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BIOLOGY

A comparative SEM study of ossicles in the Pleuronectiformes (Teleostei) of the Baltic Sea

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Abstract. The morphologies of ossicles of the European plaice *Pleuronectes platessa* Linnaeus, the European flounder *Platichthys flesus trachurus* (Linnaeus) (Pleuronectidae), and the turbot *Scophthalmus maximus* (Linnaeus) (Scophthalmidae) are described and compared. The examined material of these flatfishes (Pleuronectiformes) originates from Estonian waters, the eastern Baltic Sea. The cycloid and ctenoid scales, tubercles, lateral line scales and segments, and swivel-joint platelets noticeably differ among the three studied species. Because of characteristics of ctenii and development of the ctenial pattern, the scales of the flounder have been separated into a new subtype, the overgrowing ctenoid scales belonging to a general type of ctenoid scales. Two different appearances of tubercles, ctenoid and circuloid, are described for the flounder and turbot, respectively. Although the ossicles of the European plaice and European flounder differ in many details, they are not as highly modified as are the circuloid tubercles of the turbot. In the European plaice, a strong difference also occurs between the scales of males and females. In the European flounder and turbot, nodular ultrasculpture on the external surface of ossicles and structure formed by Mandl's corpuscles on their internal surface, known to be caused by mineralization processes, are imaged under scanning electron microscope and also described.

Key words: Pleuronectiformes, scales, tubercles, platelets, Baltic Sea, SEM study.

INTRODUCTION

Fish scale morphology is steadily gaining more attention in taxonomic and systematic investigations. Early descriptions of fishes were accompanied by simple drawings; for example, Benecke (1881) gave for each fish from Prussia (= part of Poland, Kaliningrad District, Russia, and Lithuania) a drawing of its scale or tubercle. Light microscopy and photography allowed much more detailed studies, which mainly focused on different teleost fish scales (Cockerell 1913; Kobayashi 1951; Haque 1955; Batts 1964; Patterson et al. 2001). The introduction of scanning electron microscopy (SEM) initiated a new approach in the study of teleost fish scale morphology and surface sculpture worldwide (DeLamater and Courtenay 1974; Hughes 1981; Sire 1986; Bonwick et al. 1989; Roberts 1993; Sire and Meunier 1993; Jawad 2005a, b; Jawad and Al-Jufaili 2007; Märss et al. 2010a, b; Lees et al. 2012). In addition to facilitating morphological studies, SEM also revealed the finest sculptural elements on the surface of scales, which were not visible previously owing to their small dimensions. Such a fine sculpture, measured in tens of micrometres only, became known as ultrasculpture in fossil fish investigations (Märss 2006).

Several researchers have turned their attention to the study of bony elements in flatfishes. The famous palaeoichthyologist R. H. Traquair (1840–1912) devoted his first study to the asymmetry of the Pleuronectidae, in which he also discussed and perfectly illustrated the mucous canals of the lateral lines on the heads of the turbot and plaice (Traquair 1865, pl. 32, figs 2, 3). He

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referred to the tubular scales of the lateral line as 'ossicles', a term that later was adopted by our team for all small bony elements despite their origin or function (Märss et al. 2010a, b; Lees et al. 2012). Ossicles were defined as very small bony structures, including scales, tubercles, lateral-line elements, and swivel-joint platelets, usually overlooked or given only a cursory treatment in morphological studies of fishes. Only small bony elements were under focus in these studies. The authors showed that the ossicles of various taxa of fishes from the Baltic Sea had a wealth of interesting details and diagnostic features. The ossicles of cottiforms, syngnathiforms, and gasterosteiforms have been characterized and differences in scale modifications of female and male specimens of the shorthorn sculpin documented in Märss et al. (2010a, b) and Lees et al. (2012). Variations in scales between sexes of different flatfishes have been documented by Norman (1934) and Tomiyama (2013). Batts (1964) ascertained the main morphological scale characteristics to distinguish the 15 species of flatfishes of Puget Sound, Washington. The morphology of dermal ossicles of the turbot *Scophthalmus maximus* was examined by Zylberberg et al. (2003). In recent years, several papers dealing with the bony elements [lateral line scales (l.l.s.), the head sensory canal system] in flatfishes, were published by Voronina (2007, 2009, 2010).

Nevertheless, the morphology of scales and other ossicles of pleuronectiforms, especially of the Baltic Sea, remains insufficiently known. When studying the structure of the seismosensory system of scophthalmids, Voronina (2010, p. 699) stressed that the small number of papers on the morphology of scales of Pleuronectiformes complicates the use of characters of scale structure in cladistic analyses. Even though claims have been made for the taxonomic value of scale morphology and surface features, the simple drawings and light photographs do not show the details of their sculpture clearly enough.

The present SEM study was undertaken to assess the potential usefulness of ossicles of pleuronectiforms for distinguishing among the taxa and for identifying characters of value in systematic and phylogenetic research. Our task is to show that ossicle morphology and sculpture may provide a novel tool for taxonomic studies. We demonstrate similarities and dissimilarities in ossicles of three pleuronectiform taxa obtained from the Baltic Sea. The three studied pleuronectiform species represent two closely related forms, the European plaice *Pleuronectes platessa* Linnaeus, 1758 and the European flounder *Platichthys flesus trachurus* (Linnaeus, 1758) (Pleuronectidae), and one more distantly related form, the turbot *Scophthalmus maximus* (Linnaeus, 1758) (Scophthalmidae). We here describe and SEM image the morphology and sculpture of scales, tubercles, and lateral line units (scales and segments), as well as the very small platelets of the distal radials of fins called also swivel-joint platelets. Special attention is paid to the occurrence of ultrasculpture on the same bony elements.

Our results can be used in the studies of the feeding preferences of fishes and sea birds, and the formation of the fish fauna during the development of the Baltic Sea. In archaeology, fish ossicles can reveal which fishes were used by ancient settlers, allow the estimation of the length and weight of those fishes, and indicate the season of capture as supposed by Slyke (1998). As hybrids are well known in flatfishes (Kijewska et al. 2009), our data on ossicle morphology and sculpture in the ancestors may help to clarify whether hybridization of flatfish species is expressed in their ossicle features. This character could be used for the identification of hybrid specimens along with the modern but expensive methods of molecular biology.

MATERIALS AND METHODS

The examined material of flatfishes originates from Estonian waters, the eastern Baltic Sea. The ossicles were taken from 20 specimens each of *Pleuronectes platessa* Linnaeus and *Platichthys flesus trachurus* (Linnaeus) of the family Pleuronectidae, subfamily Pleuronectinae, and *Scophthalmus maximus* (Linnaeus) of the family Scophthalmidae. Only adult specimens were studied. Both male and female specimens were investigated; however, the sex of a few scaled and cleaned specimens bought at the market remained unidentified. The fishes were mostly caught with a gillnet or pelagic trawl in the course of commercial trawl catches and during the monitoring of fish catches, while a few were bought at a fish market between April 2008 and January 2014. The material originates geographically from the waters of Pakri Bay and Paslepa Bay (Gulf of Finland), Küdema Bay (north of Saaremaa Island), and Pärnu Bay (Gulf of Riga) (Fig. 1). A few specimens of *Pleuronectes platessa* from southern Sweden and from the Bornholm Basin of the southern Baltic Sea were obtained for comparison but not described herein. The collected specimens of taxa (GIT 584-collection number; TL – total length, cm; F – female; M – male/if available/) are as follows:

Scophthalmus maximus (Pakri Bay GIT 584-17, TL = 14.0; Küdema Bay GIT 584-32, M, TL = 18.2; GIT 584-116, M, TL = 24.7; GIT 584-117, F, TL = 27.0; Pärnu Bay GIT 584-88, F, TL = 31).

Platichthys flesus trachurus (Pakri Bay GIT 584-20, TL = 29.7; Küdema Bay GIT 584-51, M, TL = 10.6; GIT 584-52, F, TL = 30.0; Pärnu Bay GIT 584-48, M, TL = 18.7; GIT 584-49, F, TL = 16.2; GIT 584-93, TL = 16.0; GIT 584-115, TL = 35.0; GIT 584-118, TL = 36.0).

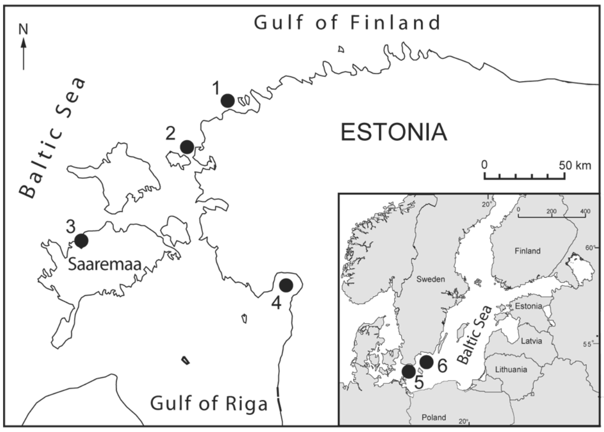

Fig. 1. Location of fishing grounds: 1, Pakri Bay; 2, Paslepa Bay; 3, Küdema Bay; 4, Pärnu Bay; 5, southern Sweden; 6, Bornholm Basin.

Pleuronectes platessa (Paslepa Bay GIT 584-121, F, TL = 29.0; GIT 584-122, M, TL = 26.5; Pärnu Bay GIT 584-89, M, TL = 27.0; southern Sweden GIT 584-94, TL = 34.5; GIT 584-97, TL = 34.0; Bornholm Basin GIT 584-98, F, TL = 36.0; GIT 584-99, M, TL = 29.0).

The preparation methods used in this study are the same as those described in Märss et al. (2010a, b) for cottiforms and in Lees et al. (2012) for gasterosteiforms and syngnathiforms. Fish bodies were washed and measured. The skin pieces were removed and put into the solution of 30% hydrogen peroxide (H_2O_2), and distilled water, and buffered with 25% ammonia water (NH_4OH) with the ratio of 200 : 100 : 50 mL, respectively. After chemical manipulation the dermal units were washed with tap-water in the sieves with the mesh size of 0.01 mm, and deposited in 65% ethanol. Before SEM imaging the better preserved specimens were kept in 30% ethanol for a short time, semi-dried in open air, set on SEM stubs covered with double-sided sticky tape, dried, and then coated with gold. The specimens were searched and photographed.

The skin samples were taken as 1 cm wide strips along the lateral lines from the blind side and eye-side (Fig. 2). The samples from the whole length of the caudal fin comprise both the blind side and eye-side material, i.e. elements from both sides together. The samples from the basal parts of dorsal and anal fins were also taken through both sides. The trunk lateral line was divided into three or four slices depending on the fish length, while the caudal fin was sampled as one slice through the fin. Some whole-skin samples from our earlier studies were also used for this paper. After employing different sampling methods, we decided that the best way to sample the fish was as shown in Fig. 2. Still, we do not have a good method to take and chemically treat single l.l.s. and swivel-joint platelets, and thus we have done it set by set. The fine tubuli of some l.l.s. were studied using a Nikon Microscope ECLIPSE 50i and images of scales in 60% alcohol were taken with Nikon camera DS-Fi1 using transmitted illumination.

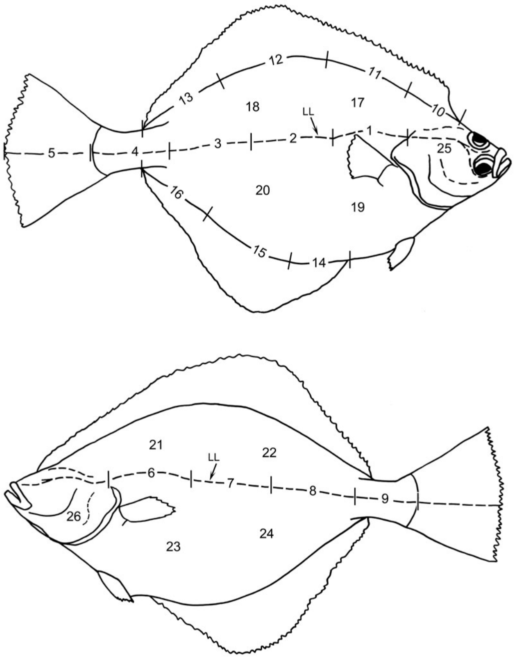

Fig. 2. Sampling areas on the body: 1–4, lateral line on the eye-side; 5, lateral line on the caudal fin; 6–9, lateral line on the blind side; 10–13, dorsal fin base; 14–16, anal fin base; 17–20, skin samples from the eye-side; 21–24, skin samples from the blind side; 25, skin from the head of the eye-side; 26, skin from the head of the blind side. LL, lateral line.

The specimens are deposited in the Institute of Geology at Tallinn University of Technology in the vertebrate collection under the number GIT 584, followed by the articulated specimen number, and by the unit number of that specimen.

TERMINOLOGY

The terminology of scale features follows Traquair (1865), Roberts (1993), Zylberberg et al. (2003), Jawad (2005a), Voronina (2007, 2009, 2010), Voronina and Hughes (2013), and Märss et al. (2010a, b). The *Dictionary of Ichthyology* by B. W. Coad and D. E. McAllister (http://www.briancoad.com/dictionary/complete%20dictionary.htm) and *FishBase* (www.fishbase.org, version 10/2013) by R. Froese and D. Pauly (eds, 2013) were used for definitions below. The terminology for fine morphological descriptions of ossicles is still not satisfactory, especially for the tubercles, and, in addition, the fine tubuli (longitudinal, perpendicular, oblique, or reticular network) in l.l.s. need to be named.

The main types of the studied ossicles of *Pleuronectes platessa*, *Platichthys flesus trachurus*, and *Scophthalmus maximus* are shown in Fig. 3. The ossicles are oriented with their anterior to the left if not

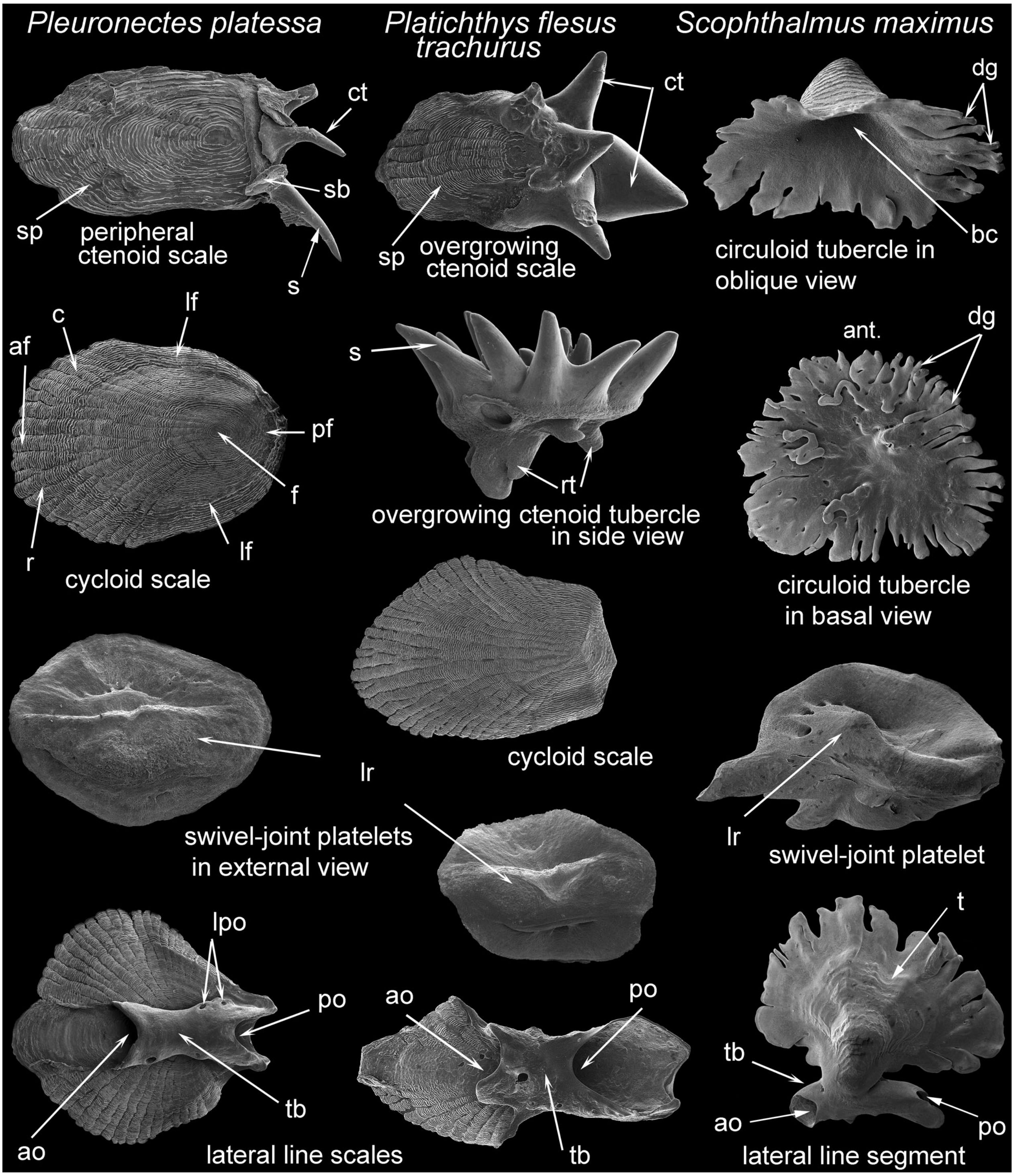

Fig. 3. Terminology of dermal units of three pleuronectiforms used in this work; anterior is to the left for the units of both body sides unless shown by an abbreviation (ant). Abbreviations: af, anterior field; ant, anterior; ao, anterior opening of the tube of the lateral line scale; bc, basal cavity; c, circuli; ct, ctenius; dg, digits; f, focus; lf, lateral field; lpo, lateral pore opening; lr, longitudinal ridge; pf, posterior field; po, posterior opening of the tube of the lateral line scale; r, radii; rt, 'rootlets'; s, spine; sb, spine base; sp, scale plate; t, tubercle; tb, tubular part (or tube) of a lateral line unit.

indicated otherwise. The main terms used in the text are as follows (see also the explanation to Fig. 3): **anchoring device**, horizontal digits or oblique or vertical 'rootlets' in tubercles; **anterior field**, a section of a scale between the focus, antero-lateral corners, and anterior margin; **basal cavity**, cavity in the base of a tubercle; **basal plate/scale plate**, disk of the elasmoid scale or disk forming the base of a tubercle; **circuli**, elevated, usually concentric lines on the scale plate or dermal tubercle; **circuloid tubercles**, conical dermal elements with outstanding circular growth structures on their external surfaces; **ctenii**, tiny discrete projections on ctenoid scales, each ctenius being composed of a spine and its base; **ctenoid scales**, scales with discrete, separately ossified ctenii; **elasmoid scales**, thin, usually imbricating cycloid and ctenoid scales; **focus**, initial part of a scale delineated by the first circulus; **lateral field**, area on both lateral sides of a scale, postero-lateral to the anterior field; **lateral line scale**, a single unit of the chain-like scales of the trunk sensory line; **lateral line segment**, chain-like unit of the trunk sensory line in fully tuberculated taxon; **longitudinal ridge**, the most prominent ridge that runs along the plate; **overgrowing ctenoid scales**, ossicles with ctenii seemingly continuous with the ossicle basal structure and occurring on the posterior, lateral, and partly on the anterior field; **overgrowing ctenoid tubercles**, ossicles with ctenii covering most of the basal plate, and with a completely overgrown edge of the basal plate (the circular part of that plate, if it exists, does not extend visually to the edge); **posterior field**, area between the focus, posterolateral corners, and the posterior margin of a scale; **radii**, unmineralized grooves radiating from the initial scale part (primary radii) or being outside it (secondary radii); **spine**, needle-shaped element on the ctenial base or basal plate of an ossicle; **spinoid scales**, scales bearing spines continuous with the main body of the scale (Roberts 1993); **swivel-joint platelets**, paired perichondral ossifications of the ovoid or ball-shaped distal radial located between the proximal ends of the paired hemitrichs of dorsal and anal fins; **tube** or **tubular part**, tunnel-like canal of the lateral line scale; **tubercles**, usually sculptured, with rounded to multiangular ground plan convex ossicles; **tube vault**, roof above the sensory canal in the lateral line scale; **tubuli**, the finest canals in the l.l.s.; **ultrasculpture**, the finest sculptural elements on the surface of ossicles, measured in tens of micrometres.

RESULTS

The scale morphology and sculpture in the Teleostei are highly variable and only part of them have been systematized and named by Roberts (1993). He distinguished four major types of scales in the Teleostei: cycloid, ctenoid (with three subtypes), crenate, and spinoid (ibid., pp. 62–65). Among 27 species of 20 genera of the Pleuronectiformes, he refers to the presence of all scale types except the crenate type. Among the wide variety of ossicles we describe herein a new subtype of ctenoid scales, overgrowing ctenoid scales, and two kinds of tubercles – overgrowing ctenoid tubercles and circuloid tubercles.

Order PLEURONECTIFORMES
Family PLEURONECTIDAE Rafinesque, 1810

Pleuronectes platessa Linnaeus, 1758
Figures 4, 5

Distribution of ossicles

Three specimens, male GIT 584-89, 27.0 cm long, from Pärnu Bay; female GIT 584-121, 29.0 cm long, and male GIT 584-122, 26.5 cm long, both from Paslepa Bay, were studied thoroughly, while four specimens (GIT 584-94, GIT 584-97, GIT 584-98, GIT 584-99) from the southern Baltic Sea were also oxidized to get ossicles for comparison. The main part of the body is covered with cycloid scales, but unlike females, males have also ctenoid scales on the head on the eye-side and on the trunk behind the head and close to dorsal and anal fins. Most of the ctenoid scales originate from the trunk along the midpart of both dorsal and anal fins. The scales are deeply embedded and do not overlap; the spines of ctenoid scales protrude to the skin surface (Ojaveer and Drevs 2003, p. 359, and our own observations).

Description of ossicles

In our male specimen GIT 584-89 the shape of the scale plate varies from rectangular to triangular or to oval (Fig. 4): cycloid scales tend to be more quadrangular or roundish (Fig. 4A, B) and ctenoid scales mainly quadrangular (Fig. 4F, J–L), oval (Fig. 4H, I), or triangular (Fig. 4M, N).

Cycloid scales. The scale plate edge is smooth posteriorly and laterally, but uneven anteriorly to varying degrees. The posterior and lateral fields take up about one-third of the scale plate; the rest is occupied by the anterior field. The anterior field of the scale surface is segmented by up to 22 narrow radii, of which up to 8 are primary ones (Fig. 4A); new radii appear stepwise at about four levels. The radii converge towards the focus, which is situated rather far posteriorly, at about 3/4 the length of the scale plate. The specimens caught from Estonian waters have narrower radii than specimen GIT 584-94 (length 34.5 cm) from southern Sweden, in which radii are groove-like. Circuli are distinctly


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developed between the radii; they are discontinuous anteriorly due to disruption by radii, and continuous laterally and on the posterior field following the outline of the scale margin. The circuli on lateral fields are somewhat wavy. The visceral side of cycloid scales is relatively smooth, with a knobby area in the middle of the scales (Fig. 4B). Peculiar scales come from the caudal peduncle (shown in visceral view in Fig. 4D, E). In the residue they catch the eye with a constriction that follows the boundary between the anterior and lateral fields. The cycloid scales of a female and a male as seen in GIT 584-121 and GIT 584-122, respectively, also have the focus far back. The presence of radii on the posterior field is evident in the sample taken from the female specimen dorsally behind the head from the blind side. In the other five samples just very short radii occur far back but there is always a narrow sector without them. The male specimen has a few (two from twelve) scales with radii on the posterior field dorsally behind the head on the blind side, while the other five samples lack radii there.

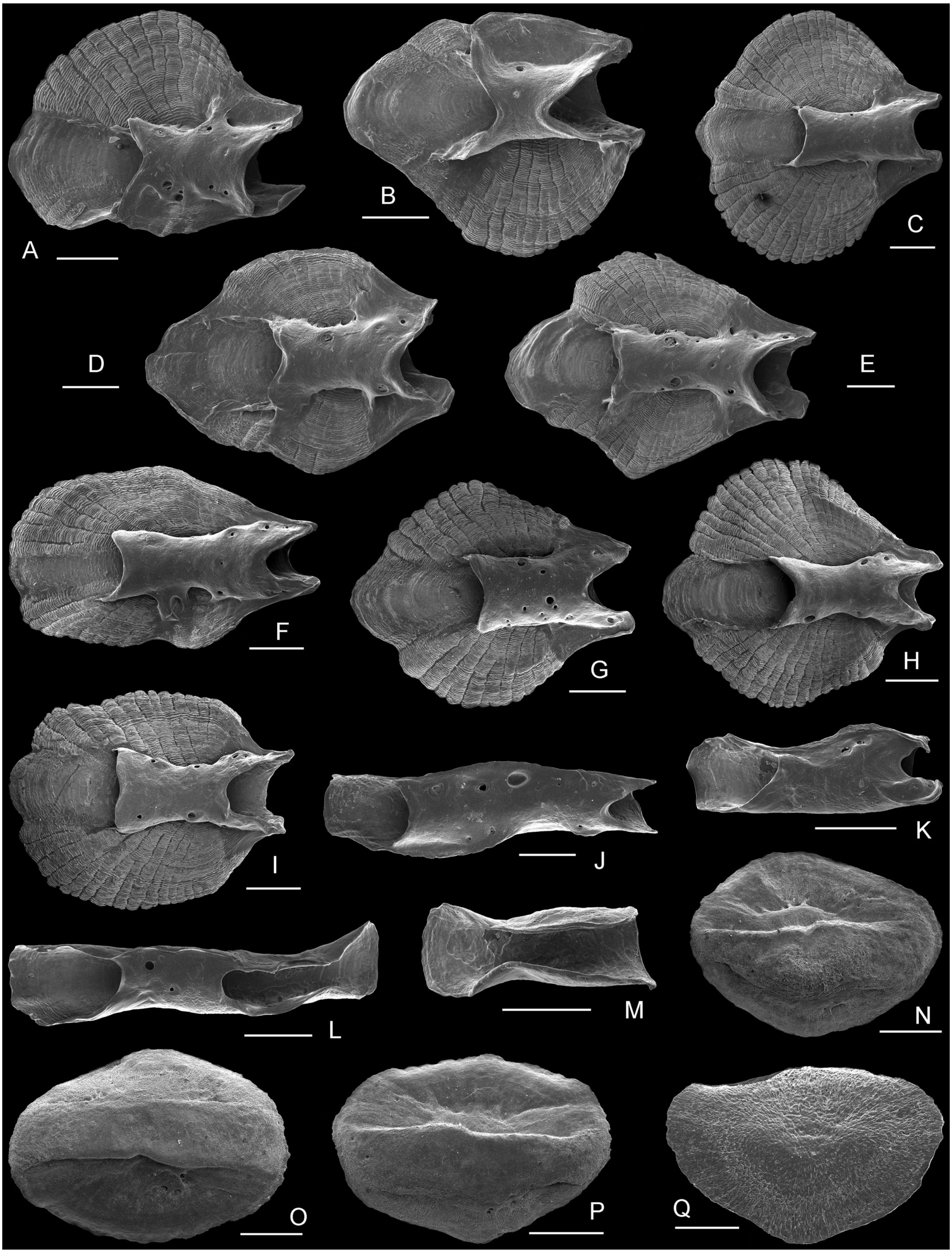
Ctenoid scales. The anterior and lateral fields are distinct on the scale plate as is the focus of the scale, which is situated far posteriorly, close to the posterior field and midway between the lateral fields (Fig. 4C, I–N). The focus is oval in outline, with the longer axis a little oblique to the longitudinal axis of the scale. Circuli are distinct but somewhat curvy and disrupted by the radii on the anterior field. They run capito-caudally on the lateral fields but posteriorly, where there is supposed to be the posterior field, they are replaced by a smooth, thin layer (band), which forms the base for the ctenial row. Radii, two to six in number, are relatively rare, occurring only on the anterior field. The radii on ctenoid scales are much less numerous than those on cycloid scales (compare with Fig. 4A). The visceral side of ctenoid scales (Fig. 4F, H) is relatively smooth, with a knobby surface posteriorly. The ctenii of scales of males are arranged in one ctenial row along the posterior edge of the posterior field; the number of ctenii is three to four, mostly three (Fig. 4F, H–N) for specimen GIT 584-89. Some scales, shown with less ctenii in Fig. 4C, K–N, have lost them during chemical treatment (see Discussion). Each ctenius has a spine, and the base for anchoring it at the posterior scale plate (Fig. 4F–N). Such scales belong to the subtype of peripheral ctenoid scales (Roberts 1993).

Lateral line scales (l.l.s.). A sample taken from the eye-side of specimen GIT 584-89 from the curved lateral line slice directly behind the head contains scales in which lateral fields occur asymmetrically either on the dorsal or ventral side (Fig. 5A, B), or else occur symmetrically (Fig. 5D, E). The asymmetrical scales have a shorter tubular part, and much more strongly expressed radii and circuli on one side; the radii run from the elongate centre of the scale also towards the postero-lateral margin; the opposite side of such scales has a rather smooth surface. Anterior to the mineralized tube, the scale plate surface is also rather smooth, with weakly developed circuli. This smoother part of the l.l.s. is protruding anteriorly. It seems that the tube had continued somewhat anteriorly (the remains of the tube wall can be seen in Fig. 5A, D, E). Numerous pores of varying diameter (up to eight in Fig. 5A) are found in the tube wall mainly on the sides of the tube. In the Pleuronectidae the free posterior (caudal) edge and ctenii of l.l.s. are absent, as already noted by Voronina (2009, p. 951). The symmetrical scale in Fig. 5C originates from the mid-body. It is a relatively wide l.l.s. with a smooth tubular part and of almost the same width over its whole length; some of the primary radii run postero-laterally from an inferred point situated beneath the tube. Lateral line scales become elongate towards the posterior part of the body. On the caudal peduncle the l.l.s. are symmetrical and well elongated (Fig. 5F), being about twice as long as wide (Fig. 5F).

The l.l.s. from the blind side are exemplified by scales taken behind the head (Fig. 5G) and from the mid-body (Fig. 5H, I). Asymmetrical scales were not found on the blind side. A remarkable feature is the large number of small pores in the tubular part of scales (Fig. 5G). In the mid-body region the l.l.s. are rather similar to those on the eye-side, with quadrangular scale plates (Fig. 5I); radii occur rather far posteriorly on the scale sides around the tubular part. There is not one midpoint (focus) but an elongate centre in the posterior l.l.s., with subparallel radii on the sides (Fig. 5I).

A dramatic change in l.l.s. morphology occurs in the transition from the caudal peduncle to the caudal fin. It

Fig. 4. European plaice, *Pleuronectes platessa* Linnaeus, specimen GIT 584-89. **A**, **B**, **D**, **E**, cycloid scales; **C**, **F**, **H**–**N**, peripheral ctenoid scales; **G**, two loose ctenii. All elements originate from the areas taken from the eye-side as shown in Fig. 2: **A**, area 3; **B**, area 1; **C**, area 12; **D**, **E**, area 4; **F**–**H**, area 17; **I**–**N**, area 14. A, C, I–N, scales in external view; B, D–F, H, scales in visceral view. A, GIT 584-89-10; B, GIT 584-89-3; C, GIT 584-89-42; D, GIT 584-89-12; E, GIT 584-89-13; F, GIT 584-89-58; G, GIT 584-89-36; H, GIT 584-89-59; I, GIT 584-89-34; J, GIT 584-89-35; K, GIT 584-89-30; L, GIT 584-89-31; M, GIT 584-89-32; N, GIT 584-89-33. The specimen was caught from Pärnu Bay, depth 3.5 m. Scale bar 200 μm.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should be noted that the l.l.s. set includes the scales from both the blind side and the eye-side. Along the midline of the caudal fin the l.l.s. are very narrow (width up to 2 mm) with a complete, closed (Fig. 5J–L) or incomplete, open (Fig. 5M) tubular part, and with a strongly reduced scale plate (Fig. 5J, K) or without a scale plate (Fig. 5L, M). A few circuli (14 in Fig. 5L), placed anterior to the tubular part, are weak (Fig. 5L).

Lateral line scales of both the eye- and blind sides of *Pleuronectes platessa* are rather similar. Both the blind side and the eye-side l.l.s. have a relatively short mineralized tube. It does not reach the anterior end of the scales where only the remains of the tube wall are preserved (Fig. 5A, D, E, H). The eye-side l.l.s. of *P. platessa* have a more pronounced protruding area of the anterior field than the blind side l.l.s. (compare Fig. 5B, D and Fig. 5G–I).

Swivel-joint platelets. The platelets in Fig. 5N–Q originate from the middle part of the anal fin. They occur in symmetrical pairs between the proximal parts of fin rays and their internal supports. The platelets of this taxon are rather simple. They have a convex external side. The upper longitudinal ridge on that side is just slightly curved, relatively wide, with a few pores on both sides (Fig. 5N–P). The other, lower ridge is partly covered by the upper one. The internal side of platelets is smoothly concave, with an irregular radial pattern on its surface.

Fine structures. The nodular structure occurs on the visceral side of all scales (Fig. 4B, D–F, H), on the external side on posterior bands, and on the bases of ctenii of ctenoid scales (Fig. 4C, I–N). The nodules are larger on the visceral side of the scales.

Platichthys flesus trachurus (Linnaeus, 1758)
Figures 6, 7

Distribution of ossicles

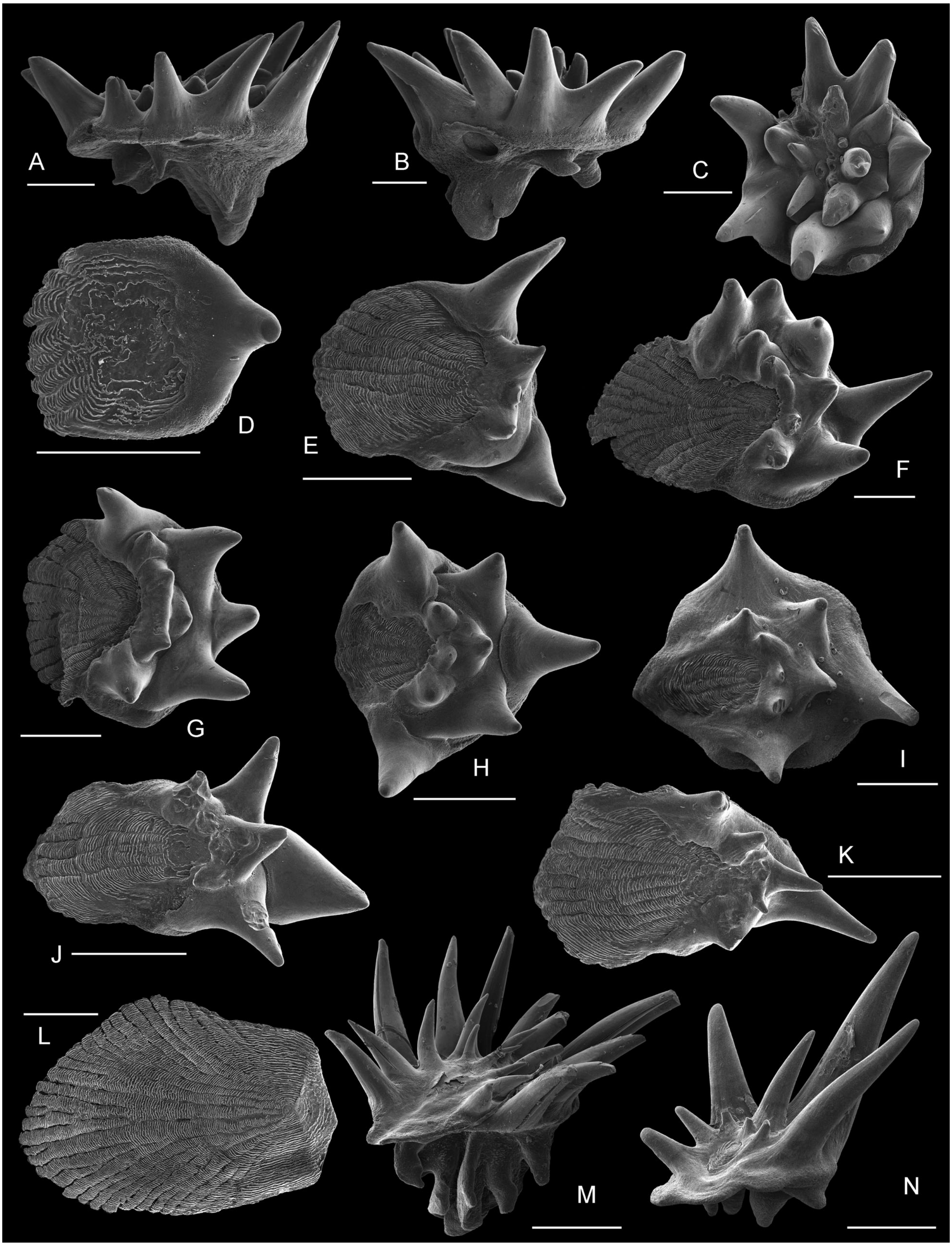
Eight specimens from the western Estonian waters, with body lengths between 10.6 and 36.0 cm, were studied. Ossicles of three 16.0, 29.7, and 30.0 cm long specimens (GIT 584-93, GIT 584-20, and GIT 584-52, respectively; of these, GIT 584-20 was examined as a whole skin sample) were studied under SEM. Cycloid scales, overgrowing ctenoid scales and overgrowing ctenoid tubercles, occur on the body of this species. Such tubercles cover the strips along the lateral lines and the bases of dorsal and anal fins of both the eye-side and blind side. On the eye-side they start between the bases of the 15th and 16th rays and extend as far as the bases of the 42nd and 43rd dorsal fin rays, but are absent in the anterior and posterior skin portions of the trunk close to the fins. Along the anal fin, the first tubercles occur between rays 2 and 3 and the last one is between rays 25 and 26. The tubercles are tightly spaced also on the head and on the area between the head, lateral line, and pectoral fin. On the blind side the tubercles occur at the bases of the longest dorsal and anal fin rays but are absent anteriorly and posteriorly of those areas. Scattered tubercles are found on the trunk. The tubercles most richly equipped with spines are distributed on both sides of the lateral lines and along the fin bases. The biggest tubercles are placed on the eye-side, behind the head and close to the lateral line.

Description of ossicles

Cycloid scales. An example of cycloid scales of *Platichthys flesus trachurus* from the Baltic Sea is shown in Fig. 6L. Its anterior, posterior, and lateral fields are well defined; lateral fields are short, the anterior margin is crenate, the circuli are distinct and just slightly wavy, and their course is interrupted by well-expressed radii. The focus is placed at the point where the fields meet posteriorly on the scale. A slightly higher ridge occurs between the lateral and posterior fields due to an artificial wrinkle of the scale.

Overgrowing ctenoid tubercles and overgrowing ctenoid scales. The flounder has a highly variable distribution pattern of ctenii on both the tubercles and ctenoid scales (Fig. 6). The ctenial base and spine of their ctenii are not distinctly separated, the transition is very smooth, the lower part of spines just becoming larger and joining with the scale or tubercle basal plate.

Fig. 5. European plaice, *Pleuronectes platessa* Linnaeus, specimen GIT 584-89. **A**–**M**, lateral line scales; **N**–**Q**, swivel-joint platelets. **A**, **B**, **D**, **E**, area 1; **C**, area 2 and **F**, area 4 of the eye-side; **G**, area 6 and **H**, **I**, area 7 of the blind side; **J**–**M**, area 5 through the caudal fin; **N**–**Q**, area 15 through the base of the ventral fin. All elements in external view except Q, which is in visceral view. A, GIT 584-89-7; B, GIT 584-89-6; C, GIT 584-89-8; D, GIT 584-89-4; E, GIT 584-89-5; F, GIT 584-89-11; G, GIT 584-89-22; H, GIT 584-89-25; I, GIT 584-89-26; J, GIT 584-89-17; K, GIT 584-89-15; L, GIT 584-89-16; M, GIT 584-89-14; N, GIT 584-89-38; O, GIT 584-89-41; P, GIT 584-89-40; Q, GIT 584-89-37. The specimen was caught from Pärnu Bay, depth 3.5 m. Scale bar 200 μm.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In the overgrowing ctenoid tubercles the high marginal spines with their apices are pointing obliquely upwards (Fig. 6A–C), while the medial ones lie more vertically. The number of spines may exceed 20. The tubercle basal plate is viscerally either flat or with a vertical attachment device, resembling roots (Fig. 6A–C). The 'roots' are present on the tubercles that are arranged in longitudinal rows along the dorsal and anal fins, but are lacking on tubercles on the head and along the lateral lines. The 'roots' are of different depths (Fig. 6A, B, M, N). The deepest 'roots' are found in tubercles with the longest/highest spines. Some overgrowing ctenoid tubercles have peculiar round structures on the lower side of the basal plate (Fig. 6A, B). A tubercle of the 16.0 cm long specimen GIT 584-93 (Fig. 6M) has a very complicated arrangement of spines on the basal plate and also very deep 'roots'. The element in Fig. 6I has an anteriorly overgrown edge and thus is treated as a tubercle.

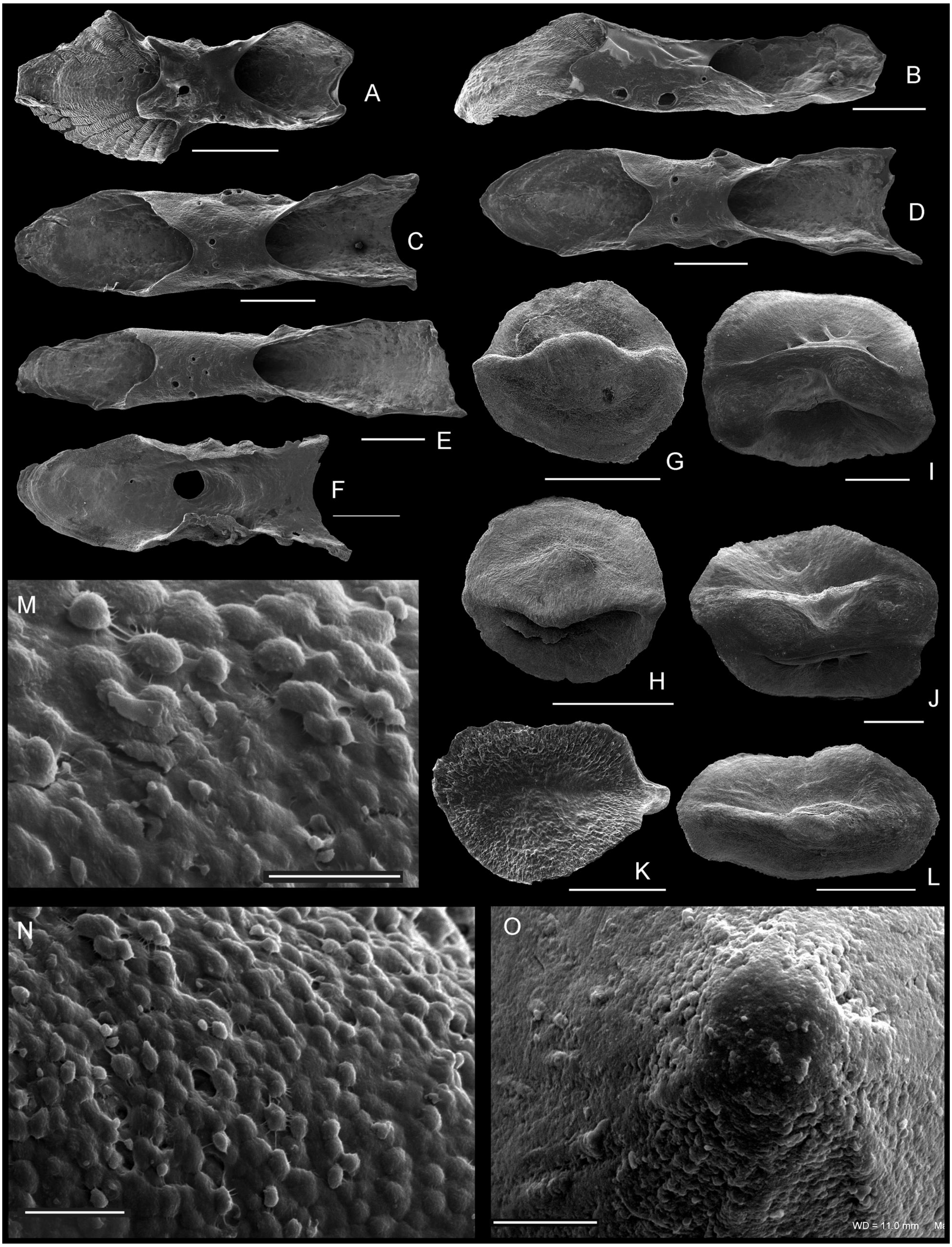
In overgrowing ctenoid scales a solitary spine may be found at the posterior margin (Fig. 6D; this one is a regenerated or replacement scale with the characteristic central area lacking circuli and also seen in other fishes), or indistinct two to three curved rows of spines may occur mainly posteriorly and laterally but also antero-laterally of the focus (Fig. 6E–H, J, K). The spines of ctenii are strong and roundish in cross section. The ctenii lie close to each other at the scale basal plate and do not separate even if there is a shallow slit between the ctenial and scale base (e.g. Fig. 6F, H, J). In some cases they seem to arise as continuous spines with the overgrowing ctenoid scale main body (Fig. 6G, H, lateral ctenii, and as also seen in the tubercle in Fig. 6I). In such occurrence the contact between the ctenial base and scale base is well hidden. The ctenii have been added in a complex order, for example, the latest ctenii may have been added posteriorly and antero-laterally until the focus became almost fully embraced by them (Fig. 6H). Several SEM images (Fig. 6E, G) revealed a very narrow strip of the scale basal plate with a few circuli behind the posteriormost ctenii, meaning that during growth the ctenii did not occupy the posterior-most margin of the scale plate.

Lateral line scales (l.l.s.). In GIT 584-20 and 584-52 the tubular part of the scales has a very thin wall and is always poorly preserved. Most scales have a closed tube vault (Fig. 7A–E), while just a few lack that vault (Fig. 7F). As seen in other specimens (GIT 584-115 and GIT 584-118), on the eye-side the trunk l.l.s. have an anterior field with well-developed radii and circuli anteriorly of the tubular part (Fig. 7A). Pores in the tube wall are relatively large (Fig. 7A–E). Both the anterior and posterior openings of the tube are strongly stretched out, especially on the caudal peduncle (Fig. 7C–E). The l.l.s. on the blind side behind the head may have an open tube vault (Fig. 7F) or a closed one. A very large pore, an opening for a nerve, occurs on the bottom of the tubular part. The tube vault of all segments of the supratemporal canal on the eye-side is open.

Swivel-joint platelets. The platelets from the anterior part of the dorsal fin (Fig. 7G, H) are very simple, well-rounded in configuration, with a wavy longitudinal ridge along about the middle of the external surface. The mid-curve of that ridge is of variable length (Fig. 7G, H). The ridge rises smoothly towards the centre of the platelet. No pores are detected in the platelet's external surface. The swivel-joint platelets from the anterior third of the anal fin are quadrangular to oval, and relatively long (Fig. 7I–K). Both edges of the medial longitudinal ridge are risen and distinct; the ridge width is about 1/3 the width of the platelet. The ridge is tied to its basal plate by narrow bands (Fig. 7I). The internal (lower) side of platelets is concave, with a longitudinal groove; its surface is knobby in the middle and has a radial pattern towards the margins (Fig. 7K). An elongate platelet in Fig. 7L comes from the middle part of the anal fin where there are the longest fin rays. Its medial longitudinal ridge is rather narrow with a broadening in the middle.

Fine structures. Fine nodules occur on the lateral margins of the external surface of overgrowing ctenoid scales (Fig. 6D = Fig. 7M, N), on the basal parts of ctenial bases (Fig. 6E, J), and rarely also on spine peaks (Fig. 6H = Fig. 7O). The shape of nodules (mineralized globules by Zylberberg et al. 2003) in our material varies from spheritic to ovoid, with a flattened basal part. The size of the nodules varies between 0.6 and 4.2 µm (Fig. 7M, N). The outgrowths of nodules occur at an early stage of their development (Fig. 7M, N).

Fig. 6. European flounder, *Platichthys flesus trachurus* (Linnaeus). **A**–**C**, **I**, **M**, **N**, overgrowing ctenoid tubercles; **D**–**H**, **J**, **K**, overgrowing ctenoid scales; **L**, cycloid scale with the posterior field with an artificial wrinkle. **A**–**H**, **J**–**L** from the whole skin sample of GIT 584-20; **I**, area 10 through the base of the middle part of the dorsal fin and **N**, area 15 through the mid-anal fin base of specimen GIT 584-52; **M**, from the mid-anal fin base of GIT 584-93; A, B, M, N, elements in side view; C–L, elements in external view. A, GIT 584-20-3; B, GIT 584-20-1; C, GIT 584-20-2; D, GIT 584-20-10; E, GIT 584-20-7; F, GIT 584-20-15; G, GIT 584-20-6; H, GIT 584-20-14; I, GIT 584-52-10; J, GIT 584-20-8; K, GIT 584-20-11; L, GIT 584-20-4; M, GIT 584-93-5; N, GIT 584-52-9. Fish GIT 584-20 was caught from Pakri Bay, GIT 584-52 from Küdema Bay, and GIT 584-93 from Pärnu Bay. Scale bar equals 500 µm.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Family SCOPHTHALMIDAE Chabanaud, 1934

Scophthalmus maximus (Linnaeus, 1758)
Figures 8–11

Distribution of ossicles

Five specimens of the turbot from the Baltic Sea with total length between 14.0 and 31.5 cm were studied, with emphasis on the morphological variability of circuloid tubercles, lateral line segments, swivel-joint platelets, and ultrasculpture (Figs 8–11). Common elasmoid scales are absent in the turbot; unexpectedly, the lateral line on both body sides is not protected by tubercle rows as in the European flounder in this study. Tubercles are in rows only on the caudal peduncle of the eye-side.

Description of ossicles

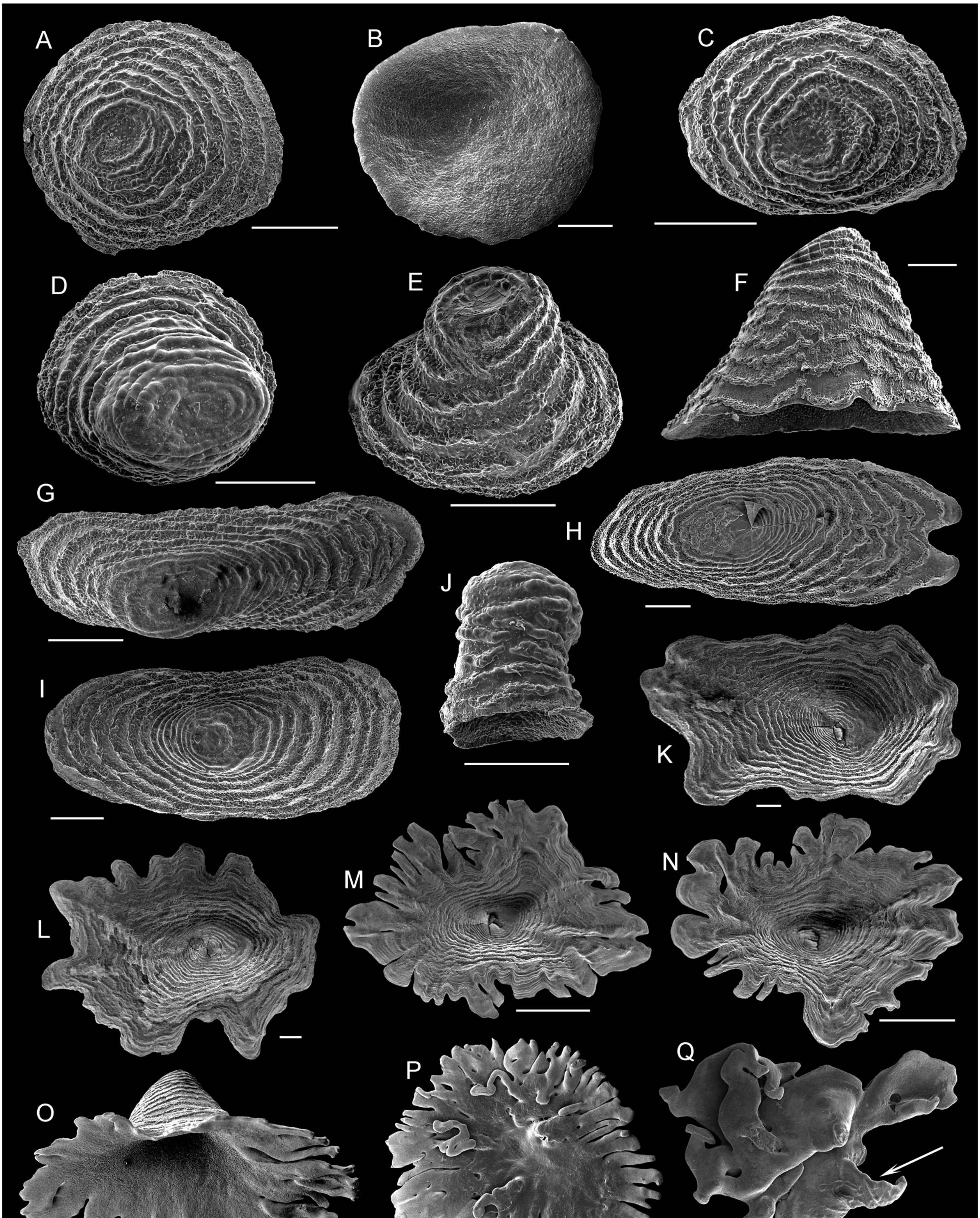
Circuloid tubercles. Tiny to large circuloid tubercles are scattered on the head and posterior to it (Drevs 2003; Zylberberg et al. 2003). The structure of tubercles (Zylberberg et al. 2003) and their morphology change greatly during growth. The circuloid tubercles of our smallest, with TL 14.0 cm, specimen GIT 584-17 (Fig. 8A–C, E–I) are conical in shape, with a regular round base, or oblate from the sides and tapering upwards. The peak of the circuloid tubercles is never pointed but has a small, roundish, flat surface in the middle. The circuloid tubercles are covered with seven (Fig. 8C) to 17 (Fig. 8H) relatively regular concentric nodular higher ridges, which alternate with low and smooth intervals; sometimes the ridges join or diverge (Fig. 8A, C, E, F). Tiny nodules can also be found in between the ridges (Fig. 8A, E), or these intervals are covered with vertical ridgelets (Fig. 8F). These tubercles have a wavy, nodular, circular ridge at the lower margin (e.g. Fig. 8F). The lower surface of circuloid tubercles is concave and porous (Fig. 8B), forming a basal cavity. The middle part has more pores, which are larger than more marginal ones.

Most of the circuloid tubercles of 18.2 cm long specimen GIT 584-32 (Fig. 8K–O) have digits (terminology of Zylberberg et al. 2003), although distinctions occur here as well. Rather few circuloid tubercles are small, with a round or roundish base, and have 7–11 nodular circular ridges like specimen GIT 584-17 described above. However, most of the circuloid tubercles have elongate, and quadrangular or irregular base configuration. The margins of circuloid tubercles may be shallowly (Fig. 8K) or deeply crenate (Fig. 8M–O). In the latter case they are called digits, which can occur either on both ends in elongate specimens or all around roundish tubercles. The number of nodular circular ridges is close to 25 (Fig. 8M). Such circular ridges are also observed on the digits, being interrupted by the space between the digits, and becoming indistinct distally on the longest prominent digit (Fig. 8M, N). The basal cavity can be rather deep in the middle of the circuloid tubercle but smoothly shallowing towards the margins (Fig. 8O).

In specimen GIT 584-88, with total length of 31.5 cm, the circuloid tubercles may be very tiny, with roundish configuration and 0.2 mm diameter (Fig. 8D, J), to rather large and elongate, 7.5 mm long as a maximum. In larger circuloid tubercles with a rather flat lower surface, the ‘roots’ of irregular distribution appear at the base (Fig. 8P). The element in Fig. 8Q is an aberrant form, in which the upper and lower sides of the tubercle and l.l.s. features are conjoined.

Lateral line segments (l.l.se.). The turbot shows a high number of morphological varieties of l.l.se., some of which are illustrated herein (Figs 9A–M, P, 10A–D). A whole skin sample of the 14.0 cm fish contains l.l.se. with a relatively wide plate (Fig. 9A), reduced, slight extensions of the plate (Fig. 9B), or without any plate (Fig. 9C). The tubular part of these segments is very porous. One l.l.se. of a slightly larger specimen (18.2 cm) is shown in Fig. 9P with the tubular part and a tubercle, whose surfaces are imaged in Fig. 11E, G, respectively. The segment in Fig. 9D was taken from the eye-side of the head, and those in Fig. 9E, F came directly from behind the head. They all have small to large tubercles on one side of the tubular part; a large pore may be found between the tubercles (Fig. 9F). The l.l.se. from the linear part of the lateral line on the blind side (Fig. 9G, H) and from the eye-side (Fig. 9I) have similar morphology and are of the same length. Specimen GIT 584-88 has

Fig. 7. European flounder, *Platichthys flesus trachurus* (Linnaeus). **A**–**F**, lateral line canal scales; **G**–**L**, swivel-joint platelets; **M**–**O**, nodular external surface of tubercles; note the outgrowths of nodules (Fig. 7M, N). **A**, **B**, from the whole lateral line samples; **C**–**E**, area 4; **F**, area 6; **G**, **H**, area 10; **I**–**K**, area 14; **L**, area 15. A–J, L, elements in external view; K, element in visceral view. A, GIT 584-20-9; B, GIT 584-20-5; C, GIT 584-52-13; D, GIT 584-52-14; E, GIT 584-52-15; F, GIT 584-52-16; G, GIT 584-52-11; H, GIT 584-52-12; I, GIT 584-52-2; J, GIT 584-52-3; K, GIT 584-52-6; L, GIT 584-52-7; M = N, GIT 584-20-10; O, GIT 584-20-14. Fish GIT 584-20 was caught from Pakri Bay, fish GIT 584-52 from Küdema Bay. Scale bar for A–L equals 500 μm and for M–O, 10 μm.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unusually long l.l.se. on the caudal fin (4.9 and 4.6 mm, Fig. 9L, M, respectively). They have straight tubes with 3–4 large openings arranged in a line (Fig. 9M), or with some shifted aside (Fig. 9L).

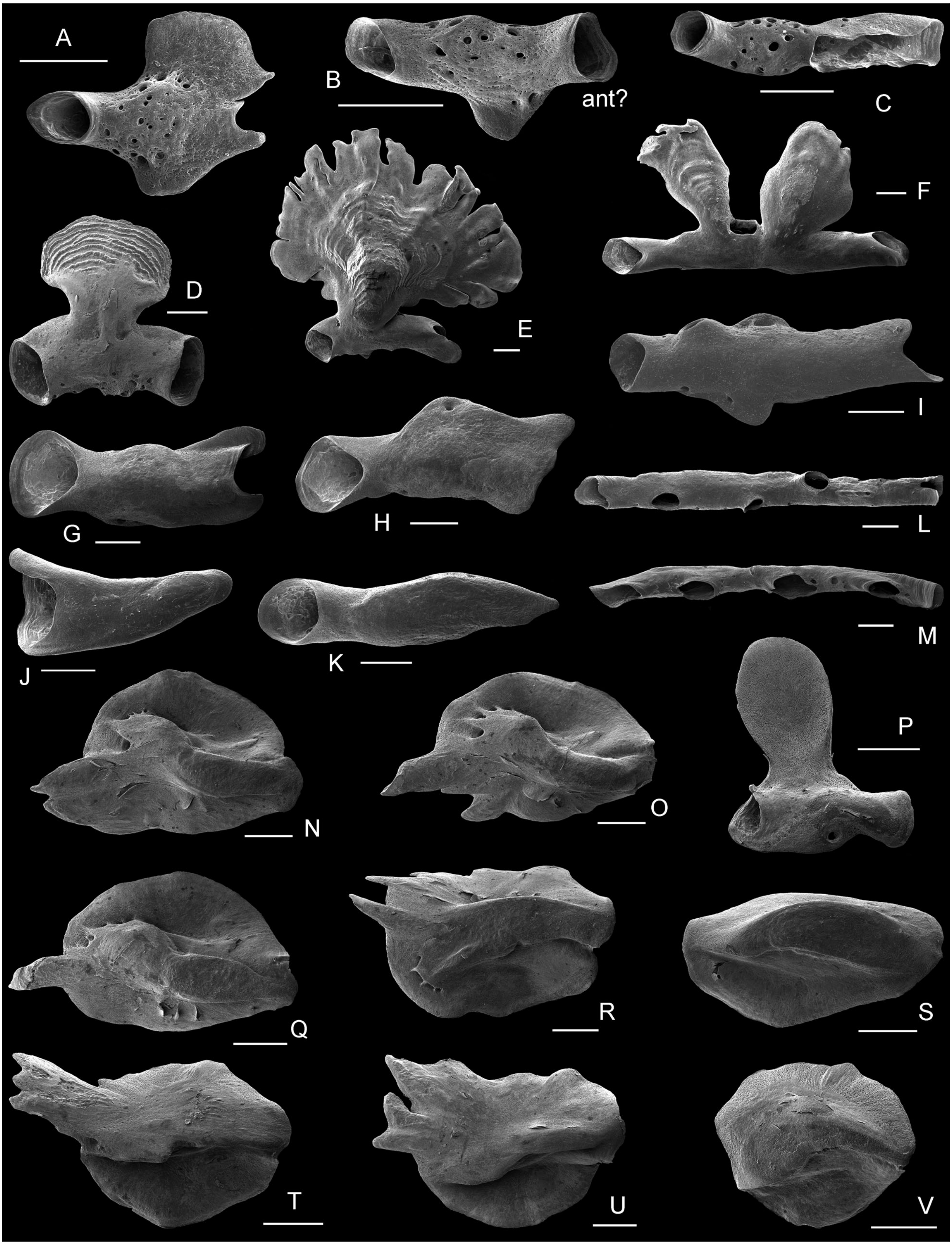
Besides the common l.l.se., the skin of the turbot bears l.l.se. of special interest, found in a skin sample taken from the posterior part of the dorsal eye-side, the caudal peduncle (Fig. 9J, K). Notably, some of them have a wide anterior opening of the tubular part but such an opening is missing posteriorly. In transmitted light (Fig. 10A–D) a complex pattern of the finest tubuli is observed inside such segments. The tubuli are rather numerous and of various shapes and sizes. Anteriorly there are two tubuli (Fig. 10A, D), or one wide tube (Fig. 10B), or four fine tubuli (Fig. 10C). Besides the 2–4 tubuli anteriorly in the tubular part, each such segment has meandering tubuli before they join into a medial widened chamber (Fig. 10B–D), and 1–3 radial tubuli on both sides, directed towards the l.l.se. margins (Fig. 10A–D), some of which open on the lateral surface as fine pores. In about the posterior third, some tubuli join again into one main tube which opens through the basal plate posteriorly (Fig. 10C, D) either in the midline (Fig. 10D) or on one side (Fig. 10C). In some l.l.se. this opening is very small or closed up, giving an impression of a blind l.l.se.

Swivel-joint platelets. Such platelets (Fig. 9N, O, Q–V) of this taxon are rather complicated paired elements, much more complicated than those in the European plaice and European flounder. Some of the platelets were studied when they were attached to the ball-shaped structure, the distal radial, while the paired bases of the fin rays and their internal supports, the pterygiophores, were also present. The anterior–posterior orientation of platelets in the scattered material is ensured by the deepest depression in the posterior half of the platelet, which is attached to the roundish process in the proximal end of one of the paired fin rays. The most characteristic feature of the platelets is the longitudinal ridge on the external surface (Fig. 9N, O, Q–V). That ridge has 1–2 larger spines in its anteriormost end; on the medial part, it is seemingly covered by a branch proceeding arch-like posteriorly up to the end of the platelet. That branch anteriorly gives 5–6 outgrowths to the platelet surface. The longitudinal ridge rather often outreaches the plate anteriorly. That longitudinal ridge together with the branch in the mid-plate gives an imagination of a winglet. Very thin platelets both with ‘winglets’ and simple, or without ‘winglets’ occur at the anterior third of the dorsal fin (Fig. 9N, O, Q–S) but also in the mid-part of this fin. Swivel-joint platelets with ‘winglets’ are absent from the posterior third of the dorsal fin. Platelets in Fig. 9T–V come from the anterior half of the anal fin and mostly contain ‘winglets’, half the length of the platelet (Fig. 9T) or even more in length. This sample contains also simple swivel-joint platelets. The posterior half of the anal fin has mainly simple platelets (except one pair, which may originate from the more anterior sample slice). The visceral side of platelets is shallowly concave.

Fine structures. The external and visceral sides of ossicles of the turbot exhibit somewhat different structures (Fig. 11A–H). The external surface carries nodules of different sizes (mineralized globules of Zylberberg et al. 2003). Most often the nodular structure is in the shape of elongate, discontinuous rows between the circular growth rings (Fig. 11A, B). Sometimes the nodular structure is in the shape of flows or dripping or in the shape of long icicles (Fig. 11C), while some structures are more hummocky (Fig. 11D). In close-ups (Fig. 11E) the nodules have very different sizes (2–12 µm), some having outgrowths (Fig. 11E on the bottom).

The visceral surface bears Mandl’s corpuscles. Figure 11F–H shows small nodules of the tubercle illustrated in Fig. 8O and a tubercle of the lateral line unit given in Fig. 9P. The corpuscles in our material have rhomboid to ellipsoid shapes (Fig. 11G, H). The corpuscles are attached vertically with their longer axis when forming the walls of canals but take a horizontal circular arrangement farther around these canals; the mouths of the canals, which form groups (Fig. 11F), are higher than the main surfaces (Fig. 11G). The fusion of corpuscles is evident also in our material (Fig. 11H).

Fig. 8. Turbot, *Scophthalmus maximus* (Linnaeus). **A–P**, circuloid tubercles; **Q**, aberrant lateral line tubercle unit in visceral? view, the arrow points to the tubular part of l.l.se. **A–C**, **E–I**, circuloid tubercles from the whole skin sample of specimen GIT 584-17, and **K–O** from specimen GIT 584-32; **D**, **J**, **P**, **Q**, from specimen GIT 584-88; **D**, **J**, area 5, and **P** area 17. A, C, G–I, K–N, tubercles in external view; B, P, tubercles in visceral view; D, E, O, tubercles in oblique view; F, J, tubercles in side view. A, GIT 584-17-5; B, GIT 584-17-14; C, GIT 584-17-15; D, GIT 584-88-18; E, GIT 584-17-7; F, GIT 584-17-3; G, GIT 584-17-10; H, GIT 584-17-2; I, GIT 584-17-6; J, GIT 584-88-19; K, GIT 584-32-4; L, GIT 584-32-5; M, GIT 584-32-8; N, GIT 584-32-7; O, GIT 584-32-6; P, GIT 584-88-33; Q, GIT 584-88-13. Fish GIT 584-17 was caught from Pakri Bay, GIT 584-32 from Küdema Bay, and GIT 584-88 from Pärnu Bay. Scale bar for A–L, Q equals 200 µm and for M–P, 1 mm.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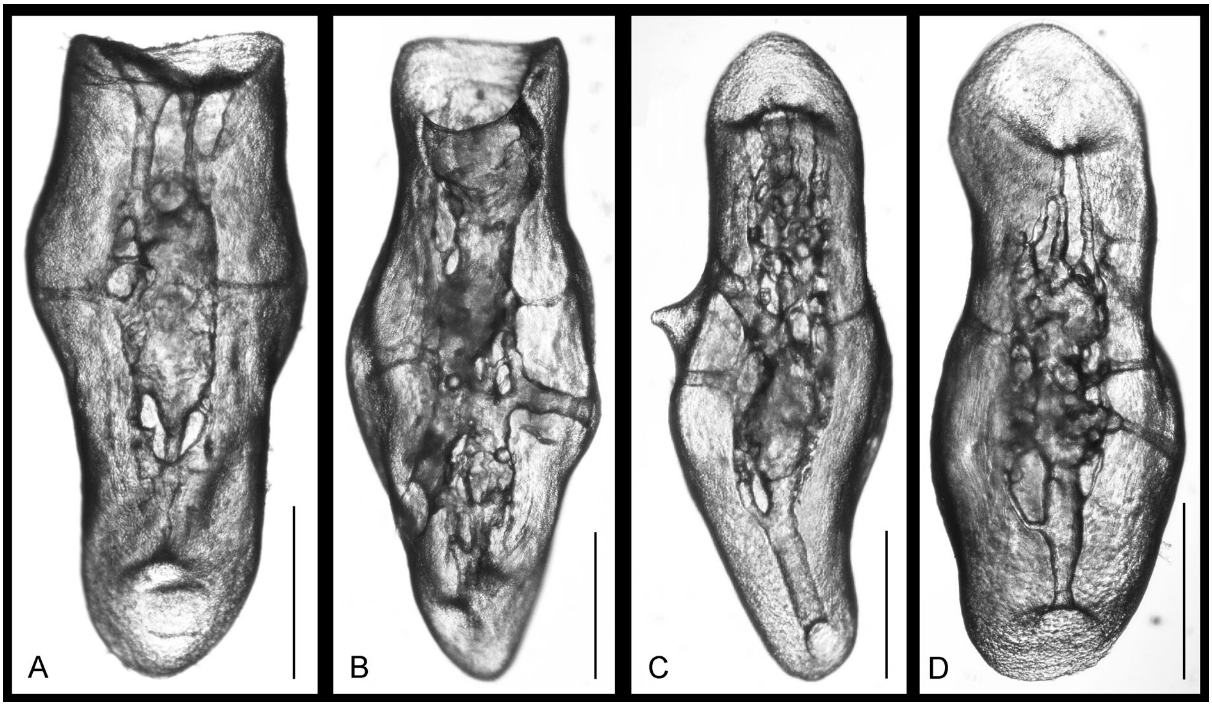


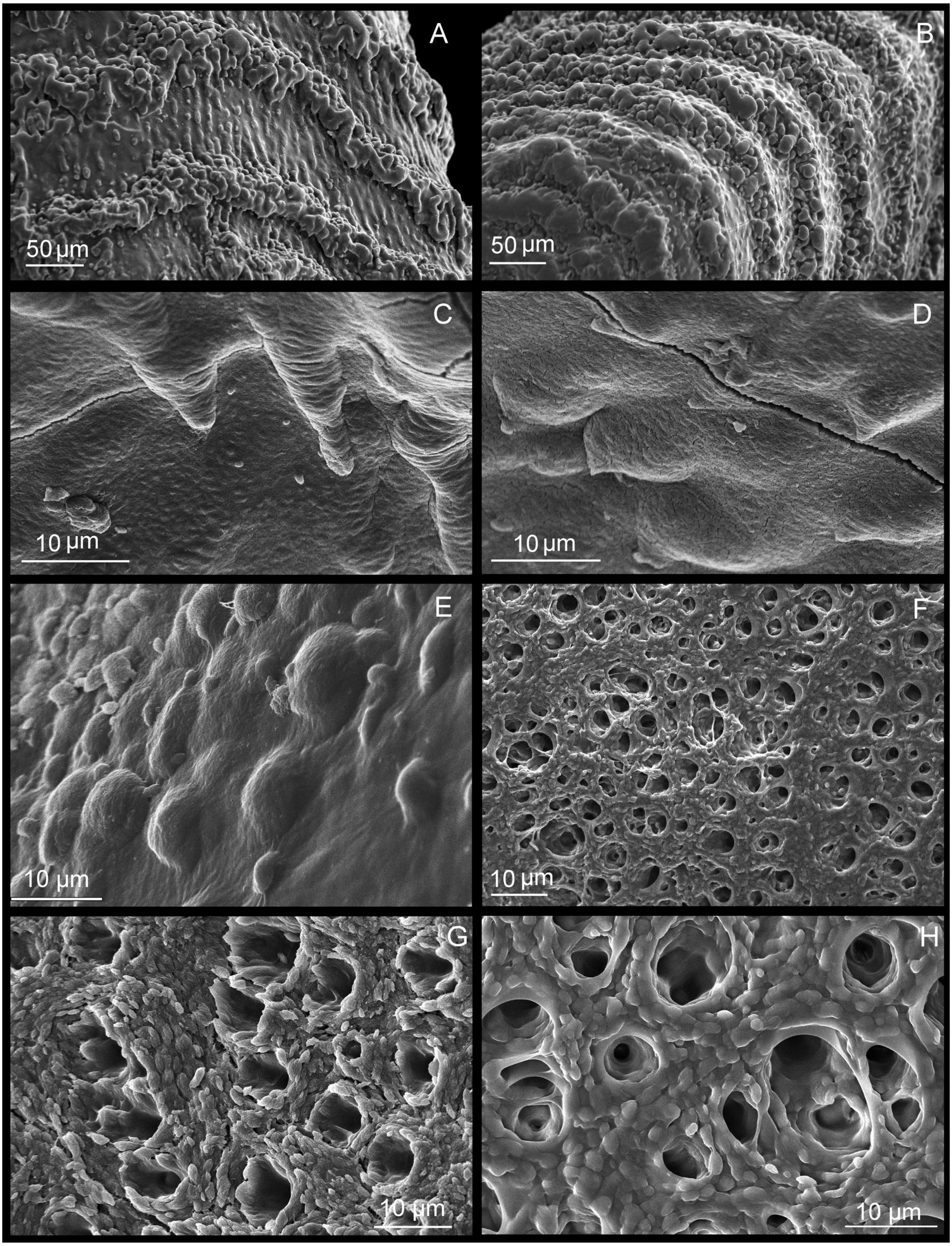
Fig. 10. Turbot, *Scophthalmus maximus* (Linnaeus). Lateral line units from the blind side of area 9. Photos were taken in transmitted light. Note the multiple fine tubuli inside each tubular part. **A**, GIT 584-88-38; **B**, GIT 584-88-39; **C**, GIT 584-88-40; **D**, GIT 584-88-41. Fish GIT 584-88 was caught from Pärnu Bay. Scale bar equals 500 µm.

DISCUSSION

Earlier studies of extinct taxa have shown that the scale features change among different specimens, across body areas, and during the growth of individuals, but there are always distinct species-specific characteristics of the scales, which can be used for the taxonomic identification of fishes and also for phylogenetic, palaeogeographical, and other reconstructions (e.g. Märss et al. 2006, 2007).

Lepidological studies of modern fishes are not very numerous. In this paper we have presented our results on three members of the Pleuronectiformes (flatfishes): the European plaice *Pleuronectes platessa* and the European flounder *Platichthys flesus trachurus*, both in the family Pleuronectidae, and the turbot *Scophthalmus maximus* of the family Scophthalmidae. The present investigation is the fourth in a series describing ossicles in fishes of the Baltic Sea. The scales and their modifications in pleuronectiforms are easily distinguished from comparable ossicles in teleosts we studied earlier (Cottidae, Cyclopteridae, Liparidae, Gasterosteidae, Syngnathidae), though there are interesting comparisons between the spined and lateral-line scales as well as tubercles of pleuronectiforms and cottiforms (cf. Märss et al. 2010a, b).

Fig. 9. Turbot, *Scophthalmus maximus* (Linnaeus). **A**–**M**, **P**, lateral line canal segments; **N**, **O**, **Q**–**V**, swivel-joint platelets; **A**–**C**, from specimen GIT 584-17; **D**–**H**, **J**–**O**, **Q**–**V**, from specimen GIT 584-88; **I**, from specimen GIT 584-116; **P**, from specimen GIT 584-32. The ossicles come from the following areas: **A**–**C**, **P**, from the whole skin samples; **D**, area 25; **E**, **F**, area 6; **G**, **H**, area 7; **I**, area 2; **J**, **K**, area 9; **L**, **M**, area 5 through the fin; **N**, **O**, **Q**, **R**, dorsal fin base slice 11; **S**, dorsal fin base slice 12; **T**–**V**, anal fin base slice 14 + 15. A, GIT 584-17-1; B, GIT 584-17-19; C, GIT 584-17-18; D, GIT 584-88-1; E, GIT 584-88-10; F, GIT 584-88-11; G, GIT 584-88-31; H, GIT 584-88-29; I, GIT 584-116-7; J, GIT 584-88-15; K, GIT 584-88-14; L, GIT 584-88-16; M, GIT 584-88-17; N, GIT 584-88-22; O, GIT 584-88-23; P, GIT 584-32-1; Q, GIT 584-88-24; R, GIT 584-88-21; S, GIT 584-88-25; T, GIT 584-88-27; U, GIT 584-88-26; V, GIT 584-88-28. Fish GIT 584-17 was caught from Pakri Bay, GIT 584-32 from Küdema Bay, GIT 584-88 from Pärnu Bay. Scale bar equals 500 µm.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Variations in scales between sexes are known in different flatfishes. Norman (1934, p. 33) discovered that differences between sexes, especially in some species of the family Bothidae, may be found in scales, rostral and orbital spines, interorbital widths, form of the fins, coloration, and (in one species) teeth. According to him, only two genera of the Pleuronectinae, *Liopsetta* and *Pleuronectes*, exhibit sexual dimorphism which in both is connected with scale characters. He also found that the scales of the European plaice (*Pleuronectes platessa*) are mostly cycloid, but not infrequently some of them are feebly ctenoid in the male, especially in the small Baltic race. Our material of the European plaice, even if containing a rather small number of specimens, confirms differences in male and female scales and for the first time these differences are illustrated in SEM images. Scales of the other two studied species (European flounder and turbot) did not show variations between sexes. Females of the European plaice in our samples carry cycloid scales only, while males have both cycloid and ctenoid (peripheral ctenoid) scales.

During the chemical treatment of samples of the European plaice with H_2O_2, the disintegration of ctenii in ctenoid scales started at the sides of the ctenial row. In case of three or more ctenii, the most lateral ones break away easily, leaving a smooth posterior margin (band), and finally the median ctenius disintegrates. We believe that the formation of the ctenial row proceeds *vice versa*. The scales of the European plaice of the eastern Baltic Sea have only few ctenii which disintegrate and fall into sediment, differing in that aspect from the scales of many Atlantic pleuronectids, which produce a large number of such elements (T. M. pers. obs., 2014).

Generally the spined scales for the European flounder and European plaice are ctenoid scales. According to Roberts (1993), the ctenoid scales have spines (ctenii) ossified separately from the scale plate and have been grouped into three subtypes: peripheral ctenoid scales with whole spines in one row at the posterior margin; transforming ctenoid scales with whole spines in two or three alternating rows marginally and truncated spines submarginally; whole ctenoid scales with marginal and submarginal whole spines forming several approximately alternating rows in the posterior field.

As shown above, the European plaice has spined scales which belong to the peripheral ctenoid subtype. The ctenoid scales in the European flounder appreciably differ from the peripheral ctenoid scales in the European plaice, while some difficulties arise with the specification of the scale subtype for the flounder. The following characters of the scales serve as a base for defining the scale subtype. The scales in the flounder bear ctenii that are strong and roundish in cross section, the transition from spine to ctenial base is smooth, i.e. the ctenial base is not as distinct as in ctenii of the plaice. The ctenii of scales of the flounder fuse with the scale plate, i.e. they do not disintegrate from the scale plate even if some ctenii show a very narrow break between the ctenial base and the scale base. When ctenii in the European plaice are pointing backwards, the European flounder has scales with predominantly upwardly projecting spines. The difference can also be seen in the arrangement of spines. Spines in the European flounder do not form a distinct single row at the scale posterior margin but form irregular curved rows which are located posteriorly and laterally and even antero-laterally of the focus; spines are higher and bigger marginally than closer to the focus. Truncated spines which are characteristic of the transforming ctenoid subtype are missing. Because of the differences listed above, the scales of the European flounder do not fit into the subtypes of Roberts (1993) but form a new subtype among ctenoid scales. Based on the fact that the ctenii can overgrow the lateral and even the anterior fields of the scale plate, we propose herein the new subtype, the overgrowing ctenoid scales.

Similarly, the ctenoid tubercles in the European flounder have upwardly projecting spines, arranged in one or several somewhat arch-shaped rows, and also in tubercles these spines do not disintegrate. The bases of ctenoid tubercles that are distributed along the basal parts of dorsal and anal fins are equipped with vertical ‘roots’, the largest ones are situated on the eye-side at the bases of the largest fin rays. However, the ctenoid tubercles on both blind and eye-sides above and below the trunk sensory canal have a flat or slightly concave basal surface, as have the tubercles on the head and around the pectoral fin base. As there seem to be many similarities and transitions between the scales and tubercles of the European flounder, we suggest calling the latter elements overgrowing ctenoid tubercles.

Fig. 11. Turbot, *Scophthalmus maximus* (Linnaeus). **A**–**D**, fine structures on the external surface of tubercles; **E**, fine structure on the tubular part of a lateral line unit, and **G**, surface of the tubercle of that unit shown in Fig. 9P; **F**, **H**, visceral surface of the tubercle shown in Fig. 8O. A, GIT 584-17-3; B, GIT 584-17-2; C, GIT 584-32-7; D, GIT 584-32-8; E, G, GIT 584-32-1; F, H, GIT 584-32-6. Fish GIT 584-17 was caught from Pakri Bay, GIT 584-32 from Küdema Bay.

The overgrowing ctenoid scales and overgrowing ctenoid tubercles may be quite similar at a certain stage of development. According to our definition, the ctenii on the European flounder cover most of the basal plate on the tubercles. The edge of the basal plate is completely overgrown and circuli (if they exist) do not extend visually to the edge of the basal plate; ‘roots’ are present. According to our interpretation, at least one ctenius must be visible on the posterior and anterior field of the overgrowing ctenoid scales. Judging from the overgrowing ctenoid tubercles (Fig. 6I, N), which still have elements of cycloid scales (circuli, radii) in the middle, it can be assumed that in ontogeny at least some of the overgrowing ctenoid scales transform into overgrowing ctenoid tubercles.

Unlike in the European flounder, the tubercles in the turbot lack spines. Zylberberg et al. (2003) described the tubercles of the turbot *Scophthalmus maximus* collected in France and compared the organization and structure of the tubercles with those of typical elasmoid scales. The three-layered composition of the tubercles of *Scophthalmus maximus* is similar to the thin and flat elasmoid scales of many teleosts, and therefore the tubercles should be considered as modified elasmoid scales (Zylberberg et al. 2003). Because of the need for terminology in morphological descriptions, we propose the term ‘circuloid tubercles’ (conical tubercles with nodular circular growth rings) for the tubercles of the turbot.

Lateral line units, the scales (l.l.s.) and segments (l.l.se.), of the three examined species differ significantly. In her studies of flatfishes, Voronina (2007) observed that l.l.s. of pleuronectiforms have high morphological variety: in some cases the features of l.l.s. are species-specific, but important distinctions can be made at supraspecific ranks, and the morphological diversity of l.l.s. depends on the level of development of scales and the interaction of the scale plate and its tubular part (Voronina 2009).

Our study also revealed differences in the morphology of l.l.s. depending both on the taxon and on the location on the body. Compared to the other two species, the European plaice has the most developed scale plate and a rather thick tube wall on trunk l.l.s. The curved lateral line slice directly behind the head on the eye-side contains both symmetrical and asymmetrical l.l.s. Asymmetrical l.l.s. were not found on the blind side. Lateral line scales become elongate on the caudal peduncle. The scale plate of l.l.s. or l.l.se. in the European flounder and turbot, respectively, is not developed or is only slightly developed. The European flounder has very thin l.l.s. and their thin anterior part is seldom preserved; the tubular posterior part has a long, lengthwise opening. The turbot may have just a slight extension on one or both sides of the l.l.se. tube; sometimes also tubercles are attached to the tube. Because the elasmoid scales in the turbot are modified into tubercles, it can be assumed that modifications have also affected the development of the scale plate of l.l.se. The posterior opening of l.l.se. on the caudal peduncle of the turbot may be almost closed (or overgrown); this feature was not observed in the European flounder and European plaice. As our samples from the caudal fin of all three taxa contain the l.l.s. of both the blind side and the eye-side, we leave detailed characterization and comparison open at present.

Swivel-joint platelets have neither been described in detail nor SEM-imaged previously for the three examined species. Such paired symmetrical platelets of small/young individuals of the European plaice and European flounder are similar, being smooth and having a wide, flat longitudinal ridge. Besides becoming thicker and having a wider longitudinal ridge, swivel-joint platelets of large/old turbot also develop the distinctive, medial, very complicated, bifurcate longitudinal ridge with outgrowth.

One of the objectives of the study was to find out the occurrence or absence of ultrasculpture on the ossicles, which has proven to be important in studies of fossil fishes. The nodular appearance on the external surface of the ossicles of the European flounder and turbot is the result of their mineralization process, which was described for turbot tubercles by Zylberberg et al. (2003, p. 91: mineralized globules). The interesting feature not described so far is the finest outgrowths of nodules at their margins. Such outgrowths occur at an early stage of development of nodules. The coalescence of nodules on the external surface is somewhat similar to the coalescence of Mandl’s corpuscles on the visceral surface of tubercles, both forming a mineralization front described for many taxa (e.g. Meunier 1981, 1984, 2009; Sire and Meunier 1981; Francillon-Vieillot et al. 1990; Meunier and Brito 2004). The shape of Mandl’s corpuscles may vary in different taxa (Schönbörner et al. 1981).

CONCLUSIONS

- The ossicles of three pleuronectiforms, the European plaice *Pleuronectes platessa*, the European flounder *Platichthys flesus trachurus*, and the turbot *Scophthalmus maximus* from the eastern Baltic Sea, were described and compared for the first time using SEM imagery. The studied species can be distinguished by the morphology of their ossicles. Consequently, the ossicles can be used in taxonomic, systematic, and phylogenetic studies of these pleuronectiforms.
- The distribution of cycloid and ctenoid scales in the European plaice exhibits sexual dimorphism, which was not detected in the other two studied species.

- The European flounder has cycloid and ctenoid scales as well as ctenoid tubercles. Because of characteristics of ctenii and development of the ctenial pattern, we have included the scales of the flounder in a new subtype of scales, overgrowing ctenoid scales. Together with the peripheral ctenoid, transforming ctenoid, and whole ctenoid subtypes (Roberts 1993), this subtype belongs in a general type of ctenoid scales. Because of the transitional morphologies from scales to tubercles in the flounder, we feel justified to call their tubercles overgrowing ctenoid tubercles. The examined scophthalmid lacks any spined scales, having only highly modified circuloid tubercles. Their dermal ossicles have been known to have similarity with cycloid scales (Zylberberg et al. 2003).
- Lateral line scales (l.l.s.) of the Pleuronectidae and lateral line segments (l.l.se.) of Scophthalmidae species differ significantly. The European plaice has the most developed scale plate among the three species studied. The scale plate of l.l.s. in the European flounder is weakly developed and the l.l.se. in the turbot is not developed or is weakly developed. Because the elasmoid scales in the turbot are completely modified into tubercles, it can be assumed that modifications have also affected the development of the lateral line units.
- Paired symmetrical platelets, which occur bilaterally in the swivel joints situated between the dorsal and anal fin rays and pterygiophores, have not been described previously in detail but their morphologies in pleuronectiforms reveal both common and taxon-specific features.
- A fine nodular ultrasculpture was found on the external surface of ossicles of the European flounder and turbot, while another type of nodular structure, Mandl's corpuscles, was described on the visceral surface of tubercles of the turbot.
- Morphological differences of fish scales have been known and used in many earlier taxonomic researches. We suggest that detailed images obtained from further SEM studies would help in ascertaining the distinguishing features of related species also in other regions. We predict that the results of our study can be used not only in fish taxonomic, systematic, and phylogenetic studies, but also in fish biology, archaeology, and palaeoichthyology.

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Läänemere lestaliste (Pleuronectiformes, Teleostei) väikeste luuliste elementide võrdlev SEM-uuring

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On kirjeldatud ja võrreldud merilesta *Pleuronectes platessa* Linnaeus, lesta *Platichthys flesus trachurus* (Linnaeus) ning kammelja *Scophthalmus maximus* (Linnaeus) väikeste luuliste elementide morfoloogiat. Uuritud kalamaterjal pärineb Läänemere idaosast, Eesti vetest. Tsükloid- ja ogasoomused (*spined scales*), tuberkulid, küljejoonekanalite lülid (soomused ning segmendid) ja luustunud sümmeetrilised paarilised plaadikesed selja- ning pärakuuime liigenditelt erinevad nende kolme taksoni puhul märgatavalt. Kuigi merilesta ja lesta luulised elemendid erinevad paljudes detailides, pole kummagi soomused võrreldavad tugevasti modifitseerunud kammelja tuberkulitega. Samuti erinevad teineteisest isase ja emase merilesta soomused, kusjuures isase soomused kannavad ogasid tagaääres, emase soomustel need puuduvad. Mineraliseerumise protsessidest põhjustatud nodulaarne ultraskulptuur kirjeldati lesta ja kammelja luuliste elementide välisküljedel ning korpuskulaarne struktuur nende sisemistel pindadel. Kõigi kolme liigi luulised elemendid on rikkalikult illustreeritud SEM-piltidega.