Miniaturization and its effects on cranial morphology in plethodontid salamanders, genus *Thorius* (Amphibia: Plethodontidae). I. Osteological variation

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Cranial skeletal morphology, ontogeny and variation are examined in five species of *Thorius*, a genus of diminutive plethodontid salamanders that are among the smallest, extant, tailed tetrapods. The skull of adult *Thorius* is characterized by: (1) limited development or absence of several ossified elements and dentition; (2) increased inter- and intraspecific variability; (3) novel morphological configurations of the braincase and jaw suspensorium. Posthatching cranial mineralization in all species of *Thorius* is truncated precociously with respect to that typical of larger and more generalized plethodontid genera, such as *Pseudoeurycea*. These features implicate paedomorphosis as a predominant mechanism responsible for the evolution of decreased size in *Thorius* from larger plethodontid ancestors. Interspecific differences in cranial morphology are evident; species may be characterized by greater or lesser degrees of truncated development. However, there is no consistent relationship between degree of paedomorphosis and mean adult body size in interspecific comparisons. Adult morphology of several individual elements represent potentially useful taxonomic characters for distinguishing species.

Reduction, increased variability, and morphological novelty are common to many lineages of dwarfed taxa. They represent a null hypothesis for examination of the developmental mechanisms and morphological consequences of miniaturization in other groups.

KEY WORDS:　Amphibia – Caudata – Plethodontidae – *Thorius* – skulls – morphological variation – paedomorphosis – dwarfism.

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INTRODUCTION

Two primary goals of evolutionary morphology are (1) identification of the developmental processes by which often complex morphological features are produced during ontogeny, and (2) analysis of the way these processes are altered or perturbed during phylogeny to produce morphological diversity. Gradually, evolutionary biologists have come to appreciate the relevance of scale and relative size to considerations of these problems (see reviews by Bonner, 1968; Gould, 1966; Rensch, 1959). Many recent authors have directed particular attention to the apparent significance of phylogenetic size decrease to macro-evolutionary trends and the appearance of morphological novelty. Stanley (1973:14) alluded to "the tendency for major adaptive breakthroughs to occur at relatively small body size," defending this claim with numerous examples from fossil invertebrates. Swedmark (1964:1), in reviewing the biology of the tiny "interstitial" invertebrate fauna that lives in marine sand, described the common occurrence of "morphologically aberrant species, which sometimes represent entirely new types of organization". [See also examples in Gould (1977), Rensch (1948) and below.] Similarly, in vertebrates, decreased adult body size has been implicated as a critical factor in the morphological evolution of the earliest reptiles (Carroll, 1969) and Recent amphibians (Carroll & Holmes, 1980).

Phylogenetic size decrease is well documented in vertebrates (Maglio, 1973; Marshall & Corruccini, 1978; Prothero & Sereno, 1982) and invertebrates (Britton & Stanton, 1973; Hallam, 1975; Snyder & Bretsky, 1971; Sprinkle & Bell, 1978; Swedmark, 1968). So too is the frequent association between greatly decreased adult body size, here termed miniaturization, and morphological novelty. Yet the way in which ontogenetic patterns in an ancestor are altered to produce a smaller descendant, and how size decrease in effect may promote the appearance of novel morphological design, remain unexplored in most groups.

Among vertebrates, the lungless salamanders (family Plethodontidae) present an excellent opportunity to examine in detail both the mechanisms and consequences of phylogenetic size decrease. Reduced body size has evolved repeatedly in several genera (Wake, 1966); two of these, *Thorius* and *Parvimolge*, consist exclusively of salamanders of tiny adult body size—as little as 14 mm standard length (SL snout to posterior end of vent) in *Thorius*—which ranks them among the smallest, extant, tailed tetrapods. Furthermore, the frequent availability of large samples of adults allows consideration of population-wide estimates of variation in morphology—information that is unavailable for many other groups.

I recently examined morphological and genetic aspects of miniaturization in *Thorius*, which comprises a distinct group of arboreal or terrestrial salamanders of montane forests in southern Mexico (Hanken, 1980). An initial analysis of electrophoretic variation in nearly 70 populations confirmed the existence of at least 15 species (many of which are not yet described), and provided a hypothesis of phylogenetic relationship, independent of morphology, with which to detect and evaluate morphological trends (Hanken, 1982a, 1983a). A subsequent study considered appendicular skeletal morphology and variation primarily in five species in the context of skeletal growth regulation and adult size determination (Hanken, 1982b). In this and a later paper I examine the consequences of miniaturization for cranial morphology. Part I is a descriptive and

comparative analysis of patterns of variation of cranial osteology, including (1) a general comparison of the skulls of *Thorius* and larger, related genera, (2) quantitative estimates of inter- and intraspecific variation in several anterior cranial elements in adults, and (3) the ontogeny of posthatching cranial mineralization. In part II (Hanken, 1983b) the consequences of cranial miniaturization for the relative size and arrangement of the brain and special sense organs; the influence of these components on skull shape and orientation; and the role of these and other "soft" tissues in skull morphogenesis and evolution will be considered. The results identify how cranial morphology and development have been modified to produce an adult skull of drastically reduced size, and facilitate evaluation of the consequences of size decrease in other plethodontid genera as well as vertebrates generally.

MATERIALS AND METHODS

Cranial osteology was examined in five species of *Thorius* that collectively span the range of adult body size in the genus [mean SL (mm) for adult males and females, respectively]: *T. pennatulus* (18.9, $N = 10$; 18.9, $N = 10$); *T. macdougalli* (19.5, $N = 10$; 22.3, $N = 10$); *T. minutissimus* (22.6, $N = 9$; 24.1, $N = 9$); *T. schmidti* (23.5, $N = 8$; 25.0, $N = 10$); *T. narisovalis* (25.4, $N = 10$; 27.8, $N = 10$). Each species sample comprised approximately 30 specimens (10 adult males, 10 adult females and 10 juveniles, when available) collected either at a single locality or at adjacent localities usually separated by no more than a few kilometres of continuous suitable habitat (Appendix 1); an additional 10 juveniles supplemented the sample of *T. macdougalli*. Specimens were cleared and differentially stained for bone and cartilage using an alizarin red S–alcian blue procedure (Dingerkus & Uhler, 1977; Wassersug, 1976) as modified by Hanken & Wassersug (1981). Sexual maturity was judged by the presence of well developed testes and convoluted vasa deferentia in males and ripe follicles and/or convoluted oviducts in females.

Variation in anterior skull morphology was assayed by scoring all animals for the following nine characters (states for characters 2–9 are arranged in the order in which they appear during ontogeny) (Figs 1A, 2–4).

(1) Fusion of ascending processes of premaxilla. (a) Processes separate, (b) processes barely articulate, internasal fontanelle large, (c) processes fused along less than one-half of their length, internasal fontanelle moderate, (d) processes fused along more than one-half of their length, internasal fontanelle small.

(2) Premaxilla-maxilla overlap. (a) Premaxilla and maxilla separate, do not overlap in ventral view, (b) premaxilla and maxilla overlap slightly in ventral view but do not articulate directly, (c) premaxilla and maxilla overlap broadly in ventral view but do not articulate directly, (d) premaxilla and maxilla overlap in ventral view and articulate directly.

(3) Nasal development. (a) Nasal absent, (b) nasal thin, rod-like, at posterior edge of nasal capsule, (c) nasal broad, extending somewhat anteriorly over nasal capsule.

(4) Nasal-maxilla overlap. (a) Nasal and maxilla separate, (b) nasal and maxilla articulate, (c) nasal and maxilla fused.

(5) Prefrontal development. (a) Prefrontal absent, (b) prefrontal separate from nasal, (c) prefrontal articulates with nasal, (d) prefrontal fused with nasal.
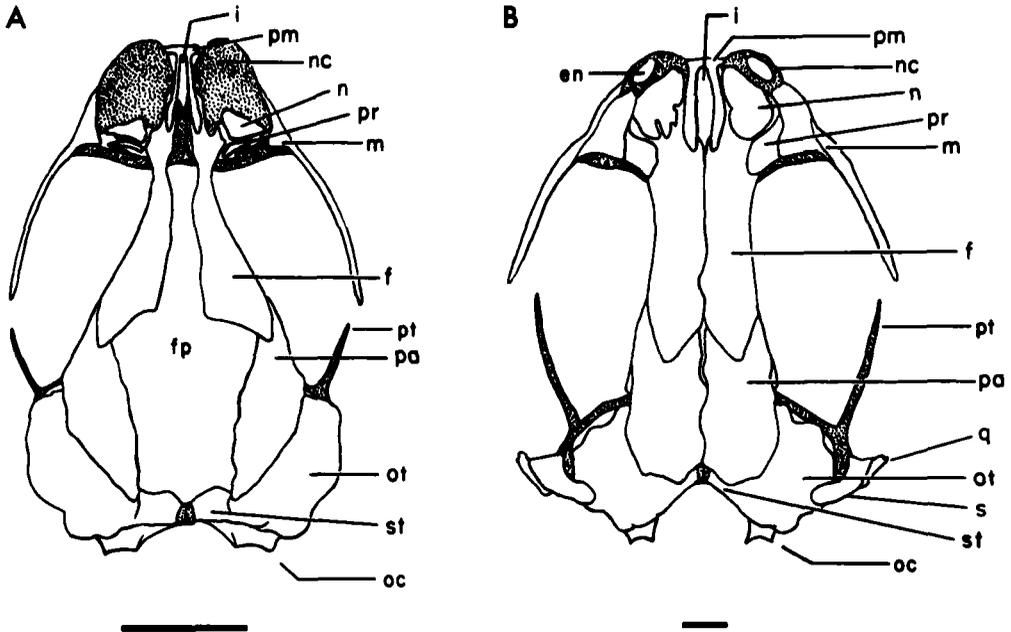
Figure 1. A, skull of *Thorius narisovalis* (M 4449; sl = 27.4 mm); B, skull of *Pseudoeurycea goebeli* (MVZ 130543; sl = 54.9 mm). Dorsal views, mandible is not figured; cartilage is stippled. Scale bars = 1 mm. Abbreviations: en, external naris; f, frontal; fp, frontoparietal fontanelle; i, internasal fontanelle; m, maxilla; n, nasal; nc, nasal capsule; oc, occipital condyle; ot, otic capsule; pa, parietal; pm, premaxilla; pr, prefrontal; pt, pterygoid process; q, quadrate; s, squamosal; st, synotic tectum.

(6) Prefrontal-maxilla overlap. (a) Prefrontal and maxilla separate, (b) prefrontal and maxilla articulate.

(7) Septomaxilla development. (a) Septomaxilla absent; (b) septomaxilla barely visible; (c) septomaxilla distinctly visible along posterior rim of external naris.

(8) Premaxillary teeth. (a) Absent, (b) present.

(9) Maxillary teeth. (a) Absent, (b) present.
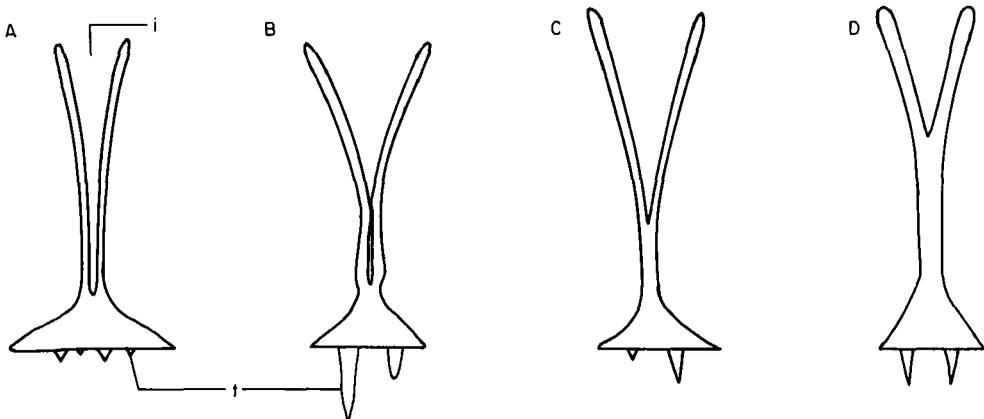
Figure 2. Anterodorsal view of the premaxilla, illustrating variation in the degree of articulation of the paired ascending processes (character 1). A–D, four character states described in text. In B note the extremely enlarged premaxillary tooth (t) which protrudes through the upper lip of this specimen.
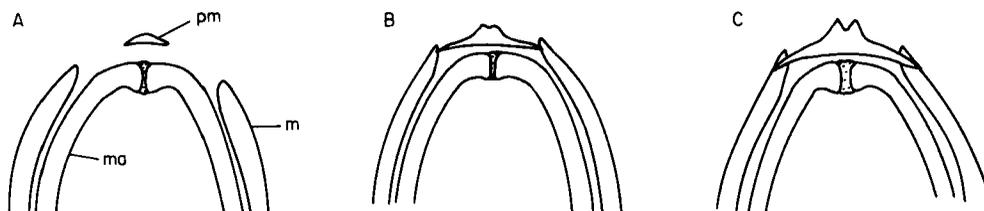
Figure 3. Ventral view of anterior skull region, illustrating variation in the degree of premaxilla-maxilla overlap (character 2). Ascending processes of the premaxilla, and other cranial elements, are not figured; ma, mandible. A–C, character states a–c described in text. In B and C the premaxilla remains separated from the maxilla (in lateral view) by fibrous connective tissue. Character state d (not illustrated) resembles C, but the premaxilla and maxilla are in direct contact (in lateral view).

Many characters vary continuously between states, thus few character states represent discrete developmental stages. Instead, the particular states chosen represent easily identifiable reference points along the continuum of variation. Right-left asymmetry—common for several of the "paired" characters (2–7)— was assessed by scoring both sides of each specimen.

RESULTS

General observations

The skull of adult *Thorius* is poorly ossified relative to that of other, more generalized plethodontid salamanders such as *Pseudoeurycea* (Wake, 1966) (cf. Fig.
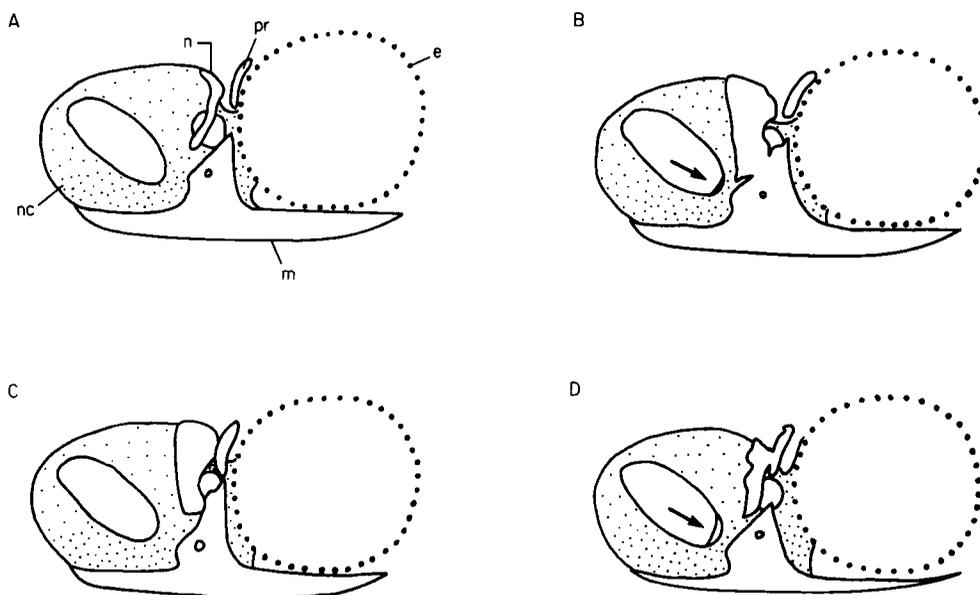
Figure 4. Lateral view of anterior skull region, illustrating variation of cranial elements (characters 3–7). Premaxilla and frontal are not figured; e, outline of eye. A, nasal thin and separate from maxilla, prefrontal separate from nasal and maxilla, septomaxilla absent; B, nasal broad and fused to maxilla, prefrontal separate from nasal and maxilla, septomaxilla (arrow) barely visible; C, nasal broad and articulates with maximilla, prefrontal articulates with nasal and maxilla, septomaxilla absent; D, nasal thin and separate from maxilla, prefrontal fused to nasal but separate from maxilla, septomaxilla (arrow) distinctly visible.

1A,B). Several bones, the septomaxilla for instance, are variably present in adults; others, such as the columella, fail to appear in any species. Many additional elements, though present, are extremely reduced in all species. This is particularly true of anterior elements (e.g. maxilla, nasal, prefrontal, premaxilla) and dentition. Dorsally, the frontal and parietal are very thin and have irregular medial margins; the failure of these paired elements to meet in the midline leaves a roughly diamond shaped opening—the frontoparietal fontanelle—in the adult skull roof. Ventrolaterally, the orbitosphenoid is reduced and, instead of articulating dorsally with the frontal and parietal to form a rigid bony braincase as is typical of generalized plethodontid salamanders, it is separated from these elements by membrane. Ventral and lateral braincase elements are further modified, resulting in a change in braincase shape (in dorsal view) from a more or less parallel-sided structure, which is typical of larger salamanders, to one that is narrow anteriorly (especially between the prominent eyes) and wide posteriorly. Prominent cartilaginous nasal capsules, which typically are invested by bone in adult plethodontid salamanders, are retained in all adult *Thorius*.

In contrast to the extreme reduction of anterior skull elements, posterior skull elements are relatively well developed. Dorsal and lateral occipito-otic elements articulate solidly with the ventral parasphenoid, forming a vertical bony ring that encircles the rear of the brain stem. Otic capsules are greatly enlarged. The jaw suspensorium, comprising the quadrate and squamosal, descends ventrally from its articulation with the underside of the otic capsule to the lower jaw, instead of the ventrolateral displacement as in larger salamanders; thus it is no longer visible from dorsal view (Fig. 1A). Accompanying the reorientation of the jaw suspensorium is the appearance of a posteriorly directed process or spur on the squamosal. This process forms the site of attachment of the quadropectoralis muscle, the origin of which in *Thorius* has shifted from the quadrate to the squamosal (Tanner, 1952).

Character variation in adults

Premaxilla and maxilla

Anteriorly, the premaxilla consists of a single, narrow, horizontal tooth-bearing segment from which a reduced shelf extends posteriorly to partly underlie the nasal capsule. Unlike the condition seen in most plethodontid salamanders, the premaxilla typically fails to establish direct contact with the maxilla laterally; however, these elements are joined by fibrous connective tissue that is calcified in a few specimens. Variation in the degree of premaxilla-maxilla overlap is common in all species, and ranges from clear separation of the two elements to broad overlap (without direct contact) (Fig. 3); the two elements met in only three of more than 150 specimens examined, and on only one side in each (Table 1). Interspecific differences in the pattern of variation are evident: the premaxilla and maxilla are well separated in nearly all *T. pennatulus*, whereas they overlap in more than half of the *T. narisovalis* sampled; each remaining species shows an intermediate degree of overlap.

Paired ascending processes extend dorsally from the tooth-bearing segment of the premaxilla, bow anteriorly and medially as they curve between the nasal capsules, and continue dorsally and posteriorly to a solid articulation with anterior extensions of the frontals. The processes are separate along their entire

Table 1. Frequency (%) of variation in development of the premaxilla and premaxilla-maxilla overlap in adult *Thorius**

Species	Ascending processes of premaxilla				Premaxilla-maxilla overlap			
	Separate	Articulate, fontanelle large	Fused, fontanelle moderate	Fused, fontanelle small	Separate, no overlap	Slight overlap	Broad overlap	Overlap, articulate
T. pennatulus (N = 20)	90	10	—	—	90	—	10	—
T. macdougalli (N = 20)	10	20	40	30	75	12	8	5
T. minutissimus (N = 18)	55	17	22	6	61	30	6	3
T. schmidti (N = 18)	83	17	—	—	61	28	11	—
T. narisovalis (N = 20)	25	20	35	20	47	16	37	—

* Frequencies calculated separately for right and left sides were averaged to yield a single value in each species.

length in nearly all *T. pennatulus* and *T. schmidti*, but variation in degree of articulation and fusion is common in each of the remaining three species (Fig. 2; Table 1).

Nasal

In adults of larger, generalized plethodontid salamanders, the nasal is a major dorsal bony element in the snout where it occupies most of the area between the premaxilla, maxilla, frontal and prefrontal. This condition is never seen in *Thorius*; instead, the nasal is extremely reduced and invests only a small portion of the cartilaginous nasal capsule (Fig. 4). In *T. pennatulus*, *T. macdougalli* and *T. minutissimus*, the three smallest species considered in the present study, the nasal usually comprises only a bony splint that lies along the posterolateral wall of the nasal capsule between the anterior tip of the frontal (dorsally) and the facial lobe of the maxilla (ventrally); it is absent from a single adult *T. macdougalli* (Table 2). In *T. schmidti* and *T. narisovalis*, two larger species, the nasal extends further anteriorly along the dorsal and lateral surfaces of the nasal capsule (Fig. 1A). This condition was observed only once in any of the three smaller species (*T. macdougalli*).

The nasal is absent from small juvenile specimens of all species but it always develops before sexual maturity (see below). When it first appears, the nasal assumes the thin, rod-like condition (character state b) which is retained in some adults; the broadened condition (character state c) results from continued growth anteriorly. Intrapopulational variation in nasal morphology is evident in all species, and may include all three character states (e.g. *T. macdougalli*).

Nasal development is correlated with the degree of nasal-maxilla overlap (Fig. 4; Table 2). When well developed, the nasal is lengthened ventrally and frequently meets the facial lobe of the maxilla. Thus *T. schmidti*, in which the nasal is relatively well developed, also exhibits the highest frequency of nasal-maxilla articulation. Conversely, when poorly developed the nasal often fails to articulate with the maxilla and remains isolated from all other bony elements on the surface of the nasal capsule, as in *T. minutissimus*. Only *T. pennatulus* is invariant with

Table 2. Frequency (%) of variation in development of the nasal and nasal-maxilla overlap in adult *Thorius**

Species	Nasal development			Nasal-maxilla overlap		
	Absent	Thin, rod-like	Broad, expanded anteriorly	Separate, no overlap	Overlap, articulate	Overlap, fuse
T. pennatulus (*N* = 20)	---	72	28	100	—	—
T. macdougalli (*N* = 20)	5	90	5	75	17	8
T. minutissimus (*N* = 18)	—	75	25	80	14	6
T. schmidti (*N* = 18)	—	11	89	17	83	—
T. narisovalis (*N* = 20)	—	55	45	60	37	3

* Frequencies calculated separately for right and left sides were averaged to yield a single value in each species.

Table 3. Frequency (%) of variation in development of the prefrontal and prefrontal-maxilla overlap in adult *Thorius**

| Species | Prefrontal development | | | | Prefrontal-maxilla overlap | |
	Absent	Separate from nasal	Articulates with nasal	Fused with nasal	Separate	Articulate
T. pennatulus (*N* = 20)	10	67	10	13	100	—
T. macdougalli (*N* = 20)	--	75	25	—	100	—
T. minutissimus (*N* = 18)	—	80	17	3	100	—
T. schmidti (*N* = 18)	—	50	39	11	94	6
T. narisovalis (*N* = 20)	--	57	30	13	95	5

* Frequencies calculated separately for right and left sides were averaged to yield a single value in each species.

respect to nasal-maxilla overlap; three of the remaining four species (*T. macdougalli*, *T. minutissimus* and *T. narisovalis*) each display the full range of adult variation in this character observed in the genus.

Prefrontal

The prefrontal lies immediately behind the nasal at the anterior border of the orbit. It extends dorsomedially from the facial lobe of the maxilla to articulate with the frontal. Like the nasal, the prefrontal is absent from small juveniles in all species. It appears first as a thin rod separate from the nasal; with continued development it will broaden and sometimes meet or even fuse with the nasal. All species demonstrate variation in adult prefrontal morphology (Fig. 4; Table 3). Variation is most extreme in *T. pennatulus*; the 20 adults sampled collectively display the full range of variation seen in the genus. As the prefrontal grows ventrally it approaches the facial lobe of the maxilla. However, unlike nasal-maxilla overlap, which is relatively common, the prefrontal and maxilla were observed to articulate in only single specimens each of *T. schmidti* and *T. narisovalis*.

Septomaxilla

The septomaxilla develops on the surface of the nasal capsule along a restricted part of the posterior rim of the external naris adjacent to the nasolachrymal duct (Fig. 4). Like the nasal and prefrontal, it is absent from small juvenile specimens. The septomaxilla fails to develop in most adults, while in others it may comprise only a speck of calcified tissue. All five species demonstrate variation in adult septomaxilla morphology (Table 4). Septomaxillae are absent in virtually all *T. macdougalli*; they are most common in *T. pennatulus*, yet even in this species nearly two-thirds of the specimens sampled lacked them.

Premaxillary teeth

Premaxillary teeth are absent from most small juvenile specimens but they are present in nearly all adults (Table 4). Tooth number rarely exceeds four or five

Table 4. Frequency (%) of variation in development of the septomaxilla
and premaxillary teeth in adult *Thorius**

Species	Septomaxilla			Premaxillary teeth	
	Absent	Present, small	Present, large	Absent	Present
T. pennatulus (N = 20)	62	3	35	—	100
T. macdougalli (N = 20)	97	3	—	40	60
T. minutissimus (N = 18)	86	8	6	—	100
T. schmidti (N = 18)	89	3	8	—	100
T. narisovalis (N = 20)	85	—	15	5	95

* Frequencies calculated separately for right and left sides were averaged to yield a single value in each species.

per specimen in any species. Adult teeth varied greatly in size; enlarged premaxillary teeth are more frequent in adult males, especially those of larger species such as *T. narisovalis*. In these males, enlarged, ventrally directed premaxillary teeth pierce the upper lip and are visible anterior to the closed mouth (Fig. 2B). *Thorius macdougalli* is the only species in which a moderate proportion of adults (40%) lack premaxillary teeth; absence of teeth is especially common among adult females (Fig. 5). Although all small juveniles lack teeth, presence of teeth in adults of this species is only poorly correlated with body size— both the largest and smallest adults lack teeth.

Maxillary teeth

Thorius schmidti is one of only two species in the genus that have maxillary teeth (Hanken, 1980). Teeth appear prior to sexual maturity and are present in all specimens except the smallest juveniles (SL < 14 mm).
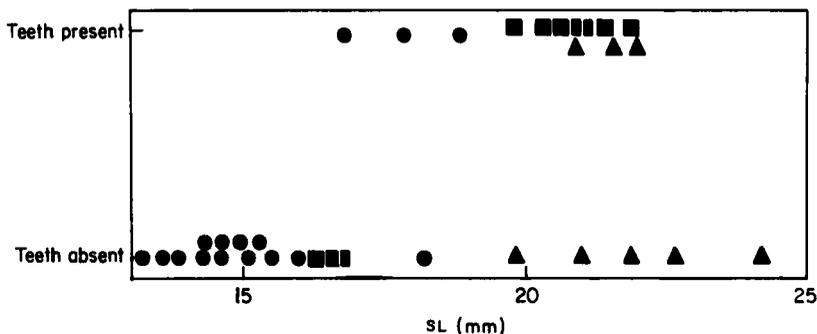

Figure 5. Presence of premaxillary teeth in *Thorius macdougalli*. Teeth are absent from the smallest specimens; they develop before sexual maturity in some specimens but fail to appear in others, including some large adults. Symbols: ●, juvenile; ■, adult male; ▲, adult female.

Asymmetry

Five of the six paired osteological characters (2–7) may show asymmetric development between right and left sides of a given specimen (Table 5). Prefrontal and septomaxilla are most variable; more than one-third of both *T. pennatulus* and *T. narisovalis* are asymmetric for the former, at least one-quarter of both *T. pennatulus* and *T. minutissimus* are asymmetric for the latter. No asymmetry was observed in prefrontal-maxilla overlap (character 6), but this is not surprising in view of the slight variation detected overall (Table 3). Most cases of asymmetric septomaxilla development involve specimens in which the bone is present on one side and absent on the other.

One to five characters may be asymmetric in a given species; septomaxilla is the sole asymmetric character in *T. schmidti*, whereas moderate asymmetry is observed in all five characters in *T. minutissimus*.

Ontogeny of cranial mineralization

The sample of *T. macdougalli* constitutes the most numerous and complete juvenile to adult size series; it therefore was used to determine the posthatching ontogenetic sequence of appearance of mineralized tissues (Fig. 6). Premaxilla, maxilla, parietal, frontal, orbitosphenoid, parasphenoid, vomer, squamosal, quadrate, operculum, occipito-otic series and mandibular elements are present in even the smallest specimen examined ($sL = 13.1$ mm). Remaining elements appear in the following sequence: prefrontal, nasal, premaxillary teeth, septomaxilla (the latter present in only a single adult). Whereas the successive appearance of bony elements is correlated generally with increase in body size (sL), there is substantial variation in the timing of mineralization with respect to body size (Fig. 6). Several juvenile specimens possess both prefrontals and nasals whereas some larger animals including an adult male have prefrontals but lack nasals. Additional variation is contributed by the absence of premaxillary teeth and/or septomaxillae from the largest adults, whereas these elements are present in at least some smaller individuals. In general, mineralization is more extensive

Table 5. Frequency (%) of right-left asymmetry* in five paired osteological characters in adult *Thorius*. No asymmetry was observed in prefrontal-maxilla overlap

Species	Premaxilla-maxilla overlap	Nasal	Nasal-maxilla overlap	Prefrontal	Septomaxilla
T. pennatulus ($\mathcal{N} = 20$)	10	5	—	35	25
T. macdougalli ($\mathcal{N} = 20$)	20		15	5	5
T. minutissimus ($\mathcal{N} = 18$)	17	11	17	11	28
T. schmidti ($\mathcal{N} = 18$)		. .		—	11
T. narisovalis ($\mathcal{N} = 20$)	10		15	35	. .

* Unequal character state scores for the two sides of a given specimen.

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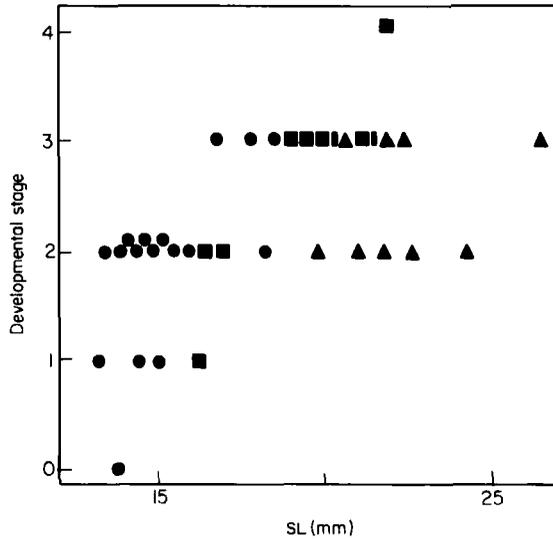
Figure 6. Posthatching ontogeny of cranial mineralization in *Thorius macdougalli*. Developmental stages: 0, prefrontal, nasal, premaxillary teeth, and septomaxilla absent; 1, prefrontal only; 2, prefrontal and nasal; 3, prefrontal, nasal, and premaxillary teeth; 4, prefrontal, nasal, premaxillary teeth, and septomaxilla. Symbols: ●, juvenile; ■, adult male; ▲, adult female. The inferred mineralization sequence is prefrontal, nasal, premaxillary teeth, septomaxilla.

in males; only four of nine adult females have either premaxillary teeth or a septomaxilla, whereas seven of 10 adult males have one or both. Furthermore, the appearance of a given element is delayed (i.e. occurs at a larger size) in females, which typically attain a larger adult body size than males.

Mineralization sequences of *T. narisovalis*, *T. minutissimus* and *T. pennatulus* appear to be similar to that of *T. macdougalli*, though the frequency of the septomaxilla is greater in these species (Table 4). *Thorius schmidti* is exceptional among the five species considered in this study in that all adults and many juveniles have maxillary teeth, yet the septomaxilla is as rare in this species as in the other four. Assuming that the infrequent presence of a septomaxilla indicates that this element is the last to appear in the ontogeny of this species, the posthatching mineralization sequence of *T. schmidti* differs from that of *T. macdougalli* and the remaining species such that maxillary teeth appear before the septomaxilla (Fig. 7 and see below).

DISCUSSION

Consequences of size decrease in Thorius

The skull of *Thorius* is highly derived. Three general characteristics are apparent: reduction, variability and morphological novelty.

Reduction

Many skull elements commonly found in morphologically generalized plethodontid salamanders (e.g. *Pseudoeurycea*) are greatly reduced or absent altogether in *Thorius*. Moreover, the adult morphology of several elements closely
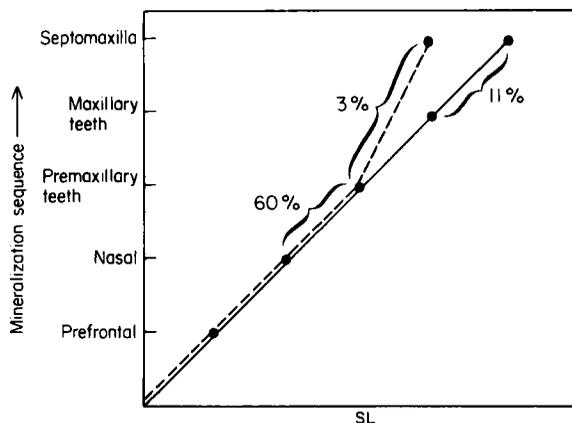
Figure 7. Posthatching mineralization sequence in *Thorius schmidti* (——) and *T. macdougalli* (– – –). All adults of *T. schmidti* have maxillary teeth; only some adults have one or both septomaxillae, which presumably appear last. The sequence of *T. macdougalli* (and most other species) is different: maxillary teeth fail to develop, but septomaxillae are found in a few adult specimens. Premaxillary teeth also are absent in many adult *T. macdougalli*. For illustration purposes, sequences are standardized to the same body size coordinates; limited samples prevent precise correlation of mineralization events with a given body size for either species (see text).

resembles that seen in juveniles in other genera. For example, the characteristic and usually predominant frontoparietal fontanelle in *Thorius* may be ascribed to a failure of the paired frontals and parietals to abut medially before skeletal growth ceases at sexual maturity; frontoparietal fontanelles are typical of early ontogenetic stages in many amphibians (Alberch & Alberch, 1981; Bonebrake & Brandon, 1971; Wake & Hanken, 1982; Wake *et al.*, 1983). Similarly, adult configurations of the nasal and prefrontal result from failure of these elements to invest the cartilaginous nasal capsules during ontogeny. In this way, typical plethodontid skull development is truncated precociously in *Thorius*. This confirms the interpretations of Cope (1869, 1889) and Wake (1966) that the adult skeletal morphology of *Thorius* is largely a result of paedomorphosis (*sensu* Gould, 1977). Paedomorphosis has been implicated in skull evolution in other plethodontid salamander genera. However, extreme skeletal reduction as is the norm in *Thorius* is approached only by relatively few species of the genera *Batrachoseps*, *Bolitoglossa*, *Chiropterotriton* and *Oedipina* (Alberch, 1983; Alberch & Alberch, 1981; Marlow *et al.*, 1979; Wake, 1966; Wake & Elias, 1983).

Through reduction or loss of many bony elements, the skull in effect has shrunken around the now predominant brain and special sense organs (Hanken, 1983b). This is most evident anteriorly. As a result of their reduced size many individual elements, such as the nasal, prefrontal, and septomaxilla, typically fail to articulate with any other, even in adults; apparently they contribute little to skull support or rigidity. In the case of the nasal and septomaxilla, their structural role in the adult skull may have been usurped by the prominent cartilaginous nasal capsule, on the surface of which these elements remain isolated and which they would normally—in larger, more generalized salamanders—invest during ontogeny. Similarly, the widely separated frontals and parietals provide little protection to the brain dorsally. The upper, outer dental arcade in most species is reduced to two or three median premaxillary teeth below the snout; the

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premaxilla, and especially the typically edentulous maxilla, obviously can have little direct role in either prey capture or prey processing. This is supported by the functional analysis of Lombard & Wake (1977) who described the elaborate tongue projection mechanism that is characteristic of all neotropical plethodontid salamanders, including *Thorius*, and which is used to capture prey at relatively great distance from the mouth.

Cranial skeletal reduction is less extreme posteriorly. The contrast between anterior and posterior regions is particularly conspicuous in small juveniles, which may lack the septomaxilla, nasal and prefrontal, yet possess the full adult complement of rear skull elements. In plethodontid salamanders, rear skull elements mineralize earlier in ontogeny than anterior elements (Wake, 1966; Wilder, 1925). Consequently, the greater tendency toward reduction and loss displayed by anterior elements also may be attributed to paedomorphosis, which would be expected to effect more pronounced alterations on late-appearing structures than on those that appear relatively early. However, in the absence of functional analysis of skull structure it is not possible to exclude possible mechanical explanations of the more complete ossification in the rear of the skull.

Species are characterized by differing degrees of truncated development. In *T. macdougalli*, for example, nasal development typically ceases before it contacts the maxilla; the prefrontal usually is separate from the nasal and always is separate from the maxilla; premaxillary teeth commonly are absent in adults; and maxillary teeth are lacking entirely (Tables 2–4). In *T. schmidti*, on the other hand, the nasal typically is broad and articulates with the maxilla; the prefrontal commonly articulates or even fuses with the nasal; and premaxillary and maxillary teeth are present in all adults. Individuals of different species may closely resemble each other in certain aspects of skull morphology because of often extensive intraspecific variation (see below); nevertheless, interspecific differences in adult cranial skeletal morphology are evident. There is, however, no consistent association between relative degree of paedomorphosis and relative mean adult body size in interspecific comparisons; species of *Thorius* do not form a simple, linear array of progressively smaller adults with correspondingly greater degrees of cranial reduction.

Mineralization sequence data provide further demonstration of this pattern of differential paedomorphosis. Elements that appear late—septomaxilla, premaxillary and maxillary teeth—exhibit greater variation among species in terms of presence/absence than do those elements that appear earlier in ontogeny (Tables 2–4). This is expected if species differ in the extent to which they complete the ancestral mineralization sequence.

Presence of maxillary teeth is a primitive feature in *Thorius* based on outgroup comparisons to other plethodontid genera (Wake, 1966). The mineralization sequence of *T. schmidti*, adults of which all have maxillary teeth, thus may resemble the ancestral sequence more closely than that of species that lack maxillary teeth. In *T. schmidti* one or both septomaxillae are found in only 11% of adult specimens (Table 4), which suggests that maxillary teeth appear prior to the septomaxilla in this species (see Results). There are at least two possible mechanisms of achieving maxillary tooth loss in most remaining species, all of which retain the septomaxilla at low frequency. First, deletion of maxillary teeth from the ancestral mineralization sequence while retaining normal timing of septomaxilla formation. Second, reversal of the relative time of appearance of the

septomaxilla and maxillary teeth such that cranial mineralization, which is truncated precociously, ceases prior to maxillary tooth formation. Additional information that compares the timing of cranial mineralization among several species is required to evaluate these alternatives.

Variability

All species of *Thorius* demonstrate extensive variation in adult cranial characters (Tables 1–5). Moreover, as each species sample was collected from a restricted geographic area (see Materials and methods), observed levels of variation reflect the extent of local (intrapopulational) variation in each species. Quantitative estimates of cranial skeletal variation in natural populations of amphibians generally, and salamanders in particular, are rare; therefore it is not possible to present direct, statistically testable comparisons between *Thorius* and other genera (see discussion of this problem and presentation of quantitative data for anurans in Trueb, 1977). Nevertheless, based on qualitative comparisons with skeletal variation described for other plethodontid genera, variation seen in several characters in many species of *Thorius* must be considered extreme. In three characters—premaxilla development, nasal-maxilla overlap, prefrontal development—the range of intrapopulational variation in a given species of *Thorius* may nearly match the range of variation of each character in other plethodontid genera (Wake, 1966). Variation in other characters, though extensive, is not unique. The septomaxilla, for instance, is highly variable in several species, for example *T. pennatulus* (Table 4), yet variation of this magnitude is common in the neotropical plethodontid genera *Chiropterotriton* (Lynch & Wake, 1975, 1978) and *Bolitoglossa* (Alberch, 1983; Wake, 1966).

It is difficult to postulate any direct selective factor that would establish and maintain such variation, especially asymmetry. Instead, and as was observed in the appendicular skeleton (Hanken, 1982b), increased variability in *Thorius* seems to represent a 'by-product' of skeletal reduction. Indeed, it may reflect a relaxation of functional constraints that serve to stabilize the morphology of individual elements in skulls of larger salamanders. The poorly developed ossified elements in *Thorius*, which seemingly play only a minor functional role in the miniaturized skull, in effect are free to accumulate variation that is not restricted by normalizing selection for anatomical and functional integration.

Morphological novelty

Several cranial features are unique to *Thorius* and are derived with respect to more generalized skulls of most larger plethodontid salamanders (Fig. 1).

(1) Vertical orientation of the jaw suspensorium. Ventrolateral orientation of the jaw suspensorium from its origin on the ventral surface of the otic capsule is typical for larger plethodontid salamanders; a fully vertical orientation is seen only in *Thorius* (juvenile and adult), though it is approached in adults of smaller species of several genera (e.g. *Chiropterotriton*) and in juveniles of many larger species.

(2) Posteriorly directed squamosal process or spur. While typical of *Thorius*, the process is virtually unknown in other plethodontid salamanders; Wake (1966) reported its presence elsewhere in only a single, large, adult *Oedipina complex*.

(3) Anteriorly constricted braincase. This represents concerted size and shape change of several cranial elements which together comprise the roughly parallel-sided structure of other genera.

Novel features also characterize a number of other functional systems in the head and postcranial skeleton of *Thorius*. Lombard & Wake (1977) described two extreme modifications of the hyoid skeleton and musculature: disarticulation of the first ceratobranchial and basibranchial during tongue projection to form a linear array of the hyobranchial apparatus, and novel arrangement of the rectus cervicis profundus muscle. Wake (1970) described the unique vertebral morphology which distinguishes *Thorius* from all other plethodontid genera. Uzzell (1961) and Hanken (1982b) noted excessive mineralization of the adult limb skeleton, including ossification of the characteristically (in larger salamanders) cartilaginous mesopodial elements and long bone epiphyses, which is a component of a novel pattern of overall determinate skeletal growth that is coincident with sexual maturity. Hanken (1982a, b) also reported the presence of numerous unique carpal and tarsal patterns. Grunwald (1981) and Roth (pers. comm.) have identified unique neuroanatomical configurations, including both gross brain morphology and neuronal dendrite arborization pattern in the optic tectum. Thus morphological novelty is the rule, rather than the exception, in *Thorius*.

Unlike skeletal reduction, the presence of most novel morphological features in *Thorius* may not be explained simply as a result of truncated development and the retention of 'juvenile' traits; many features are seen in no other plethodontid salamander, adult or juvenile, including those of comparable size. [Cope (1889: 124) recognized this nearly a century ago, and cited the skeletal anatomy of *Thorius* as an example of "inexact parallelism"; that is, a heterogeneous assemblage of paedomorphic and derived traits.] Many authors have stressed the importance of scaling effects whereby structural reorganization accompanying size change is necessary to maintain functional efficiency (e.g. Gould, 1975; Pilbeam & Gould, 1974; Schmidt-Nielsen, 1975). Accordingly, in studies of many vertebrate lineages, including groups as diverse as the plethodontid salamander genus *Chiropterotriton* (Rabb, 1960; Wake, 1966) and amphisbaenid reptiles (Gans, 1974), novel morphological features that are associated with the evolution of decreased size have been interpreted as adaptations that compensate for the presumed reduced mechanical efficiency of other structures due to their decreased size. This functional explanation may also hold for the derived cranial morphology of *Thorius*, but it need not be the sole, or even the primary, factor responsible for the appearance of the novel features. Instead, many novel structures and arrangements appear to represent only secondary consequences of physical rearrangements effected by the prominent brain, otic capsules, eyes and nasal capsules during head development at reduced size (Hanken, 1983b). This alternative hypothesis is not incompatible with the idea of mechanical compensation, as the imposed rearrangements must still function. Instead, it only provides an additional way in which pronounced size decrease may effect—or in a sense demand—structural modification and the appearance of morphological novelty.

A null hypothesis of miniaturization

Lauder (1981: 430) urged the use of "emergent organizational properties of structural and functional systems" in analyses of morphological evolution. He stressed particularly the need to identify null hypotheses of morphological change

in a lineage in order to distinguish truly unique attributes from more general trends. In *Thorius*, three general features of cranial skeletal morphology are associated with the evolution of decreased body size. (1) Precocious truncation of development relative to the presumed ancestral ontogeny which produces a reduced, paedomorphic morphology. (2) Increased variability—interspecifically, intrapopulationally, and within individuals. (3) Morphological novelty. These features also are characteristic of postcranial skeletal morphology in *Thorius* (see references above). I therefore propose these features as a null hypothesis for the morphology of miniaturized taxa.

This null hypothesis predicts general features of the morphology of dwarfed taxa—predictions which are observed in many lineages of vertebrates and invertebrates. Extreme reduction of the ossified skeleton is characteristic of many species of dwarfed fishes (Balon, 1980; Fink, 1981; Roberts, 1981), frogs (Tyler *et al.*, 1981), and several genera of plethodontid salamanders (Alberch, 1981; Alberch & Alberch, 1981; Wake, 1966); in each case, paedomorphosis has been implicated as the mechanism responsible for size decrease. Few studies have compared levels of morphological variation of dwarfed taxa with that of ancestors. Marshall & Corruccini (1978), however, reported increased variation of several molar tooth dimensions as having accompanied dwarfing in three lineages of Australian marsupials, and Alberch (1983) documented extensive intraspecific polymorphism for prefrontal development in several small species of the neotropical plethodontid genus *Bolitoglossa*. Lastly, novel skeletal morphology has been observed repeatedly in lineages of dwarfed fish (Roberts, 1981) and anurans (Grandison, 1981; Griffiths, 1959; Lynch & Ruiz-Carranza, 1982) (see also earlier references to invertebrates).

A second strength of this null hypothesis lies in its ability to evaluate alternate mechanisms of morphological change. Parallel morphological evolution in related groups may be explained as a consequence of their sharing common developmental pathways which are truncated in an analogous manner, albeit independently. In the case of closely related taxa, parallelism may even be *expected*. This is observed repeatedly in the Plethodontidae; species of small adult body size in several genera, e.g. *Chiropterotriton* (Rabb, 1960) and *Bolitoglossa* (Alberch & Alberch, 1981; Alberch, 1983; Wake, 1966), evince recurrent trends of reduced cranial ossification that are analogous to that in *Thorius*. Derived features that are characteristic of these species include absence of the septomaxilla, prefrontal and maxillary teeth; diminution of the nasal, frontal and parietal; and a prominent frontoparietal fontanelle. In those dwarfed taxa in which these predictions are not met, paedomorphosis as a possible mechanism of size decrease therefore is unlikely and an alternate mechanism must be sought (e.g. proportioned dwarfism—Gould, 1977). An example of such a taxon may be the plethodontid genus *Parvimolge*, the adult skeleton of which is not characterized by extreme reduction or novelty relative to larger salamanders (Wake, 1966, and pers. comm.); the developmental mechanism by which size decrease has evolved in *Parvimolge* remains poorly known.

This view of miniaturization unites as a general trend phenomena that recur in many lineages. However, the particular array of reduced, variable, and novel structures obviously will differ among lineages, reflecting the differing functional and developmental constraints that impinge on morphological evolution in these groups. In *Thorius*, many reduced, variable or novel

morphological features in the cranium do not appear to be the primary or direct focus of selection. Instead, they may be only indirect consequences of adult size decrease. The primary focus of selection may be a particular ecological or life history attribute (e.g. decreased age at sexual maturity) that is closely tied to body size. [Alberch (1983) proposed a similar explanation for cranial reduction and increased variability that have accompanied size decrease in *Bolitoglossa*.] Knowledge of the role of natural selection in promoting analogous changes in other groups, together with identification of the developmental mechanisms responsible for them, will further our understanding of the importance of phylogenetic size decrease to the evolution of morphological diversity.

Taxonomic implications

For a long time following the original description of *Thorius* by Cope (1869), the combination of reduced and novel skeletal traits obscured phylogenetic affinities of the genus *vis-à-vis* other urodeles; Cope (1869, 1889) referred *Thorius* to the monotypic family Thoriidae, and as recently as 1944, Taylor (1944) placed it in the subfamily Thoriinae, separate from all other plethodontid genera. Now, however, *Thorius* is recognized as a *bona fide* member of the neotropical assemblage of plethodontid salamanders of the tribe Bolitoglossini (Wake, 1966; Wake & Elias, 1983).

Systematics within the genus have been equally confusing. Early taxonomic workers were unable to identify unambiguous morphological characters that reliably distinguish species, hence they perceived a general lack of substantial morphological differentiation in *Thorius* (discussed in Hanken, 1983a). In addition, formal descriptions of the nine described species have been difficult to apply to identification of preserved specimens (Wake & Lynch, 1976). Detailed consideration of several cranial characters in this study revealed often pronounced morphological differences among five species. However, extensive intraspecific variation in most characters (Tables 1–4) limits the discriminatory ability of any one by itself. Nasal development and nasal-maxilla overlap each provide nearly complete separation of some species pairs (e.g. *T. schmidti v. T. macdougalli*); other characters, such as septomaxilla development, discriminate poorly. Only one character, maxillary teeth, provides unequivocal discrimination among species and only in distinguishing *T. schmidti* from the remaining four. Cranial osteology, including the full range of inter- and intraspecific variation, thus likely will be of value to future analyses of the systematics of *Thorius*, but especially when used with an independent means of assessing taxonomic identity and phylogenetic relationship, such as electrophoresis (Hanken, 1980, 1983a).

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APPENDIX 1

Specimens examined and localities*

Thorius pennatulus
   Mexico: Prov. Veracruz: Cerro Chicahuaxtla, Cuautlalpam; M. 2803, M 2834, M 5111–5118, M 5120, M 5143, M 5143a, MVZ 85733, MVZ 85737, MVZ 85742, MVZ 85749, MVZ 85829, MVZ 85835.
   Mexico: Prov. Veracruz: 1.4 mi SW Huatusco, MVZ 84850.

Thorius macdougalli
   Mexico: Prov. Oaxaca: Cerro Pelon, 18–51 km N Guelatao along Mexican Hwy. 175; M 3268, M 3271, M 3282, M 3285, M 3313–3315, M 3317, M 3322, M 3323, M 3329, M 3331, M 3334–3336, M 3365, M 3366, M 3368–3371, M 3379, M 3380, M 3382, M 3460, M 3465, M 4911, M 4934, M 4935, M 4940, M 4941, M 4969, M 4975, M 5022, M 5025, M 5059, M 5069, M 5073.

Thorius minutissimus
   Mexico: Prov. Oaxaca: 18.5 km S Sola de Vega: M 4570, M 4571, M 4573–4578, M 4580, M 4582, M 4583, M 4585, M 4587–4590, M 4594, M 4596, M 4607, M 4608, M 4621, M 4628–4630, M 4632, M 4653, M 4657, M 4658, M 4660b.

Thorius schmidti
   Mexico: Prov. Veracruz: Volcan Orizaba, 1.5 km W Texmola; M 3012, M 3024.
   Mexico: Prov. Veracruz: Volcan Orizaba, 1.5 km SE El Berro; M 3052, M 3053, M 3055–3057, M 3062–3065, M 3067, M 3070–3076, M 3078, M 3080.
   Mexico: Prov. Veracruz: Volcan Orizaba, Xometla: M 5173, M 5176, M 5181, M 5185, M 5187, M 5190, M 5192, M 5207, M 5215.

Thorius narisovalis
   Mexico: Prov. Oaxaca: Cerra San Felipe, 4 km W LA Cumbre; M 3471, M 3473, M 3476, M 3477, M 3479 M 4515, M 4517–4521, M 4523, M 4524, M 4526–4528, M 4544, JH 787–791.
   Mexico: Prov. Oaxaca: Cerro San Felipe, 9 km W La Cumbre; M 3489, M 4443, M 4444, M 4449, M 4453–4457.
   Mexico: Prov. Oaxaca: Cerro San Felipe, 15 km W La Cumbre; M 3554, M 3558, M 3560, M 3572.

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*MVZ = Museum of Vertebrate Zoology, University of California, Berkeley; M = MVZ Mexican field tag series; JH = James Hanken field tag series. All specimens are or will be deposited in the permanent MVZ collection.