The post-cranial body armor of the armored Agonidae fishes - How far do the morphological scale modifications go?

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Abstract

The family of the Agonidae is characterized by the presence of modified bony scales that form a protecting armor against predators. Despite some very intensive research concerning the skeletal cranial and postcranial anatomy of different Agonids, little information is available in the literature concerning the morphology of these bony scales. In this paper, three common species of the Northeastern Pacific Ocean are microscopically (both stereo and scanning electron microscopy) investigated to show that these bony plates are (a) morphologically different from regular fish scales, (ii) build for strength but also for minimizing the weight of the armor plates and (iii) morphologically different between different species.

Introduction

The family of the Agonidae (Teleostei, Scorpaeniformes) consists of 47 bottom dwelling species that inhabit shallow waters throughout the world (Nelson 1994, Nowroozi et al. 2009). One characteristic that typifies this group of fishes is the reformation of their scales into a series of fused or overlapping bony plates (Busby 1998). Being occasionally ornamented with caudally directed spines, this armor serves to protect the organism against predation. However, having no swimbladder (Aleev, 1969), the presence of re-enforced bony scales may cause a trade-off between how far these species can go in modifying their scales to create a sufficiently strong armor while trying to prevent loss of movability due to a too heavy armor. Although some work has been done on the skeletal cranial and postcranial anatomical differences between representatives of the Agonidae family (Kanayama 1991), very little information is available on the morphology of these modified scales (Nowroozi et al. 2009).

The goal of this research is to explore the morphological variation of these scales within and between species of the Northeastern Pacific Ocean. Research questions are (i) how are these armored plates different from the regular fish scale, (ii) is the expected trade-off between protection on the one hand and movability and buoyancy on the other hand visible at the level of the scale and (iii) do all investigated Agonidae species use the same strategy to build their armored plates?

In order to answer these questions, individual scales of three common Agonids of the Northeastern Pacific Ocean, being the Northern Spearnose Poacher (*Agonopsis vulsa*), the Gray Starsnout (*Bathyagonus alascana*), and the Smooth Alligatorfish (*Anoplagonus inermis*), are microscopically examined and morphologically compared. Two of these species, *A. vulsa* and *B. alascana*, have spined plates, while *A. inermis* has a complete smooth appearance. However, looking at the phylogenetic relationship between these three species, *B. alascana* is more closely related to *A. inermis* than to *A. vulsa* (Kanayama 1991).

Material & Methods

Specimens

Five specimens of *Agonopsis vulsa* (Jordan & Gilbert, 1880) were collected at Jackson beach (Friday Harbor, Washington; 48.520270, -123,009777) while seining (6/17/2014). One specimen of *Anoplagonus inermis* (Günther, 1860) and seven specimens of *Bathyagonus alascana* (Gilbert, 1896) were collected during three consecutive trawls in the vicinity of Friday Harbor (Washington; 48,543583, -123,010817) (6/20/2014).

Sample preparations

All specimens were euthanized using a lethal dose of tricaine mesylate (MS-222) (07/05/2014) and frozen afterwards. Body scales were removed in representatives of the three species using a dissecting microscope (Nikon SMZ-2B, Japan). All scales on the left side of the body, with the exception of the lateral line scales, were removed in four different body regions. From anterior to posterior, these regions include (i) the region anterior to the first dorsal fin, (ii) the region between both dorsal fins, (iii) the region posterior to the dorsal fin were the two dorsolateral plates fuse medially to form one mid-dorsal plate and (iv) the region halfway between the previous point and the caudal fin.

After dissection, most of the covering tissue was manually removed using a dissection needle. Individual scales were subsequently placed overnight in a pepsin solution (0.4% pepsin in 10 mM hydrochloric acid) and cleaned using an ultrasonicator.

Microscopy

Photographs of individual scales were made with a stereo and zoom microscope (SteREO Discovery.V20, Carl Zeiss Microscopy GmbH) mounted with a high-resolution microscope camera (AxioCam HRc, Carl Zeiss Microscopy GmbH) and operated by the digital image processing software AxioVision (Release 4.8.2 SP1 (12-2011), Carl Zeiss Microscopy GmbH). In preparation for scanning electron microscopy (SEM) (JEOL NeoScope JCM-5000, Nikon Instruments Inc.), scales were dehydrated with ethanol and critically point dried (Tousimis Samdri-790) before being sputter-coated with gold (PSI Sputter). All scales were photographed in a dorsal view and three measurements were obtained on every image using ImageJ. These measurements include: (i) the length of the scale, measured along the longitudinal axis of the spine, (ii) the width of the plate, measured perpendicular to the obtained length with the posterior margin of the spine as intersecting point, and (iii) the distance between the anterior margin of the scale and the posterior margin of the spine, measured parallel to the obtain length.

3D-reconstructions

The four investigated body regions were additionally cut out as a whole in one specimen of *Agonopsis vulsa* in order to be CT-scanned (University of Washington). Subsequently, these scans were three-dimensionally visualized in Amira 5.3.3 (3D Visualization software, FEI Visualization Sciences Group) allowing segmentation of all individual scales in their original composition using the segmentation editor.

Results

Scale morphology

1. Agonopsis vulsa

Scales are removed from two specimens of *Agonopsis vulsa* (SL: 86.2 and 85.7mm). Stereo-microscopic pictures of these scales are taken for both specimens (data not shown). Scanning electron microscopy (SEM) images of the scales of one specimen (SL: 86.2) are given in figure1 and figure2.

Four scales (the dorsal (fig1a-d), supralateral (fig1e-h), infralateral (fig1i-l), and ventral scale (fig1m-p)) per dissected area (see material and methods) are depicted in Figure 1. Due to preparation problems, one scale (the infralateral scale fig1i) in the first dissected region and three scales (the dorsal (fig1b), infralateral (fig1j) and ventral (fig1n) scales) in the second dissected body region have lost pieces of the covered part of scale. Therefore, their SEM-images are present in the figure, but are incomplete.

Based on the obtained images and their measurements (Table1), the following trends with relation to the shape and general morphology of these scales can be observed. First, no spines are present at the any of the dissected ventral plates. Second, scale length does not change significantly when comparing the scales within the same body region and when comparing scales between body regions. Third, the scale length to scale width ratio increases with a more posterior location along the body. Fourth, the position of the posterior margin of the spine shifts posteriorly when closer located towards the rostral end of the body. Fifth, the part of the scales that is not covered by surrounding scales is occupied by trabeculae (figure2a) that start at the margin of the scale (figure2b) and are building up towards the actual spine. These trabeculae are interconnected, creating crevices and holes between different neighboring trabeculae and resulting in a web-like structure (figure2c) connecting the apparently solid tip of the spine onto the concentric layers of the underlying scale (figure2a,d). Although these trabeculae are present within most of the observed scales, the web-like structure does not appear to be as elaborate in the ventral plates. The latter do exhibit the trabeculae, which are composed of a series of short elevations of bone, whose interconnectivity creates small crevices between the trabeculae.

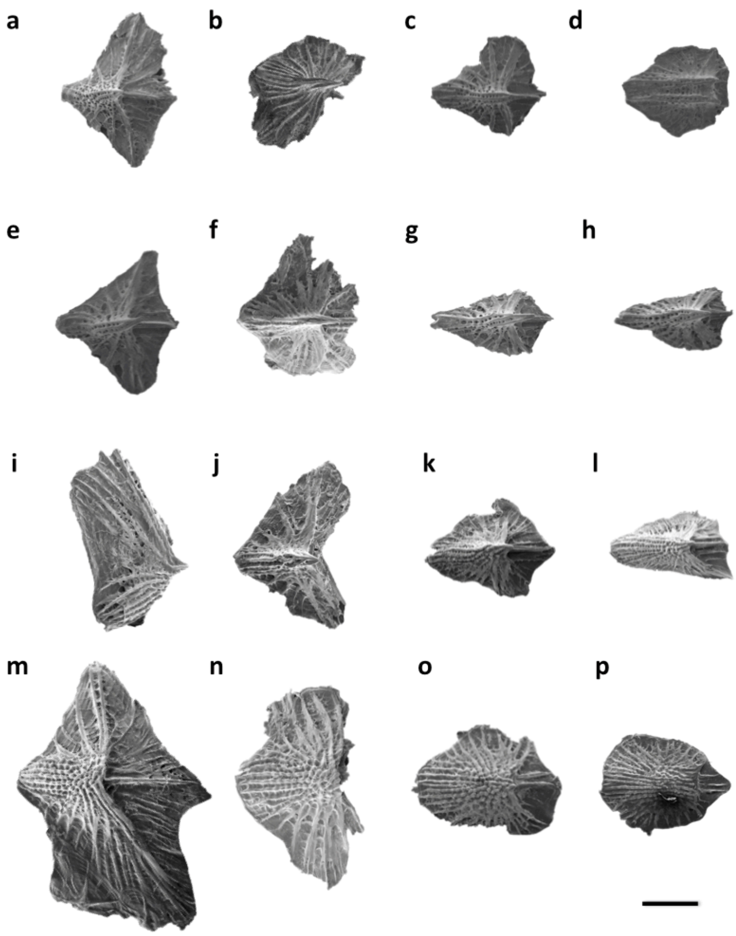

Figure 1 – Overview of scanning electron microscopic images of the scale morphology of *Agonopsis vulsa* along four different regions of the body. (a,e,i,m) anterior to the dorsal fin, (b,f,j,n) between both dorsal fins, (c,g,k,o) behind dorsal fin, (d,h,l,p) halfway between the dorsal and the caudal fin. (a-c) dorsolateral scales, (d) mid-dorsal scale, (e-h) supralateral scales, (i-l) infralateral scales, (m-n) ventrolateral scales, (o-p) mid-ventral scales. Scalebar 1mm

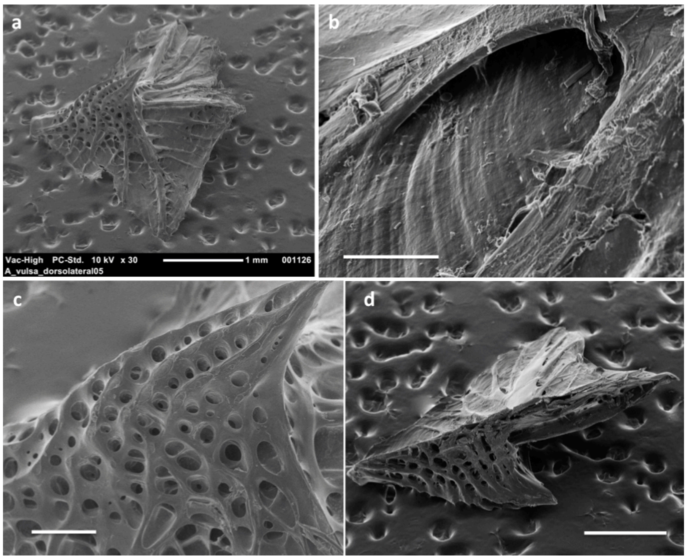

Figure 2 – SEM images of the dorsolateral scale in the region before the dorsal fin in *Agonopsis vulsa*. (a) left-lateral view on the scale, (b) detail of the edge of the scale (scalebar 100µm), (c) detail of the spine (scalebar 200µm), (d) cross section through the dorsolateral scale (scalebar 1mm).

	Width (mm)	Length (mm)	Spine (mm)	L/W	Spine/L
DL01	3.95	2.62	1.33	0.71	0.57
SL01	3.14	2.46	1.38	0.78	0.56
IL01	3.52	2.70	1.13	0.77	0.42
VL01	4.10	5.76	1.84	1.10	0.41
DL02	3.40	3.42	2.44	1.01	0.71
SL02	3.50	2.99	2.02	0.85	0.67
IL02	3.72	2.57	1.58	0.76	0.59
VL02	3.94	2.50	1.99	0.69	0.64
DL03	2.48	2.23	2.21	1.17	0.65
SL03	1.12	1.93	1.31	1.73	0.68
IL03	2.84	3.50	2.05	1.23	0.63
MV03	2.46	3.15	2.10	1.23	0.64
MD04	2.43	3.20	2.27	1.19	0.81
SL04	2.02	3.18	2.24	1.78	0.73

IL04	1.91	3.22	2.18	1.77	0.69
MV04	2.57	3.50	2.60	1.36	0.74

Table 1 – Averages of the measurements on the scales of two specimens of *Agonopsis vulsa*. Spine = the distance between the anterior tip of the scale and the posterior margin of the base of the spine. Scale-abbreviations in the four dissected regions of the body (01-04) DL: Dorsolateral, IL: Infralateral, MD: Mid-dorsal, MV: Mid-ventral, SL: Supralateral, VL: Ventrolateral.

3D-reconstruction

From the obtained CT-scans, both the lateral scales as well as the dorsal and ventral scales of the region directly behind the dorsal fin are three-dimensionally reconstructed. Figure 3a shows these scales in their original composition while figures 3b-c illustrate the complex network of holes, corridors and crevices that run through the basis of the spine.

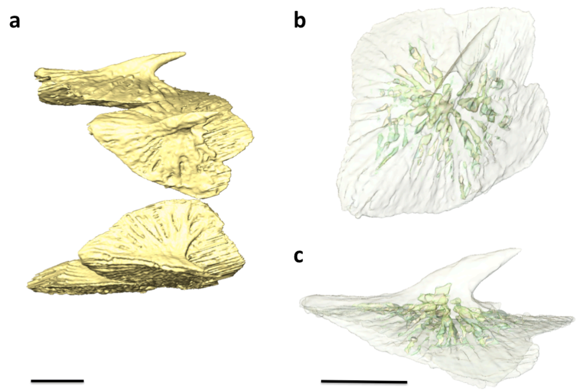

Figure 3 – 3D-reconstruction of body scales *Agonopsis vulsa*. (a) the dorsolateral, supralateral, infralateral, and ventrolateral scale in the region just behind the dorsal fin in their natural orientation, (b) dorsal view on the supralateral scale with indication of the network of corridors running through the scale, (c) lateral view on the supralateral scale with indication of the network of corridors running through the scale. Scalebar 1mm.

2. Bathyagonus alascana

Scales are removed from two specimens of *Bathyagonus alascana* (SL: 103.9 and 104.5mm). Stereo-microscopic pictures of these scales are taken for both specimens (data not shown). Scanning electron microscopy (SEM) images of the scales of one specimen (SL: 103.9) are given in figure4 and figure5.

Four scales (the dorsal (fig4a-d), supralateral (fig4e-h), infralateral (fig4i-l), and ventral scale (fig4m-p)) per dissected area (see material and methods) are depicted in Figure 4. Due to preparation problems, no SEM images are available of the supralateral scale of the third body region (fig4f) and the dorsal (fig4d) and supralateral (fig4h) scale of the fourth body region. Based on the obtained images and their measurements (Table2), the following trends with relation to the shape and general morphology of these scales can be observed. First, no spines are present at the ventral plates of the second, third, and fourth dissected body region. Second, scale length increases gradually from a dorsal towards a ventral location of the scale, but does not change significantly along the longitudinal axis of the body. Third, the scale length to scale width ratio increases with a more posterior location along the body. Fourth, the position of the posterior margin of the spine shifts posteriorly when closer located towards the rostral end of the body. Fifth, the part of the scales that is not covered by surrounding scales is ornamented with a uniform pattern of short elevations of bone that are interconnected by shallower ridges, giving the scale a spotted-like appearance (figure5).

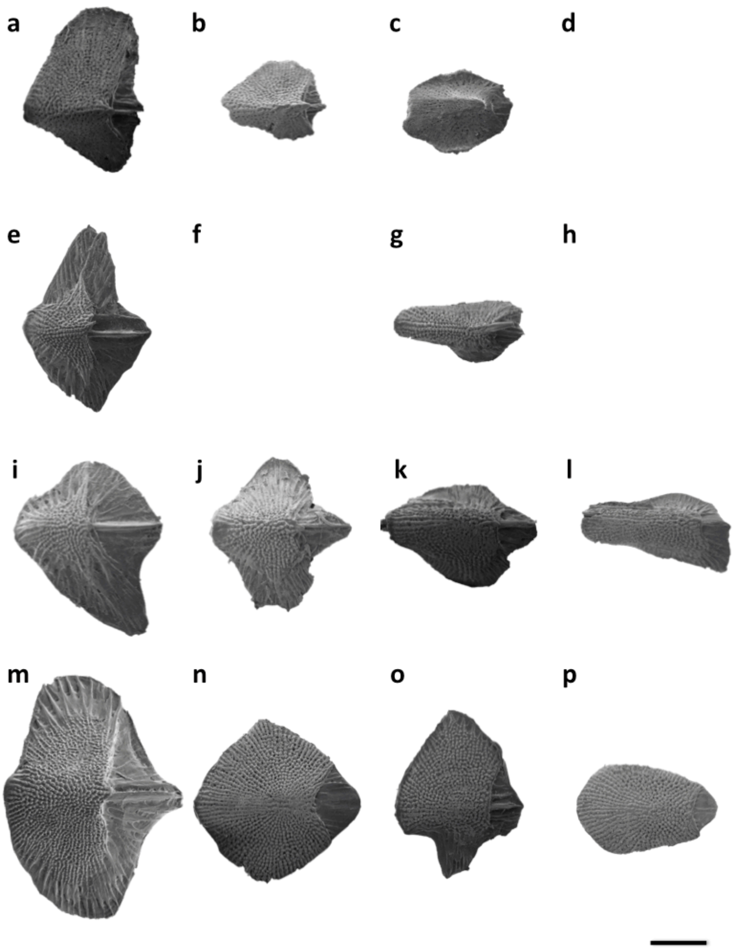

Figure 4 – Overview of scanning electron microscopic images of the scale morphology of *Bathyagonus alascana* along four different regions of the body. (a,e,i,m) anterior to the dorsal fin, (b,f,j,n) between both dorsal fins, (c,g,k,o) behind dorsal fin, (d,h,l,p) halfway between the dorsal and the caudal fin. (a-c) dorsolateral scales, (d) mid-dorsal scale, (e-h) supralateral scales, (i-l) infralateral scales, (m-n) ventrolateral scales, (o-p) mid-ventral scales. Scalebar 1mm

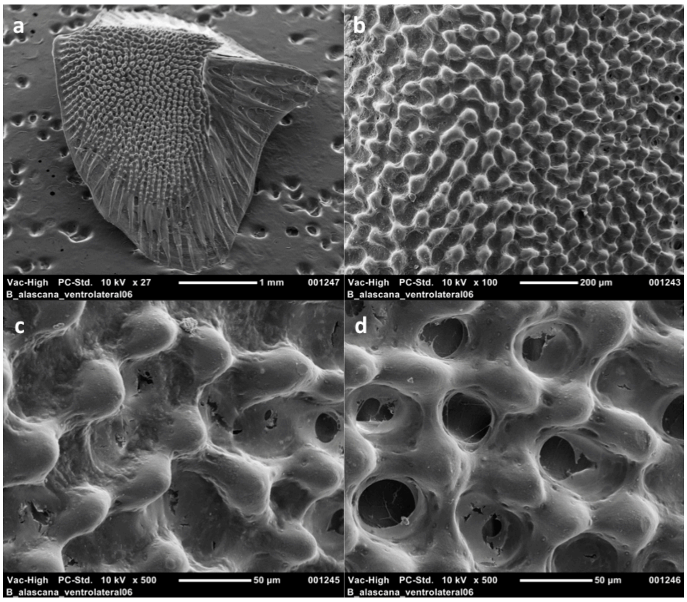

Figure 5 – SEM images of the ventrolateral scale in the region before the dorsal fin in *Bathyagonus alascana*. (a) left-lateral view on the scale, (b) dorsal view on the spine, (c) detail at the level of the spine, (d) detail at the level of the wing of the scale.

	Width (mm)	Length (mm)	Spine (mm)	L/W	Spine/L
DL01	2.88	2.05	1.46	0.71	0.71
SL01	3.83	2.65	1.34	0.69	0.50
IL01	3.34	2.83	1.45	0.85	0.51
VL01	4.99	3.55	1.99	0.71	0.56
DL02	1.63	2.02	1.60	1.25	0.79
SL02	1.24	2.58	1.96	2.08	0.76
IL02	3.14	2.70	1.61	0.86	0.59
VL02	3.23	2.82	2.06	0.88	0.73
DL03	1.57	2.07	1.66	1.32	0.80
SL03	1.26	2.57	1.95	2.05	0.76
IL03	1.83	2.77	2.01	1.52	0.73
MV03	3.19	2.83	2.07	0.88	0.73
MD04	-	-	-	-	-
SL04	1.50	2.32	1.74	1.55	0.75
IL04	1.41	2.71	2.17	1.92	0.80
MV04	1.74	2.65	2.22	1.52	0.84

Table 2 – Averages of the measurements on the scales of two specimens of *Bathyagonus alascana*. Spine = the distance between the anterior tip of the scale and the posterior margin of the base of the spine. Scale-abbreviations in the four dissected regions of the body (01-04) DL: Dorsolateral, IL: Infralateral, MD: Mid-dorsal, MV: Mid-ventral, SL: Supralateral, VL: Ventrolateral.

3. Anoplagonus inermis

Scales are removed from one specimen of *Anoplagonus inermis* (SL: 104.9mm). Stereo-microscopic pictures of these scales are shown in figure6. This figure depicts the four scales (the dorsal (fig6a-d), supralateral (fig6e-h), infralateral (fig6i-l), and ventral scale (fig6m-p)) per dissected area (see material and methods) along the body. Based on these images and their obtained measurements (Table3), the following trends with relation to the shape and general morphology of these scales can be observed.

First, no spines are present on any of the dissected scales. Second, scales on the dorsal and ventral side of the body are longer at the rostral end of the body than the scales on the lateral side. However, as the dorsal and ventral scales diminish in length along the longitudinal axis of the body while both of the lateral scales increase in length, all scale are approximately equally long at the caudal end of the body. Third, the scale length to scale width ratio increases for all scales when located more posteriorly along the body. Fourth, apart from the lateral scales at the rostral end of the body, the position of the posterior margin of the area that is not covered by surrounding scales shifts posteriorly when closer located towards the rostral end of the body. Fifth, the uncovered part of the scales is ornamented with a uniform pattern of short elevations of bone that are interconnected by shallower ridges, creating a spotted-like appearance (figure7).

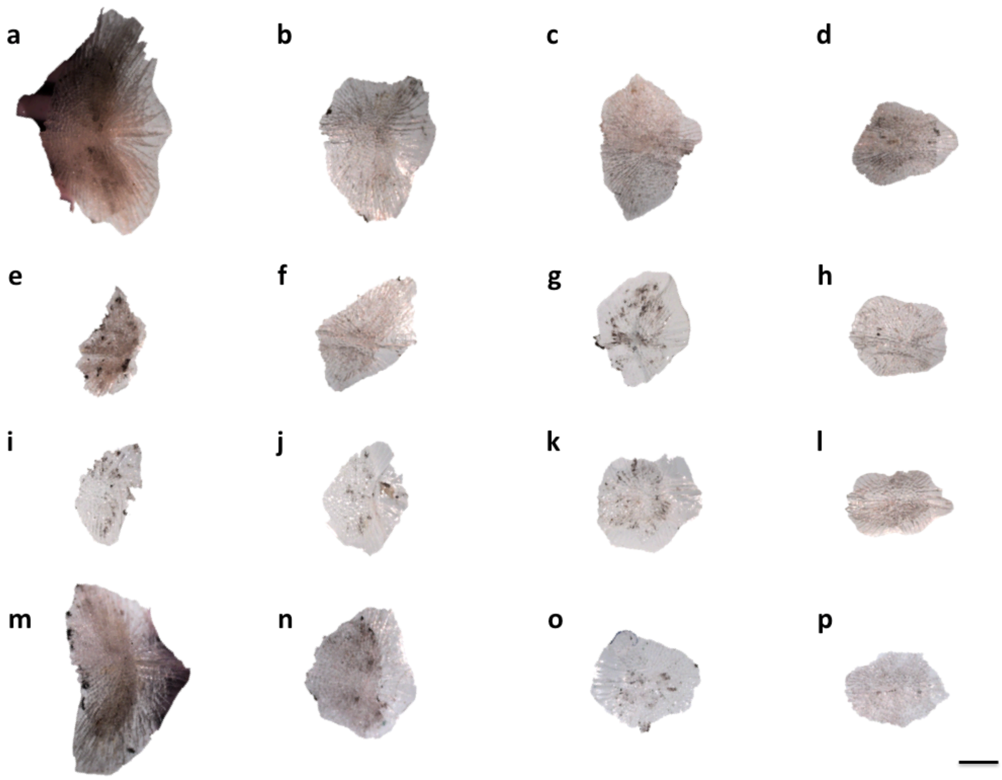

Figure 6 – Overview of microscopic images of the scale morphology of *Anoplagonus inermis* along four different regions of the body. (a,e,i,m) anterior to the dorsal fin, (b,f,j,n) between both dorsal fins, (c,g,k,o) behind dorsal fin, (d,h,l,p) halfway between the dorsal and the caudal fin. (a-c) dorsolateral scales, (d) mid-dorsal scale, (e-h) supralateral scales, (i-l) infralateral scales, (m-n) ventrolateral scales, (o-p) mid-ventral scales. Scalebar 1mm

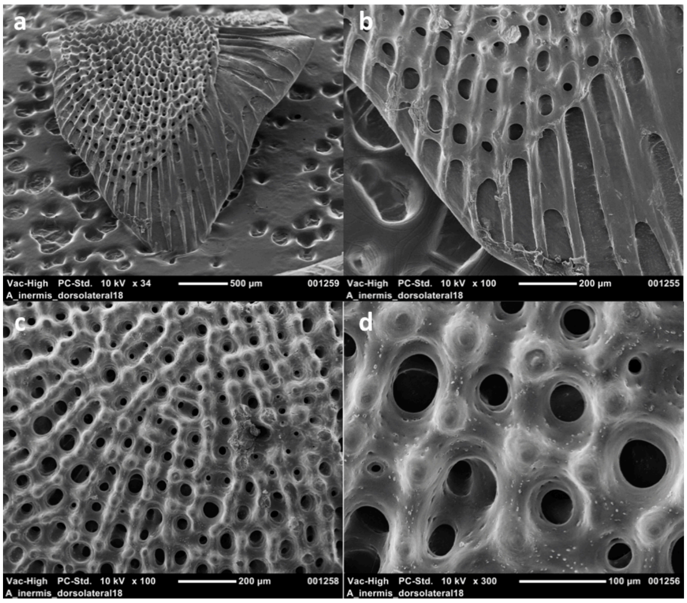

Figure 7 – SEM images of the dorsolateral scale in the region before the dorsal fin in *Anaplagonus inermis*. (a) left-lateral view on the scale, (b) detail of the edge of the scale, (c) detail of the wing of the scale at the level of the spine, (d) detail of the SEM-image from (c).

	Width (mm)	Length (mm)	Spine (mm)	L/W	Spine/L
DL01	6.53	3.52	1.77	0.54	0.50
SL01	2.47	2.18	1.75	0.88	0.80
IL01	3.03	1.90	1.33	0.63	0.70
VL01	5.03	3.08	1.55	0.61	0.50
DL02	4.18	3.11	1.87	0.74	0.60
SL02	3.00	2.71	1.75	0.90	0.64
IL02	3.31	2.58	1.62	0.78	0.62
VL02	3.35	2.86	1.85	0.85	0.64
DL03	3.89	2.98	1.94	0.76	0.65
SL03	3.31	2.61	1.90	0.78	0.72
IL03	2.87	2.78	1.92	0.97	0.68
MV03	3.37	3.08	2.20	0.91	0.71
MD04	2.32	2.84	2.27	1.22	0.80
SL04	2.53	2.46	1.96	0.97	0.80
IL04	1.70	2.83	2.14	1.66	0.76
MV04	2.13	2.89	2.28	1.35	0.79

Table 3 – Averages of the measurements on the scales of one specimen of *Anoplagonus inermis*. Spine = the distance between the anterior tip of the scale and the posterior margin of the base of the spine. Scale-abbreviations in the four dissected regions of the body (01-04) DL: Dorsolateral, IL: Infralateral, MD: Mid-dorsal, MV: Mid-ventral, SL: Supralateral, VL: Ventrolateral.

Discussion

Based on the three investigated species, one general trend with relation to the morphology and shape of the scales in Agonidae can be derived. Plates on the dorsal side of the body namely appear to be smallest in size, while scales at the ventral size of the body are biggest in size. Since scale size will influence the flexibility of the body, it can be expected that these poachers are more flexible in their dorsal movement than in their ventral movement. Although no empirical data on flexibility was obtained in this research to support this hypothesis, having a greater flexibility in your dorsal compared to your ventral movement may be advantageous when you are a bottom dwelling species.

Additionally, three research questions are addressed in this paper. First, microscopic evidence is used to find out how the armored plates of the three investigated Agonidae species are modified compared to a regular fish scale. In order to get this comparison, the scanning electron microscopy pictures in this paper (Figures 2,5,7) are compared to the works of Zhu et al. (2011) and Roberts (1993) on modern and spined fish scales, respectively. This comparison shows that the three investigated Agonids, in varying degrees, build trabeculae on top of the original 'typical' concentric layered fish scale. Neighboring trabeculae interconnect with each other, creating an entire network of web-like bony support structures that work their way up to a spine in both *Agonopsis vulsa* and *Bathyagonus alascana*. Surprisingly, the same armor blueprint can be seen in *Anoplagonus inermis*, although this species lacks spines on its modified scales. Remarkably, no scale can be found in the work of Roberts on spined scales throughout the Teleostei that resembles the observed network of trabeculae in the Agonidae armor plates.

Second, the expected trade-off between protection and movability/buoyancy is indeed observed in the scales of *Agonopsis vulsa*. Cross-sections (Figure 2d) and 3D-reconstructions (Figure 3b-c) of the armor plates namely show an entire network of corridors and holes running parallel with the base of the scale, while some of these are projected towards the spine. However, none of these projections make it all the way up to the top (Figures 2d, 3c). As a result, the solid tip of the spine is supported by the network of trabeculae, which is an efficient way of distributing any force applied to the distal side of the scale and/or the tip, while keeping the amount of extra weight that these modifications bring along as low as possible. Unfortunately, neither cross-section nor 3D-data is currently available for the two other investigated species to support this observation.

With relation to the question whether or not all research species use the same strategy to build up their armor plates, two distinct morphologies can be observed within the three investigated species. *Agonopsis vulsa* has the interconnecting trabeculae creating a whole network of ridges and holes (figure 2c), while the scales of both *Bathyagonus alascana* and *Anoplagonus inermis* are covered by regularly places elevations of bone that are interconnected to each other. When mapping these species onto the phylogenetic tree provided by Kanayama (1991), *B. alascana* and *A. inermis* are closer related to each other than both are to *A. vulsa*. Combining the existence of two different scale morphologies with the classification of the three investigated species into two separate subgroups within the Agonidae, it can be suggested that different subgroups indeed have different strategies of building up their armor.

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