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Received: 9 November 2000

Revised and Accepted: 5 May 2002

Chelonian Conservation and Biology, 2003, 4(3):682-684
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Comparative Ultrastructural Carapace Morphology in Three Freshwater Turtles

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Several researchers have reviewed the topographic features of the outermost layer of reptile scale epidermis (Hutchinson and Larimer, 1960; Maderson, 1965; Monroe and Monroe, 1967; Porter, 1967; Ruibal, 1968; Dowling et al., 1972; Stewart and Daniel, 1972, 1975; Burstein et al., 1974). This outermost layer, termed the Oberhautchen, is approximately 1 μm thick and is composed of distinctive β -keratin, in comparison to subsequent layers (Stewart and Daniel, 1972). In hard shelled turtles, the shell (carapace and plastron) is composed of dermal bony plates covered with keratinized epidermal scales (Zangerl, 1969). The ultrastructural surface features, denoted as microornamentations, that are derived from such epidermal modifications have recently become useful taxonomic tools, as well as ecologi-

cal indicators for some species. Although several squamate taxa have been examined, the literature fails to thoroughly examine the microornamentation of chelonians. Zangerl (1969) examined the morphological variation in the epidermal and dermal shields of the carapace, but based comparisons primarily on patterning and organization. Proctor (1958) noted that the growth of epizoophytic algae (*Basidiocladia* spp., Cladophoraceae) was primarily due to carapace morphology. Although the lamellar surface features of *Graptemys*, *Chrysemys*, *Pseudemys*, and *Deirochelys* spp. were distinct in comparison to *Chelydra* and *Kinosternon* spp. (Proctor, 1958), the ultrastructural microornamentation of these taxa was not examined. This study was designed to examine the ultrastructural carapacial microornamentation of three freshwater turtles and to ascertain its potential usefulness as a diagnostic tool for taxonomy and ecological significance within this group of reptiles.

Materials and Methods. — Twenty-five turtles (*Chelydra serpentina* [$n = 10$], *Chrysemys picta* [$n = 10$], and *Emydoidea blandingii* [$n = 5$]) were collected from pond systems located at the Chippewa Nature Center, Midland County, Michigan, during the summers of 1997 and 1998. Using a scalpel, carapace surface samples (ca. 1 cm^2) of the epidermal laminae were extracted from the right fourth costal scute of each turtle (if damaged, an adjacent scute was used). Samples were stored in 2% glutaraldehyde and refrigerated. The samples were later dehydrated by graded four minute ETOH washes of 30, 70, 95, and 100% (3x). Once dehydrated, samples were dried in a critical-point dryer at 1200 psi and mounted on scanning electron microscope (SEM) stubs with double-sided carbon tape. Samples were subsequently sputter-coated with 25 nm of gold and stored in a dessicator until examination.

Prepared samples were viewed using a JSM-840A scanning electron microscope. The exterior layers of the laminae were examined for microornamentation, both in terms of distinct ultrastructure and relative surface area. Electron micrographs of the ultrastructure were quantitatively compared for the relative densities of pronounced features (i.e., ridges and canals) in a randomly chosen 1 μm^2 area. Although canals are a direct product of pronounced ridges, they were scored independently because both represent microornamentation and past studies have shown that canals or fissures can be present without ridges (Stewart and Daniel, 1975). The quantified amount of ultrastructure within this given area, or total ultrastructural value (TUV), was averaged for each species and compared using a Kruskal-Wallis test.

Results. — All turtles examined exhibited some form of carapacial microornamentation. Most of these structures were visible as ridges and adjacent canals that were irregular in orientation. *Chelydra serpentina* samples possessed a loose stratification of laminae in the upper layers; beneath this layer the lamellae were tightly compacted and possessed pronounced microornamentation (Fig. 1).

Examination at higher magnification (10000x) revealed that each species possessed distinctive microornamentation

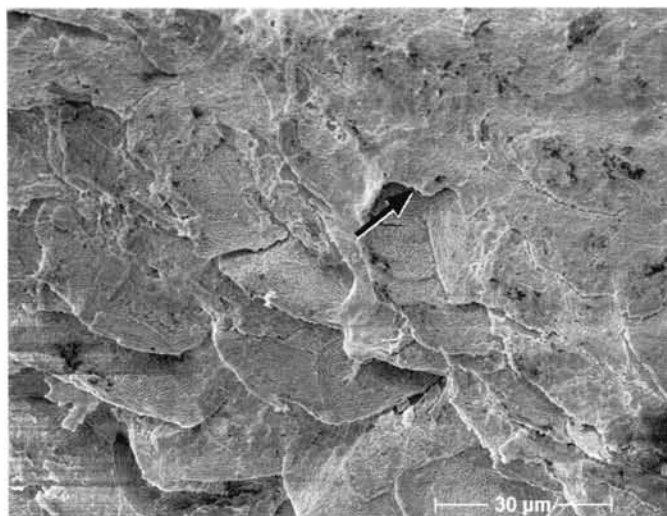
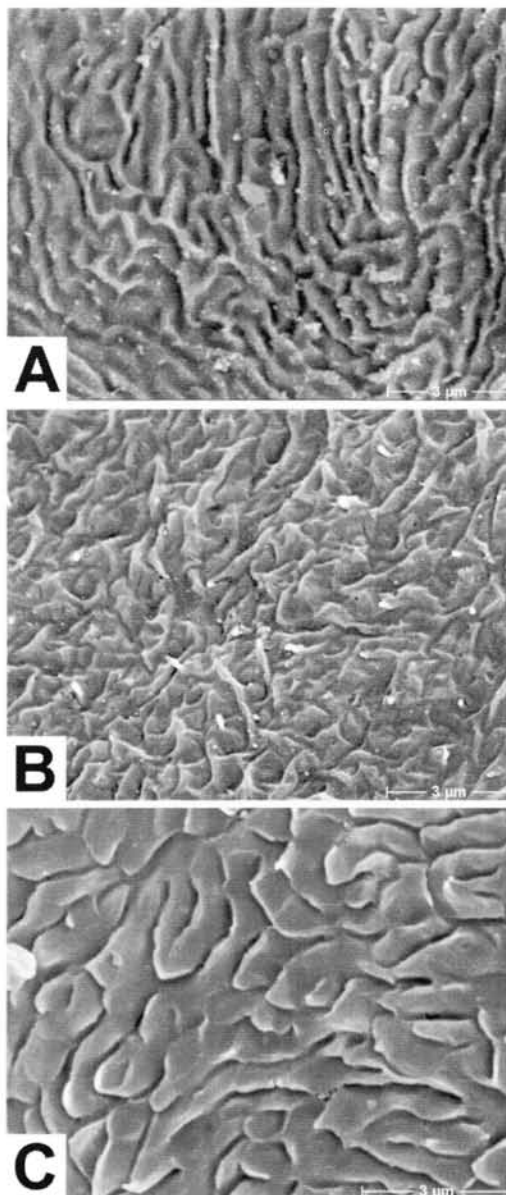


Figure 1. Carapace laminae of *Chelydra serpentina* showing the loosened laminae on the Oberhautchen and subsequent compact layering of the underlying laminae with pronounced microornamentation.

both qualitatively and quantitatively (Fig. 2). *Chelydra serpentina* possessed ultrastructural ridges that were apparently irregular in orientation (Fig. 2A). The ridges were variable in length, ranging from approximately 1–4 μm , and approximately 0.5–1 μm in height. *Chrysemys picta* also possessed microornamentation composed of ridges and canals (Fig. 2B), however, the top edges of these ridges were shorter (approximately 0.5–3 μm) in comparison to *C. serpentina* and showed greater irregularity in orientation than other turtles examined. Dispersed among the ridges were lamellar projections that were spine-like, but could not be accurately described as spinules (Fig. 2B). These structures, along with the relatively short ridge margins, created a microornamentation pattern for *C. picta* that appeared jagged. In contrast, *Emydoidea blandingii* had characteristically smoother microornamentation than either *C. serpentina* or *C. picta* (Fig. 2C). The Oberhautchen of *E. blandingii* was folded into bulbous structures that were relatively irregular in orientation. The adjacent canals, in regards to their topographic morphology, were either broad or crevice-like depending on the proximity of the two neighboring folds. The depth of these canals was not determined. In all species examined, no spinules or pitted structures, both common to squamate microornamentation, were identified. Likewise, all ridge edges examined were relatively smooth and did not possess teeth-like structures typical of several taxa of squamates.

The TUV for *Chelydra serpentina* ($\bar{x} = 5.8$) was not significantly different from that of *Chrysemys picta* ($\bar{x} = 7.1$). However, both were significantly different from *Emydoidea blandingii* ($\bar{x} = 4.2$). Thus, quantitatively the microornamentation of *C. serpentina* and *C. picta* possessed greater surface area than that of *E. blandingii*.

Figure 2. Microornamentation patterns for turtles examined at 10000x; (A) *Chelydra serpentina*, (B) *Chrysemys picta*, and (C) *Emydoidea blandingii*. All surface features consisted of ridges and canals, but varied interspecifically with respect to quantity and overall morphology.



Discussion. — *Chrysemys picta* and *E. blandingii*, though grossly similar in terms of their macrocarapacial morphology, had microcarapacial ultrastructural features that differed both qualitatively and quantitatively. Despite macrocarapacial features of *C. serpentina* being more pronounced than those of *C. picta*, their ultrastructural microornamentation patterns were similar. Thus, presumptions of microornamentation based on macrocarapacial features can be inaccurate and warrant individual examination.

Chelydra serpentina was found to possess relatively high surface area microornamentation. Such intricate substrata may be of ecological significance in relation to the attachment of epizoophytic algae. Several authors (Edgren et al., 1953; Proctor, 1958) have noted increased algal colonization of *C. serpentina* and attributed this to carapacial features. The loosened upper lamellae of Chelydridae and Kinosternidae is a product of continual scute sloughing and allows for colonization of *Basiacladia* algae beneath the Oberhautchen (Proctor, 1958). In addition to carapacial morphology, the aquatic and sedentary behavior of *C. serpentina* (Ernst, 1968; Ernst and Barbour, 1972; Obbard and Brooks, 1981) may induce a favorable environment for *Basiacladia* growth. The ecological significance of carapace microornamentation and algal growth to *C. serpentina* may be related to feeding. Harper (1950) suggested that the role of carapacial algae as camouflage is irrelevant since adult *C. serpentina* have no natural predators. However, *C. serpentina* is an ambush predator (Feuer, 1971; Punzo, 1975) and may therefore benefit by having natural camouflage from prey. Consequently, juvenile *C. serpentina* do not possess *Basiacladia* due to rapid lamellar sloughing (Edgren et al., 1953; Proctor, 1958).

Several authors have reviewed the role of microornamentation in thermoregulation of squamates (Hutchinson and Larimer, 1960; Porter, 1967; Monroe and Monroe, 1967; Ruibal, 1968; Stewart and Daniel, 1975). Although the significance of microornamentation in chelonian thermoregulation has not been examined, the thermal variability induced by ultrastructure could theoretically be similar.

Taxonomic relationships between several species of squamates have been based upon comparative microornamentation. Ruibal (1968) distinguished the Iguanidae, Lacertidae, and Gekkonidae from the Scincidae on the basis of epidermal spinules. Within the genus *Sceloporus*, microornamentation proved significant at the subspecies and interspecific level, but was inconclusive for taxonomic determinations at higher levels (Burstein et al., 1974). Stewart and Daniel (1975) found a distinction between ascalabotan and autarchoglossan lizard families based on similarities in scale microornamentation. For the present study, the differing microornamentation between *C. serpentina*, *C. picta*, and *E. blandingii* may indicate interspecific distinctiveness. Preliminary observations of *Graptemys geographica*, *Glyptemys insculpta*, and *Terrapene carolina* also support species-specific distinctiveness, but remain inconclusive due to small sample size.

Acknowledgments. — This study was supported by the Biology Department at Central Michigan University and the facilities at the Chippewa Nature Center, Midland County, Michigan. I particularly thank Paul Elsner and Geoff Williams for assistance with the SEM portions and James Gillingham for his expertise in turtle behavior, experimental design and his assistance with manuscript review. I also thank John Rowe for manuscript review and my colleagues at Central Michigan University for their cooperation in facilities use and overall support.

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Received: 20 November 2000

Revised and Accepted: 28 June 2002