JOURNAL OF MORPHOLOGY 206:25–43 (1990)

Structure of the Scales of *Dermophis* and *Microcaecilia* (Amphibia: Gymnophiona), and a Comparison to Dermal Ossifications of Other Vertebrates

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ABSTRACT The structures of the dermal scales and the cells surrounding the scales in two species of gymnophione amphibians were studied using histochemistry and light, scanning and transmission electron microscopy. Scales are composed of a basal plate of several layers of unmineralized collagenous fibers topped with mineralized squamulae. Squamulae are composed of numerous mineralized globules and mineralized, thick collagen fibers. Mineralization is therefore both spheritic and inotropic. Isolated flattened cells lie on the outer surface of the squamulae and seem to be involved in mineral deposition. Cells that line the basal plate synthesize the collagenous stroma of the plate. Each scale lies in a thin connective tissue pocket, and a large connective tissue pouch includes several scales in each annulus.

The similarities of gymnophione scales to elasmoid scales of osteichthyans are largely superficial. Aspects of mineralization and of pocket development differ considerably. There are also similarities, as well as differences, in the gymnophione scales and osteoderms of amphibians and of reptiles. We consider that such dermal structures have arisen many times in diverse lineages of vertebrates, and that these are expressions of properties of dermal collagen to support mineralization by specialized dermal cells. However, we recommend that the term "dermal scale" be used for the mineralized dermal units of osteichthyans and gymnophiones, and "osteoderm" for the dermal structures of frogs and squamates, with the understanding that the terminology recognizes certain *convergent* attributes of shape and structure, but not of process.

The presence of dermal scales that are composed of an unmineralized base plate of collagenous fibers and a superficial layer of mineralized squamulae is unique to members of the amphibian Order Gymnophiona among all living tetrapods. Because gymnophione scales are structurally similar to those of teleost fishes, they have been assumed to be homologous (Zylberberg et al. '80; Ruibal and Shoemaker, '84). However, recent examination of new data on gymnophione scales, together with an assessment of the morphology of fish scales, the dermal ossifications of frogs, and the osteoderms of lizards, causes us to question the assumption of homology of dermal scales. This analysis indicates that *all* dermal ossifications share a number of features that are structural properties of the particular organization of the dermis, and that the similarities *and differences* in scale and osteoderm structure found in various lineages constitute evidence for convergent evolution, in response to the structural constraints of the tissue involved.

There have been several descriptions of the scales of gymnophione taxa in the last 150 years. Mayer (1829a,b), Rathke (1852), and Leydig (1853) commented briefly on scale morphology. Sarasin and Sarasin (1887–90) described the scales of *Ichthyophis glutinosus* from Sri Lanka, and several subsequent analyses have corroborated their conclusions about the relationship of the scales to other dermal and to epidermal structures. Cockerell ('11, '12), Phisalix ('10, '12), Datz ('23), Ochotorena ('32), Marcus ('34), Gabe ('71a,b), Casey and Lawson ('79), Zylberberg et al. ('80), Perret ('82), and Fox ('83) described the scales of diverse species, usually *Ichthyophis* or *Hypogeophis,* at various levels of resolution. Taylor ('72) assembled an "atlas of scales" composed of photographs and descriptions of single scales from virtually all of the species of gymno-

phiones. He indicated that features such as scale size, shape, numbers and distribution, and pattern of mineralization might be useful for systematic analysis. However, Wake and Nygren ('87) demonstrated marked individual, ontogenetic, and sexual variation in nearly all scale parameters for *Dermophis mexicanus* from a single population, and suggested that scales are of little use as taxonomic characters to diagnose gymnophione species. Although Feuer ('62) proposed that scales might provide information about ages of individuals, Zylberberg et al. ('80) presented evidence to the contrary.

The greatest numbers of scales occur in taxa of gymnophiones considered primitive based on other characters (osteology, myology, reproductive biology; *Ichthyophis, Caudacaecilia, Rhinatrema, Epicrionops*—see Taylor, '68, '72; Nussbaum and Wilkinson, '89). Scales are embedded in virtually all of the annuli (i.e., body rings) of primitive species, and these taxa have several annuli per body segment. In gymnophiones, there is a trend toward the reduction of numbers of annuli and, concomitantly, of scales. Taxa considered highly derived based on other characters (e.g., typhlonectids and scolecomorphids) characteristically lack secondary annuli and scales (though Wake, ['75] and Moodie, ['78] found scales in some *Typhlonectes*).

However, the scales of few taxa have been examined in detail with comparison to outgroups in mind. We present new data on the morphology of scales of *Dermophis mexicanus* and *Microcaecilia unicolor* (both Gymnophiona: Caeciliaidae [*fide* Nussbaum and Wilkinson, '89]) to augment the detailed data available for *Ichthyophis kohtaoensis* (Gabe, '71a,b; Zylberberg et al., '80; Fox, '83) and *Hypogeophis rostratus* (Casey and Lawson, '79; Zylberberg et al., '80). The morphology of the scales of *D. mexicanus* and *M. unicolor* is compared with that of those scales described previously, and with that of dermal ossifications in other vertebrates. Our comparative morphological approach elucidates the similarities and differences in mineralization patterns of such dermal derivatives as scales and osteoderms and allows assessment of homology versus convergence of teleost scales, caecilian scales, and anuran and reptilian osteoderms. The study also provides a basis for continued research into the questions of development and homologies of dermal structures in vertebrates.

MATERIALS AND METHODS

Scales of *Dermophis mexicanus* from San Marcos Prov., Guatemala, and Chiapas, Mexico, and *Microcaecilia unicolor* from Guyana were processed for analysis by several techniques. The specimens of *Dermophis mexicanus* from which scales were taken are deposited in the collections of the Museum of Vertebrate Zoology, University of California, Berkeley, and the *M. unicolor* in the Museum National d'Histoire naturelle, Paris. Scales were stripped from the annuli of several formalin-fixed, alcohol-preserved specimens of different sizes (and presumably ages) and sexes; all scales per annulus were stained with alizarin red-S (see Wake and Nygren, '87, for details). Quadrants of skin including several annuli were fixed in neutral buffered formalin or in Bouin's mixture, embedded in paraffin, and sectioned sagittally or frontally. Stains and histochemical reactions are summarized in Tables 1 and 2. Scales were prepared for scanning electron microscopy by excising, mounting on stubs, critical-point drying, and sputter-coating with gold or gold–palladium alloy. Scales were examined and photographed with an ISI-DS-130 dual-stage scanning electron microscope or with a JEOL 8S M 35 SEM at an operating voltage of 25 kV.

For transmission electron microscopy, small pieces of skin were removed from several regions of the body, fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 or 3 hr at room temperature; specimens were washed in 0.1 M cacodylate buffer containing 10% sucrose, and postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer. Some samples were decalcified in the fixative with 0.1 M ethylene-diamino-tetraacetic acid (EDTA) added for 1 or 2 days at 4°C, and then washed and postfixed. Ruthenium red (Martino et al., '79) was used as an electron microscopic stain for extracellular polyanions (Luft, '71a,b) such as acid mucosubstances and acid phospholipids. Ruthenium red was added to the fixative containing EDTA, the wash buffer, and the osmium tetroxide solution. All the fixed samples were dehydrated in ethanol and cleared in 1–2 epoxy–propane. They were left in a 1:1 epoxy–propane/Epon mixture overnight or longer, and then transferred to fresh resin. Polymerization was carried out at 60°C. Thick sections (~1 μm) were stained with toluidin blue for light microscopy. Thin sections of selected areas were cut with a Reichert ultramicrotome using a diamond knife with the tissue block oriented obliquely to the knife to improve sectioning of hard fibrous tissues (Allizard and Zylberberg, '82). Sections were mounted on collodion-coated grids and stained with uranyl acetate and lead

TABLE 1. Histological stains employed for analysis of scales

Histological stain		Reference
Azan		Heidenhain in Gabe ('68)
Mallory's azan		Humason ('79)
Hematoxylin & eosin		Humason ('79)
One-step trichrome		Gabe and Martoja in Gabe ('68)
Histochemical reactions	**Specificity**	**Reference**
Proteins		
Danielli's coupled tetrazolium reaction	General reaction	Gabe ('68)
Ferric ferricyanide reaction	Reducing groups	Adams in Gabe ('68)
Carbohydrates		
Periodic acid-Schiff (PAS)	Neutral mucosubstances	MacManus in Gabe ('68)
Alcian blue pH 2.5 (AB 2.5)	Acid mucosubstances	Mowry in Gabe ('68)
Alcian blue pH 0.5 (AB 0.5)	Sulfated mucosubstances	Mowry in Gabe ('68)
AB 2.5 + PAS	Neutral and acid mucosubstances	Mowry in Gabe ('68)
AB 0.5 + PAS	Neutral and sulfated mucosubstances	Mowry in Gabe ('68)
Toluidine blue pH 4.2	Neutral vs. acid (carboxyl-rich and sulfated mucosubstances)	Lison in Gabe ('68)
Paraldehyde–fuchsin–Alcian blue pH 2.5	Sulfated mucosubstances	Gabe ('68)
Danielli's reaction + AB 2.5	Protein and mucosubstances	Lillie and Turner in Gabe ('68)
Calcium		
Alizarin red-S	Calcium	Wake and Nygren ('87)

TABLE 2. Histological and histochemical characteristics of scales of Dermophis mexicanus and Microcaecilia unicolor

	Scale			
	Basal plate	Squamulae		
Reaction		Inner	Outer	Dermis
Azan	Red	Blue	Blue	Blue
Mallory's azan	Blue	Red	Red	Blue
Hematoxylin & eosin	Pink	Purple	Purple	Pink
One-step trichrome	Red	Green	Green	Green
Picro-ponceau	Yellow-pink	Dark yellow	Red	Pink
Danielli's reaction	++	+++	+−	++
Ferric ferricyanide	−	−	−	−
PAS	+−	+++	++	+
AB 2.5	−	++	++	−
AB 0.5	−	+	+++	−
Toluidine blue	Blue	Blue or purplish-blue	Purplish-blue	Blue
PAS + AB 2.5	Red	Purplish-blue	Blue	Red
PAS + AB 0.5	Red	Purplish-red	Purplish-blue	Red
Paraldehyde–fuchsin + PAS	Red	Reddish-purple	Purple	Red
Danielli's reaction + AB 2.5	Purple	Purple and Blue	Blue	Purple
Alizarin red-S	—	Red	Red	—

citrate (Reynolds, '63). The grids were examined in a Philips EM 300 electron microscope at an operating voltage of 80 kV with a cooled anticontamination device.

RESULTS

General organization of the scales

The most anterior scales in *Dermophis mexicanus* are located in the tenth to twentieth primary annulus (counting posteriorly from the head); their sizes and numbers increase posteriorly in both primary and secondary annuli to mid-body, from which point numbers are relatively constant, although sizes within each annulus show much variation, until they diminish in the most posterior annuli (see Wake and Nygren, '87, for data). In both taxa examined, the scale rows are covered by a thin layer of epidermis (Figs. 1a, 2), hence are not exposed to the environment. Scales are somewhat irregular in size and shape, and a diversity of scale sizes and shapes is found in each scale row (Wake and Nygren, '87). Excised scales treated with alizarin

red-S have red-stained structures atop an unstained base plate, indicating specificity for calcium in the mineralized squamulae; the latter also have been called denticles. We prefer the more precise term squamulae, to avoid possible confusion of these structures with the "denticles" of sharks, for example.

In parasagittal sections of skin with several annuli, the scale pouches are embedded in each annulus and form a ring that partly or completely encircles the body. Characteristically, the distal end of the scale pouch lies in the dermis below the epidermis (Figs. 1a, 2, 3). The distal margin of the pouch may be deep to small dermal mucous glands; the proximal end of the pouch is broader and located at the boundary between the superficial and the deep dense dermis that overlies the body wall musculature (Figs. 1a, 2). Each scale pouch usually is associated with large mixed granular (so-called "poison") glands both dorsally and ventrally. The pouch is a thin, connective tissue structure (Figs. 2, 3). Each scale lies in its own pocket or sac within the pouch (Figs. 2, 4–6). Fibroblasts line the inner surface of each pocket (Figs. 7, 8), and, within each pocket, the fibroblasts (scleroblasts) that form the scale are arranged in layers surrounding each scale. The scleroblasts constitute an uninterrupted layer on the basal surface of the scale. At the scale margin, the collagenous stroma of the scale aligns with the connective tissue of the scale pocket (Figs. 1b, 25). Numerous scales, contained in their pockets, overlap within a pouch (Fig. 1, 2). All scales lie similarly in the pouch with the denticulate surface facing outward. Some large scales are folded back on themselves in the scale pockets (Fig. 4). Scale structure varies with size of scale and with region on the scale (central vs. peripheral). Large scales have a thick base plate composed of as many as seven layers, or plies, of fibers. The orientation of the fibers alternates regularly (Figs. 1c, 5, 13). Mineralized squamulae lie atop, and slightly embedded in, the upper layer of the base plate (Figs. 1c, 3, 13). Each squamula is a discrete structure (Figs. 3, 13).

The squamulae

At low magnification, central squamulae on the base plate appear to be square to round shaped, whereas more peripheral squamulae are more elongate. Squamulae are arrayed in irregularly concentric circles (Figs. 9, 10) and are highly irregular in shape. The longitudinal sectional profiles of the squamulae vary; they are relatively flat but bear points and ridges (Figs. 2–5). Squamulae are flattest centrally; points and ridges of more peripheral squamulae are higher (Figs. 9, 10). At moderate magnification (~× 1,000), they show numerous ridges and folds (Figs. 11, 12). At higher magnification (Fig. 13), it is clear that each squamula is an array of round, mineralized deposits of varying heights. Some of the deposits are fused superficially or linearly; however, the bases of the mineralized structures rarely are fused.

The points and ridges that form the uppermost surface of each squamula include a loose framework composed of acid mucosubstances that stain with Alcian blue (Fig. 14), whereas abundant neutral mucosubstances and proteins are located more centrally within the squamula (Figs. 14, 15; Table 2). The uppermost surface of a squamula is composed of numerous mineralized globules that appear as isolated structures, particularly at the periphery of the squamula (Figs. 7, 24, 25), or that aggregate to form large concretions in the central part of the squamula (Figs. 13, 16). The mineral deposit is composed of crystals that have a radial arrangement within the globules (Figs. 18, 22). In demineralized sections, organic matrix cannot be distinguished in many globules. They appear as electron-lucent circular spaces (Fig. 17). In other globules, a thin fibrillar network forms a central core surrounded by an electron-lucent space (Fig. 19). An electron-dense opaque material that displays high contrast with ruthenium red lines the surface of the globules (Fig. 19). The central part of the squamula contains both mineralized globules and mineralized, thick collagen fibrils that arise from the basal plate (Figs. 16, 23). The crystals are oriented along the collagen fibrils, and invade the fibrils somewhat. At the inner surface of the squamulae, mineralized globules are inserted in the network formed by the collagenous stroma (Fig. 23).

Isolated, flattened cells are located on the outer surface of the squamulae (Fig. 13). They contain a voluminous central nucleus, and are more numerous in young animals than in older ones. In young animals, the cells have long cytoplasmic processes that cover the surfaces of the squamulae, and the processes are near the outer mineralized globules (Fig. 13). A fuzzy organic material is located near the plasma membrane facing the squamula where the first crystal deposits appear (Fig. 20), suggesting that these cells are involved in the production of the mineralized globules. The crystals first deposited in these globules have no apparent orientation (Fig. 20). The radial organization of the crystals becomes apparent in globules still in the vicinity of the sclero-

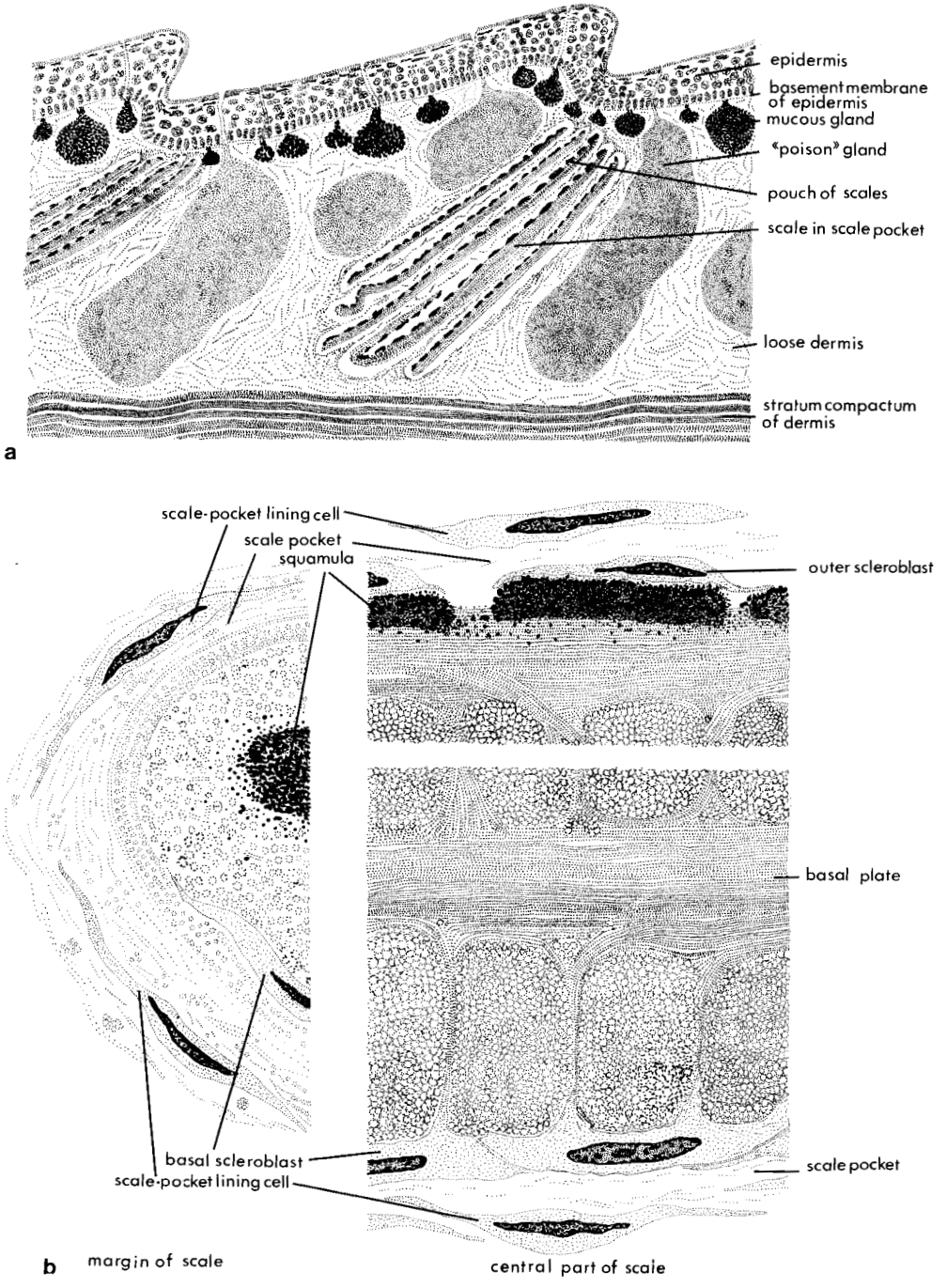
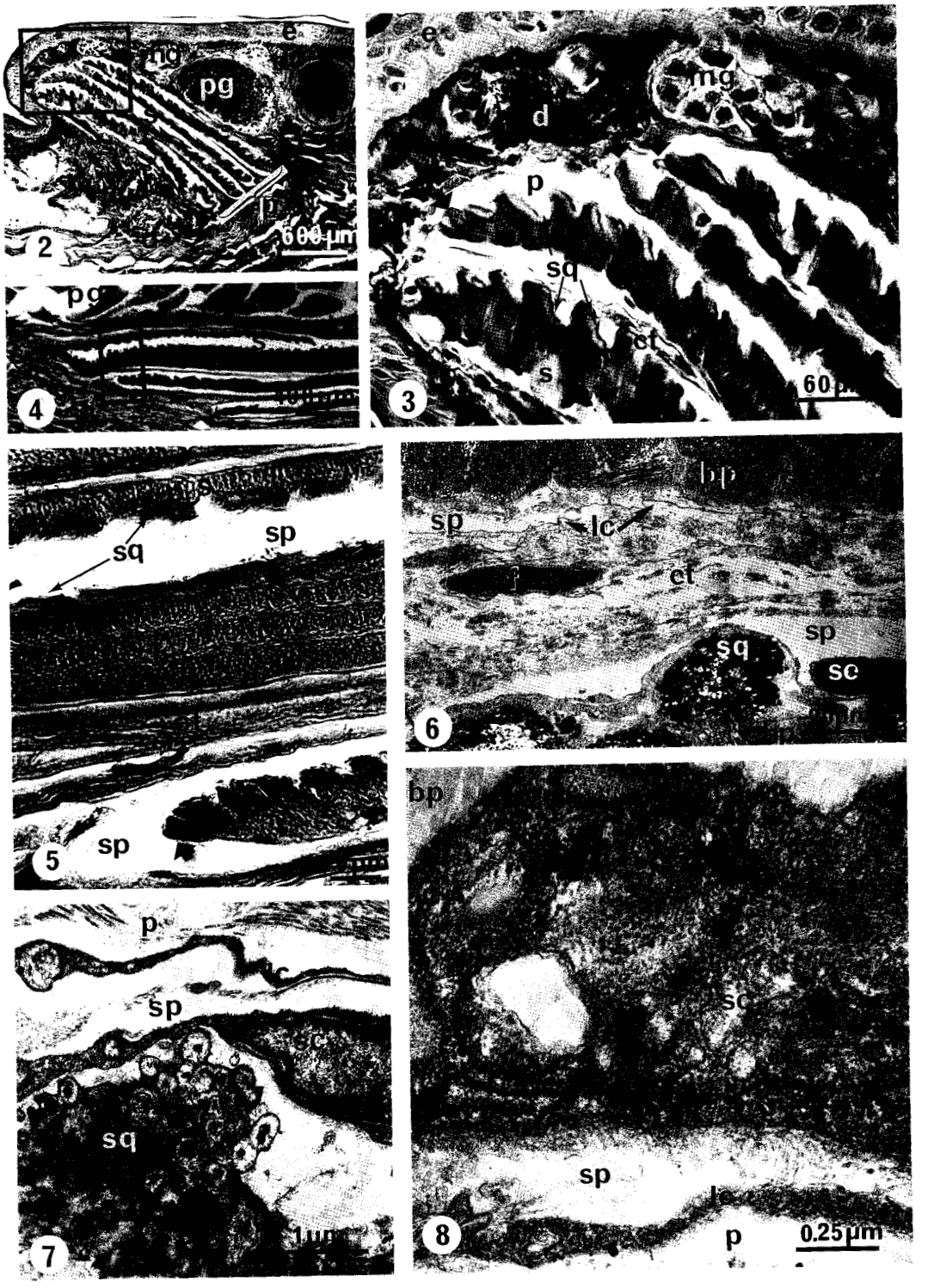


Fig. 1. Diagrammatic representation of a caecilian scale. **a:** Scales in scale pocket relative to "poison" glands, mucous glands, and annuli. **b:** Margin and central part of scale, showing cells lining scale and the arrangement of the basal plate and the squamulae.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Figures 2–8

blast (Fig. 21). The globules subsequently aggregate to form larger concretions.

The basal plate

The basal plate is the most extensive part of the scale, and is made up of superimposed plies of collagen fibrils. A diameter of ~100 nm is achieved by most of the newly synthesized collagen fibrils that lie in the vicinity of the plasma membrane (Figs. 28, 29, 30). The collagen fibrils of the basal plate are distinctly thicker than those of the dermis (i.e., 100 vs. 30-nm dia; Figs. 35, 36). In each ply, the collagen fibrils are packed in thick bundles, oriented in parallel (Figs. 13, 26), and often, the periodic structure of groups of fibrils is in register (Fig. 34). The direction of the fibrils varies from one ply to another; the angle of rotation between two adjacent plies is about 90°. Thus, the plies constitute an orthogonal plywood-like structure. The collagen fibrils of the basal plate mineralize only within the squamulae.

The collagenous stroma of the basal plate is synthesized by the cells that line the basal surface of the scale. These flattened cells have a voluminous central nucleus and form a continuous sheet that is considered a pseudoepithelium composed of scleroblasts (Figs. 26, 27). The rough endoplasmic reticulum (RER) is composed of short saccules. Mitochondria are not abundant and the Golgi areas are not well developed (Fig. 13). Microfilaments are abundant; in some cells, they appear to be aligned with microtubules and with the newly synthesized collagen fibrils (Figs. 28, 29), whereas in other cells such alignment is not observed (Fig. 30). The scleroblasts have long cytoplasmic processes that insert among the collagen fibrils, thereby separating the fibrils into distinct bundles (Figs. 13, 27). Collagen fibrils arise from these processes perpendicularly to the collagenous plies (Fig. 13). When a new ply is formed, the orientation of the first collagen fibrils synthesized is at a right angle to that of the preceding ply (Fig. 31); then an isolated bundle is formed (Fig. 32), and finally the whole newly synthesized ply is formed by collagen fibrils aligned in the new direction (Fig. 33).

DISCUSSION

Comparative morphology of dermal ossifications

The gymnophione condition

The presence of dermal scales is unique to gymnophiones among extant tetrapods. Various workers (Taylor, '72; Zylberberg et al., '80; Perret, '82) have described the variation in scale morphology (e.g., size, shape, depth of base plate, distribution and shape of squamulae) among caecilian taxa. Certain features of scalation, such as overall distribution and presence or absence, have been used as reliable systematic characters.

We describe microscopic features of scale morphology that might be taxonomically useful. Among adults, the shapes of the squamulae on the scales and the pattern of deposition of mineralized material seem to be fairly consistent. Such features are best examined by scanning electron microscopy, so that details of structure can be assessed; large samples of scales have yet to be evaluated for any single taxon. A comparison of the scanning electron micrographs of *Hypogeophis* (Casey and Lawson, '79), *Ichthyophis* and *Hypogeophis* (Zylberberg et al., '80), *Geotrypetes* and *Herpele* (Perret, '82), *Dermophis* (Wake and Nygren, '87), and those in Taylor's atlas ('72) reveals considerable variation in squa-

Fig. 2. Longitudinal section perpendicular to the surface of the skin at the level of an annulus in *Dermophis mexicanus* (light micrograph [LM]). The pouch (p) contains four overlapping scales (s) and lies below the epidermis (e). The scale pouch and the glands (mg = mucous gland, pg = "poison" gland) lie within the loose dermis (d) and do not penetrate the dense dermis.

Fig. 3. Enlargement of outlined area of Figure 2 showing the outer part of the pouch (p) with the four scales (s). The mucous glands (mg) are located in the dermis (d) between the epidermis (e) and the scale pouch. A thin layer of connective tissue (ct) separates the scales in their pocket. The squamulae (sq) lie on the dorsal surface of the scales.

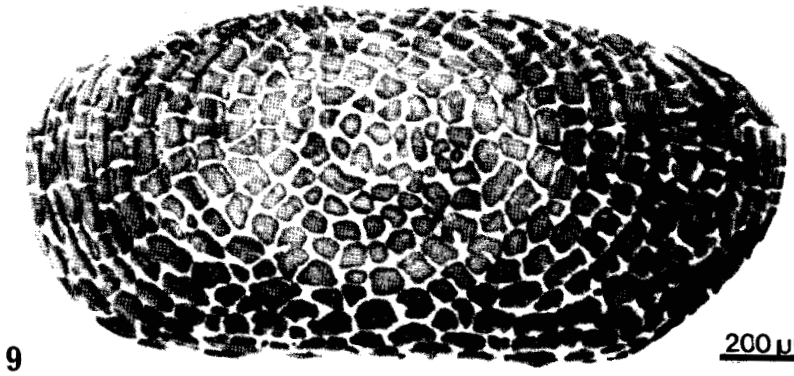
Fig. 4. Longitudinal section (LM) of the inner part of a pouch containing a folded scale (s) in *Dermophis mexicanus*. pg = poison gland.

Fig. 5. Enlargement of outlined area in Figure 4. The two scales (s) are inserted in their own pocket (sp), surrounded by connective tissue (ct). The thick central part of the scale has six plies, whereas the thin, folded part has only one ply. The squamulae (sq) top the basal plate. The margin of the scale (solid arrow) is not lined by scleroblasts.

Fig. 6. The thin layer of connective tissue (ct) between two scales separates the pockets (sp); transmission electron micrograph (TEM); longitudinal section (LS). Fibroblasts (lc) line the pocket. The scale is surrounded by the scleroblasts (sc). *Dermophis mexicanus*. bp = basal plate; sq = squamula.

Fig. 7. Detail of the dorsal part of a pocket lined by a fibroblast (lc) in *Microcaecilia unicolor* (TEM; LS). The scleroblast (sc) lies on the squamula (sq). p = pouch; sp = scale pocket.

Fig. 8. Detail of the basal part of a pocket in *Dermophis mexicanus* (TEM; LS). The scale pocket (sp) is lined by a fibroblast (lc). bp = basal plate; p = pouch; sc = scleroblast.



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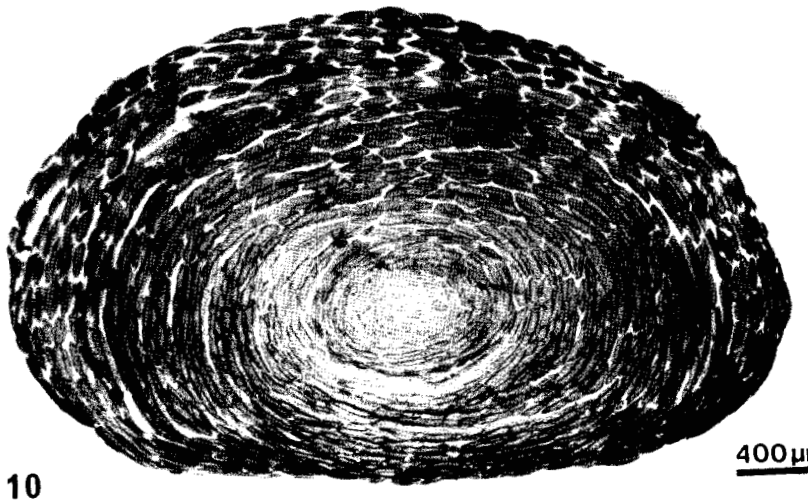

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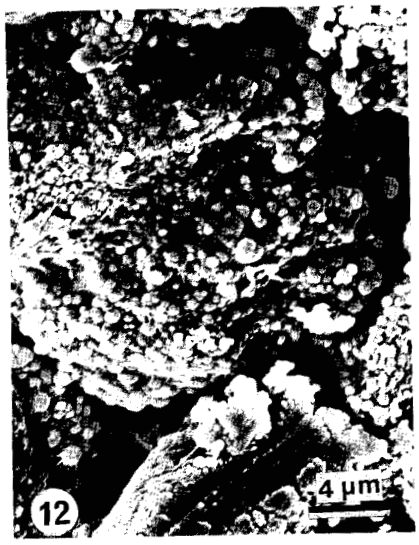


Figures 9–12

mular shape and structure. The rectangular shape of peripheral squamulae in *Ichthyophis* (Zylberberg et al., '80) contrasts markedly with the rounded shapes of *Geotrypetes* (Perret, '82) and *Dermophis* (Wake and Nygren, '87). It should be noted that these contrasts are greatest among peripheral squamulae; examination of the published photomicrographs indicates that the centers of scales bear squamulae that are both rounder and more irregular in shape. A common feature of squamulae of virtually all species examined is that mineralized material is deposited in a globular manner (see photomicrographs cited above). The only exception to this pattern that we have observed is in *Caecilia tentaculata,* in which deposition is essentially smooth (Wake and Zylberberg, unpublished data), and we are examining this situation further.

Comparison with osteichthyan scales

The pouch and the pocket

Our data confirm that gymnophione scales have peculiarities apparently related to their location in the segmentally arranged annuli, and that they share more structural similarities with the scales of extant osteichthyans than with the osteoderms of extant amphibians and reptiles. Because of their position relative to the annuli, the overlapping scales in gymnophiones occur in narrow transverse rows that partly or completely encircle the body. In osteichthyans, the imbricate scales are distributed over the body completely or in patches, without an obvious segmental association. Our data indicate that every scale in both species examined lies within its own pocket, as do the elasmoid scales of osteichthyans (Whitear et al., '80). Recent descriptions, including those at the ultrastructural level, have mentioned that several overlapping scales lie in a "pocket" (Taylor, '72; Gabe, '71a; Wake, '75; Zylberberg et al., '80; Fox, '83). However, as noted above, that "pocket" is actually a large connective tissue pouch associated with an annulus. The pouch contains the scales, each of which lies in a thin, connective tissue pocket, homologous to that of osteichthyans. However, the fibroblasts that line the scale pocket of gymnophiones do not differentiate as in fishes (see Whitear et al., '80). In osteichthyans, the outer surface of the elasmoid scale is connected to the superficial dermis by collagenous anchoring bundles that arise from the outer surface of the scale and extend through the dermis to the epidermal–dermal junction (Zylberberg and Meunier, '81; Sire, '85). Such bundles are absent in the gymnophione scales examined. This phenomenon may be related to the location of the scale rather than to the structure of the squamulae. In gymnophiones, the scales are well inserted within the dermis among skin glands, and they are covered by a thick pleuristratified epidermis (Gabe, '71a). Similarly, the reduced scales of the eel which are covered by a mosaic of smooth squamulae do not have anchoring bundles. These scales also are covered by a thick pleuristratified epidermis (Zylberberg et al., '84). In further contrast, the large scales of *Protopterus,* which are located in a very loose dermis close to the thin epidermis, have squamulae with well-developed collagenous anchoring bundles (Zylberberg, '88).

Squamulae

Our observations confirm that mineralization in gymnophione scales is limited to the squamulae, which are isolated plates topping the scales; mineralization does not occur in the basal plate as described by Casey and Lawson ('79). Gymnophione squamulae have two simultaneous processes of mineralization, termed spheritic and inotropic (Orvig, '68). These two processes are the result of the heterogeneous composition and organization of the extracellular matrix in the squamulae. Recent reports point out the importance of the organic matrix in controlling crystal deposition (reviewed by Weiner, '86). Where spheritic mineralization occurs, the shape of the spicules depends on the radiating arrangement of the noncollagenous matrix. The crystals associated with the collagen fibrils are aligned along the fibrils so that their long axis (the crystallographic *c* axis) is parallel to the collagen fibril axis (Schmidt, '36; Stuhler, '38). Moreover, the crystals are distributed according to the axial periodicity of the collagen fibrils as first described in bone (Hodge and Petruska, '63; Glimcher and Krane, '68; Glimcher, '81; Arsenault, '88).

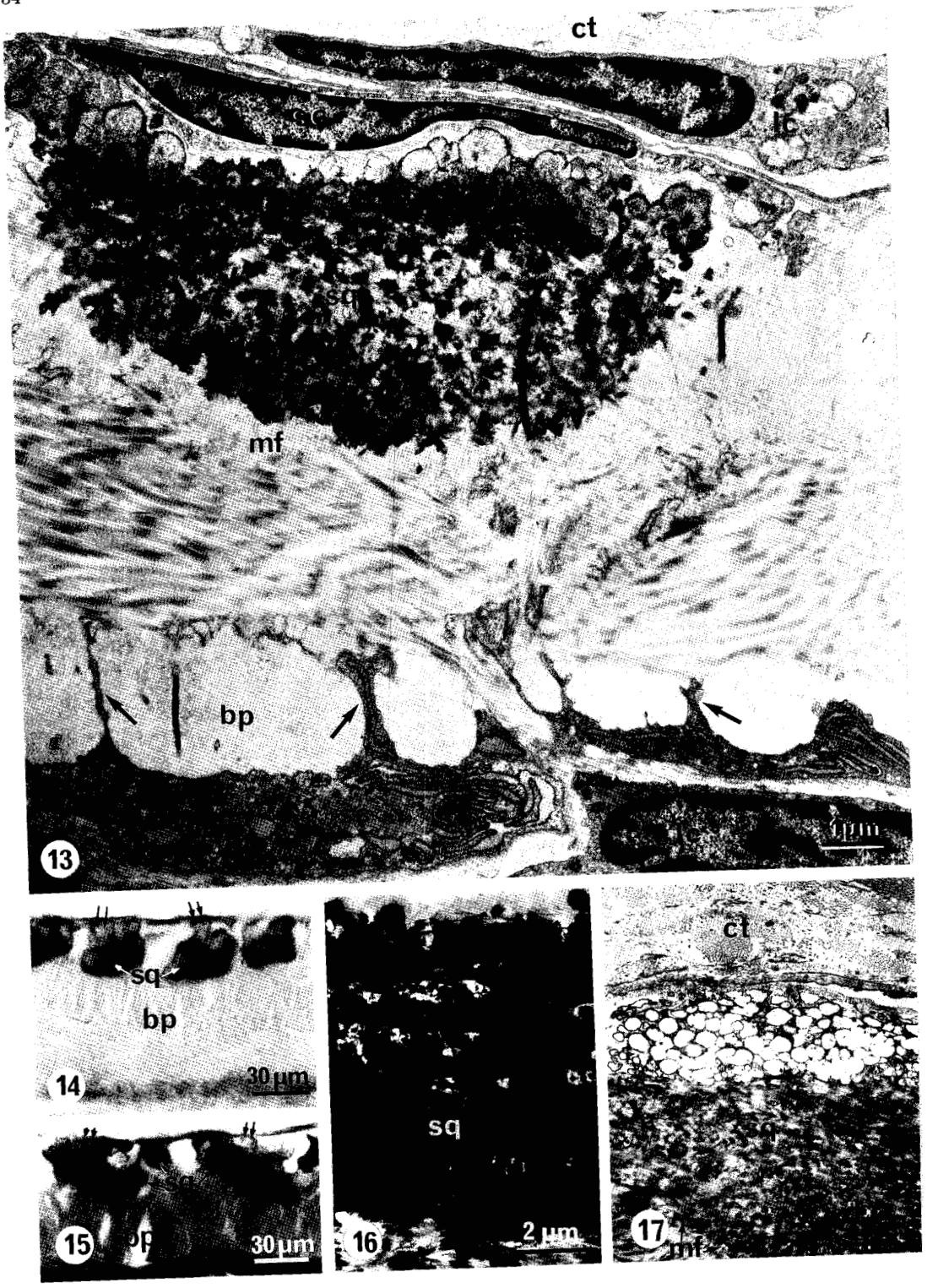
According to Orvig ('68), spheritic mineralization may be considered the phylogenetic precursor of inotropic mineralization. He considered the latter to represent the "ultimate stages" in a phyletic process of increasing complexity of "calcification mechanisms." Spheritic mineralization also may be considered an ontogenetic pre-

Fig. 9. Randomly selected scale of *Microcaecilia unicolor* stained with alizarin-red to show shape and arrangement of squamulae.

Fig. 10. Randomly selected scale of *Dermophis mexicanus* stained with alizarin-red.

Fig. 11. Scanning electron micrograph (SEM) of squamulae of *Microcaecilia unicolor.*

Fig. 12. Squamulae of *Dermophis mexicanus* (SEM).



Figures 13–17

cursor of inotropic mineralization, because during dentine ontogenesis, spheritic mineralization (which forms globular dentine) is replaced by inotropic mineralization (Keil, '39; Orvig, '67; Poole, '67). Therefore, the squamulae of gymnophiones, the outer surface of osteichthyan scales (Sire, '85; Zylberberg, '88 and unpublished data), and the osteoderms of reptiles (Levrat-Calviac and Zylberberg, '86) have retained a primitive type of mineralization that occurs concomitantly with a "more advanced" mineralization process. The mineralized spherules are more abundant on the outer surface of the squamulae, but also are found on the inner surface among the collagen fibrils. These spherules, which have a radiation arrangement of crystals, do not correspond to the Mandl's corpuscles characteristic of elasmoid scales. In Mandl's corpuscles, the crystals are oriented by the alignment of the collagen fibrils (Schonborner et al., '81). The limit between the squamulae and the basal plate is represented by the mineralizing front, though collagen fibrils arise from the basal plate and penetrate the squamulae. These fibrils are not part of the plywood-like structure of the basal plate itself. As noted by Zylberberg et al., ('80), the TEM micrographs of well-developed scales show mineral deposits both along the collagen fibrils and within the interfibrillary matrix, as well as in spherules, where they are arranged in a radiating pattern. Therefore, the site of nucleation cannot be determined (present study; Zylberberg et al., '80). Moreover, the cells that participate in the mineralization processes have not been identified.

Fig. 13. Scale of *Microcaecilia unicolor* sectioned perpendicular to its surface (TEM). The fibroblasts (lc) forming the scale pocket surround the scleroblasts (sc). The outer part of the squamula (sq) has abundant mineralized globules. In the inner part of the squamula the mineralizing front (mf) is located in the basal plate (bp). The basal scleroblasts have long processes (arrows) that separate the bundles of collagen fibrils.

Fig. 14. The inner part of the squamulae (sq) is PAS-reactive (dark color) whereas the outer part shows weak staining with alcian blue (pH 2.5) (arrows) (LM; LS). The basal plate does not react to either stain. *Dermophis mexicanus.*

Fig. 15. The inner part of the squamulae (sq) is strongly reactive to tetrazolium, though the outer part (arrows) and the base plate (bp) stain weakly (LM; LS). *Dermophis mexicanus.*

Fig. 16. The outer surface of the squamula (sq) has many mineralized globules (TEM; LS). *Dermophis mexicanus.*

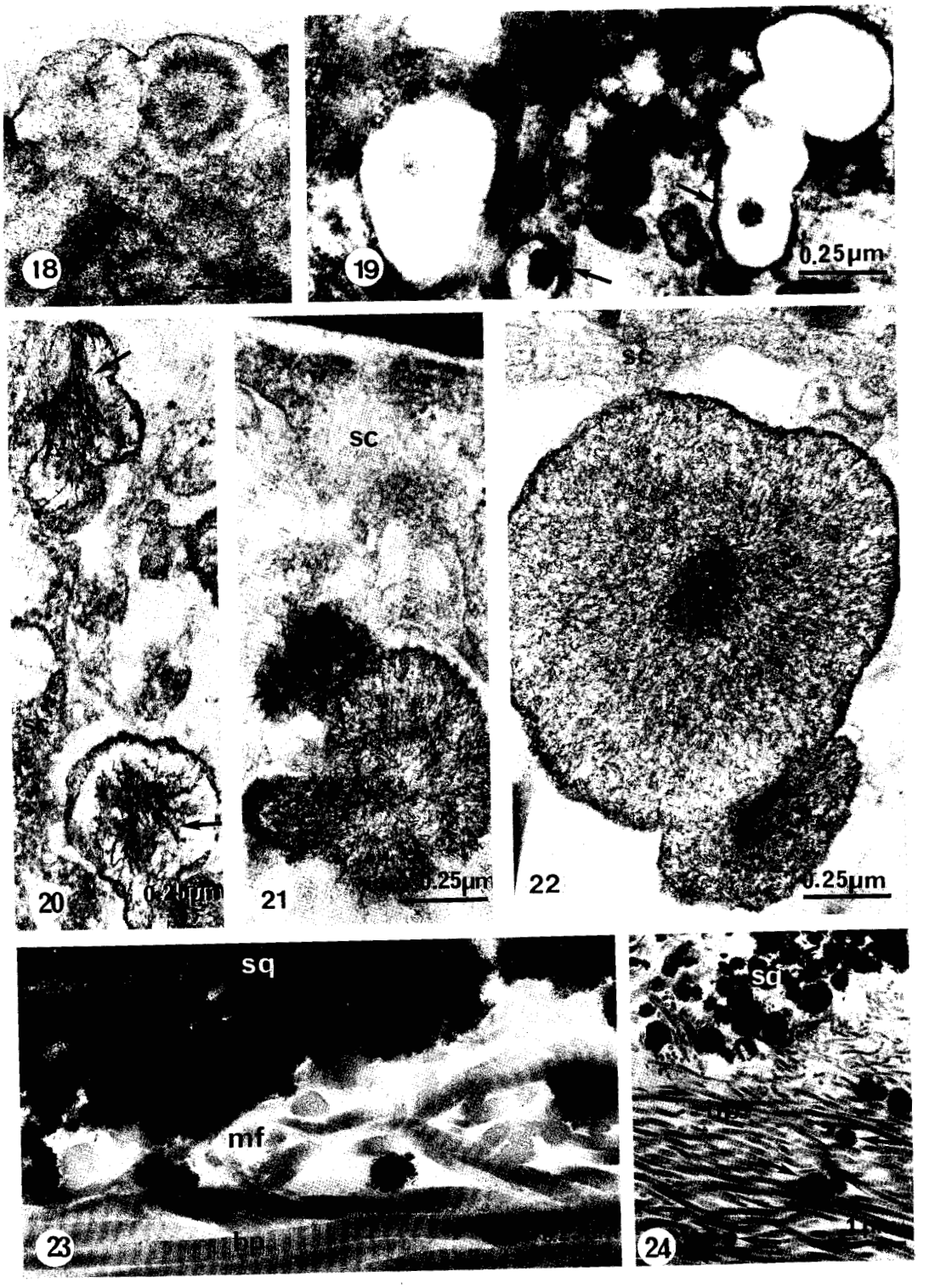
Fig. 17. Demineralized (EDTA) scale section from *Dermophis mexicanus* (TEM; LS). The globules are primarily at the outer part the squamula. They do not show any organic matrix. ct = connective tissue; mf = mineralizing front.

Basal plate

In gymnophiones, the thick collagenous fibrils forming the basal plate are organized as a plywood-like structure, but it is less regularly arranged than in the elasmoid scales of teleosts and sarcopterygians. The reduction of organization of the basal plate in gymnophiones may be associated with a generalized reduction of the dermal skeleton in both fishes and tetrapods. The most reduced osteichthyan scale observed occurs in the eel in which the basal plate has only one ply (Zylberberg et al., '84). The scales of gymnophiones are thin and the plies are much less numerous than in fishes. However, the basal plate, which shows the usual histological and histochemical characteristics of lamellar bone, does not mineralize as it does in the basal plate of osteichthyans (Meunier, '84). According to Meunier, thick basal plates made of isopedine that has lost its ability to be mineralized reflect a general trend toward reduction of the dermal skeleton.

The collagen fibrils of gymnophione scales, like those of fish, are thicker than those of the surrounding dermis, including the collagen fibrils that separate the bundles forming the plywood-like structure of the base plate and those that cross the scale perpendicularly to the plies. The latter collagen fibrils differ from the TC fibers described in the elasmoid scales of Cyprinidae. They are thinner than the collagen fibrils of the plywood-like structure (Onozato and Watabe, '79) and are involved in the first stages of mineralization of the basal plate (Zylberberg and Nicolas, '82). As in fish scales, the collagenous stroma as well as the noncollagenous extracellular matrix are synthesized by the cells surrounding the scales, which also control mineral deposition. Therefore, the gymnophione scale is an acellular tissue as defined by Meunier ('87); further, the basal plate may be considered a bony tissue which has lost its capacity to be mineralized (Meunier, '84).

The scleroblasts involved in the formation of the scales line each scale. They are contiguous only along the inner surface of the scale where they form a pseudoepithelium. Because they synthesize the thick collagen fibrils that form the basal plate, they are assumed to control fibrillogenesis and the orientation of the collagen as has been described for fish scales (see above). The long cytoplasmic processes that penetrate deeply within the basal plate are involved in the formation of the bundles that form the superimposed plies. However, the processes seem to orient the



Figures 18–24

collagen fibrils that arise from the cell processes perpendicularly to the plies. An obvious coincidence of the orientation of cytoskeletal elements (superficial microtubules and actin filaments) with the innermost collagen fibrils has been described (Zylberberg et al., '88), and indicates that cytoskeletal organization also is involved in the orientation of collagen fibrils. Such relationships between the cytoskeleton and newly synthesized collagen fibrils are not observed in the basal scleroblasts of gymnophione scales. Therefore the less well organized plywood-like structure of the basal plate in gymnophione scales may be related to reduced involvement of the cytoskeleton in the orientation of fibrils produced by the scleroblasts. The scleroblasts are connected to each other and are not able to modify their shapes, as do isolated fibroblasts (Birk and Trelstad, '84; Birk and Trelstad, '86).

Most of the cells surrounding well-developed gymnophione scales do not appear to be actively secretory, suggesting that increase in size and in thickness of developed scales is slow, if it occurs at all. In teleosts, increase of thickness of developed elasmoid scales is primarily a contribution of basal scleroblasts, which have a low rate of synthesis (Waterman, '70; Frietsche and Bailey, '80). Increase in size results from the activity of marginal scleroblasts that form a rim around the scale. A similar rim of marginal cells is absent in gymnophiones.

Fig. 18. Partially demineralized section from *Microcaecilia unicolor* (TEM; LS). The globules have concentric spheres of mineralization.

Fig. 19. Scale of *Microcaecilia unicolor* treated with EDTA and ruthenium red (TEM). The globules are lined by an electron-dense material (arrows). Some also have an electron-dense central core.

Figs. 20–22. Formation of globules of mineralization in *Microcaecilia unicolor* (TEM).

Fig. 20. The crystals (arrows) appear in globules near the vicinity of a scleroblast (sc).

Fig. 21. The crystals are arranged in a radiating pattern. The globules lie near the scleroblast (sc). Small globules are aggregating.

Fig. 22. The globule has the same location and the same organization as it increases in size. sc = scleroblast.

Fig. 23. Mineralizing front (mf) at the inner part of a squamula (sq) (TEM; LS). Mineralized globules lie among the collagen fibrils of the basal plate (bp). *Dermophis mexicanus*.

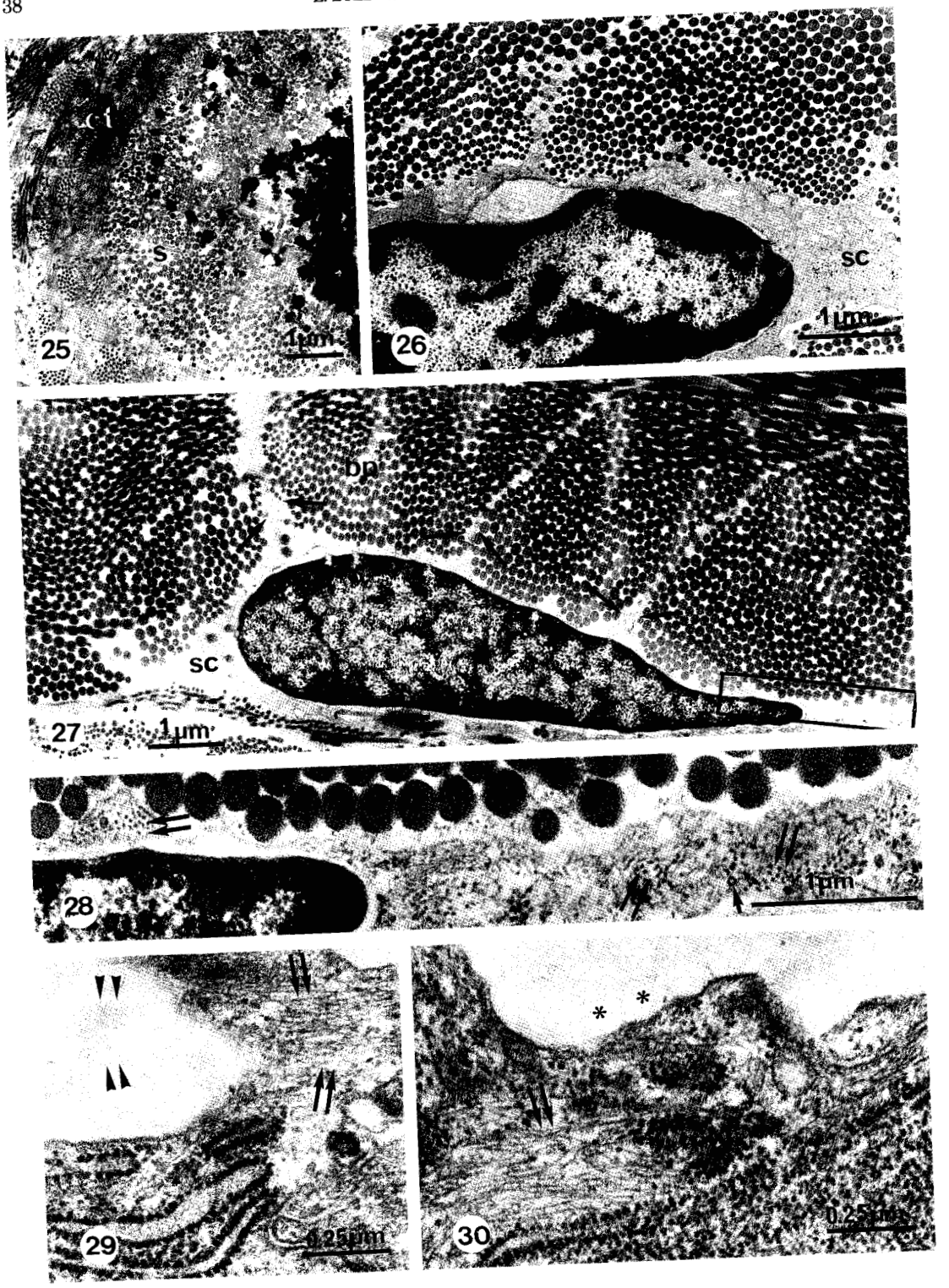
Fig. 24. Periphery of a squamula (sq) of *Dermophis mexicanus* (TEM; LS). Isolated globules are abundant (arrows) among the collagen fibrils of the base plate (bp).

Comparison with dermal ossifications in other amphibians

Some anurans and salamanders have dermal ossifications, but not scales. Members of four families of frogs independently have evolved osteoderms—bony plates that occur in the dermis on the dorsum of the body, and in some on the head and the limbs. Ruibal and Shoemaker ('84) carefully examined the structure of frog osteoderms, and reported considerable variation among families, including vascularized bony plates that bears spines that protrude into the epidermis and avascular osteoderms composed of calcified collagen fibrils in a three-dimensional arrangement. Mineralization is apparently inotropic.

Members of all three orders of amphibians possess another pattern of dermal ossification, co-ossification of the skin of the head to the skull. Trueb ('66) described co-ossification in the skull of the casque-headed frog, *Hyla septentrionalis*. Extensive bony protuberances of skull bones and of the dermis bind skull, dermis, and epidermis. In the plethodontid salamander *Aneides lugubris*, spikes of dermal bone protrude into the epidermis and there is virtually no loose dermis (D. B. Wake, personal communication). In gymnophiones, much of the dermis on the dorsal part of the skull and the margins of the jaws has been eliminated, and dermal fibers that overlie the skull and bind it to the epidermis have been mineralized (Wake, unpublished data).

Several taxa of extinct amphibians among the Labyrinthodontia, Aistopoda, and Microsauria possess scales. Colbert ('55) and Olson ('79) examined patterns of scalation in members of these groups, particularly the labyrinthodont *Trimerorhachis*. The scales of *Trimerorhachis* are thin and somewhat elongate, less round than in other Carboniferous scaled amphibians. The scales have a basal layer with a pattern of concentric rings, assumed to be growth rings by Colbert, and a superficial layer of longitudinal ridges, giving a "corrugated" appearance to the surface. Both layers are well mineralized. Olson, however, considered these structures to be layers of osteoderms having *no* resemblance to cycloid scales. There appear to us to be both terminological and analytical problems with these interpretations. Significantly, both workers suggested that trends in evolution of dermal structures were similar and parallel in amphibians and teleosts, with reduction of size, composition, numbers, and distribution of scales. Colbert sug-



Figures 25–30

gested that the evolutionary patterns represent convergences between amphibians, descended from crossopterygians with rhomboid ganoid scales, and teleosts, descended from palaeoniscids that also had rhomboid ganoid scales. Because there is virtually no evidence from which patterns of mineralization can be deduced, this debate cannot be resolved.

Comparison with dermal ossifications of reptiles

Osteoderms, or mineralized dermal plates located in the skin, occur in many squamate reptiles. Levrat-Calviac and Zylberberg ('86) reviewed the literature on such structures as part of their careful assessment of the histology and cytology of osteoderms in *Tarantola mauritanica*. They noted much variation in location, development, and correlation with epidermal scales, relationship with adjacent tissues, and general structure, though there is consistency within species. Their analysis revealed that osteoderms of *T. mauritanica* have two components. A mineralized basal layer is composed of many closely packed collagen fibrils effectively continuous with those of the dermis. An outer layer, in the superficial loose dermis, is crossed by rather few collagen fibrils that arise from the basal layer. These fibrils connect the osteoderm to the loose dermis. The outer, superficial, layer contains mineralized globules that surround the mineralized collagen bundles. Within the globules, crystals of mineralization are deposited on a matrix of microfibrils that is composed of radially oriented, tangled microfilaments that occur among the collagen bundles. Both inotropic and spheritic mineralization take place in these osteoderms. The osteoderms are strikingly continuous with the deep dermis, and are joined to each other by dense bundles of collagen fibrils. Osteoderms grow concentrically, and exhibit electron-dense growth rings, such as occur in the bones of all vertebrates (Castanet, '81).

Levrat-Calviac and Zylberberg ('86) considered osteoderms to be secondary formations. These data and conclusions largely corroborate those of Zylberberg and Castanet ('85) for the osteoderms of *Anguis fragilis,* though osteoderms of the latter differ from those of *T. mauritanica* in being small, flat, disc-shaped, and associated with the epidermal fold of the overlying epidermal scale. It is significant that osteoderm formation occurs without differentiation of a specialized tissue structure, such as the dermal papilla of the fish scale. It is likely that these observations characterize osteoderm formation and structure in many squamates, with lineage-specific variation in location, size, and shape.

Evolution of dermal ossifications

Dermal scales in gymnophiones and osteichthyans, and osteoderms in frogs and squamate reptiles, share a number of common properties. For example, squamate osteoderms bear a strong superficial resemblance to the osteoderms of frogs as described by Ruibal and Shoemaker ('84) in their dermal continuity, variable location, and apparent mineralization pattern, though they lack the dorsal spikes exhibited by many frog osteoderms. Further, the general structure with a distinct basal layer and a differently mineralized superficial or outer layer is similar among osteoderms to the dermal scales of osteichthyans and of gymnophiones. The similarity is enhanced by evidence that mineralization in all these groups usually is both inotropic and spheritic; this suggests common properties of association with collagen fibrils and their surrounding matrix. Moss ('72) commented on the series of homologous developmental events involved in the formation of diverse dermal structures (e.g., teeth), and emphasized the common properties of collagen and "ground substance." He presented a classification of integumental skeletal structures. Meunier and Geraudie ('80) presented cogent arguments about the common pattern of development of dermal ossification, with particular reference to scales. They described the spatial organization of collagen as a plywood structure in the both basement lamella and the dense dermis, including the basal layer of teleost scales. The scales have thicker fibrils

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Fig. 25. Margin of the scale in *Dermophis mexicanus* (TEM). The scale (s) is in contact with the connective tissue (ct) of the scale pocket.

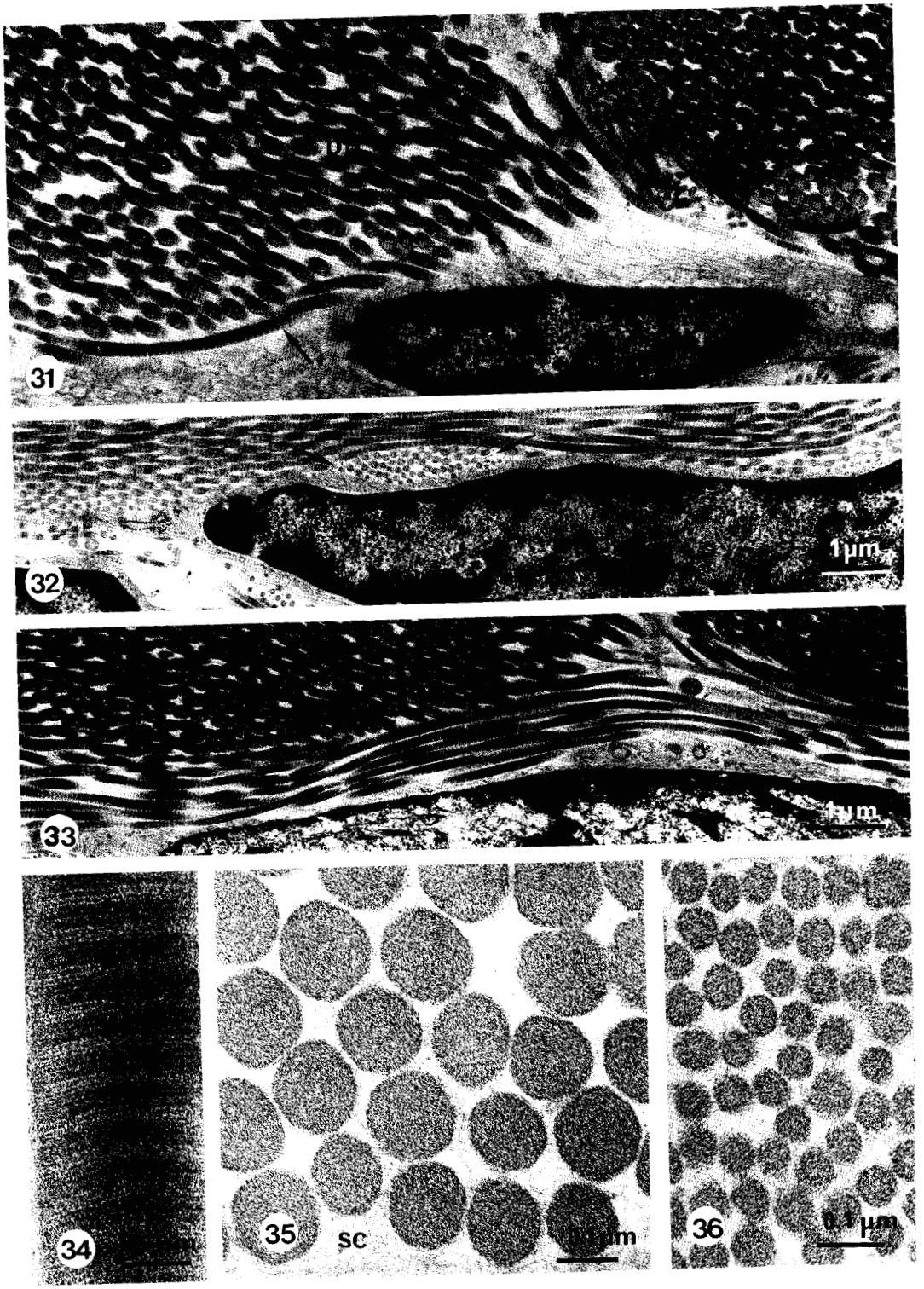
Fig. 26. Basal scleroblasts (sc) of *Dermophis mexicanus* synthesizing the thick collagen fibrils of the basal plate (bp) (TEM). The collagen fibrils oriented in parallel are not packed in bundles.

Fig. 27. Basal scleroblasts (sc) have long processes (arrows) which separate bundles of collagen fibrils (TEM). *Dermophis mexicanus.*

Fig. 28. Enlargement of outlined area of Figure 27. The collagen fibrils and cytoskeletal components (microtubules [arrow], microfilaments [double arrows]) show the same orientation. The collagen fibrils are in contact with the scleroblast.

Fig. 29. Some microfilaments (arrows) have the same orientation as the collagen fibrils (arrowheads) in contact with the cell (TEM). *Microcaecilia unicolor.*

Fig. 30. No relationship of the direction of the cytoskeleton (arrows) and of the collagen fibrils (asterisks) is discernable (TEM). *Microcaecilia unicolor.*



Figures 31–36

than in the associated dermis (we describe the same phenomena in gymnophiones). They concluded that the *contre-plaque* structure is a common property of dermis. Different taxa have different "aptitudes" for mineralization, dependent upon the macromolecular arrangement of the collagen fibrils and the biochemical environment in which they occur.

The data available on the structure of gymnophione and osteichthyan scales and frog and squamate osteoderms support the hypothesis that such mineralized dermal structures occur because of the structural properties of the dermis, common to all vertebrates. Expressions of these properties arise in a number of lineages, and have a diversity of manifestations. We consider it inappropriate to speculate about the "selective force(s)" that might have favored such new structures. Instead, we offer a structuralist, a-historical explanation for the similarity of structure of the dermal ossifications and their apparent modes of mineralization, despite the significant differences in size, shape, and location of such structures.

We are concerned that an assumption of homology of the dermal scales of osteichthyans and gymnophiones as a consequence of descent from a hypothetical ancestral dermal scale has crept into the literature (see, e.g., Ruibal and Shoemaker, '84, p. 324). We believe that assumptions of homology of osteichthyan and gymnophione scales are based only on (1) their dermal origin, and (2) their similarities of shape and some aspects of structure (despite the fact that these are shared in many ways with osteoderms). We particularly object to the notion of common ancestry. Earlier workers did not so misconstrue; even Colbert ('55), although associating gymnophione scales with those of possible extinct amphibian ancestors, called teleostean and gymnophione scales a convergence among "active" vertebrates. Kerr ('55) found a striking "convergent similarity" between the scales of *Neoceratodus* and *Amia,* but the resemblance of the scales of the other lungfish to teleost scales to be more superficial. He concluded that lungfish scales have only a general resemblance to gymnophione scales. It is clear that attributing common ancestry to dermal scales of osteichthyans and gymnophiones rests on little or no significant evidence.

It is equally difficult to associate gymnophione scales with those of extinct amphibian groups proposed as ancestral. Various groups have been suggested, but there is a paucity of evidence. Further, there is not yet an agreed-upon phylogeny for all amphibians. For example, Carroll and Currie ('75) considered microsaurs to be ancestors of gymnophiones; Gardiner ('83), based only on vertebral structure, considered nectridians to be the sister group of extant amphibians, and microsaurs to be amniotes! Because there are significant differences in structure of scales of extinct amphibians and of gymnophiones, and there is no soft tissue information available for the former, we consider it equally plausible that scales might have arisen independently in several lineages of amphibians, including gymnophiones. Clearly dermal ossifications have arisen numerous times; differences in size, shape, location, pocket structure, etc., of amphibian scales and the mineralized dermal structures of other vertebrates suggest lineage-specific variation and, probably, origin, but from a *tissue* of origin that has common structural capabilities (and constraints). Furthermore, the unique structural properties of gymnophione scales (particularly the segmental association and other aspects of location and structure) suggest a *de novo* acquisition in the lineage.

We agree with Ruibal and Shoemaker ('84) that the term "dermal scale" should be used for the mineralized dermal units of *both* osteichthyans and gymnophiones, and "osteoderm" for the dermal structures of frogs and squamates. This terminology recognizes certain convergent attributes of shape and structure (scales being structures that arise from differentiating dermal fibroblasts, lie in pockets, and are generally spheroid; osteoderms being metaplastic structures occurring in preexisting dermal tissue and strongly connected to the dense dermis), but it does not

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Figs. 31–33. Formation of a new ply in the basal plate in *Dermophis mexicanus* (TEM; LS).

Fig. 31. The collagen fibrils (arrow) in contact with the scleroblast are directed approximately perpendicular to the previously deposited layer of fibrils. bp = basal plate.

Fig. 32. A bundle of collagen fibrils (arrows) in contact with the scleroblast perpendicular to those forming the ply beneath.

Fig. 33. Collagen fibrils in contact with the scleroblast are perpendicular to those of the adjacent ply.

Fig. 34. Longitudinal section of collagen fibrils of a scale of *Dermophis mexicanus* (TEM). The periodicities of both fibrils are in register.

Fig. 35. Cross section of the collagen fibrils of the basal plate of a *Dermophis mexicanus* scale (TEM). sc = scleroblast.

Fig. 36. Cross section of the collagen fibrils forming the plywood of the dense dermis in *Dermophis mexicanus* (TEM). Note the difference in diameter of basal plate (Fig. 35) and dense dermis collagen fibrils.

carry any connotation of common ancestry or of developmental process. We conclude that, at this time, the dermal ossifications of vertebrates appropriately are regarded as diverse expressions of the common structural propensity for mineralization of the dermis itself.

ACKNOWLEDGMENTS

We thank Francoise Allizard, Enrique Lessa, Wendy Marlor, and Kristen Nygren for technical assistance, and Jean Lescure, Theodore Papenfuss, Robert Seib, David Wake, and Thomas Wake for collecting specimens used in this study. Transmission electron microscopy was performed at the Centre de Microscope électronique (CNRS, Université Pierre et Marie Curie), and the photomicrographs were printed in their Photographic Department. Scanning electron microscopy was done at CME and in the Electron Microscope Laboratory at the University of California, Berkeley. Our research was supported by CNRS UA 04 11 37 and NSF BSR 87-06135. MHW is grateful for a John Simon Guggenheim Foundation Fellowship which supported research and writing in Paris.

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