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Scanning Electron Microscope Study of Microstructure and Regeneration of Upper Pennsylvanian Cladid Crinoid Spines

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ABSTRACT

The crinoid skeleton is characterized by a complicated, highly porous microstructure known as stereom. Details of stereomic microstructural patterns are directly related to the distribution and composition of connective tissues, which are rarely preserved in fossils. However, certain portions of the crinoid skeleton have never been studied with respect to stereomic microstructure. In particular, spines are common features on the crowns of many Paleozoic crinoids but had not previously been studied in detail with respect to stereomic microstructure. This study focused on pirasocrinid cladid crinoids, a common group with numerous identifiable crown spines, including spines on the arms and anal sac. The specific goals of this project were (1) to describe the microstructure of anal sac spines and interpret aspects of soft-tissue anatomy; and (2) to describe biologically relevant stereomic patterns associated with regeneration of broken spines.

Pirasocrinid anal sac spines consist of three major zones: the primary spine shaft; a sculptured region with short, meandering protuberances; and an articular ridge, where the spine articulated to the rest of the anal sac. The spine shaft is characterized by dense stereom indicating little interpenetration by connective tissues. The protuberances of the sculptured zone are equally dense, but the intervening valleys are characterized by labyrinthine stereom, suggesting articulation by short ligamentary fibers. The complex sculpturing of this region may reflect an increase in anchorage points for connective tissues that did not penetrate into the interior of skeletal plates. The articular ridge is characterized by dense crenulae, suggesting little penetration by connective tissues, and valleys indicating penetration by short ligamentary fibers, similar to the sculptured zone. This indicates that spines were articulated at their bases to the rest of the tegmen by a combination of physical interlocking of dense crenulae and articulation by

ligaments in valleys.

Planes of regeneration are similar in both anal sac and brachial spines. Major patterns include (1) significantly larger pores between rods of stereom than the dense rectilinear stereom pattern present on unbroken or fully regenerated spines; (2) large, triangular outgrowths of stereom that eventually coalesce to form a layer extending outward from the regeneration plane; and (3) concentric "sheaths" of triangular stereomic outgrowths at similar stages of development throughout the entire diameter of the spine. These suggest that spine regeneration involved a complicated set of processes operating across the plane of regrowth rather than from the interior to the exterior.

ACKNOWLEDGMENTS

I would like to thank Dr. James R. Thomka, who served as project supervisor and guided me through the research process, as well as my committee members, John F. Beltz and Thomas J. Quick. TJQ was instrumental in operation of the ESEM and capturing of images. Access to specimens was provided by the Cleveland Museum of Natural History, specifically Douglas Dunn and Joseph T. Hannibal. Ronald D. Lewis (Auburn University) provided the images of articulated pirasocrinid crowns. Lastly, I would like to thank Joshua K. Mercier for helping me with the sorting of specimens and assistance with image collection.

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INTRODUCTION

Crinoids ("sea lilies"), like all echinoderms, have an endoskeleton consisting of numerous calcite plates known as ossicles. Although ossicles appear solid, they are actually highly porous and are constructed of a complex three-dimensional meshwork of microscopic, interconnected rods, which produces a microstructure termed stereom (Ausich et al., 1999). Soft tissues are interwoven throughout the stereom, and the fine-scale properties of stereomic microstructure are directly related to the types of connective tissues present in that portion of the skeleton (Macurda and Meyer, 1975; Roux, 1975; Smith, 1980). As a result, studies of crinoid stereom have focused nearly entirely on comparisons between modern specimens and corresponding portions of ancient specimens. The distribution of different types of connective tissues (for example, muscles vs. ligaments) in Paleozoic crinoids has been successfully identified in multiple case studies (e.g., Lane and Macurda, 1975; Gorzelak et al., 2014). However, this comparative approach is rooted in first considering particular portions of modern crinoid skeletons and then looking for similar or different stereomic patterns in ancient specimens. This means that skeletal features that are present in extinct crinoids but absent in living crinoids have been relatively infrequently studied. One important illustration of this involves spines: because modern crinoids lack spines (Ausich et al., 1999), little is known about the stereomic structure of these skeletal elements in spite of the fact that many Paleozoic crinoids have prominent and/or numerous spines.

The purpose of this study is to use scanning electron microscopy to document stereomic patterns of certain Paleozoic crinoid spines. Research focused on two primary areas: (1) description of anal sac (tegmen) spines, a portion of the crinoid skeleton that had not previously been studied with respect to stereomic structure; and (2) description and interpretation of the

microstructure of regeneration planes in anal sac and brachial (arm) spines, which had also not previously been studied in detail and which can potentially provide information on the biology of regeneration in ancient crinoids.

MATERIALS AND METHODS

Specimens used in this study consisted of isolated spines belonging to a group of extinct crinoids known as pirasocrinid cladids. This group was selected because pirasocrinids are one of the most common and geographically widespread families of crinoids in the upper Paleozoic (Webster, 2018) in addition to being among the spiniest of all crinoids (Figs. 1-2). Pirasocrinids had distinctive spines on multiple portions of the skeleton, including readily identifiable brachial spines at the base of the arms (feeding appendages; Fig. 1) and at the top of the anal sac (part of the tegmen, a structure containing the digestive system; Figs. 1-2). Both brachial and anal sac spines are easily collected and are identifiable at least to family level (Thomka and Eddy, 2018).

Material was borrowed from the invertebrate paleontology collections of the Cleveland Museum of Natural History (CMNH), specifically specimen lots CMNH 9211, 9262, and 9279. Each of these specimen lots consist of numerous (100+) loose pirasocrinid ossicles, including both types of spine. Specimens were collected from the Upper Pennsylvanian (~305 million years old) Ames Limestone member of the Glenshaw Formation from closely spaced locations in Guernsey County, east-central Ohio. This interval was selected not only because of the abundance of pirasocrinid material, but also because of the high proportion of spines showing evidence of regeneration following breakage, most likely produced by unsuccessful predation by fish (Thomka and Eddy, 2018). Regeneration planes are marked by sudden changes in the diameter of the spine, and these samples are noteworthy as containing the only currently known

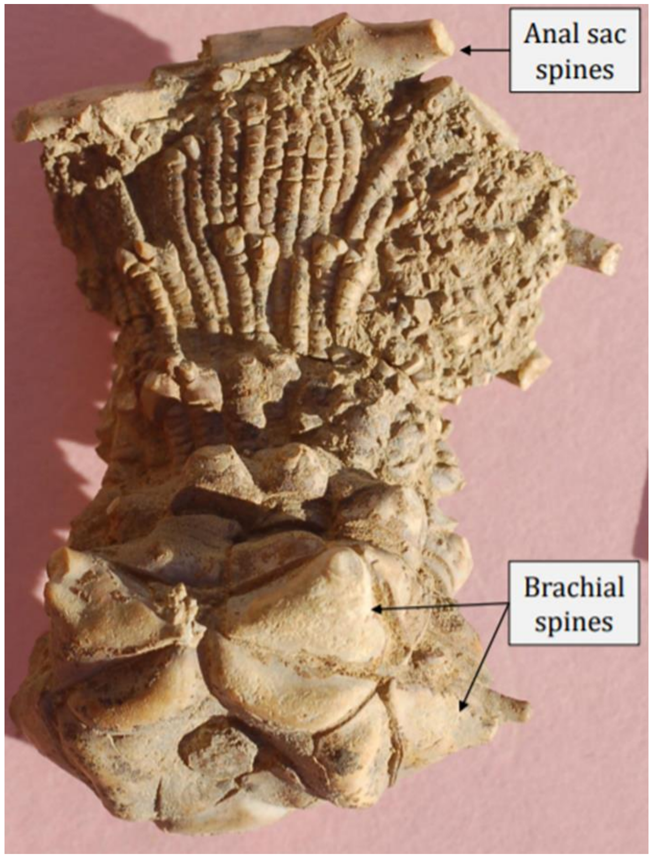

FIGURE 1: Example of an articulated pirasocrinid crown with the brachial (arm) and anal sac (tegmen) spines labeled. Note the extremely spinose morphology. Only isolated spines reflecting total disarticulation of crowns such as this were used in this study. Photograph provided by Ronald D. Lewis (Auburn University).

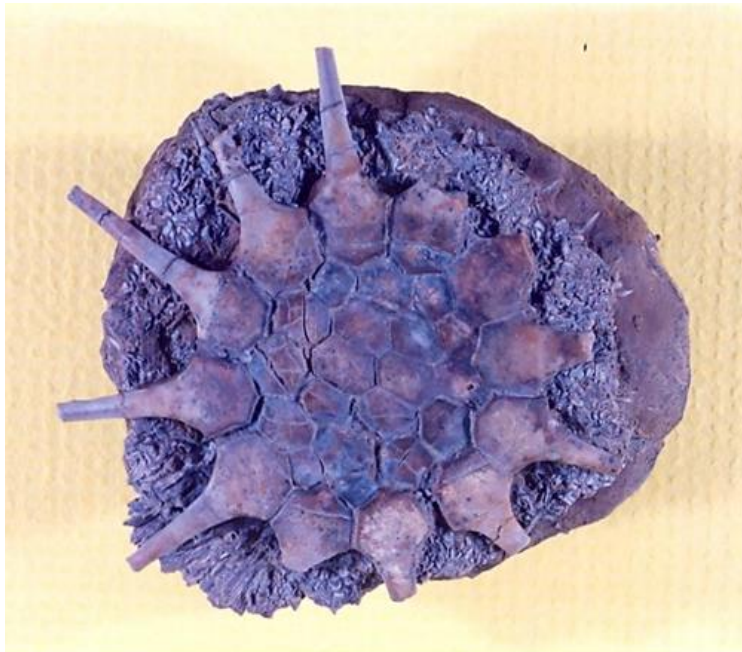

FIGURE 2: Example of an intact pirasocrinid anal sac summit, showing the radial array of spines at the very top of the tegmen. For reference, this view is equivalent to looking down from the top of the specimen in Figure 1. Photograph provided by Ronald D. Lewis (Auburn University).

spines with multiple planes of regeneration (Thomka and Eddy, 2018; Fig. 3), some of which were analyzed as part of this study.

Non-destructive, extremely high-resolution imaging of pirasocrinid spines was made possible through use of an environmental scanning electron microscope (ESEM) in Crouse Hall (University of Akron main campus). The ESEM sample stage can accommodate up to seven samples at a time. Once loaded, the ESEM is set to low vacuum mode where the atmosphere in the chamber is reduced to 0.6 Torr (80 Pascal). When the atmosphere is stable, the electron beam is energized to 30 Kv. This permitted greater than 1000x magnification, although the maximum magnification for observation of stereomic microstructure was approximately 800x. Images are displayed on a computer monitor, where they can be captured and saved. For this study, emphasis was placed on documenting the stereomic microstructure of different regions of intact anal sac spines and the characteristics of stereom at planes of regeneration on both anal sac and brachial spines. Terminology and interpretation of stereom is based on published descriptions of modern and ancient crinoids (e.g., Macurda and Meyer, 1975) as well as other Paleozoic echinoderms (e.g., Macurda, 1973; Gorzelak and Zamora, 2016). Terminology of pirasocrinid anal sac spine morphology is from Lewis (1974).

RESULTS

Stereomic microstructure of pirasocrinid spines could be readily observed via ESEM without any physical or chemical preparation, such as the techniques described by Lapham et al. (1976), Sevastopulo and Keegan (1980), and Gorzelak and Zamora (2013). Descriptions and interpretations are given below, organized into separate headings that define the two primary objectives of the study: physical description of pirasocrinid anal sac microstructure and analysis

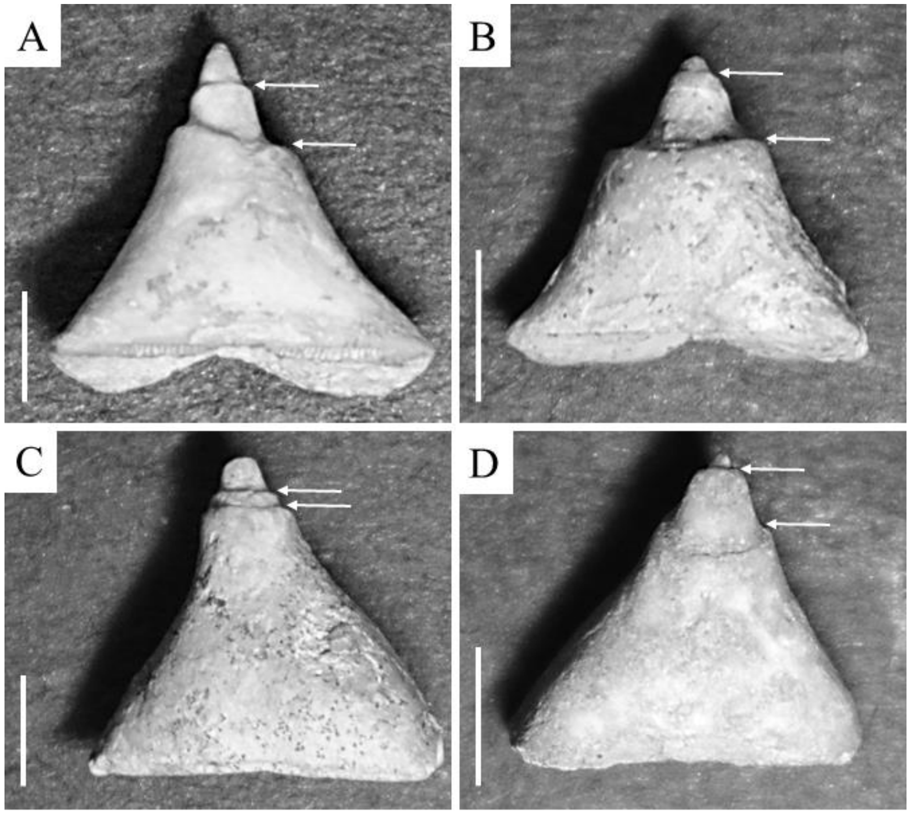

FIGURE 3: Pirasocrinid brachial spines from the study interval that contain multiple planes of breakage and regeneration, marked by arrows. All scale bars = 1 cm. From Thomka and Eddy (2018).

of spine regeneration planes.

Morphology of Anal Sac Spines

Pirasocrinid anal sac spines can be divided into three primary regions: the shaft, the neck, and the base (Lewis, 1974). The shaft is the main mass of the spine, extending outward laterally from the tegmen and terminating in a sharp point. The base is the portion where the spine attaches to the tegmen, at the opposite end of the sharp point. The neck is a transitional region between the base and shaft; this feature is poorly defined in pirasocrinid anal sac spines, being essentially the widest part of the shaft (Lewis, 1974).

When looking at the "underside" (dorsal) surface of spines, where the bottom of the spine articulates to underlying tegmen plates, three different zones associated with distinctive stereomic microstructures are present. These are the (1) spine shaft, which transitions into the base at (2) a sculptured zone characterized by numerous arcuate ridges and valleys, which terminates in (3) a crenulated ridge at the very base of the spine, where the plate interlocks with crenulae from the underlying ossicle.

The spine shaft is characterized by very dense stereom (Fig. 4) indicating little interpenetration by soft tissues, as would be expected for immobile and non-sensory structures. The stereomic rods (trabeculae) are arranged in a cross-hatched pattern, producing a distinctive microstructural pattern known as rectilinear stereom (Smith, 1980; Figs. 4-5). Rectilinear stereom is not common in the crown of crinoids (Macurda and Meyer, 1975). This stereom persists throughout the entirety of the shaft, from the neck to the outer tip.

The sculptured zone on the base of the spine contains multiple short, arcuate to meandering, anastomosing ridges that are oriented roughly parallel to the long axis of the spine

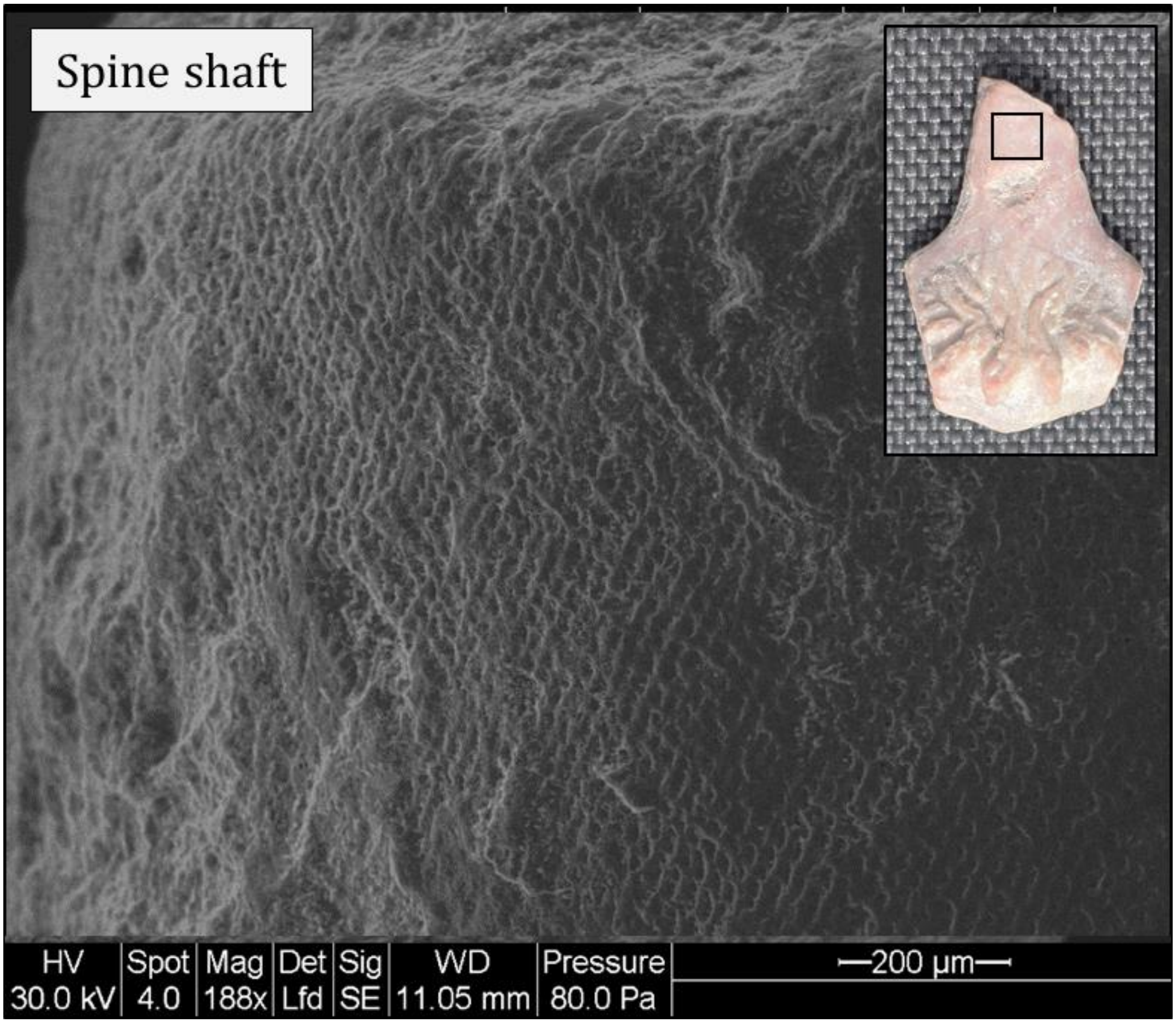

FIGURE 4: Stereom of pirasocrinid spine shafts. The inset picture of a broken spine shows the general position of shaft stereom relative to the base. Note the dense, rectilinear stereom that is present throughout the entire shaft.

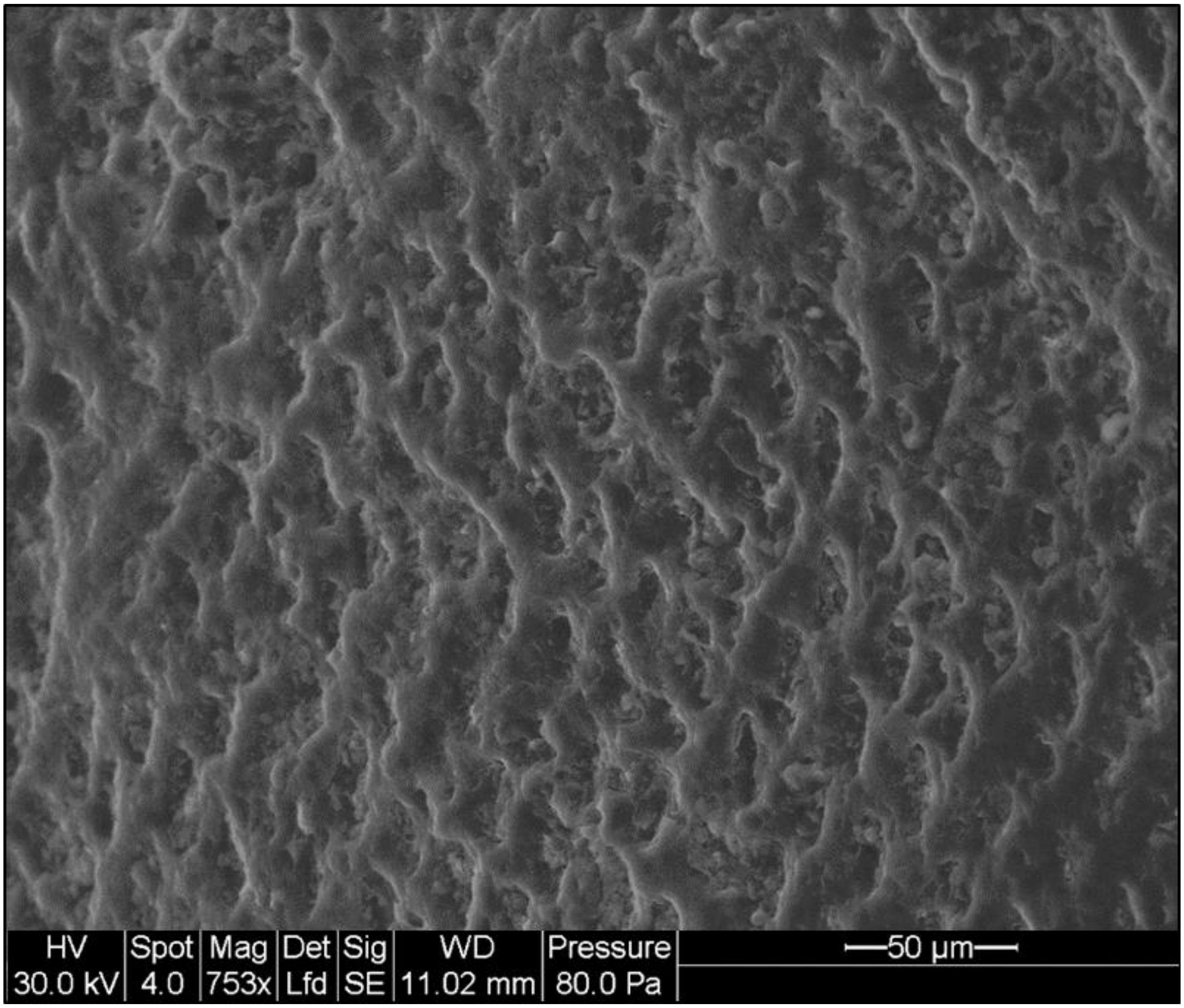

FIGURE 5: Closer version of fine rectilinear stereom of spine shafts, magnified from the same image in Figure 4. This distinctive structure suggests very little interpenetration by soft tissues.

(although those along the lateral margins are oriented radially toward the exterior). Ridges are separated from adjacent ridges by narrow valleys or canals (Fig. 6). This zone is probably the region of the "pore canals" of Lewis (1974). In contrast to the shaft, this region is characterized by differentiated stereom in the elevated portions compared to the depressed canals. The elevated ridges, which actually would have extended downward into the underlying tegmen, contain stereom that is denser and more amorphous than that of the shaft (Fig. 7), indicating that these structures basically served as solid masses and were not sites of articulation by internally penetrating soft tissues. The canals display fine labyrinthine stereom (Fig. 7), suggesting penetration by fine ligamentary fibers (Macurda and Meyer, 1975). Short, fine strands of ligamentary tissue would have connected the canals to corresponding parts of the underlying plate. The density of the stereom on ridges would have precluded this same mechanism for articulation in the elevated parts of the sculptured zone. However, the complex geometry of ridges may suggest that they were used primarily to increase the surface area of the articular region of the spine without substantially increasing volume. In this scenario, ligamentary fibers could attach to the exterior of the meandering ridges, but without penetrating them, providing further strength to the articulation between spine bases and the rest of the tegmen.

The zone of articular ridges is found further away from the spine shaft than the sculptured zone and is marked by a sharply defined linear array of parallel ridges separated by valleys, forming a crenulated structure (Fig. 8). This represents the dorsal lip of Lewis (1974), which is oriented perpendicular to the long axis of the spine and serves as a sharp boundary between the articular region of the spine and other areas not associated with articulation to underlying tegmen plates. As with the sculptured zone, there is stereomic differentiation between the elevated and depressed areas. Again, the elevated ridges display relatively dense, amorphous stereom (Figs.

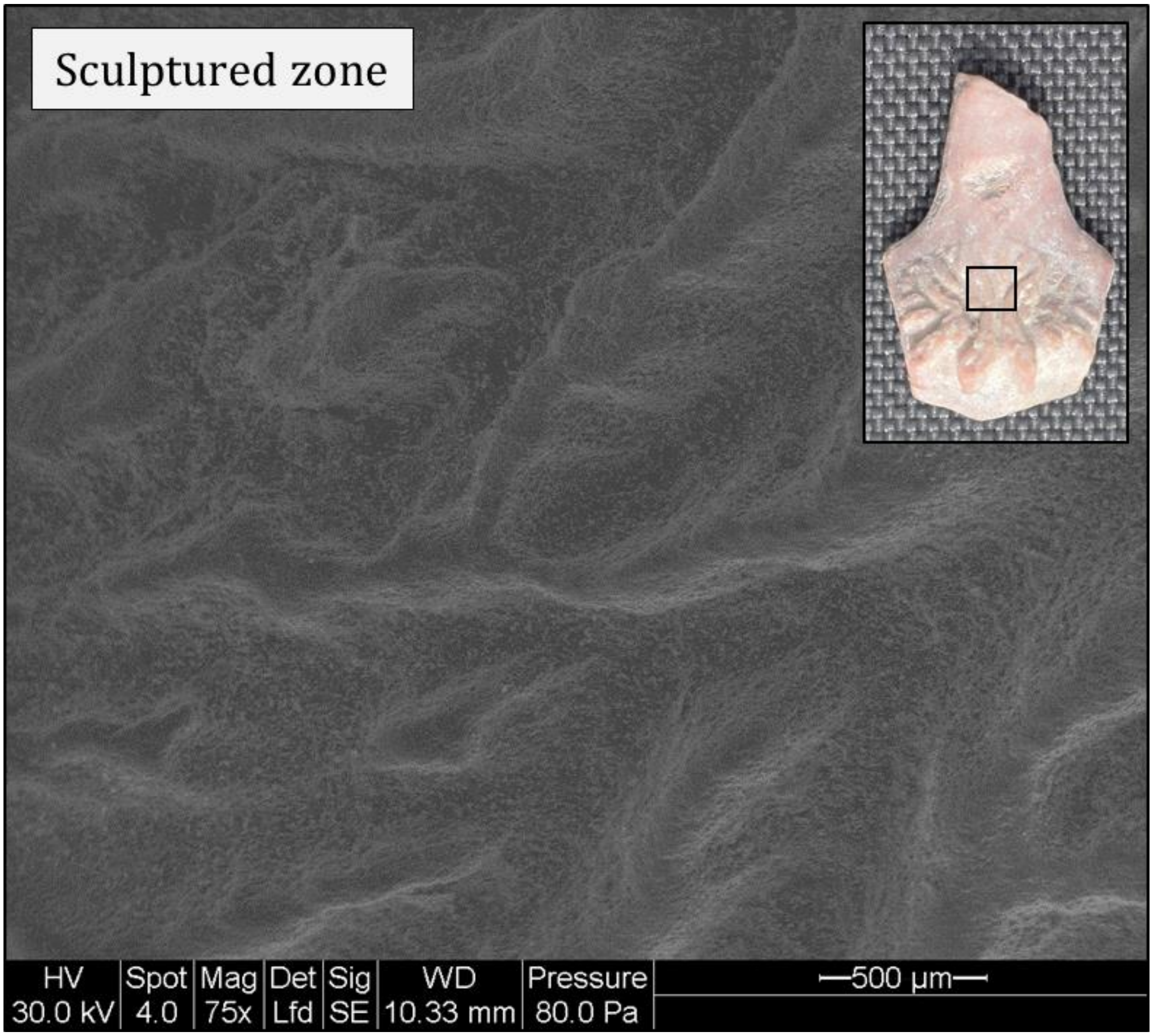

FIGURE 6: Stereom of the sculptured zone on the medial portion of pirasocrinid spine bases. The inset picture of a broken spine shows the general position of sculptured zone stereom relative to the shaft and rest of the base. This region consists of arcuate to meandering ridges of relatively dense stereom separated by valleys or canals of labyrinthine stereom.

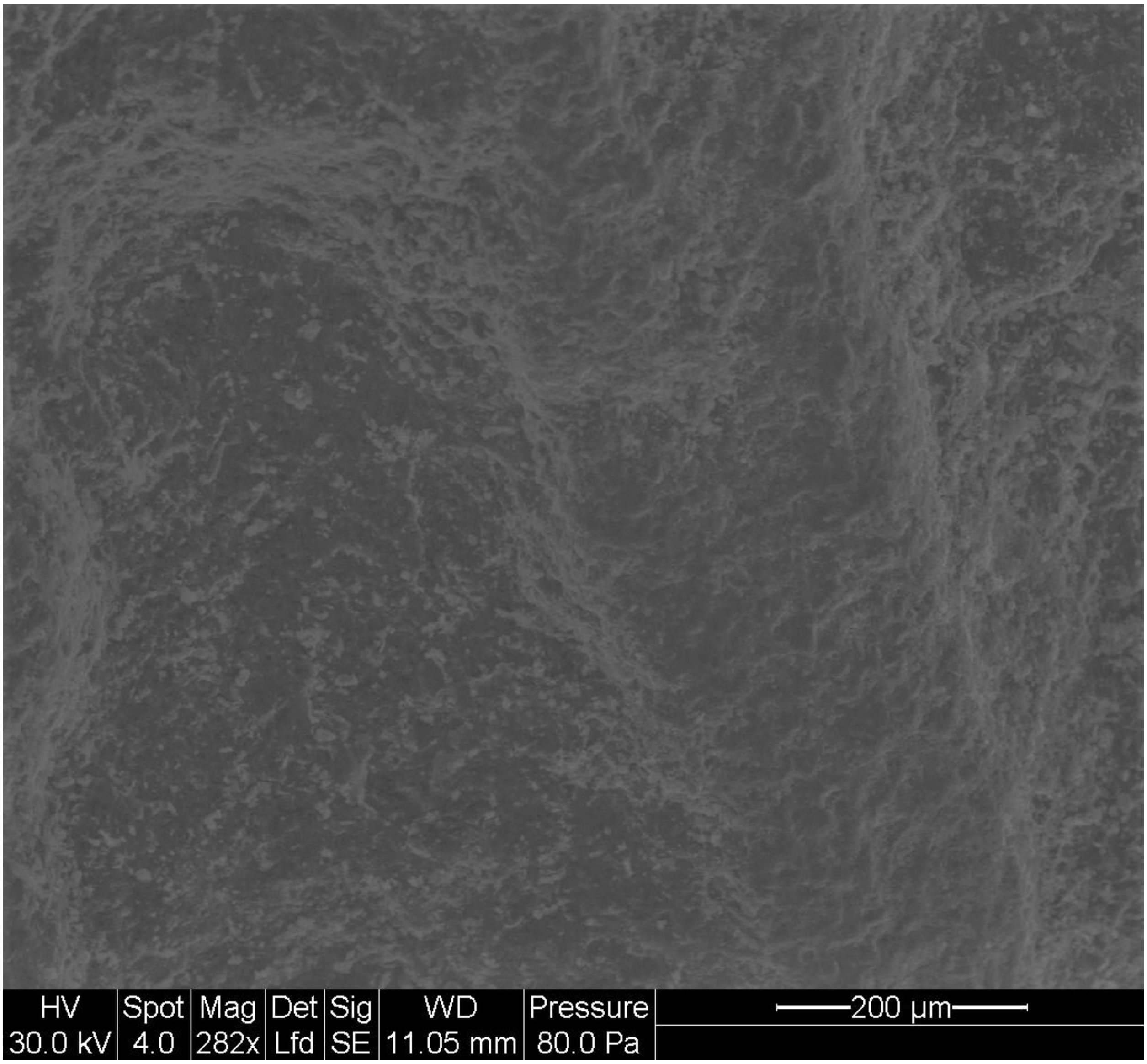

FIGURE 7: Closer view of a portion of the sculptured zone of a pirasocrinid spine. The ridge is the slightly lighter feature to the right of the image and the valley is the slightly darker feature to the left of the image. Note that the ridge is denser and imperforate whereas the valley displays more irregular (non-galleried) pores, defining labyrinthine stereom.

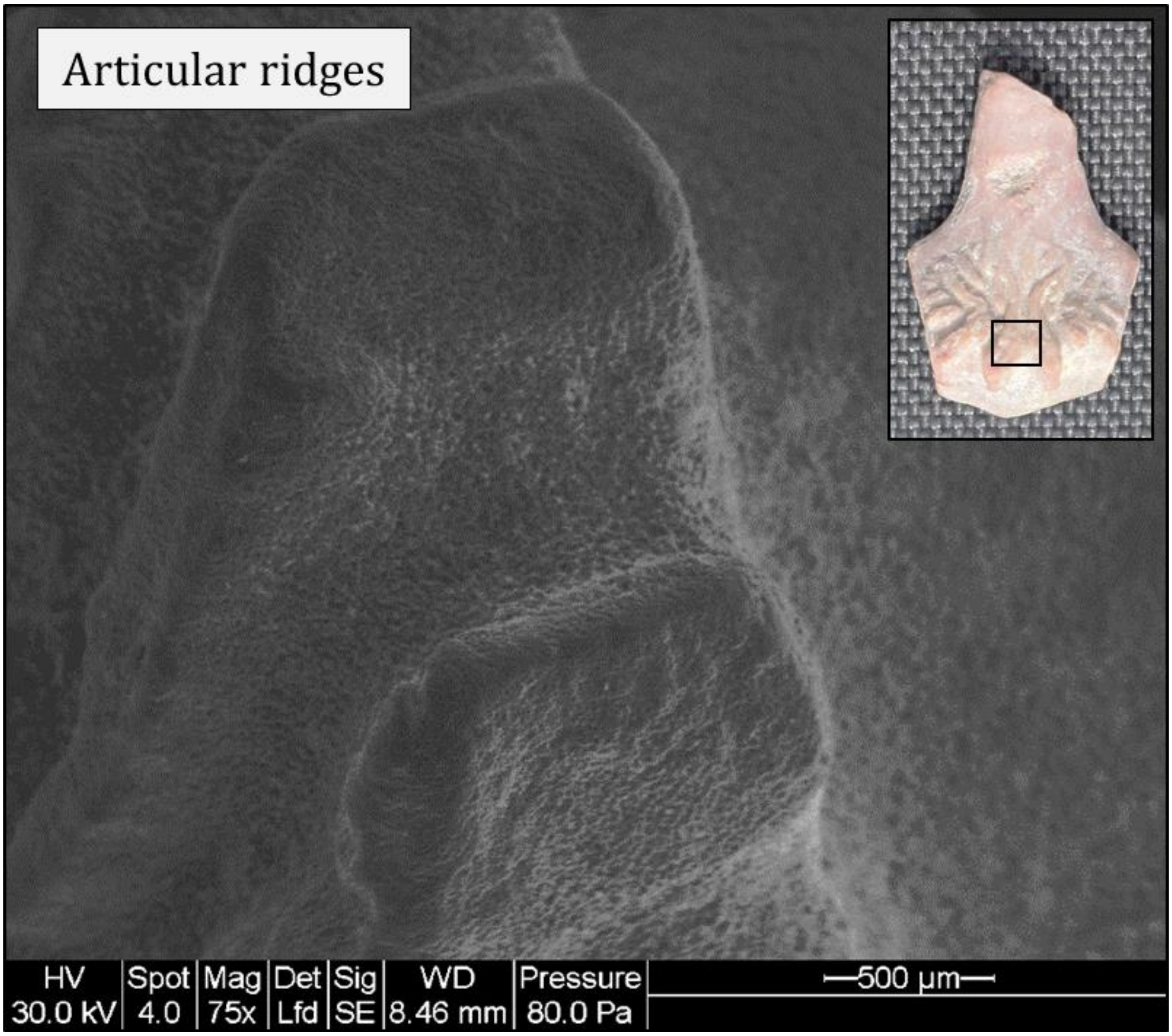

FIGURE 8: Stereom of the articular ridges on the basal portion of pirasocrinid spine bases. The inset picture of a broken spine shows the general position of ridge stereom relative to the shaft and rest of the base. This region consists of parallel ridges of relatively dense stereom that are oriented perpendicular to the long axis of the spine and separated by valley to give the region a crenulated geometry.

9-10) while the adjacent valleys display fine labyrinthine stereom (Figs. 9, 11). This indicates that the parallel ridges serve a comparable role to the meandering ridges of the sculptured zone, in that they were not penetrated by soft tissues. The parallel valleys show evidence of having been interpenetrated by fine ligamentary tissues, joining them to underlying plates (Macurda and Meyer, 1975). The ridges serve to physically interlock with structures in adjacent plates in the same way that crenellae in crinoid columnals engage with other columnals (Ausich et al., 1999). This interpretation is further supported by the presence of finer crenellae on the tip of ridges (Fig. 12), which would have allowed additional physical interconnection between the articular surface of spines and the rest of the tegmen without penetration by connective tissues.

Microstructure of Regeneration Planes

Observation of numerous brachial and anal sac spines resulted in the conclusion that both of these spine types developed stereom structures along planes of regeneration in identical ways. In addition, specimens with multiple planes of regeneration display identical patterns along both planes. For these reasons, stereomic patterns of regeneration planes are treated collectively. Three primary observations related to pirasocrinid spine regeneration are worth description and discussion: (1) development of stereomic pores at regeneration planes that are significantly larger than those observed in other parts of the spine; (2) growth of relatively large, triangular trabeculae along the regeneration plane and extending outward into the regenerating region; and (3) the presence of both of the previously mentioned features at multiple concentric layers at the regeneration plane, so that the spine increases its diameter through growth of more than just the innermost portion of the spine.

The dense, rectilinear stereom of anal sac spines is also present on the non-articular

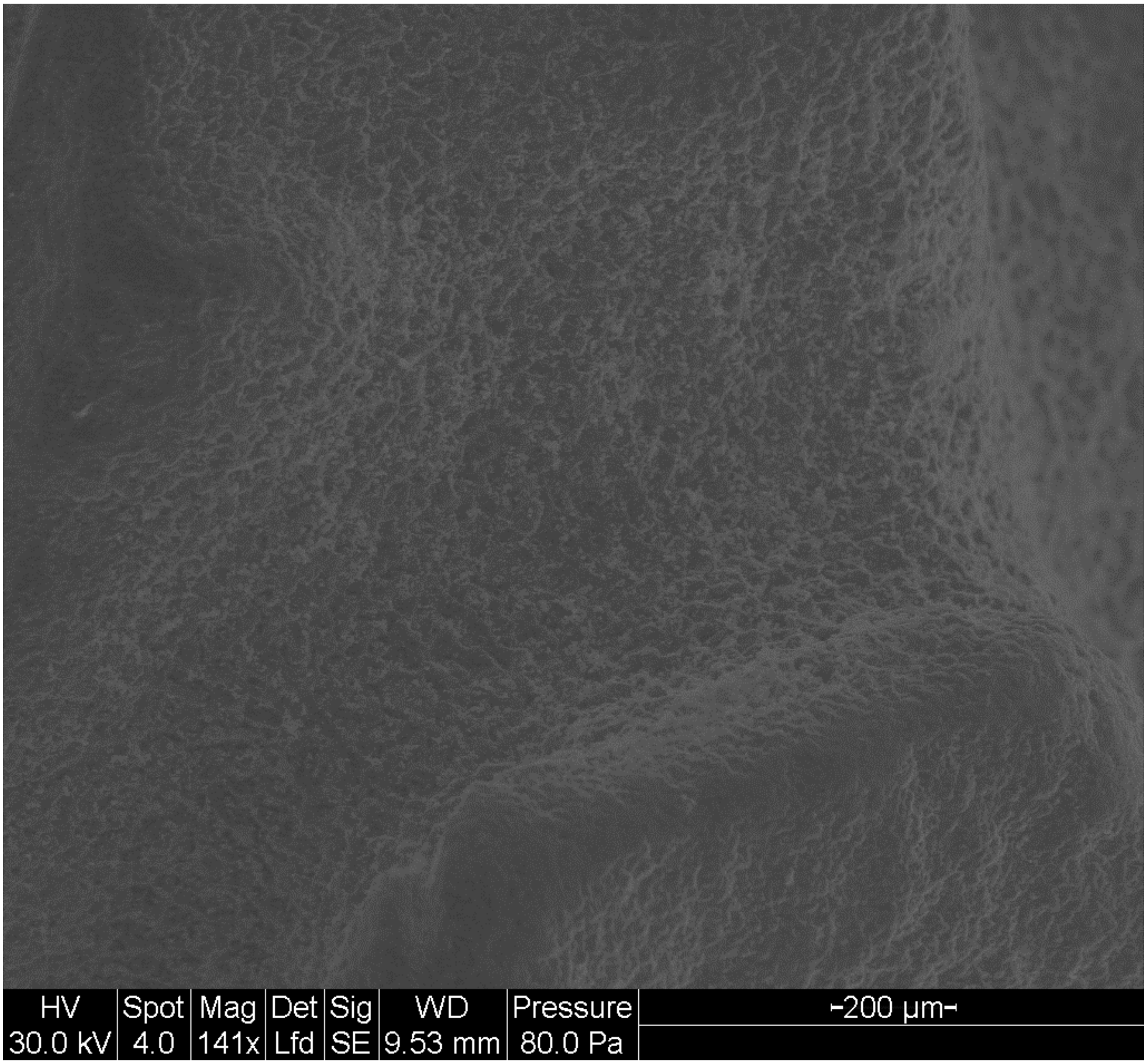

FIGURE 9: Closer view of the articular ridge region of a pirasocrinid anal sac spine, clearly showing the differentiated stereom between the ridges and valleys. The dense, imperforate ridge is visible in the foreground, with the more porous valley in the center of the image.

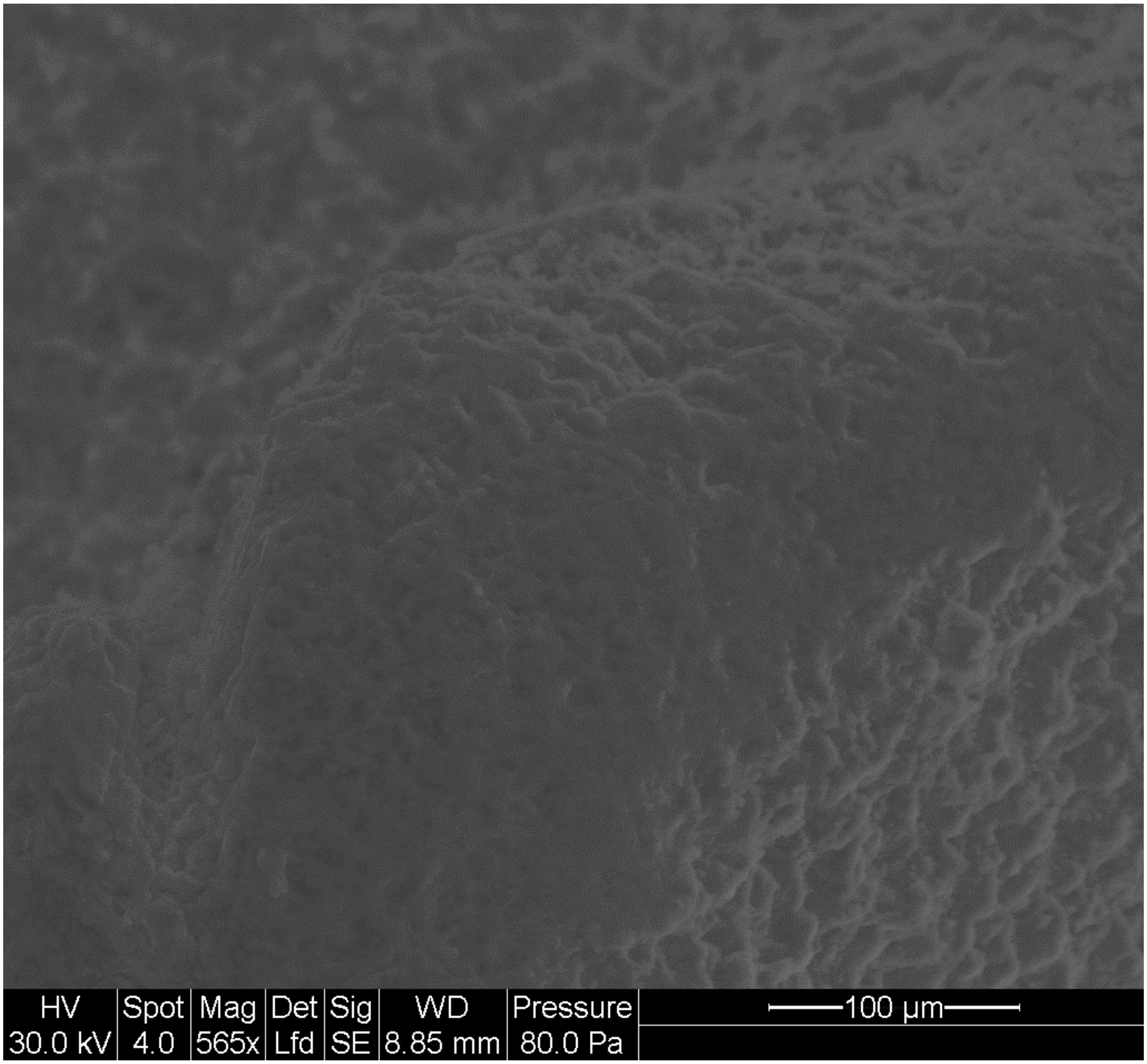

FIGURE 10: Magnified view of the image in Figure 9 showing the density of articular ridges. This indicates that connective tissues did not interpenetrate this portion of the spine base.

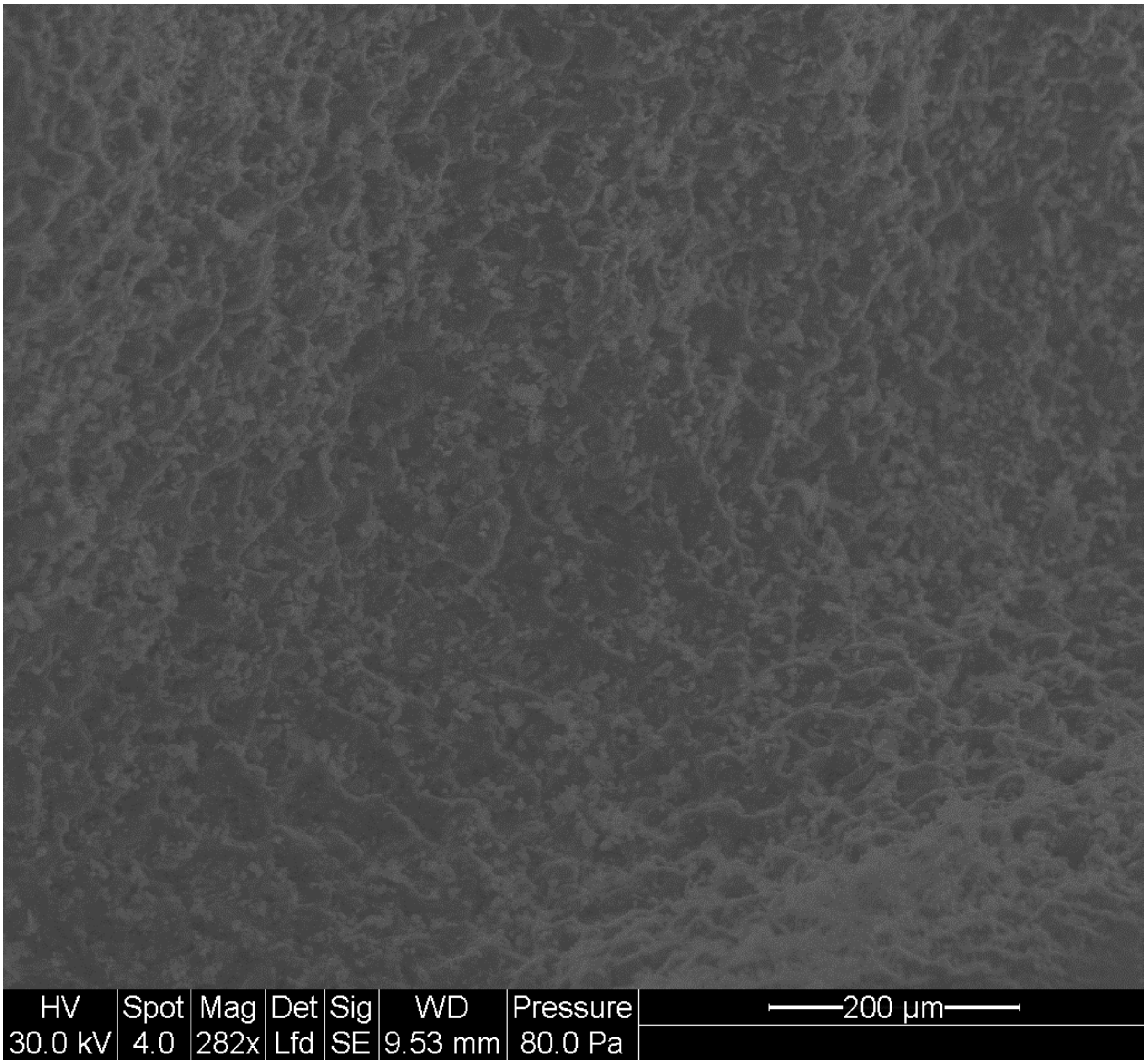

FIGURE 11: Magnified view of the image in Figure 9 showing the fine labyrinthine stereom characteristic of valleys between articular ridges. This stereom pattern suggests that fine ligamentary tissues penetrated this portion of the spine base.

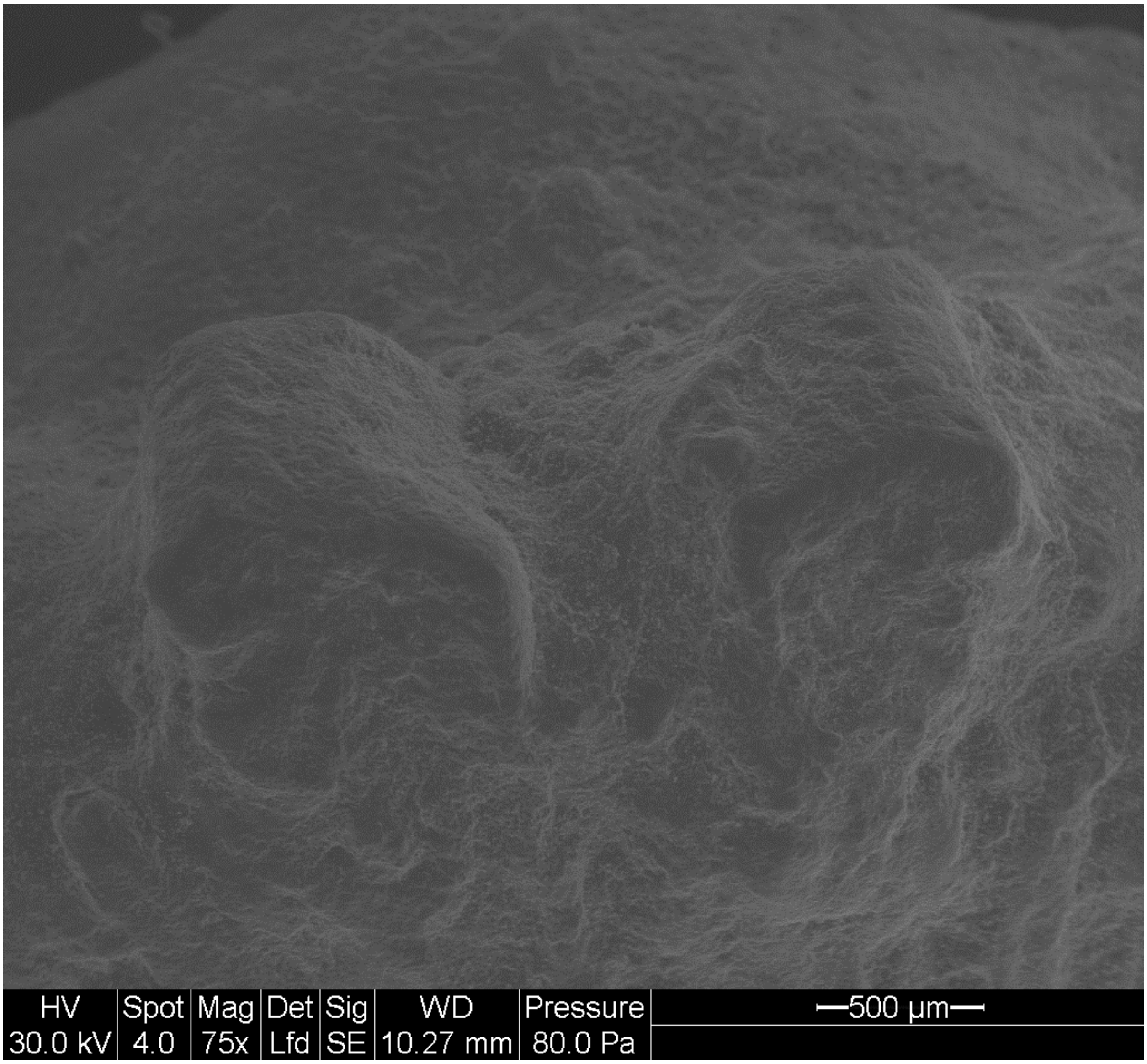

FIGURE 12: Details of articular ridges showing the presence of crenulae on the very top of each ridge. These structures would serve to further physical interlock this portion of the spine with underlying tegmen plates.

(shaft) portion of brachial spines as well (Fig. 13). This means that the size of stereomic pores in all spines is small. Pores are significantly larger in between stereomic rods precipitated right at regeneration planes (Fig. 14 and other figures below) and return to their typical small size in both directions relative to the regeneration plane. This suggests that the increase in pore size is directly related to the process of regeneration, most likely because regeneration of new spine material commenced with precipitation of a few comparatively distantly spaced outgrowths—resulting in larger pores—that each widen or thicken to fill in the pore and develop the dense rectilinear stereom characteristic of fully regenerated or unbroken spines.

The second major observation of regeneration plane stereom is the presence of relatively large, triangular stereomic rods that extend from the regeneration plane out into the regenerating region of the spine (Fig. 14). These coarse triangular structures can be so well developed that the regeneration plane can take on a “pseudocrystalline” texture, with the extending triangular rods having the appearance of euhedral, inorganically formed crystals (Fig. 14). It is these structures that appear to expand in width to close up the atypically large pores at the regeneration plane, as described above. In some instances, these triangular extensions have seemingly grown laterally into adjacent extensions to form larger plate-like structures and, with continued growth, can produce nearly continuous, thin laminae of new spine material (Fig. 15).

The third major observation is directly related to the phenomenon mentioned above, in that the development of thin laminae of new spine material at regeneration planes can be documented at multiple concentric planes along the same regeneration plane (Figs. 15-17). The increase in spine diameter is apparently driven by concurrent growth along several of these extending laminae rather than by growth from the interior of the spine outward, as might be expected. In some specimens, the triangular stereomic rods extending out from a regeneration

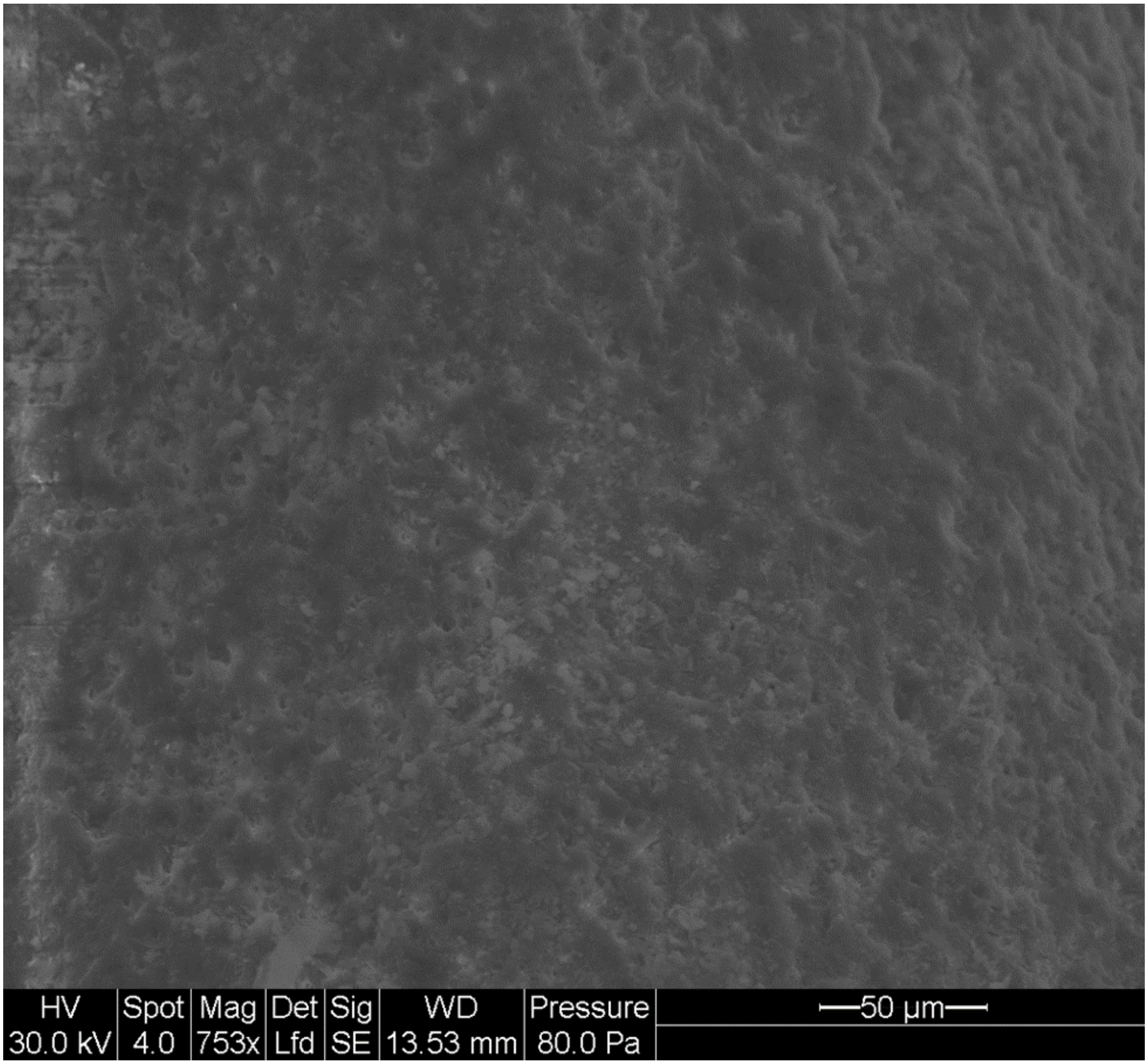

FIGURE 13: Example of weathered rectilinear stereom on a pirasocrinid brachial spine. This shows that the stereom of spines is the same regardless of whether they are anal sac spines or brachial spines.

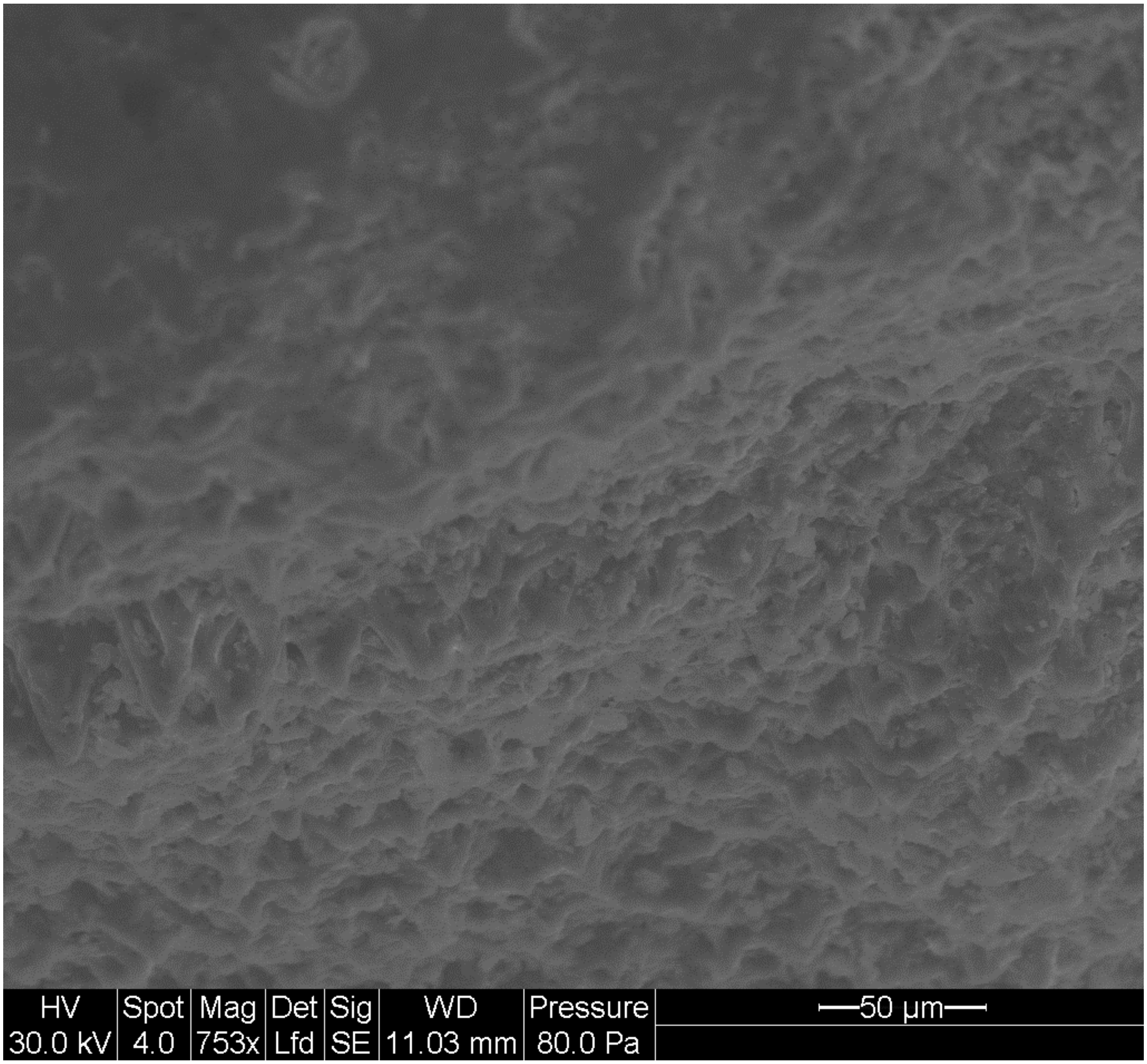

FIGURE 14: A regeneration plane on an anal sac spine with regeneration occurring from the top of the image towards the bottom. Note the relatively large pores between trabeculae (compare with Figs. 5, 13) and the prominent, large, triangular structures extending outward from the regeneration plane.

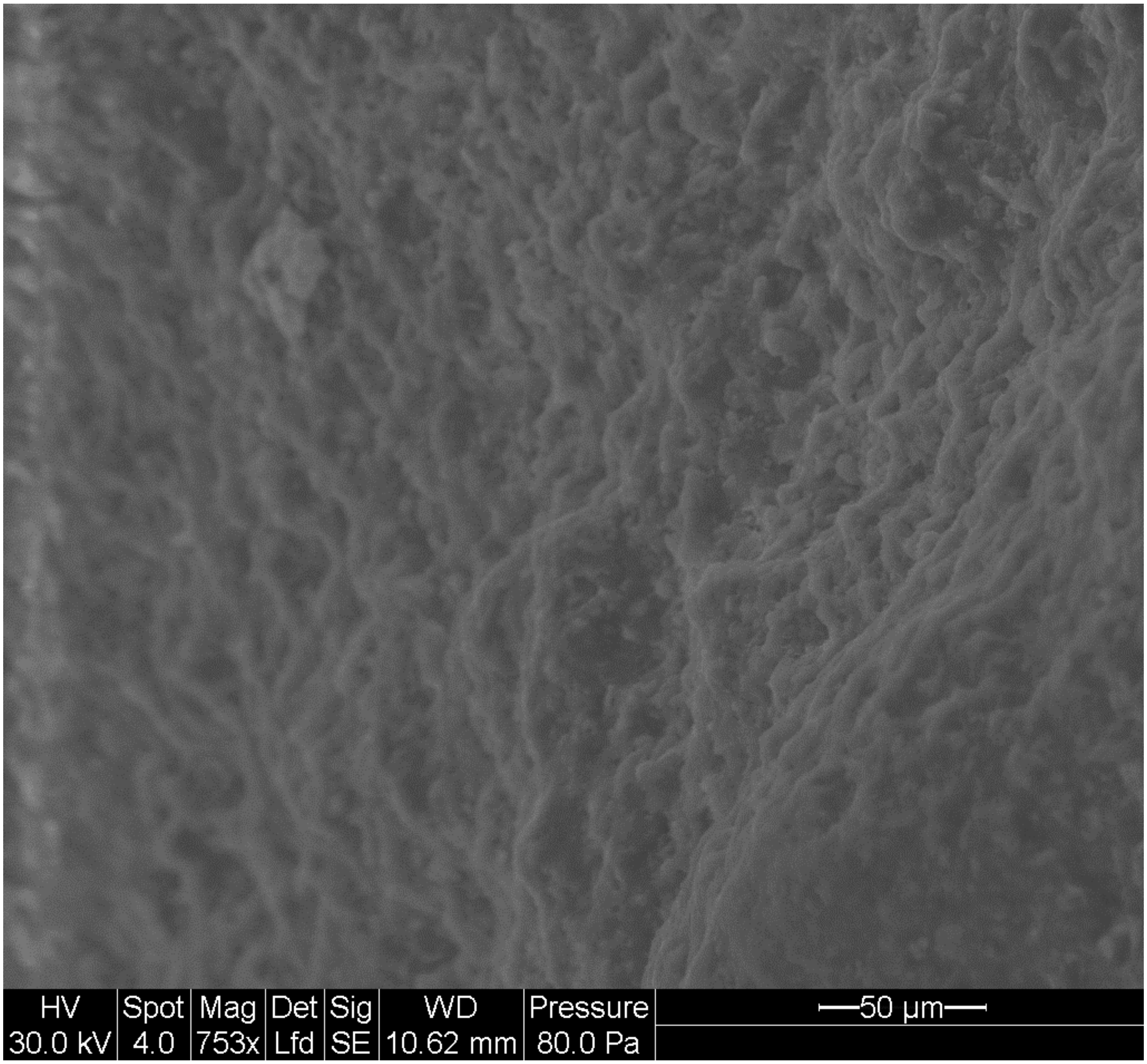

FIGURE 15: Regeneration plane in a brachial spine with regeneration occurring from the lower right to upper left. Note that the triangular extensions have coalesced laterally to form larger, plate-like structures in some places and have further coalesced to form nearly continuous laminae in others. Also note that this process of lateral expansion occurred in multiple layers concurrently, producing vertical “sheaths” of extensions of stereom.

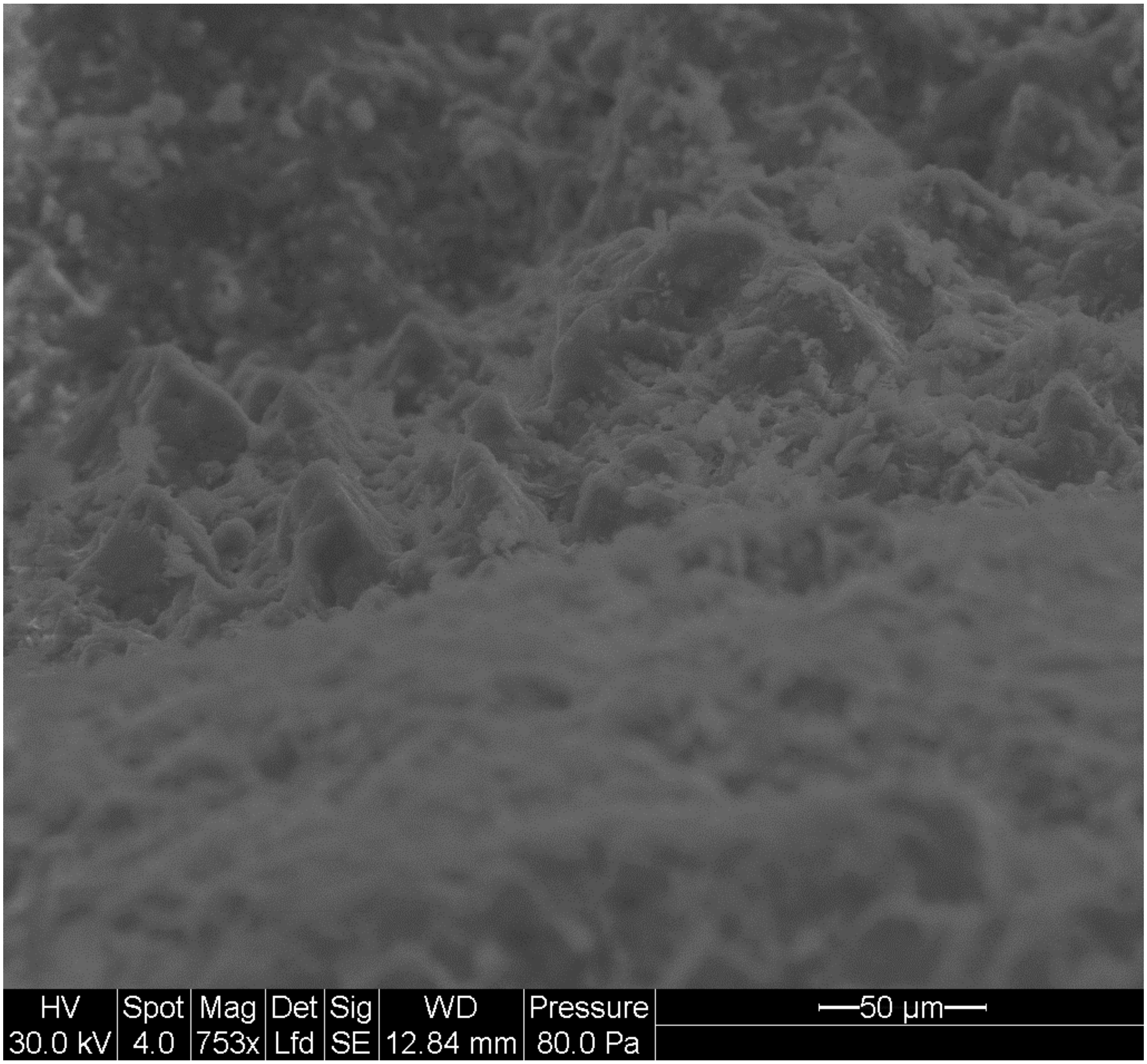

FIGURE 16: Regeneration plane in a brachial spine with regeneration occurring from the bottom of the image to the top. Note the large triangular extensions along the plane of regeneration, as also shown in Figure 14, and the growth of extensions at multiple concentric layers, as also shown in Figure 15. The growth of several parallel triangular extensions produced a "false galleried" texture, with long, continuous pores between the triangular structures.

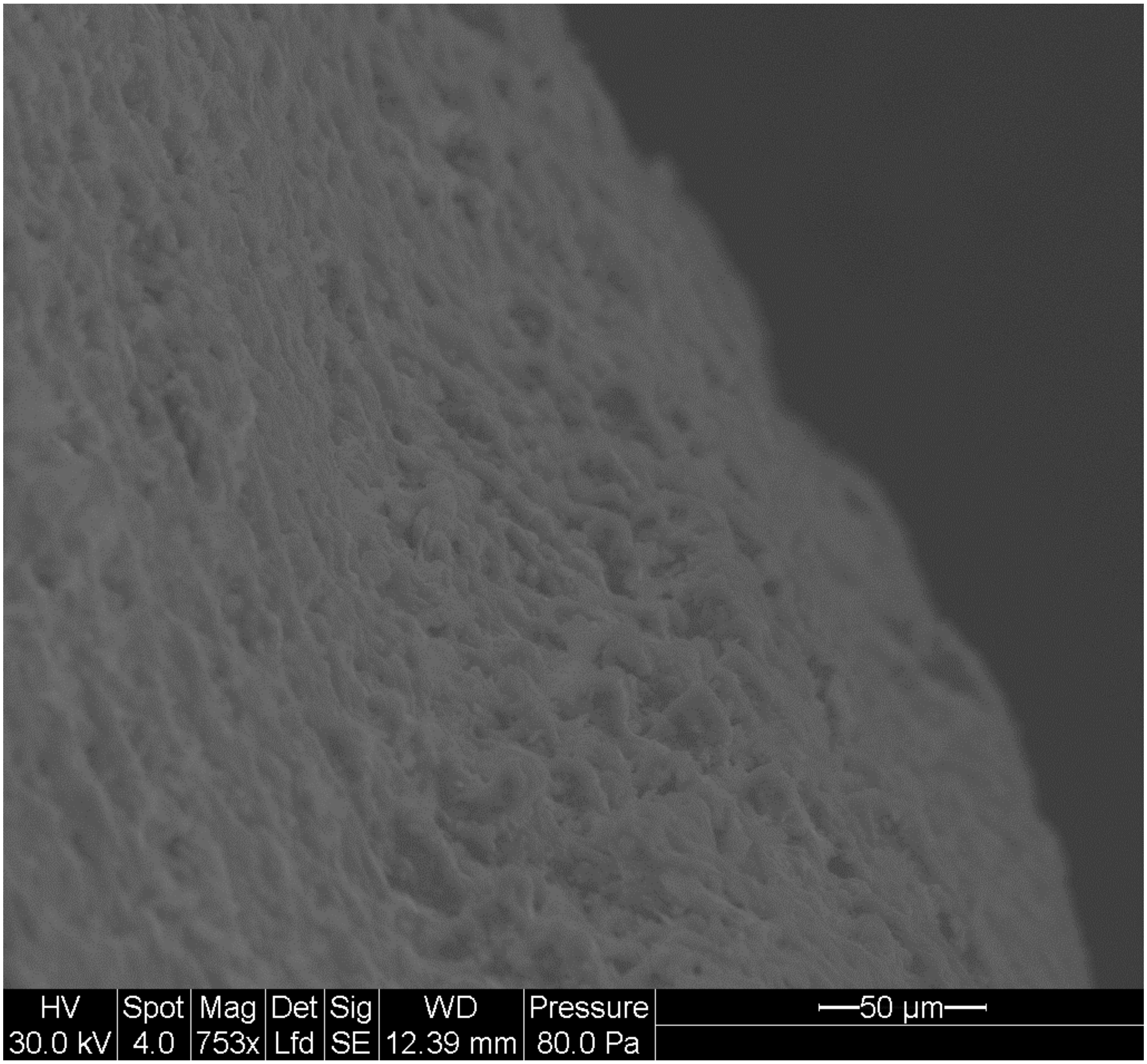

FIGURE 17: Cross-sectional view of a regeneration plane in an anal sac spine showing the concurrent development of multiple concentric laminae of new material. This suggests that spines do not increase their overall diameter by growing one lamina at a time.

plane are aligned, producing a “false galleried” stereom (Fig. 16). True galleried stereom generally reflects the presence of long strands of through-going ligamentary fibers, producing continuous pores (Macurda and Meyer, 1975), but this texture is the result of triangular extensions in adjacent concentric laminae growing concurrently (Fig. 16).

DISCUSSION

Study of pirasocrinid spine stereomic microstructure is important because it provides information on the soft-tissue anatomy of extinct crinoids. It also sheds light on the biological process of regeneration in Paleozoic echinoderms, which cannot be directly studied experimentally. The mechanism interpreted for regenerating spines following breakage, described above, is supported by observations of regions along regeneration planes where nearly complete regeneration had taken place. Regeneration was apparently more rapid in certain localized regions than in surrounding portions of the spine, resulting in lobate “regeneration fronts” (Figs. 18-19). These areas nicely show the coalescence of outward growing trabecular rods into continuous laminae and the occurrence of multiple laminae arranged into concentric sheaths (Fig. 19). Whether other groups of spine-bearing Paleozoic crinoids regenerated in an identical or similar manner remains unknown; future research comparing the results of this study to data gathered on other extinct crinoid spine regeneration patterns is suggested.

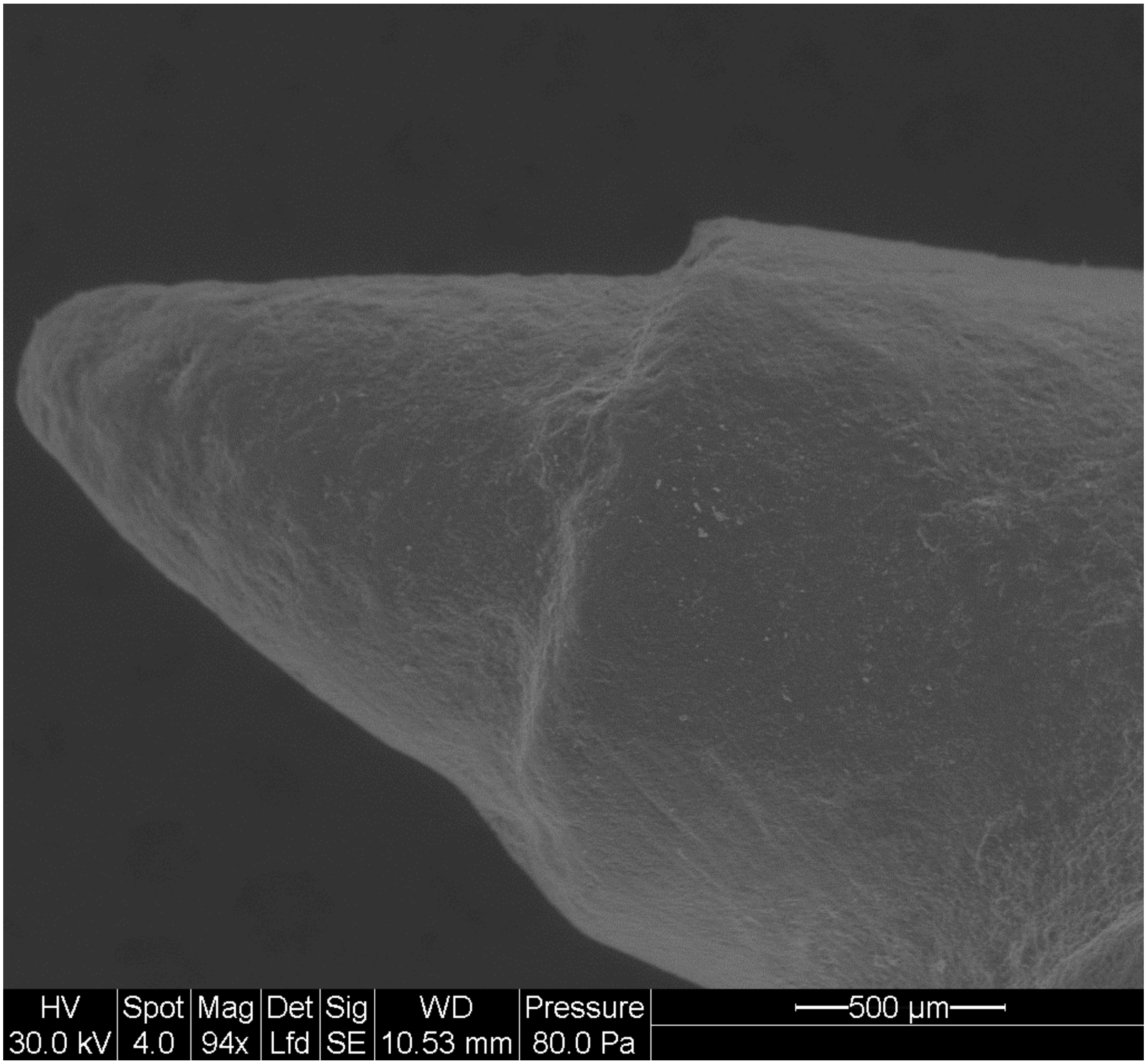

FIGURE 18: Regeneration plane on a brachial spine with a central lobate area where regeneration occurred more rapidly than surrounding areas. This portion of the spine nearly reached the full diameter of the unbroken spine shaft.

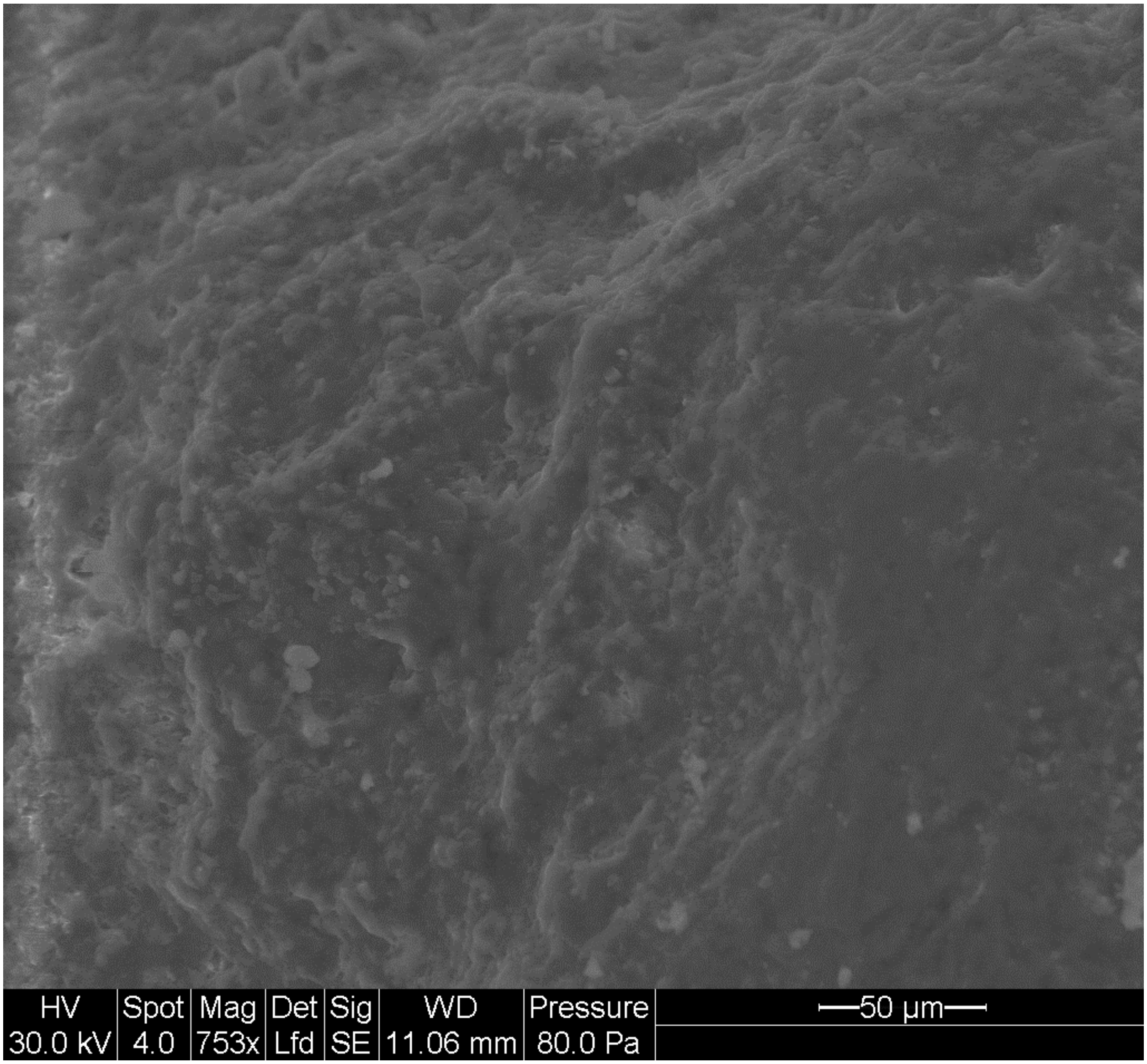

FIGURE 19: Magnified view of the lobate regeneration front shown in Figure 18. Note that this area consists of multiple concentric laminae that were growing concurrently. This is evidently how spines increase their diameter to pre-breakage levels.

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