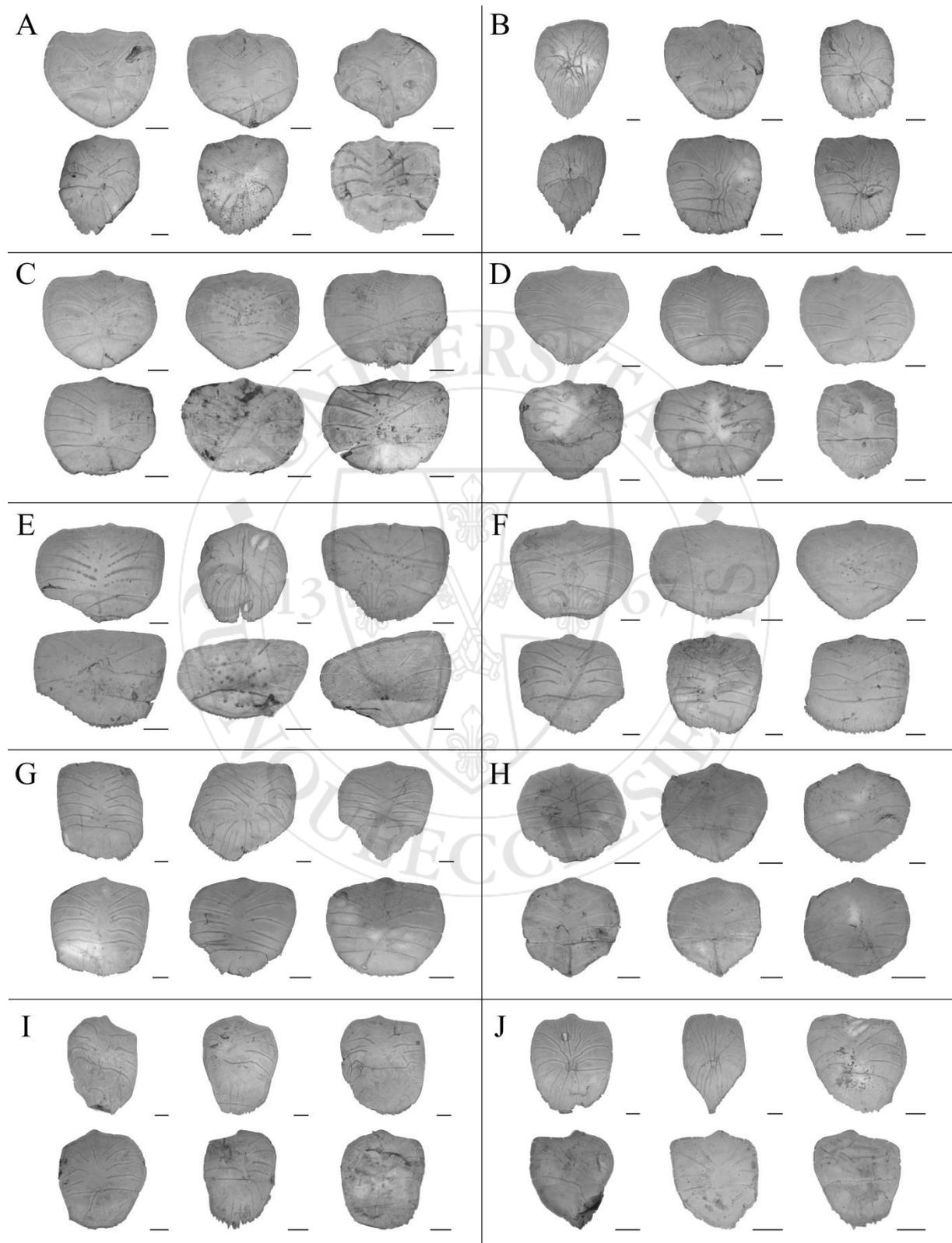
used in the average linkage dendrogram (based on Euclidean distance) to show the phenotypic relations between selected teleost species (see also TABLE 3 and TABLE 6)

Note: Scale type (ST), Scale shape (SS), Anterior field (AF), Anterior margin (AM), Lateral fields (LF), Dimension of the scale (DM), Posterior field (PF), Posterior margin (PM), Focus (FO), Position of focus (FP), Circuli (CI), Radii (RI), Spines (SP), Cteni (CT)

Species	ST	SS	AF	AM	LF	DM	PF	PM	FO	FP	CI	RI	SP	CT
EngE	1	0	0	0	0	0	2	2	0	2	2	4	0	0
AloF	1	0	0	0	0	0	1	2	0	3	2	3	0	0
CluH	1	0	0	0	0	0	1	2	0	3	2	3	0	0
SarP	1	0	0	0	0	0	1	2	0	3	2	3	0	0
SarA	1	0	0	0	0	0	1	2	0	3	2	3	0	0
SprS	1	0	0	0	0	0	1	2	0	3	2	3	0	0
CoeC	2	0	0	0	0	0	1	3	1	1	1	0	2	0
NezS	2	0	0	0	0	0	1	3	1	2	1	0	2	0
TraS	2	0	0	0	0	0	1	3	0	2	1	0	2	0
MorM	0	1	0	0	2	1	1	0	1	3	1	0	0	0
MerM	0	1	0	0	0	1	1	0	0	2	1	0	0	0
MolM	0	1	0	0	1	1	1	0	1	2	1	0	0	0
TriL	0	1	0	1	0	1	1	0	1	1	2	1	0	0
AntA	3	0	2	3	2	0	1	4	0	4	2	1	0	1
EpiA	4	1	2	3	2	1	1	4	1	2	2	1	0	2
SerC	4	4	2	3	2	0	1	4	1	3	2	1	0	2
SerS	4	2	2	2	2	1	1	4	1	3	2	1	0	2
SerD	0	0	0	3	0	0	1	0	1	2	2	1	0	0
TraT	0	0	2	3	0	0	1	0	1	2	2	1	0	0
BooB	4	3	2	2	1	0	2	4	1	2	2	1	0	2
PagA	4	3	2	2	1	0	2	4	1	2	2	1	0	2
PagE	4	3	2	2	2	0	2	4	1	2	2	1	0	2
SarS	4	4	2	2	0	0	1	4	1	2	2	1	0	2
SpaA	4	4	2	2	0	0	1	4	1	2	2	1	0	2
SpoC	4	4	2	2	1	0	1	4	1	2	2	1	0	2
MulB	4	4	2	4	0	0	1	4	0	3	2	1	0	2
MulS	4	4	2	4	0	0	1	4	0	3	2	1	0	2
CorJ	0	1	0	3	2	1	2	1	0	2	2	1	0	0
LabV	0	1	0	3	2	1	2	1	0	2	2	1	0	0
SymR	0	1	0	3	2	1	2	1	0	2	2	1	0	0
ThaP	0	1	0	1	2	1	1	1	0	2	2	1	0	0
ScoM	0	1	0	3	0	1	1	0	1	2	2	1	0	0
BotP	4	1	0	1	0	1	1	3	1	3	2	1	0	2
MicV	4	3	2	3	1	1	2	4	1	3	2	1	0	2
MonH	4	3	2	2	1	1	2	4	1	3	2	1	0	2
SolS	4	2	2	2	2	1	1	4	1	3	2	1	0	2
SymN	4	2	2	3	2	1	1	4	1	3	2	1	0	2

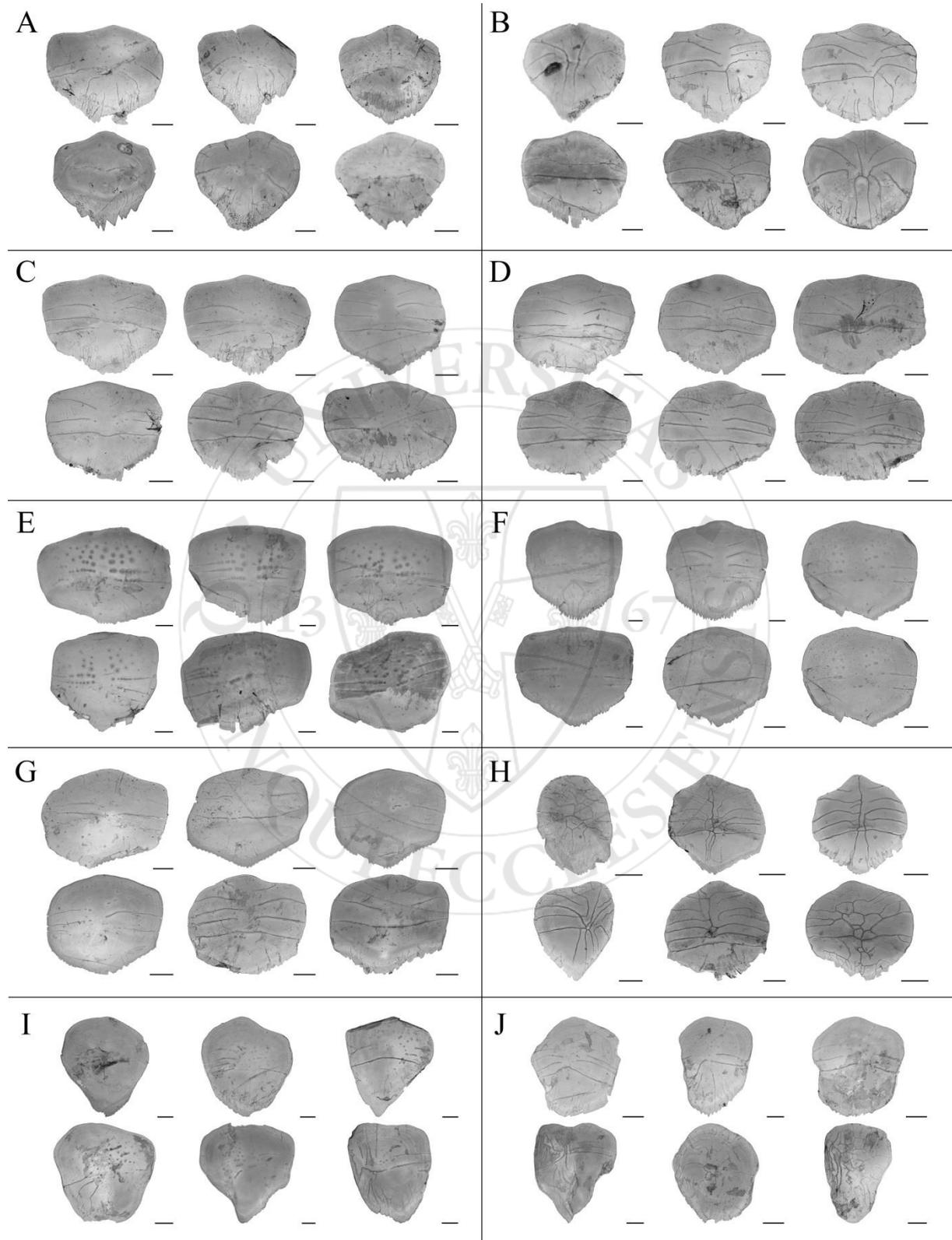
APPENDIX 3. Scale shape variability within *Sardina pilchardus* and *Sardinella aurita*

TABLE 1. Scale shape repertoire of *S. pilchardus*

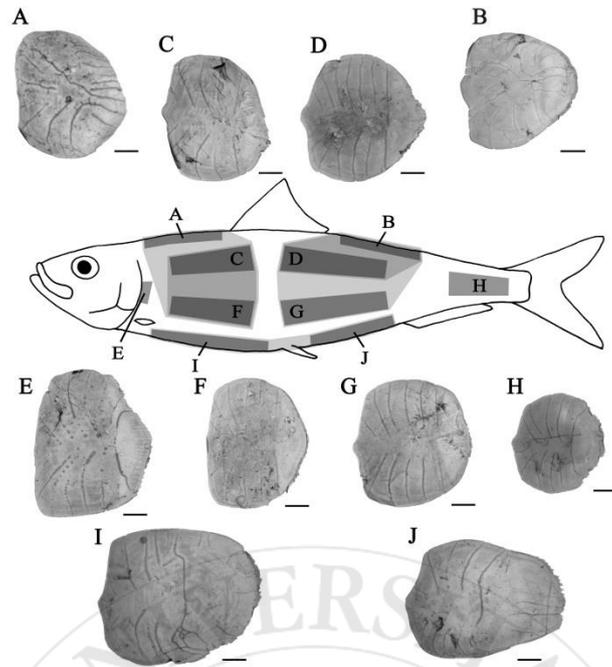


Note: scale bar = 1 mm

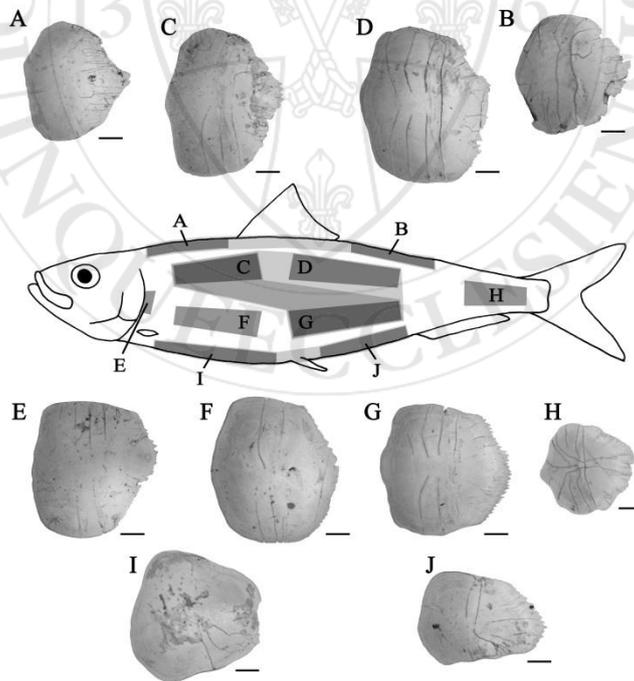
**TABLE 2. Scale shape repertoire of *S. aurita***



Note: scale bar = 1 mm



**FIGURE 1. Scale shape variability among sampling areas in *S. pilchardus***  
 Note: highlighted areas indicate similarities in scale shape indices; scale bar = 1 mm



**FIGURE 2. Scale shape variability among sampling areas in *S. aurita***  
 Note: highlighted areas indicate similarities in scale shape indices; scale bar = 1 mm

