

(Bakermann, 1965) or whether it is a mechanism to improve oxygen consumption.

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### Varanoid-Like Dentition in Primitive Snakes (Madsoiidae)

JOHN D. SCANLON<sup>1</sup>, Department of Palaeontology, South Australian Museum, North terrace, Adelaide, South Australia 5000, Australia  
MICHAEL S. Y. LEH, Department of Zoology, University of Queensland, St. Lucia, Queensland 4072, Australia

Several recent studies (Estes et al., 1988; Schwenn, 1988; Cooper, 1997; Lee, 1998; Lee and Caldwell, 2000) have suggested that snakes are related to anguilliform lizards and, in particular, are nested within varanoids. However, there are characters that contradict this arrangement. For instance, the teeth of all varanoid lizards exhibit a distinctive infolding of dentine and enamel near the base of the tooth crown. This results in a characteristic "fluted" appearance with regular vertical grooves and ridges around the entire circumference of the tooth base (Boulle, 1942). The occurrence of this "plicidentine" has previously been interpreted as a derived character uniting varanoid lizards as a monophyletic group, to the exclusion of other squamates such as snakes (Freggi et al., 1986; Estes et al., 1988; Lee, 1998). It characterizes all extant varanoid lizards (*Varanus*, *Lanthanotus*, and *Plicodontia*) and extinct terrestrial forms such as *Estesia*.

Until recently, plicidentine has not been observed in nonvaranoid lizards, apart from a weak development in some extinct anguillids and necrosaurids, which are closely related to varanoids (Estes et al., 1988). It is also absent in amphisbaenians and has been regarded as absent in all primitive living snakes (e.g., Estes et al., 1988). However, an enigmatic Eocene snake (*Atractaspis*) and some advanced snakes (caenophidians) possess externally fluted or grooved teeth, which are superficially similar to those of varanoids (Jensen, 1906; Boyett, 1943; Klaber, 1956; Vach, et al., 1985). Nevertheless, despite these occurrences, the apparent absence of plicidentine in more basal snakes (sceloporphids and "anilioids") has led to the widespread assumption that it is primitively absent in snakes. Here we describe jaw fragments of two species of primitive snakes (from the extinct family Madsoiidae) that clearly exhibit varanoid-like plicidentine. These observations, together with other recent studies of primitive snakes (see later), suggest that plicidentine

<sup>1</sup> Corresponding Author. E-mail: scanlon.john@sa.gov.au

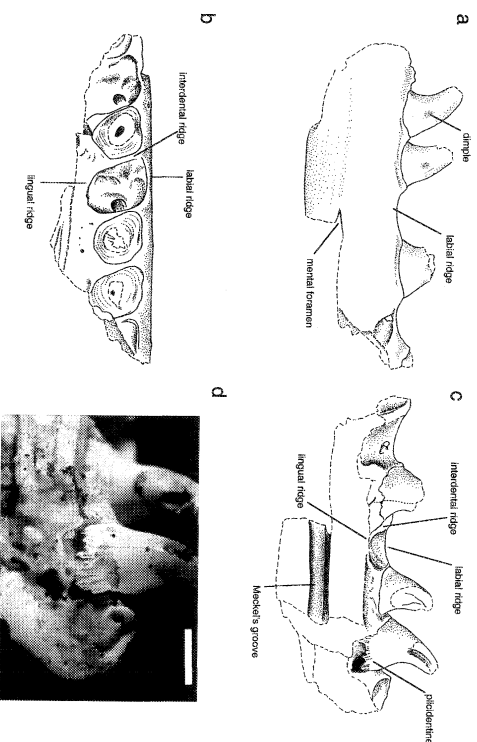


FIG. 1. Right dentary fragment of *Yurilingus* sp. QMF 36441. (a) Lateral view; (b) dorsal view; (c) medial view; (d) posteroventral view showing zone of infolding (plicidentine) around base of tooth undergoing resorption. Fragments adhering to surface of tooth at upper edge of folded zone indicate extent of bone of attachment. Scale = 5 mm.

is widespread in basal snakes and, therefore, cannot be used to exclude snakes from Varanoidae. It also supports the view that madsoiids are very primitive snakes rather than close relatives of pythons (McDowall, 1987; Scanlon and Lee, 2000; contra Underwood, 1976; Rage, 1994).

The first specimen (Queensland Museum Palaeontology, QMF 36441) is from RV Site, Godthelp Hill, Riversleigh, northwestern Queensland, Australia ("Tertiary System B," Late Oligocene or Early Miocene; Archer et al., 1997). It is part of the right dentary of a large snake (Fig. 1). No other snake material has yet been identified from RV Site, but vertebrae of the madsoiid *Yurilingus* are known from contemporaneous nearby deposits at Riversleigh (e.g., CS, MM, and DT Sites; Scanlon, 1996). The relatively wide and shallow cross-section of the bone (Fig. 1a-c) resembles dentary material of the madsoiids *Yurilingus* and *Moumbi*. It is distinct from jaws of pythons (the only other large snakes present in the Australian Tertiary), which are deeper in proportion to width. Furthermore, the size of the dentary matches the vertebrae of *Yurilingus*. The preserved fragment, with five complete and two incomplete alveoli, is 31.5 mm in length. Complete dentaries of Australian madsoiids have 17 to 25 alveoli (Barrie, 1990; Scanlon, 1997). Thus, the total length of the dentary was probably around 80 to 130 mm. Dentaries of madsoiids represent slightly more than half the total mandible length (e.g., Barrie, 1990; Scanlon, 1997). The mandible (and thus head) length of this snake can therefore be estimated as be-

tween 150 and 250 mm. This is consistent with the size of the vertebral elements of *Yurilingus*, which indicate a snake approximately 6 m long; other Riversleigh madsoiid taxa attain less than half these dimensions. *Moumbi barriei* is present in some of these sites (Scanlon, 1996; Scanlon and Lee, 2000), but based on its vertebral dimensions, and associated cranial and postcranial elements of *W. muircoensis* in Pleistocene deposits (Barrie, 1990; Scanlon and Lee, 2000), mandible length in this species would not have exceeded 80 mm. On these criteria, the specimen is assigned to *Yurilingus* sp. indet.

This fragment comprises approximately the middle third of the dentary, bearing five complete alveoli and portions of two others, one at each end. Teeth remain ankylotized to the second, fourth, and fifth complete alveoli (counting anteroposteriorly), with the relative "alternating" replacement mode typical of varanoids and snakes (Edmund, 1969). All tooth crowns are broken distally, the posteriormost being the most complete. Part of a lateral (external) foramen is preserved below the third complete alveolus, continuous with the mandibular canal, which is exposed medially by breakage. Tooth bases and empty alveoli are roughly trapezoidal in horizontal section, with a relatively sharp (acute) angle anterolaterally (proximolabially) and maximum diameter of approximately 6.5 mm. There is a shallow dimple-like concavity on the labial (lateral) surface of each tooth (Fig. 1a), which has not been observed in other material. The teeth are ankyl-

labeled by bone of attachment to the inner margins of shallow alveoli, as in most snakes, in other squamates, alveoli are either absent (amphisbaenians, most lizards) or are very deep and occupied by large bony pedestals underneath the tooth crowns (mosasauroids). Zaher and Rieppel (1999) have recently questioned the presence of discrete alveoli in mosasauroids, stating that their teeth are "in basal contact with one another" (p. 6, repeated on p. 12) and "not located in discrete sockets" (p. 12). However, mosasauroid teeth are typically not in basal contact with each other but are separated by ridges. These ridges are very high histologically continuous with the rest of the tooth-bearing element and persist even after the teeth are shed (e.g., see their fig. 3 and photographs in Langham-Schlar and Noll, 1999; Langham-Schlar, 1994), indicating they are true interdental ridges dividing the dental groove into discrete alveoli, rather than residual bone of attachment (M. W. Caldwell, L. A. Budney, and D. O. Lamoureux, 2000, unpubl.).

Interpretation of the element as representing the middle of the dentary is based on comparison with the complete dentary of *Yurlinggur* (Scanlon, 1996, 1997). The tooth row and lateral margin of the fragment are nearly straight in dorsal view, whereas noticeable curvature would be expected near the posterior (distal) and anterior (mesial) ends of the element. The dentary is deeper than the length of the teeth in the region of the preserved fragment, which again precludes an anterior position. There is also no obvious size gradient, nor any sign of the posterior lateral fossa of the dentary. Alveoli size increases anteriorly and decreases posteriorly in *Yurlinggur* (Scanlon, 1997) but remains relatively constant in the middle of the dentary. The presence of the mental foramen does not imply a more anterior position, as up to three well-spaced mental foramina occur in madisoid dentaries (Hofstetter, 1960; Scanlon, 1997; Rege, 1998).

As in most snakes, the preserved teeth taper sharply from the base and are strongly recurved posteriorly. No indications of canines are visible on the anterolateral or posteromedial surfaces of the preserved sections of the teeth. However, comparison with complete teeth of madisoids (Barrie, 1990; Scanlon, 1996, 1997) and other snakes suggests that these ridges were present on the missing distal parts.

The posterior break through the alveolar margin of the dentary exposes the base of the last tooth. This tooth was undergoing resorption of ankylosis, as indicated by a narrow fissure on the occlusal surface between the tooth and its surrounding bone of attachment, a large lacuna within the alveolus extending above and below the tooth base, and the tapering and irregular margin of the base of the tooth projecting freely into this lacuna. The basal part of the tooth clearly shows parallel vertical ridges and grooves formed by infolding of the dentine and enamel (Fig. 1c-d).

A more anterior break through the tooth in the second complete alveolus shows the same fluted structure in internal view (schematic view, Fig. 2). Thus, as in "true" plicidentine, the infolding affects both the external and internal surfaces. This tooth is at an earlier stage in its attachment cycle. There is a much smaller lacuna within the alveolus, and the tooth base

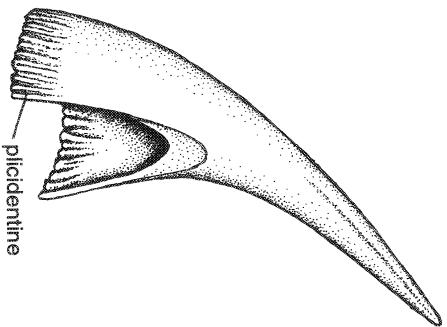


FIG. 2. A reconstruction of a madisoid tooth with plicidentine, based on specimens shown in Figures 1 and 3, and additional material discussed in the text.

is much thicker and in extensive contact with the bone of attachment.

This snake thus possessed well developed plicidentine, but it is largely restricted to the part of the tooth normally hidden within the alveolus and completely covered by bone of attachment. The observation of plicidentine in ankylosed teeth, in this case, has depended largely on fortuitous breaks exposing the basal portion of the teeth.

A second madisoid specimen is known in which the basal part of an ankylosed tooth is exposed by breakage, and also displays plicidentine. This is the posterior part of a left maxilla of the Pleistocene species *Womihia namacorrinis* (Flinders University, FL 1762) and comes from the type locality, Victoria fossil Cave, Naracoorte, South Australia. *Womihia namacorrinis* is the only large snake known from the deposit (dapsids of the extant genera *Pseudochis*, *Pseudonaja*, and *Notechis* are also abundantly present, but much smaller; Smith, 1976; JDS, pers. obs.). Comparison with complete maxillae of *W. namacorrinis* from Henschke's Cave, Naracoorte (Barrie, 1990; Scanlon and Lee, 2000) reveals an almost perfect match, and further supports referral to this taxon.

The specimen is 50 mm long and comprises the posterior end of the left maxilla (Fig. 3a). The anterior three alveoli are only partly preserved because of an oblique fracture, the remaining 10 alveoli are complete. Complete maxillae of two *Womihia* individuals from Henschke's Cave, Naracoorte (Barrie, 1990; Scanlon, 1996) are 70 and 81 mm long, with 23 and 22 alveoli, respectively. Comparison suggests a total maxilla length of about 97 mm for the present specimen, and mandible length of 160 mm (cf. figures in Barrie, 1990).

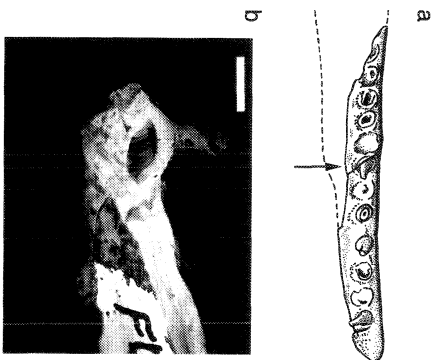


FIG. 3. Left maxilla fragment of *Womihia namacorrinis*, FL 1762. (a) Ventral (occlusal) view of whole specimen (scale = 10 mm), with arrow indicating oblique break. (b) Medial view of broken surface indicated by line in (a), showing zone of infolding (plicidentine) on internal surface of broken tooth (scale = 3 mm).

Scanlon and Lee, 2000). This size difference is consistent with the observation that the largest known vertebrae from Victoria Cave are also somewhat larger than those from Henschke's Cave (Smith, 1976; Barrie, 1990; JDS, pers. obs.). The medial edge is damaged except for the region adjacent to the four most posterior alveoli, and posterior to the last alveolus where the surface is sculptured, apparently for tendinous muscle insertion (m. *pietygoldensis*). Another fresh transverse break passes obliquely through the base of a firmly ankylosed tooth (seventh from anterior end). The medial internal view of the tooth base (Fig. 3b) shows infolding similar to the *Yurlinggur* specimen.

Resorption of part of the tooth base before replacement is typical of most squamates, including snakes (Edmund, 1969). That plicidentine has been observed only in ankylosed teeth of madisoids, and not in any of the numerous isolated (shed) teeth found at Riversleigh and Naracoorte, suggests that the basal (plicidentine) portion of the tooth is usually completely resorbed before shedding. If such a pattern were widespread among snakes, including recent forms, the occurrence of basal zone plicidentine (mostly hidden within the alveolus and resorbed before teeth are shed) could easily have escaped notice. The possibility that plicidentine is present but unrecorded in many extant snakes is suggested by the observation that plicidentine-like infolding is clearly visible on the flanges of some dapsids, which are unusual in that their alveoli are not completed by bone posteriorly (Bogert,

1943; pers. obs.). This infolded basal part, visible when ankylosed, is resorbed before the flange is shed.

The distribution of plicidentine in basal snakes is of interest. It was originally proposed that pachyophidids, that is, *Pachyophis* and *Pachyphidius*, must be outside of all other snakes (Haas, 1979, 1980a,b). Haas noted that pachyophidids displayed a curious mosaic of snake-like and varanoid-like features, although he was unsure whether they were truly related to snakes, or were convergent, he was certain that the primitive (varanoid-like) features indicated they were not nested within extant snakes. Zaher and Rieppel (1999) and Theron et al. (2000) have recently challenged Haas' conclusion and instead suggested that pachyophidids are advanced (macrosaurian) snakes. However, a more comprehensive recent study of snake relationships confirms that pachyophidids are basal to all other snakes, other basal lineages of snakes identified include madisoids, *Dinilysia* and scolecophidians (Scanlon and Lee, 2000; for review, see Coates and Ruta, 2000). Pachyophidids possess weakly infolded teeth, at least on the external surfaces (Lee and Caldwell, 1998); these were identified in the original descriptions but interpreted as possible venom grooves (Haas, 1979, 1980a). The present study reveals that madisoids possessed plicidentine. Only a few teeth are preserved in *Dinilysia*, at the posterior end of the maxilla (Estes et al., 1970), and the condition in this taxon cannot be ascertained. Scolecophidians, however, lack plicidentine. Both species of *Atelapha*, which also appear to be rather basal snakes (Scanlon, 1996), also have multiple ridges and grooves on the tooth crowns (Janney, 1996; Janney, 1998) resembling plicidentine. The presence of plicidentine in *Pachyphidius*, madisoids, and *Atelapha* means that it is probably primitive for snakes. Thus, absence of plicidentine can no longer be used as a character excluding snakes from Varanoida. If recent studies indicating that snakes are nested within varanoids are correct (e.g., Schwen, 1986; Cooper, 1997; Lee and Caldwell, 2000), the most parsimonious assumption is that plicidentine evolved once at the base of Varanoida and that its presence in basal snakes represents direct retention of the varanoid condition (Fig. 4). However, plicidentine is absent in almost all modern snakes (i.e., scolecophidians, anilioids, booids, and caenophidians) surveyed for this study (see Appendix 1); the exception was the caenophidian *Cerberus*. A few occurrences have been noted in various other caenophidian lineages (Bogert, 1943; Klumb, 1956; Voth et al., 1983). Although a more thorough survey is required, the most parsimonious interpretation is that plicidentine was lost at the base of a modern snakes, and its presence within caenophidians is a re-acquisition.

A possible reason for loss of plicidentine at the base of modern snakes involves allometry and plesiomorphic Varanoid lizard *Pachyphidius*, madisoids, and *Atelapha* are moderately to extremely large, and possess plicidentine. However, basal lineages of modern snakes (scolecophidians and anilioids) are all much smaller. The amount of infolding is highly size-dependent in extant "labyrinthodont" amphibians (e.g., Bystrow, 1936; Warren and Davey, 1992; A. Miller, pers. comm.) and living varanoids (MSL, pers. obs.). Teeth with large have more pronounced infoldings, and this trend occurs both within and be-

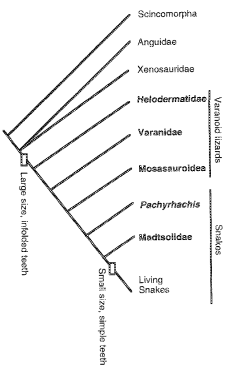


FIG. 4. The distribution of plicidentine in varanoids and primitive snakes, and its phylogenetic implications. The taxa with plicidentine are shown in bold. Taxa without plicidentine are shown in normal font. As discussed in the text, the condition in the primitive snake *Dinilysia* is not known, whereas the position of the fossil snake *Archaeophis* is not yet clear: neither is shown on the diagram. The "lizard" portion of this tree is based on Schwenk (1998) and Lee (1998); the snake portion is based on a recent phylogenetic analysis of 224 morphological characters in all the major fossil and living lineages of snakes (Scanlon and Lee 2000). The position of Pachyophis outside of all other snakes is provisionally accepted (Hase 1979, 1986a,b; Scanlon and Lee 2000, but see Zaher and Reppel 1999).

tween species. The basal zone with plicidentine is the last part of the tooth crown to be formed and would be expected to be less apparent in small teeth which cease developing early. Thus, the loss of plicidentine in snakes above *Pachyophis*, madrosoids, and presumably *Dinilysia* might have been associated with size reduction. The ability to develop this trait might have been lost by the time certain forms (boinas, pythanes) again revolved large size. Evolution by heterochrony could also account for zones of infolding or carinae appearing on the upper part of the crown in various snakes and mosasaurids, as well as (or instead of) on the tooth base; the fluted or striated tooth crowns of some carophidians might have arisen in this way (Vaeth et al. 1985), but a wide survey of tooth base morphology (not closely examined by Vaeth et al. 1985) would be required to test this idea.

Alternatively, it might be suggested that plicidentine evolved repeatedly in squamates and was never present in the ancestor of snakes. This hypothesis is unsupported in the case of snakes, as it would imply at least three origins within varanoids and two within snakes. Also, this convergence would be difficult to explain biomechanically. The varanoid origins would have been associated with strongly oblique, pleurodont attachment of teeth, whereas in snakes the angle depth of the tooth, and extent of bone of attachment are all quite different (though similar in some respects to conditions in mosasurs; Edmund, 1969). The functional significance of plicidentine does not appear to have been rigorously analyzed biomechanically. One possibility is that the basal infoldings provide a greater area of bone surface and thus strengthen the tooth

base and the tooth-to-jaw attachment. Another possibility is that the infoldings allow slight flexibility in the tooth base (analogous to movements in a concertina); this bending would help absorb shock during bites. In all these models, the forces encountered by the tooth base would presumably scale according to tooth (and skull) volume and the amount of bone in the tooth base would only scale according to tooth surface area (assuming enamel thickness remains constant), thus requiring greater infolding in larger teeth. These models are all consistent with the prevalence of plicidentine in large, kinetic ("snap") feeding predators such as temnospondyls, varanoids and snakes, where the teeth would presumably experience large shock forces during jaw closure.

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#### APPENDIX 1

*Material Examined.*—Institutional Abbreviations: AMS—Australian Museum, Sydney; QM—Queensland Museum, Brisbane.

Scoleophoridae: *Ramphephyllops* sp. QM 15326. Aniliidae: *Cylindrophis namata* AMS R13156; boister: *Aspilius melanoglyphus* QM 15616; QM 150786; *Martini trinitis* QM 15815; *Liasis fuscus* QM 1226888; *Liasis olivaceus* QM 125903; *Morita amathusina* QM 15815; *Morita spilota* QM 162828; *Carophidians* *Archaeophis antifer* QM 123718; *Bogoi irregularis* QM 12173; *Cerbertus*

*austalis* QM 12650, *Dumetia punctulata* QM 14749, *Nothofagus solandri* QM 12648, *Oxygymnias scolopendria* QM 14530, *Pseudochis collettii* QM 16280, *Pseudochis australis* QM 12906, *Pseudochis teretica* QM 12652, *J. 26668*, *Leptodactylus muniti* QM 12459.

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# **Fecal Testosterone Concentrations May Not Be Useful for Monitoring Reproductive Status in Male Blue-tongued Lizards (*Tiliqua nigrolutea*: Scincidae)**

NATALIA ATKINS,<sup>1</sup> SUSAN M. JONES,<sup>1</sup> AND ASHLEY EDWARDS,<sup>2</sup> *School of Zoology, University of Tasmania, GPO Box 252-05, Hobart, TAS 7001, Australia*

Assessment of reproductive status in animals generally depends on monitoring hormone concentrations in plasma, but blood sampling often involves significant stress to the subject. Monitoring steroid profiles by assaying excreted steroids in urine or fecal samples is noninvasive but does pose some problems. Unlike plasma assays, urinary and fecal steroid analyses are of relatively little value in monitoring rapid, short-term changes in hormone concentrations (Heistermann et al., 1993) because there is a significant delay between production and excretion of steroids. However, such assays do enable measurement of "pooled" hormone concentrations over time (Wildt et al., 1995; Brown, 1997).

Hormones do not usually appear unchanged in the urine or feces but are present as a range of metabolites produced by the liver, the major site of steroid metabolism (Klime, 1987). The proportions and forms of steroid excreted differ between the two excretory pathways and may be highly variable between species (Aldecrantz et al., 1980). In the named wolf (*Canis lupus familiaris*), 97% of testosterone (T) is excreted as unconjugated metabolites in the feces (Velloso et al., 1999), whereas in African wild dogs (*Lycaon pictus*), 60% of steroids are excreted in the feces and 40% in the urine (Montfort et al., 1993). A proportion of the excreted steroid is present as water-soluble conjugates—glucuronides and sulfates—which can complicate hormone analysis. In most mammals, however, voided feces contain a higher percentage of free than conjugated steroids (Adams et al., 1994; Masser et al., 1996; Velloso et al., 1998), so excreted steroids are measurable by standard assay procedures. However, even if the same hormones are produced, different liver enzymes may produce different metabolites for excretion (Hodges, 1986), fecal steroid assays, therefore, need to be validated separately for each species.

Most studies of fecal steroids have been conducted in mammalian species (Schwarzenberger et al., 1996),

with limited work on birds such as the kakapo (*Strigops habroptilus*; Cockrem and Rouane, 1995). This critically endangered, cryptic species is not amenable to invasive monitoring strategies, but Cockrem and Rouane (1995) have successfully used the ratio of fecal T to testosterone to assign sex in kakapo. Fecal steroid monitoring has also been used to follow the reproductive cycle in Japanese crested ibis (*Nipponia nippon*) and rock ptarmigan (*Lagopus muta*; Kikuchi and Ishi, 1997) and in the northern spotted owl (*Strix occidentalis caurina*; Masser et al., 1997; Brown et al., 1995).

There are, however, only two published reports of the application of fecal steroid monitoring to reptiles. The reproductive cycles, characterized by behavioral observations and ultrasound imaging, of four species of tortoise (*Geochelone elephantopus*, *Geochelone signata*, *Testudo graeca*, *Testudo hermanni*) have been positively correlated with profiles of fecal steroid metabolites (Casares, 1995; Dobell et al., 1992). Ideally, fecal steroid profiles should be validated against plasma steroid profiles for the same species (Pyper et al., 1993), but this was not achieved for the tortoises. No suitable reptile species has yet been monitored using fecal steroid assays, and further study of the potential of such techniques for monitoring reptilian reproductive cycles is clearly required.

Working from the premise that new techniques are best developed using a relatively common species, we collected fecal samples opportunistically during a study of the annual reproductive cycle and plasma concentrations of steroid hormones in male blotched blue-tongued lizards (*Tiliqua nigrolutea*; Edwards and Jones, 2001). Here we report on the development of a method for assay of T in feces of this species. We compare the profile of fecal T with that for plasma T through the reproductive cycle of blotched blue-tongued lizards and also examine the relative proportions of conjugated and free T in feces.

We collected fecal samples from captive male *T. nigrolutea* from March 1997–February 1999. The wild-caught animals were held at the University of Tasmania in semi-natural conditions. Male and female lizards cycle in synchrony with the wild population (Edwards, 1999; Edwards and Jones, 2001); details of annual husbandry are given in Edwards and Jones (2001). We collected fecal samples ( $N = 29$ ) passed voluntarily while animals were being observed for reproductive behaviors. These samples were classified according to reproductive stage and mean fecal T levels (Fig. 1) were calculated using results obtained by assaying these samples. Plasma T values were derived independently from the more comprehensive dataset presented as monthly mean values in Edwards and Jones (2001); standard errors are not, therefore, presented (Fig. 1).

Upon collection, each fecal pellet was frozen at  $-20^{\circ}\text{C}$  in an individual, snap-lock bag until analysis. Prior to extraction of steroids, we thawed the fecal samples and mixed each one well to control for possible heterogeneous steroid distribution within the pellet. Samples were then lyophilized for 24 h to control for varying water content (Masser et al., 1994; Thompson et al., 1998). The lyophilized samples were ground and the fecal material separated from dried plant material and snail shells to produce a powder ready for extraction. We used the fecal steroid extrac-



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<sup>1</sup> Corresponding Author. E-mail: s.m.jones@utas.edu.au