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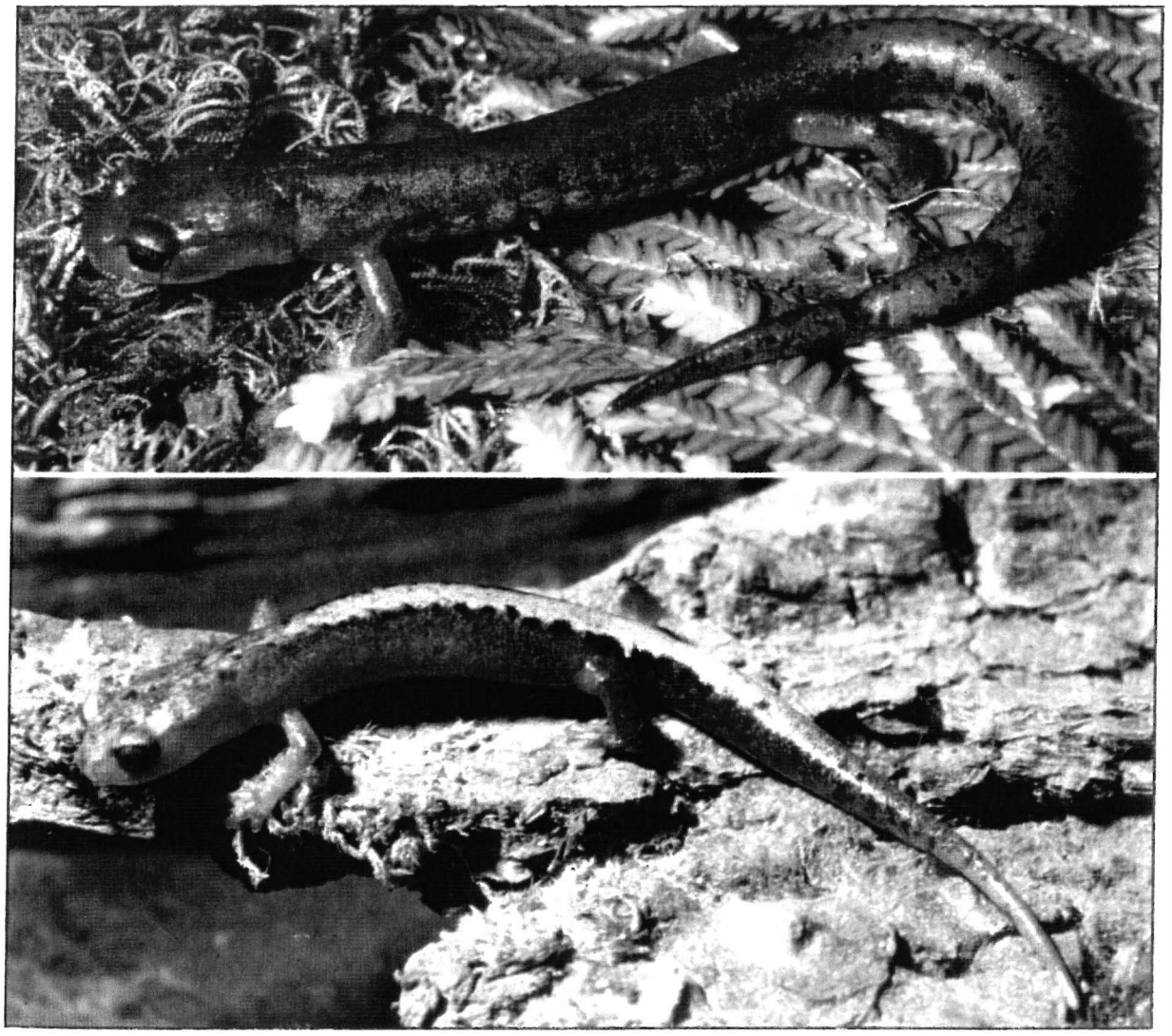

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FRONT COVER: *Pseudoeurycea juarezi* (top) and *P. saltator* (bottom) (L.Canesco-Márquez & G. Parra-Olea)

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THE AUSTRALIAN ELAPID GENUS *CACOPHIS*: MORPHOLOGY AND PHYLOGENY OF RAINFOREST CROWNED SNAKES

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The genus *Cacophis*, comprising four species endemic to eastern Australia, is uniquely derived among terrestrial Australasian elapid snakes in the temporal scale pattern, presence of a relatively high and narrow dorsal crest ('choanal process') on the palatine bone, and presence of keeled supra-anal scales in adult males. Recent analyses based on morphology and genetics do not completely resolve relationships among Australasian elapids, but support relationships of *Cacophis* with the (*Furina, Glyphodon*) and (*Aspidomorphus, Demansia*) clades, which are adopted here as outgroups for intrageneric analysis. Within *Cacophis*, morphoclines in size, head scalation, tooth numbers and colour patterns indicate that *C. squamulosus* is the sister-group to the remaining three species; among the latter, there is conflicting evidence for both (*harriettae, krefftii*) and (*churchilli, krefftii*) clades, but the latter alternative has greater support. Revised diagnoses are given for the genus and included clades, and a simple phylogeographic model proposed.

Key words: Hydrophiinae, morphology, skull, head scales, colour patterns, behaviour, phylogeography

INTRODUCTION

Numerous studies have contributed to understanding the phylogeny of Australian elapid snakes, providing evidence for monophyly of a number of genera and of several suprageneric units (e.g. Schwaner *et al.*, 1985; Mengden, 1985; Shine, 1985; Hutchinson, 1990; Greer, 1997; Keogh, 1998, 1999; Keogh *et al.*, 1998, 2000). These probable clades include: the 'subfamily' Hydrophiinae comprising all terrestrial Australasian elapids as well as marine forms (in either the sense of McDowell, 1987, or that of Slowinski & Keogh, 2000, which differ in whether *Laticauda* is included); the viviparous radiation (Shine, 1985); the true sea snakes (here regarded as a monophyletic 'tribe' Hydrophiini, despite a recent analysis suggesting diphyly; Rasmussen, 2002); and a '*Notechis* lineage' comprising chromosome groups 4, 5 and 10 of Mengden (1985). However, resolution remains poor because characters have often been inadequately defined or polarized, or insufficiently numerous to resolve the large number of species (e.g. McDowell, 1967; Storr, 1985; Wallach, 1985; Greer, 1997; Lee, 1997). I have studied external and skeletal morphology in the terrestrial Australasian elapid snakes, attempting to define and test additional characters in order to improve phylogenetic resolution (e.g. Scanlon, 1985) and as a basis for interpretation of Miocene fossils (Scanlon, 1996). An important intermediate goal is to establish the monophyly and internal relationships of groups of species (e.g. genera) which can conveniently be used as discrete units in a higher-level analysis (cf. Hutchinson, 1990). Such a 'global' analysis will not be presented here, as I concentrate on a particular genus and its putative close relatives.

Hutchinson (1990) considered diagnosis of the genus *Cacophis* problematic, and recognized it only 'tentatively' as distinct from *Furina*. *Cacophis* consists of four species of small nocturnal saurophagous (lizard-eating) snakes, all restricted to rainforest or wet sclerophyll habitats in coastal regions of eastern Australia (Queensland and New South Wales). Three of the species have long been recognized, although they were previously referred to as many as three separate genera (*Cacophis*, *Aspidomorphus* and *Glyphodon* in Worrell, 1963). McDowell (1967) suggested that these three species (*krefftii*, Dwarf crowned snake; *harriettae*, White-crowned snake; and *squamulosus*, Golden-crowned snake) formed a single natural group distinct from other genera; Cogger (1975) brought them together in *Cacophis*, and full synonymies are given in Cogger, Cameron & Cogger (1983).

The fourth species, found in the Wet Tropics of northern Queensland, was first recognized informally as '*Glyphodon* sp.' by Worrell (1963: 125 and plate 56), and subsequently as *Cacophis h.* [*harriettae*] *flavicollis* [nomen nudum] (McDowell, 1967: 536) and *Cacophis* sp. (Wilson & Knowles, 1988: 332; Gow, 1989: 84; Ehmann, 1992: 392). Cogger (in Cogger *et al.*, 1983: 219) includes the mentions by Worrell (1963) and McDowell (1967) in the synonymy of *C. harriettae*, but notes that both refer to what is probably a distinct species. The name *Cacophis churchilli* Wells & Wellington, 1985 is available for this form, though it has only recently come into wider use (Greer, 1997: 160, 178; Shea & Sadlier, 1999; Queensland Museum, 2000: 239; Cogger 2000: 771). Ehmann (1992) calls it the

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'Northern Dwarf Crowned Snake', but 'dwarf' is not especially appropriate since it attains body sizes similar to *C. harriettae* (see below).

While detailed studies of geographic and genetic variation remain to be done (J. Sumner in prep.), I regard the the identity and boundaries of these species as now being stable, and a formal revision is not given here. Rather, this paper reviews evidence for relationships between *Cacophis* and other genera, reports observations of some unusual morphological features contributing to the diagnosis of the genus, and uses readily available data to derive an explicit phylogenetic hypothesis for the four included species.

One motive for investigation of this genus is the discovery of fossil material of small elapid snakes from the Miocene of northern Australia, including a maxilla with features resembling those of *Cacophis* species (Scanlon, 1995, 1996). However, as variation in skeletal features (apart from tooth counts) within the genus is dominated by ontogenetic change in proportions (pers. obs.), the emphasis here is on external morphology.

METHODS

All data are drawn either from published sources or examination of specimens – including those in the collections of the Australian Museum, Sydney (AMS); Queensland Museum, Brisbane (QM); South Australian Museum, Adelaide (SAM); and Western Australian Museum, Perth (WAM). Some additional specimens at the American Museum of Natural History, New York (AMNH) and Museum of Comparative Zoology, Harvard (MCZ) were examined on my behalf by M. Lee.

A large number of external and skeletal morphological features have been investigated for their potential to contribute phylogenetic information for Australian elapids, and many of them show overlapping variation of continuous or discrete characters across species or more inclusive groups (Wallach, 1985; Scanlon, 1985; Lee, 1997). This is consistent with the uncontroversial hypotheses that novel characters (genetic, morphological, or behavioural) must pass through a stage of polymorphic coexistence with their alternative, plesiomorphic states before being fixed in one or more descendant populations, and that such polymorphisms may be retained for evolutionarily significant periods. In many cases, I recognize polymorphic coexistence of alternate conditions as a single, separate, intermediate state, and such characters are treated as ordered morphoclines. In other instances, polymorphic taxa are assigned to several states on the basis of relative frequency of alternate conditions observed in samples. This approach to definition and ordering of character states corresponds to a simplified version of the 'frequency bins' method, and has been shown to perform well in simulations (Wiens, 1998).

Data matrices and constraint trees were edited using MacClade version 4.0 for Power PC (Maddison & Maddison, 2000) and phylogenetic analyses carried out on a Macintosh G4 using PAUP* version 4.0b10 (Swofford, 2002), in some cases using batch commands generated using TreeRot version 2 (Sorenson, 1999).

OUTGROUP RELATIONSHIPS OF *CACOPHIS*

In order to assess the polarity of morphological characters contributing to the diagnosis of *Cacophis* and resolution of relationships among the included species, relevant outgroups must be identified. Ideally, these should include the two clades most closely related to the ingroup to allow the outgroup comparison procedure of Maddison *et al.* (1984). Previous analyses of Australasian elapid relationships support the basal position of *Laticauda* and the Solomon Island genera (McDowell, 1970; Keogh *et al.*, 1998), and the monophyly of a large viviparous lineage which includes mainly Australian terrestrial elapids and hydrophiine sea snakes (Shine, 1985; Keogh *et al.*, 1998, 2000). These results imply that the remaining Australo-Papuan oviparous genera form either one or several clades along the stem lineage of the viviparous group. This intervening part of the tree (including *Cacophis*) has been poorly resolved by prior work, which is attributable mainly to insufficient sampling of characters and (especially Melanesian) taxa, but perhaps also to the rapidity of the adaptive radiation (cf. Schwaner *et al.*, 1985; Wallach, 1985; Mengden, 1985; Lee, 1997; Greer, 1997). The selection of outgroups must therefore be provisional at this stage.

Classifications up to that of Worrell (1963, 1970) referred at least some *Cacophis* species to *Aspidomorphus* (see Mengden, 1983, for review), but it has since been considered that *Aspidomorphus* is closest to *Demansia* (McDowell, 1967; Keogh *et al.*, 1998). Also, a consensus has developed that *Cacophis* is closely related to *Furina* and *Glyphodon* (McDowell, 1967; Wallach, 1985; Hutchinson, 1990; Greer, 1997; Keogh *et al.*, 1998; Keogh, 1999). A recent analysis of DNA sequence data (Keogh *et al.*, 1998) has found support for a clade comprising *Cacophis*, *Demansia*, *Aspidomorphus*, *Furina* and *Glyphodon*. While the detailed results varied with different methods of data analysis, they 'consistently grouped these four genera in various combinations' (p. 77), with *Demansia* and *Aspidomorphus* most strongly linked. As shown in Fig.

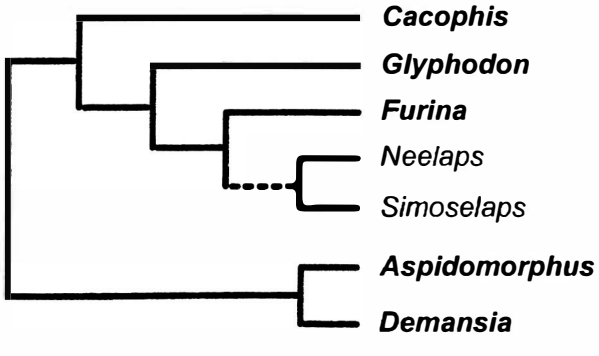

FIG. 1. Relationships assumed between *Cacophis* and other genera used in outgroup comparisons. The two outgroups *Aspidomorphus* + *Demansia*, and *Glyphodon* + *Furina*, contribute equally to the estimation of plesiomorphic characte states for *Cacophis*.

1, the two outgroups to *Cacophis* adopted in this paper are (*Furina, Glyphodon*) and (*Aspidomorphus, Demansia*), which is thus consistent with Keogh *et al.* (1998) and most previous classifications and analyses.

FURINA AND *GLYPHODON*

McDowell (1967) and Wallach (1985) concluded from morphological analyses that *Cacophis* was most closely related to *Furina* and *Glyphodon*. The latter genera were synonymized as *Furina* by Hutchinson (1990) and, as noted above, regarded only tentatively as distinct from *Cacophis*. Keogh (1999) found strong similarities among the hemipenes of *Cacophis* and *Furina* (*sensu lato*), including both in his 'Group 4' which he regards as a monophyletic group.

Synonymy of *Glyphodon* and *Furina* has been adopted by Hutchinson (1990) and others, in part to deal with the apparent problem of classifying *Glyphodon barnardi* Kinghorn, 1939. Cogger's (1975) key to genera purports to distinguish the genera on the criterion of 'nasal undivided' (*Furina*) vs. 'nasal divided' (*Glyphodon*), but in fact *G. barnardi* has the nasal undivided and would be assigned to *Furina* by this criterion. Polarity of this character is ambiguous since both states occur in related genera (see below), but other cranial and external morphological evidence suggests that *G. tristis* and *G. dunmalli* are sister taxa (e.g. in both species the parasphenoid is excluded from the optic fenestra, an uncommon derived character not observed in *G. barnardi* or other *Furina* spp.; pers. obs.), while *G. barnardi*, *Furina diadema* and *F. ornata* are more closely related to the fossorial radiation of *Neelaps* and *Simoselaps* (Scanlon, 1985, 1988, unpublished data). I therefore retain *Glyphodon* as a distinct genus for *G. tristis* and *G. dunmalli*, and refer *G. barnardi* to *Furina*.

I provisionally recognize a (*Glyphodon* (*Furina* (*Neelaps, Simoselaps*))) clade which can be diagnosed as follows: nasal and second supralabial separated from the preocular (reversing twice in fossorial lineages); ventral surface white; dorsal scales highly glossed; eyes dark (Scanlon, 1985; Hutchinson, 1990; characters discussed below). *Glyphodon* spp. lack additional derived states shared by *Furina* with *Simoselaps* and *Neelaps* spp.: postorbital bones with kinetic attachment to parietal (involved in mechanism for maxillary erection and retraction; McDowell, 1969*a*; Scanlon, 1985); frontal may contact preocular scales (rare to common variant, [Storr, 1968, 1981], never observed in *Glyphodon* or any other elapid genera, pers. obs.); black head and nape blotches contrasting with the dorsal ground colour and separated by a distinct pale spot or bar; and a reticulate dorsal pattern where each scale may have a black edge, yellow basal spot and red intermediate zone (three distinct pigments; [Storr, 1968]). *Cacophis* spp. lack most of these derived features and retain the alternate states common to most other Australasian taxa (preocular contacts second labial and frequently nasal; ventrals strongly pigmented; scales less glossy; eyes pale; postorbital lacks anteroposterior kinesis; no contact of preocular and frontal scales; occipital and dorsal ground colour similar; pale spots on dorsal scales single-coloured), and can thus be excluded from the (*Glyphodon* (*Furina* (*Neelaps, Simoselaps*))) clade.

Thus *Glyphodon* and *Furina*, either alone or together with *Simoselaps* and *Neelaps* (Scanlon, 1985, 1988: Fig. 1), form a close outgroup to *Cacophis* (Hutchinson, 1990; Keogh, 1999). Either way, the species of *Glyphodon* and *Furina* are the most appropriate taxa to estimate the ancestral states of this outgroup clade.

DEMANSIA AND *ASPIDOMORPHUS*

These two genera, suggested by McDowell (1967) to be closely related, share several probable synapomorphies, including uniquely derived features of the maxilla: tooth numbers are the highest of any elapids (Bogert, 1943; McDowell, 1967), the medial (ectopterygoid) process is elongate, and in most species the suborbital region is dorsoventrally extremely thin and (in fresh or wet-preserved specimens) flexible (pers. obs.). Mengden (1985) found that *Demansia* 'possesses a unique karyomorph not easily associated with any other Australian elapid', while the karyotype of *Aspidomorphus* has not been reported. The relatively high genetic distances found by Cadle & Gorman (1981) and Schwaner *et al.* (1985) between *Demansia* and all other genera – including *Aspidomorphus* – conflict with evidence from morphology (the skeletal characters just given, and others in McDowell, 1967) and DNA sequences (Keogh *et al.*, 1998), but can be explained by, for example, accelerated genetic change (autapomorphy) in *Demansia*, as already suggested by Cadle & Gorman (1981).

Demansia and *Aspidomorphus*, provisionally accepted as sister taxa forming a single clade, are used as one outgroup in comparisons below. Skulls of all three species of *Aspidomorphus* have been examined (see also McDowell, 1967), and a preliminary analysis suggests that *A. schlegelii* is basally related to the other two species, although *A. muelleri* is the least derived in morphology (unpublished data). The larger number of species (approximately 15 [Shea & Scanlon, unpublished data]) and morphological diversity within *Demansia* present a greater problem, but it seems likely from external characters (e.g. number of ventral scales, occurrence of posterior scale-row reduction) that *D. simplex* is basally related to all the other, larger and more elongate species (see Table 1 and characters discussed below). While cranial data have been obtained for only a few species of *Demansia*, *D. simplex* is also plesiomorphic relative to the others examined (*D. psammophis*, *D. vestigiata* and *D.* sp. cf. *olivacea*) in having a relatively broader frontal, less constricted parietal, and less developed 'interorbital septum' (Underwood, 1967) of the parasphenoid.

DISTINCTIVE FEATURES OF *CACOPHIS*

A revised diagnosis of *Cacophis* is given in a later section, in which several classes of characters are included: unambiguous autapomorphies, characterizing *Cacophis* but absent or uncommon in the outgroups and other Australasian elapids; possible apomorphies, conditions with a more restricted distribution including one or more of the outgroup genera; and likely plesiomorphies, conditions which are shared widely among Australasian elapids but lost or modified in various lineages from which *Cacophis* can thereby be excluded. Contrary to Hutchinson (1990), *Cacophis* can readily be diagnosed on the basis of autapomorphic states of external as well as cranial characters.

AUTAPOMORPHIES

A1. Parietal foramina. Most Australasian elapids, like many other colubroids, have a pair of small foramina (or sometimes closed pits, not piercing the bone) near the centre of the dorsal surface of the parietal. These are presumably not equivalent to the median pineal foramen which was lost in an ancestor of all snakes, but there do not seem to have been any descriptions of the detailed anatomy, function or phylogenetic value of the paired openings. Greer (1997: 178) noted their absence in *Cacophis* but did not discuss their occurrence in any other taxa. The foramina are present in nearly all outgroup skulls examined (but not in *D. simplex*, NTM R18625; one specimen of *F. diadema*, SAM R 6703), and absent in nearly all *Cacophis* (Figs 2, 3c; present unilaterally in one specimen of *C. krefftii*, SAM R26974, Fig. 3a). In some other taxa (e.g. *Pseudechis* spp.), the foramina may be obliterated during adult life by forward extension of median contact between the mandibular adductor muscles forming a sagittal crest, but in *Cacophis* they are typically absent even when the muscles are still widely separated.

A2. Choanal process of palatine bone. McDowell (1970, 1987) diagnosed the subfamily Hydrophiinae of 'palatine draggers' on the basis of the palatine's clasping articulation with the pterygoid, and lack of choanal and perforate lateral processes. The dorsomedial edge of the palatine is smooth and nearly parallel to the tooth row (i.e. choanal process totally absent) in most hydrophiine taxa and their probable sister group *Laticauda* (McDowell, 1970; pers. obs.; State 0). However, a number of species in the Australian radiation have a low to moderate laminar dorsal process similar to the choanal process of such forms as *Bungarus*, but it is usually nearly vertical rather than arching medially over the choana. Greer (1997) recognized this as diagnostic

TABLE 1. Comparative morphological data for species of *Cacophis* and outgroup genera: maximum known snout-vent length (max. SVL); and observed ranges of the number of ventral and subcaudal scales; and number of alveoli for tooth attachment on the maxilla (excluding the two enlarged anterior fangs), palatine, pterygoid and dentary. Scalation and size data from Brongersma (1934), Cogger (1992, 2000), Greer (1997), McDowell (1967), Scanlon (1985, unpublished data), Shea and Scanlon (unpublished data), Shine (1980*a,b*, 1981), Shine and Keogh (1996), Storr (1978), Storr *et al.* (1986). Tooth counts from skeletal material listed in Appendix, with additional data from Boulenger (1896) for *Glyphodon tristis*, and McDowell (1967) for *Cacophis harriettae*, *C. krefftii* and *Aspidomorphus* spp.

Genus	species	Max. SVL	Ventrals	Subcaudals	Maxilla	Palatine	Pterygoid	Dentary
Cacophis								
	churchilli	53.8	154-176	25-38	6	9-12	17-18	21-23
	harriettae	48.8	168-200	25-45	3-5	9-12	12-18	16-19
	krefftii	34.5	140-160	25-40	2-5	9-11	11-16	14-16
	squamulosus	71.5	165-185	30-50	6-8	11-17	19-24	21-28
Aspidomorphus								
	lineaticollis	48.0	139-174	24-40	11-18	13-16	31-34	31-32
	muelleri	62.0	160-177	29-40	10-18	16-19	36-40	31-36
	schlegeli	50.8	137-160	19-29	11-13	14	24	30
Demansia								
	psammophis	83.5	172-205	63-91	8-13	12-15	26-38	19-25
	simplex	43.6	140-158	49-66	9	12	33-34	30
	other species	47.6-154.5	160-230	63-113	8-13	10-18	22-38	19-25
Glyphodon								
	dunmalli	56.2	166-189	37-46	8-9	13	20-23	24
	tristis	77.8	171-181	44-51	6-8	9-15	16-23	16-20
Furina								
	barnardi	54.8	157-221	35-58	6-7	10-12	15-23	19-21
	diadema	34.3	156-203	35-54	4-5	8-12	15-18	14-17
	ornata	58.1	164-217	37-63	5	12	19-20	18-19

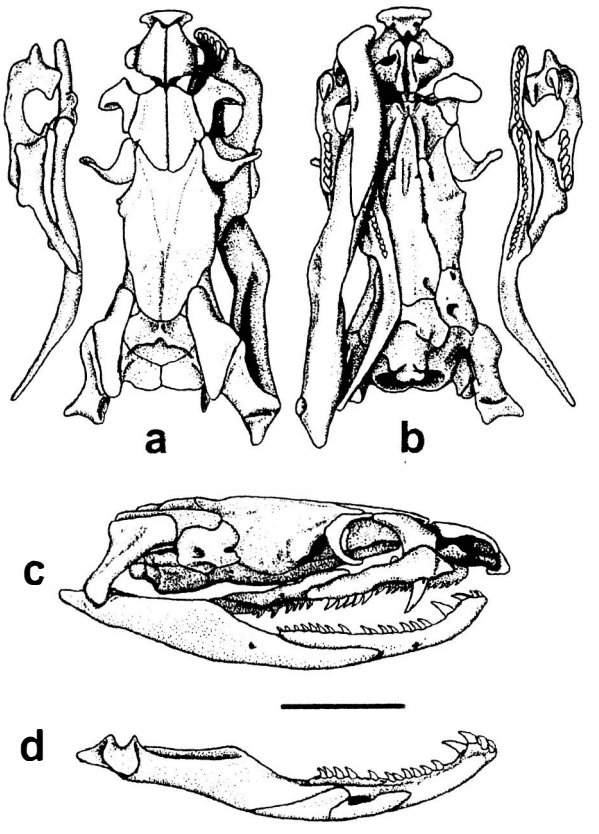

FIG. 2. Skull of *Cacophis churchilli* (QM J53282). Dorsal (a) and ventral (b) views of skull, right mandible, and displaced left palatal elements; (c) right lateral view of skull and right mandible, and (d) medial view of left mandible. While smaller than some of the *C. squamulosus* skulls examined, this relatively large adult specimen (SVL 421 mm) exceeds them in the development of bony crests for muscle attachment and other features associated with large size. Scale bar = 5.0 mm.

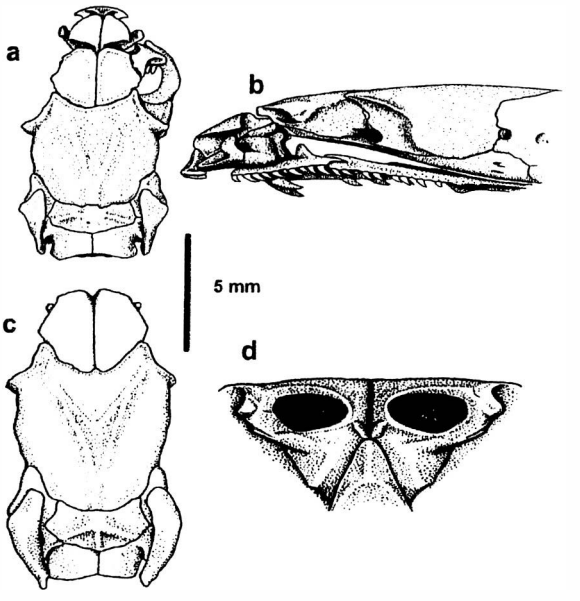

FIG. 3. Skulls of *Cacophis krefftii* and *C. squamulosus*. *C. krefftii* (SAM R26974) in (a) dorsal view, and (b) left lateral view of the anterior part of the skull; the left prefrontal and palatomaxillary arch, postorbitals, quadrates and mandibles are not shown. Braincase of *Cacophis squamulosus* (SAM R2263A) in (c) dorsal view, and (d) anterior view of the frontals and parasphenoid rostrum; the snout unit, prefrontals, palatomaxillary arches, postorbitals, quadrates and mandibles are not shown. Scale bar = 5.0 mm (for a and c only).

of *Cacophis*, but a crest-like process is also present in *Glyphodon*, *Demansia*, and *Aspidomorphus* spp. (but not in any *Furina* spp. examined) as well as a number of other Australasian taxa. The crest may be either angular or rounded dorsally, and is never as high as long in the outgroups or other hydrophiines. The 'short' choanal process is therefore considered plesiomorphic for *Cacophis* (State 1). In all *Cacophis* palatines examined there is a well-developed choanal process which is higher than long (i.e. 'tall'), and directed dorsally or slightly anteromedially (Figs 2, 3; also figured by Greer, 1997). This condition (State 2), while structurally approaching that of *Naja* and more distant outgroups, is considered derived within Hydrophiinae, and diagnostic of *Cacophis*.

Loss or reduction of the choanal process in hydrophiine elapids appears to be related to increased longitudinal mobility of the palatines relative to the vomer and snout complex, in contrast to the rotation of the palatine about its contact with the vomer in many other snakes (McDowell, 1970; cf. Cundall, 1995; Cundall & Shardo, 1995). In incompletely cleaned skulls the palatine dorsal process is seen to lie within a sheet of connective tissue connecting to the parasphenoid, prefrontal and palatine shaft, forming the medioventral wall of the orbit, but even in *Cacophis* the process remains completely free of other bones and does not appear to form a functional articulation with the snout.

A3. Anterior extent of the ectopterygoid. The anterior extremity of the ectopterygoid, nearly always its anteromedial tip, lies close to the same horizontal plane as the palatine-pterygoid joint, and slightly lateral to it, so these landmarks are readily comparable. The common and primitive condition appears to be a longitudinal overlap with the palatine, i.e. the ectopterygoid extends anteriorly somewhat past the joint (State 0). This occurs in some *Furina*, some *Aspidomorphus*, and all *Demansia* examined apart from *D. simplex*. An intermediate state can be recognized where the ectopterygoid extends to approximately level with the joint (or within the region of overlap), which characterizes the remaining outgroup taxa (State 1). The most derived state, where the ectopterygoid fails to reach the palatine (State 2), is not found in the outgroups and is hence considered apomorphic in *Cacophis*, where it is the only state observed.

A4. Supra-anal keels. A patch of keeled lateral scales is present in the cloacal region in males of all four *Cacophis* species (a series of each examined at the AMS). This secondary sexual character is sporadic but quite widespread among colubroids (e.g. Blanchard, 1931; Mertens, 1936; Gyi, 1970; Roze, 1996), but has

not been seen in the outgroups or any other Australasian elapids examined. Extent of the keeling is variable when present, ranging from barely detectible (one or two keeled midlateral scales on each side in *C. krefftii* AMS R77370, SVL 237 mm) to extensive (from 12th-last ventral to 20th subcaudal, and extending from lowest laterals to the paravertebral scale rows in *C. squamulosus* AMS R37187, SVL 410 mm). Two individuals with weak keeling (*churchilli,* AMS R11512, R12480) appear to be female based on tail shape, and some are unlikely to be mature based on size (*churchilli* AMS R11340, SVL 145 mm; *squamulosus* R28232, SVL 187 mm). Conversely, no keeling was detected in some likely adult males of *krefftii* (e.g. AMS R81158, SVL 235 mm), but generally this seems to be a useful indicator of sex and maturity in each species. Similar variability of supra-anal keels is reported within *Micrurus* by Roze (1996; see her Fig. 7).

A5. Parietal and postocular scales: occurrence of contact. In most elapids with two (or sometimes three) postoculars, the uppermost contacts a temporal (or sometimes labial) scale below the parietal (State 0). In some of each species of *Cacophis*, the parietal contacts the lower postocular, separating the upper postocular from the temporals (State 1). Among the outgroups, this state characterizes only *Aspidomorphus* spp. (it also occurs as a variant in some derived *Simoselaps*, e.g. *S. morrisi* Horner, 1998). Hence, it is parsimoniously interpreted as an apomorphy of *Cacophis*, convergent in *Aspidomorphus* (and again in *Pseudonaja* and *Oxyuranus*, which form a more distantly related clade). Variation in frequency of contact within *Cacophis* is treated as a further binary character (C7).

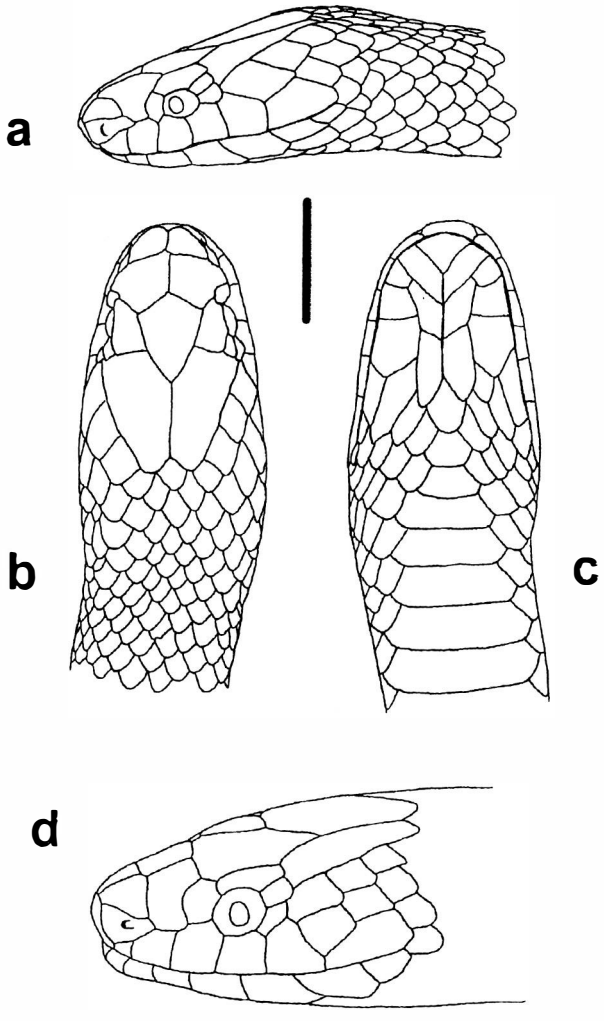

FIG. 4. Head of *Cacophis churchilli* (SAM R22392) in (a) lateral, (b) dorsal, and (c) ventral views, and (d) *Furina ornata* (AMS R110357) for comparison of head scales. Scale bar = 5.0 mm (for a-c only).

A6. Temporal scales. The practice of writers on Australian elapids has generally been to recognize two series of temporal scales, anterior and posterior, and give a formula such as '2+2' or '3+4'. McDowell (1967: 500) described the widespread 2+2 condition in Australasian elapids, and introduced the term 'temporolabial' for the lower anterior temporal "which appears to be the homologue of the penultimate supralabial of elapids with seven supralabials, but which has been squeezed out of contact with the oral border". Describing the temporolabial as present or absent (e.g. Wallach, 1985) is an oversimplification, but a number of distinct characters can be defined using a more detailed notation. Storr (1968) used a three-term formula (e.g. '1+1+2'; see also Aplin & Donnellan, 1999), but without adequate explanation. The following definition gives counts consistent with Storr's on the same specimens (Scanlon, 1985), and is applicable to most Australasian elapids.

The postsupralabial is the scale at the corner of the mouth, overlapped by the most posterior supralabial and infralabial but not counted in either series (this follows the usage of Greer & Cogger, 1985, for skinks, and is consistent with supra- and infralabial counts given by most authors). The anterior (or primary) temporal row includes the temporolabial, when distinct – regardless of whether it reaches the lip or contacts a postocular – and any other scales between supralabials and parietal which do contact the postoculars. The oblique row of scales connecting the parietal to the postsupralabial, but excluding the latter, is considered to be the last row of temporals (2nd, 3rd or 4th in Australian elapids), and the formula is given to a corresponding number of terms. Individual scales can be identified by row and position as 1°1, 2°1, 3°2 etc.; thus, the temporolabial (TL) is 1°2 in typical Australian elapids.

Using this definition, all of the outgroup species (and most other Australian elapids) normally have 2+2+3 (Fig. 4d), while the condition in *Cacophis* spp. can be written as 1+3 or 1+2 (Fig. 4a). McDowell (1967: 535) suggests the temporolabial is fused to the sixth labial in *Cacophis*, but this does not explain the reduction from three to two rows of temporals. Rather, it seems simplest to assume that the single large anterior temporal of *Cacophis* represents the four scales of the ancestral primary and secondary temporal rows (as suggested by Greer, 1997). If fusion of adjacent scales (failure of a suture to develop; Resetar & Marx, 1981) represents a single evolutionary 'step', the inferred transformation

from four scales to one could have involved two or more steps, but these are not counted as separate characters here because ingroup variation is so limited. Variants in *C. churchilli* and *C. krefftii* do corroborate the suggestion of fusion among temporals (loss of sutures rather than loss of scales). For example, AMS R75961 (*krefftii*) has '2+2' on each side, but on the right side of the head the anterior temporals are both elongate and contact the postorbitals (interpreted as 1°1=2°1 and TL=2°2, where '=' indicates fusion), while on the left there is a large L-shaped scale (representing TL=1°1=2°1) with a smaller adjacent scale (2°2) widely separated from the postoculars. Similar conditions occur asymmetrically in R81158, R90609, and R114956 (*krefftii*), and in R11512 and R11362 (*churchilli*). Accepting this evidence for fusion of the two anterior temporal rows, we may write the formula for the common *Cacophis* conditions as 1=1+3 or 1=1+2. The variation in the posterior row is used below as evidence for relationships within the genus (C6).

Many specimens of *Simoselaps* and *Vermicella* spp. also have 1=1+2, but the most common condition in these genera is 1+1+2 (Storr, 1968; Greer, 1997: 169; Horner, 1998: Fig. 2), and when fusion occurs between primary and secondary the resulting scale is either trapezoidal (deep anteriorly and tapering posteriorly) or long and shallow, in either case quite different from that in *Cacophis*. Moreover, contrary to McDowell (1969*a*), the temporolabial does occur as a separate element in this group (*Simoselaps warro* normally retains the ancestral 2+2+3, while 2+1+2 is a common variant in the *S. semifasciatus* group), which has not been observed in *Cacophis*.

Some specimens of *Demansia* spp. (like all species of *Pseudonaja,* and some *Oxyuranus microlepidotus*; Storr *et al.*, 1986; pers. obs.) have a temporal condition even more similar to *Cacophis* superficially ('1+2'). However, the complete formula in these cases is 1+2+3 or 1+2+4, indicating retention of three distinct rows. This results from a single fusion between the temporolabial and 6th supralabial, as shown by the concave upper edge and frequent partial suture of the labial scale, and comparison with normal (or in *Pseudonaja*, occasional atavistic) individuals with 2+2+3.

Conditions precisely equivalent to those of *Cacophis* (1+3 or 1+2, with a deep single anterior temporal) are found in the primitive marine hydrophiines *Ephalophis*, *Parahydrophis; Hydrelaps* and *Disteira*, and a further fusion to 1+1 occurs in some *Parahydrophis* (McDowell, 1969*b*, 1972, 1974; Burger & Natsuno, 1974; figures in Storr *et al.*, 1986; Cogger, 1992, 2000). Most other sea snakes have an increased number of temporals, often quite irregular and presumably secondarily fragmented.

The state seen in *Cacophis* is here considered functionally analogous to those of other lineages with a reduced number of temporal sutures, but to have been derived independently from the common ancestral condition 2+2+3. Head-scale fusions in snakes have been interpreted as adaptations to fossoriality, related to reduction in head width and the minimization of soil accumulation along sutures (e.g. Resetar & Marx, 1981; Savitzky, 1983). While *Cacophis* spp. are not strictly fossorial in habit, they utilize crevices and cavities in moist soil as refugia and in foraging nocturnally for inactive prey, mainly skinks (Wells, 1980; Shine, 1980a; Ehmann, 1992). Similar selective pressures would apply to the primitive sea snakes, which capture gobiid fish within burrows on intertidal mud flats (Storr *et al.*, 1986; accounts cited by Greer, 1997).

A7. Pale iris. Non-melanin pigmentation of the iris is rapidly affected by preservatives or freezing, and observations should be based on live specimens or clear photographs (cf. Gillam, 1979). On the other hand, 'dark' and 'pale' eyes can usually be distinguished in well-preserved material, so two characters are used here (see also C17 below). *Glyphodon* and *Furina* have very dark brown or black eyes (Hutchinson, 1990), while most other elapids, including the other outgroup taxa, have the dark pigment varied by a lighter ring, spot or variegations (combined as State 0). A specimen of *Demansia flagellatio* Wells & Wellington, 1985 (a valid species – Shea & Scanlon, unpublished data) at Riversleigh, north-west Queensland, had bright red eyes in life (pers. obs.), so apparently, like *Cacophis*, has little or no melanin in the iris. Hence *Demansia* is scored as polymorphic, although most species – including *D. simplex* – have only a narrow pale ring. The almost uniformly 'pale' iris of *Cacophis* (State 1) appears to be diagnostic of this genus (Hutchinson, 1990); further comparison might justify defining an intermediate state for *squamulosus*, which appears to have more speckling or variegation than its congeners.

POSSIBLE APOMORPHIES SHARED WITH OUTGROUP TAXA

A number of characters of *Cacophis* spp., despite being possibly or actually derived within the Australasian radiation, also occur in both outgroup clades and are likely to be locally plesiomorphic (Table 2, B1-10; see also generic diagnosis below, and Appendix 2). These characters will not be discussed further here.

CHARACTERS VARIABLE WITHIN *CACOPHIS*

Characters which vary among the four species of *Cacophis* (referred to here by their species names alone) provide the basis of a phylogenetic analysis carried out below. I include several autapomorphies of terminal taxa, one of which is behavioural rather than strictly morphological, in order to provide adequate diagnoses for species as well as higher groups. The distribution of character states in outgroup genera and ingroup species is given in Table 3.

C1. Maxillary tooth number. Observed ranges of tooth (alveolus) counts for the maxilla, palatine, pterygoid and dentary of ingroup and outgroup taxa are given in Table 1. The number of maxillary teeth behind the fangs is often relatively high among Australasian and

TABLE 2. Distribution of character states (characters labelled 'A' and 'B' in main text and Appendix 2) in outgroup genera and *Cacophis*. Abreviations for character types: b, binary; u, unordered; 012 (etc.), ordered multistate. Characters marked * are cladistically uninformative for analyses performed here.

Character	A	1	2	3	4	5	6	7	B	1*	2*	3*	4*	5*	6*	7*	8*	9*	10
type		b	012	012	b	b	b	b		b	b	b	b	b	b	b	b	0123	012
Aspidomorphus		0	1	01	0	1	0	0		1	1	1	1	1	01	1	01	3	2
Demansia		01	1	01	0	0	0	01		0	01	0	1	1	01	01	01	3	01
Glyphodon		0	1	1	0	0	0	0		1	1	1	1	1	0	1	1	01	12
Furina		0	0	01	0	0	0	0		1	0	01	1	1	01	1	01	23	1
Cacophis		1	2	2	1	1	1	1		1	1	1	1	1	1	1	1	3	2

TABLE 3. Distribution of character states (characters labelled 'C' in main text and Appendix 2) in outgroup genera and *Cacophis* spp. Abreviations for character types: b, binary; u, unordered; 012 (etc.), ordered multistate. Characters marked * are cladistically uninformative for analyses performed here.

Character (C)	1	2	3	4	5	6	7	8	9*	10	11	12	13	14	15*	16*	17	18	19*
type	b	b	b	b	012	b	b	b	012	0123	0123	b	b	b	b	b	012	b	b
Aspidomorphus	0	0	0	0	0	0	1	0	1	123	23	0	0	0	01	01	0	0	?
Demansia	0	0	0	0	0	0	0	01	01	013	0	0	01	01	01	0	0	1	0
Glyphodon	0	0	0	01	2	0	0	0	01	01	0	0	-	0	0	01	-	0	?
Furina	01	1	01	1	2	0	0	1	12	012	0	0	-	0	0	0	-	0	0
C. churchilli	0	1	1	0	1	1	1	0	1	2	3	0	1	1	0	0	2	1	0
C. harriettae	1	1	1	1	1	1	0	1	1	0	1	0	1	0	0	1	2	0	0
C. krefftii	1	1	1	1	0	1	0	1	2	3	2	1	1	1	0	0	0	1	0
C. squamulosus	0	0	0	0	0	0	1	0	0	1	0	1	0	0	1	0	1	0	1

marine elapids (Hydrophiinae), whereas no African, Asian or American elapids are reported to have more than four (Bogert, 1943). Some counts in the literature are probably unreliable, and dentigerous elements other than the maxilla have been mostly neglected by previous workers, but tooth counts on all bones of many elapids have recently been tabulated by Greer (1997).

Nine skulls of *squamulosus* examined show a range of 6-8 maxillary alveoli behind the fangs (Worrell, 1963 reported 7-10); *harriettae* usually has 5 (seven of eight specimens examined), but only 3 in one specimen; *churchilli* has 6 in both skulls examined; and *krefftii* 3-5 in three skulls (but Worrell, 1963; McDowell, 1967 and Greer, 1997, all report 2 in this species, so the range can be given as 2-5). Based on these figures, two non-overlapping ranges can be recognized: 6-8 (State 0) and 2-5 (State 1). State 0 is regarded as plesiomorphic, being similar to counts in *Glyphodon*, overlapping with *Demansia* and *Furina*, but lower than any in *Aspidomorphus*. The higher counts in *Demansia* and *Aspidomorphus* are a likely synapomorpy of these genera, while reduction has probably occurred independently in *Furina*, as in several other Australian lineages.

C2. Palatine tooth number. Despite overlapping ranges, *C. squamulosus* clearly tends to have a higher number of palatine teeth than the other species, and thus two states are recognized: usually more than 11 (State 0), usually 11 or fewer (State 1). The mostly high counts in *Aspidomorphus*, *Demansia* and *Glyphodon* imply that State 0 is plesiomorphic, while *Furina* tends to haver lower tooth numbers (as on the maxilla).

C3. Pterygoid tooth number. Two states can be recognized on the basis of non-overlapping ranges: 19-24 (State 0) and 11-18 (State 1). The outgroup species all have 19 or more except for *F. diadema* and *F. barnardi*, which overlap both ranges, so State 0 is considered plesiomorphic.

C4. Dentary tooth number. Again, two non-overlapping states can be defined for *Cacophis*: more than 20 (State 0) and fewer than 20 (State 1). Most of the outgroup species exhibit State 0, but *Glyphodon tristis* and *Furina* spp. fall mainly in the range of State 1, so polarity is equivocal.

C5. Nasal and preocular: frequency of contact. These scales are either in contact or narrowly separated (by contact between the prefrontal and a supralabial) in *Cacophis*, but variable within each species so that states can be defined based on frequencies. State 0 (usually contacting) occurs in *krefftii* (15 bilateral and 1 unilateral of 16 specimens; on the remaining side, nasal and preocular separated by a distinct 'loreal') and *squamulosus* (11 and 3 of 14; again one with a loreal on one side), and can be identified with the state in *Aspidomorphus* and *Demansia* (separation rare or absent). State 1 (usually narrowly separated) characterizes *churchilli* (0 bilateral contact, 1 unilateral of 15) and *harriettae* (1 bilateral of 15; indeterminate unilaterally in another where the preocular and prefrontal are fused). In *Glyphodon* and *Furina* the scales are widely separated (State 2). While State 0 is the only ingroup state shared with outgroup taxa, polarity cannot be inferred if the character is interpreted as an ordered morphocline (0-1-2).

C6. Posterior temporal scales. As noted above (character A6), variation is observed in the number of scales in the last row of temporals. Three posterior temporals is the usual condition in all outgroups and most other elapids (State 0); in a sample of *C. squamulosus* examined, three posterior temporals occur in 13 of 14 specimens, so this species is assigned the primitive state. The frequent occurrence of only two posterior temporals is recognized as an apomorphy (State 1) shared by the remaining species: *churchilli* has two in 12 of 15 (and unilaterally in another), *harriettae* in 14 of 15, and *krefftii* in every one of 16 specimens. The relative sizes of the scales indicate that it is the upper two of the three scales which fuse (3°1=3°2).

C7. Parietal and postocular scales: frequency of contact. Contact of parietal and lower postocular scales is a shared derived condition of all *Cacophis* species (see A5), but two distinct levels of frequency are apparent in samples examined for this trait. Relatively low frequency is coded as plesiomorphic (State 0), present in *harriettae* (contact bilateral in 1, and unilateral in 5 of 15 specimens) and *krefftii* (1 and 3 of 16). High frequency (State 1) characterizes *churchilli* (11 and 3 of 15) and *squamulosus* (12 and 1 of 14).

C8. Division of nasal scale: frequency. The nasal scale may be either single (pierced by the nostril) or divided (separated into anterior and posterior scales by grooves or sutures above and below the nostril). Complete separation of the nasal by the nostril extending its full depth, from supralabial to internasal, occurs in some other elapids (including some outgroup species), but is not observed in *Cacophis*. Division of the nasal is intraspecifically variable within *Cacophis*, so two states are recognized on the basis of frequency of division: high (State 0) in *churchilli* (bilateral in 10 and unilateral in 3 of 15 specimens) and *squamulosus* (13 and 0 of 14); and low (State 1) in *harriettae* (2 and 0 of 15) and *krefftii* (0 and 0 of 16). Because of the pattern of variation in the outgroups, polarity can not be assigned to this character.

C9. Body size (maximum snout-vent length [SVL]). The species of *Cacophis* vary considerably in size (Table 1; means might be preferable as the basis for this character, but good samples are not available for all species). A linear size increment close to the cube root of two (1.26 approx.) has been reported for sympatric species-pairs in numerous animal lineages by Hutchinson (1959) and others (see Sweet, 1980). In the three southern (sympatric) species of *Cacophis*, maximum SVL differs by ratios greater than 1.4 (implying ratios of 2.75 or more in mass), so that three distinct character states can be recognized. Most *churchilli* are relatively small, but the largest examined (QM J67837; SVL 538 mm, tail 58 mm) is slightly longer than the maximum recorded for *harriettae* (Shine, 1980*a*), so these two species are assigned the same intermediate state (1). Among the outgroups only *Glyphodon tristis* and some species of *Demansia* reach greater lengths than *squamulosus*, so large size (State 0, SVL > 70 cm) is probably apomorphic for this character. *C. krefftii* is one of the smallest of elapids (the largest specimen examined, AMS R13000, has SVL 345 mm, tail 39 mm), and all outgroup species except *Furina diadema* have a greater maximum SVL, so that it also represents an apomorphic extreme of the genus (State 2, SVL <35 cm). As each of the apomorphic states occurs in a single species, this character contributes no cladistic information within *Cacophis*. However, this 'uninformativeness' depends on the particular outgroup arrangement adopted here, and could possibly change if *Cacophis* were later determined to have a different pattern of relationships with other taxa.

C10. Ventral scale number. Ventral and subcaudal ranges of *Cacophis* species and outgroups are shown in Table 1; detailed frequency distributions would be preferable (cf. Wiens, 1998) but are not currently available for most species. All outgroup genera, and nearly all outgroup species, have ranges overlapping from 170 to 175 (all lower in *Aspidomorphus schlegelii* and *Demansia simplex*, all higher in some other *Demansia* species). Three species of *Cacophis* also overlap in this 'core' range, so the exception (*krefftii*) is regarded as an apomorphic extreme. The high ventral counts characterizing some *harriettae* may also be apomorphic, as they are outside the ranges of *Glyphodon* and *Aspidomorphus*. In order to utilize the maximum possible cladistic information from the data on ranges, each ingroup species is assigned a distinct state, and the four states are assumed to form a morphocline in the same order as the maximum and minimum observed ventral counts (State 0, *harriettae*; 1, *squamulosus*; 2, *churchilli*; 3, *krefftii*). Outgroup species are assigned the same state(s) as that of the ingroup species with which it most strongly overlaps; polarity remains indeterminate.

C11. Subcaudal scale number. As long-bodied snakes may have short tails (and *vice versa*), the ventral and subcaudal scale counts are considered independent characters. This character can be defined in the same way as the previous one – the four states ordered as the maxima for the ingroup species (State 0, *squamulosus*; 1, *harriettae*; 2, *krefftii*; 3, *churchilli*) since three of the minima are equal. Among the outgroups, counts below 35 occur only in *Aspidomorphus*; State 0 is parsimoniously considered plesiomorphic for *Cacophis*.

C12. Ventral melanin pattern. This character concerns only the distribution of dark brown or black, alcohol-insoluble pigment on the ventral surface; variation in dorsal colour is more continuous, and attributable to the combination of melanin and carotenoid patterns with schemochromes or structural colours (the latter responsible for whites as well as the bluish colour common in *krefftii*, and as a component of greens in some outgroup species; cf. Fox, 1953; Bechtel, 1978).

The outgroups vary considerably in ventral colour; *Demansia* spp. range from dark grey to immaculate white or yellowish, often with a median dark line or zone. The venter is usually white in *Furina* and *Glyphodon*; 'smoky' grey, peppered more or less

densely with melanin granules, in *Aspidomorphus* spp. and some individuals of *Glyphodon* spp. The dark slate-grey or black venter of *harriettae* and *churchilli* is similar to the conditions in some *Demansia* (*vestigiata*, some *torquata*) and *Aspidomorphus muelleri*, and approached by some *Glyphodon*, so uniformly distributed dark pigment is here presumed plesiomorphic (State 0). Southern (NSW) *C. squamulosus* have irregular black spots and blotches across the base of each ventral, and under the tail the black blotches form a continuous zig-zag line, while in many Queensland specimens (and at least as far south as Liston, northern NSW; pers. obs.) the black blotches on the ventrals are narrow and also form either a midventral line, or three distinct longitudinal rows. I have also seen a uniformly 'peppered' condition (precisely as in some *Aspidomorphus*) in a Sydney specimen of *squamulosus*, but this is rare. The state in *krefftii* is also contrasting, usually with a median line under the tail, but more regular on the body than *squamulosus*; the ventrals have a yellowish base and black posterolateral corners, typically forming a double saw-tooth pattern, but often joined as a continuous dark border across the free edge of some or most scales (as in the holotype of *C. fordei* Krefft, 1869). Despite the differences in detail, *squamulosus* and *krefftii* are coded with the same apomorphy (State 1).

C13, C14. Facial pattern and collar shape. The colour pattern on the face and nape is very similar in the four species of *Cacophis*; the dark upper surface of the head is bordered by a more or less continuous pale band extending from the rostrum, through and over the eyes and onto the nape; the pale stripe is broken up by dark markings at scale boundaries in the labial and temporal regions. Similar patterns are found in *Aspidomorphus* and *Demansia* (most complete in some *A. lineaticollis* and *D. torquata*), but patterns in *Glyphodon* and *Furina* are unlike these, with more discrete light and dark areas. A dark comma-shaped or 'bridle' marking from the eye to the lip is present in all *Cacophis* and some *Aspidomorphus* and *Demansia* (also some *Pseudonaja*).

In *squamulosus* the pale facial band is continuous with longitudinal stripes on the neck, somewhat expanded towards the midline but separated from each other by dark-pigmented vertebral and paravertebral scale rows (sometimes greatly elongated as shown by Gow, 1989: 95 and Greer, 1997: 178, rarely connecting to form a complete collar). This is quite similar to the 'upper light line' present in some *Aspidomorphus* populations (McDowell, 1967) which are most similar to *Cacophis* in pigmentation, and possibly also comparable to the pale or reddish dorsolateral streaks in some *Demansia* spp. (common in *D. psammophis*). The 'broken' collar is thus regarded as the plesiomorphic condition for *Cacophis*. One species of *Demansia* (*D. torquata*) has a complete, narrow pale collar continuous with a pale facial stripe; pale or dark collars in other outgroups are less similar (involving contrast between head and dorsal ground colour, or not continuous with facial markings). The collar is complete across the midline in the other species of *Cacophis*, but varies in width: about four scales wide in *harriettae*, i.e. similar in extent to that of *squamulosus* but without a dark median zone, and one or two scales wide in *krefftii* and *churchill*. Two binary characters are used, for separation vs. contact of the lateral pale markings, and width of the collar. *Furina* and *Glyphodon* are coded as not comparable for the first character, because of the very different distribution of dark pigment.

C15. Carotenoid pigment on body. Carotenoid pigments are highly soluble in alcohol, so best studied in live animals; I have not examined living *Aspidomorphus*, but O'Shea (1996) has photographs of two species in life. In *Glyphodon*, *Furina* and *krefftii* (in between dark markings) the venter is white or very pale yellow (carotenoid very faint or absent); white or yellow also occurs in some *Demansia* spp. including *D. simplex*. *Aspidomorphus lineaticollis* and some other *Demansia* have pink or orange ventral colours, and in *squamulosus* the venter varies among individuals from orange or pink to deep red, the same colour also suffusing the light centres on the dorsal scales and sometimes the collar. In one specimen from a variable population at Greenwich, NSW, the venter was a very deep red posteriorly, and there were dark orange to red centres on all of the dorsal scales of the body and tail (pers. obs.). The dark-bellied species of *Cacophis* and *A. muelleri*, appear to lack red pigments since none are visible on the sides or dorsum. Since red is present in only one ingroup species, this character is cladistically uninformative.

C16. Carotenoid pigment on face and collar. The facial stripe and collar are normally yellow in three of the species (State 0), but usually white (sometimes faintly yellow) in *harriettae* (State 1); in this species the pale centres of the dorsal scales are also whitish (an extreme condition is shown by a specimen illustrated in Wilson & Knowles, 1988: pl. 723). Yellow markings on the face and nape are considered plesiomorphic as they are usually present in nearly all outgroup species. In *Glyphodon*, yellow pigment is nearly or completely absent except for the nape patch of *G. tristis*, while in *Aspidomorphus lineaticollis* the face stripe is white in the specimen shown by O'Shea (1996: 149).

C17. Iris colour. This character concerns variation not due to melanin (see A7 above). Because of the density of melanin in the irides of *Furina* and *Glyphodon*, the presence or colour of other pigments in these taxa has not been observed; their relatives *Simoselaps bertholdi* and *S. littoralis* have white eyes, but may have apomorphically reduced carotenoid as well as melanin. Iris colour in life is not known for all *Demansia* and *Aspidomorphus* species, but in most of them, reddish pigments combine with the melanin to produce orange-brown eyes (e.g. *A. lineaticollis*, O'Shea, 1996; *Demansia* spp. illustrated in Storr *et al.*, 1986; Wilson & Knowles, 1988; Gow, 1989; Ehmann, 1992; Cogger, 1992, 2000). Red eyes, which also occur in *krefftii*, are therefore assumed to be plesiomorphic for *Cacophis* (State 0). The iris is predominantly yellow in

squamulosus (State 1), and at least partly white in *harriettae* and *churchilli* (State 2). Because the states can be ranked in order of intensity of colour, they are provisionally treated as ordered (0-1-2).

C18. Pupil shape. Pupils are strongly vertically elliptical in *squamulosus* and *harriettae*, as in *Glyphodon*, *Furina* and most *Aspidomorphus* (only weakly so in *A. muelleri*; McDowell, 1967) (State 0), but weakly oval or quite round in the other species of *Cacophis*, and round in all *Demansia* (State 1).

C19. Defensive threat display. In the threat displays of all *Cacophis* spp. the head is raised and angled downward (displaying the pale collar); from this position they may strike forward and downward, but almost never actually bite. In three of the species the neck is held straight and the jaws not or only slightly expanded during the display, while *squamulosus* is distinctive in two ways: the neck is formed into S-shaped lateral curves and the quadrates and rear of the mandibles are spread laterally, making the head much wider than the neck (e.g. Grigg, Shine & Ehmann, 1985: pl. 5; the narrow dark zone interrupting the pale collar, and longitudinal pattern on the lateral neck scales, tend to exaggerate this visual effect). All three *Furina* spp. have stiff-necked displays most like the smaller *Cacophis* spp. (e.g. Greer, 1997: 161), and *Demansia* spp., although relying on speed and venom in defence and thus apparently lacking a comparable 'bluff' display, have a similar raised-head 'alert' posture while foraging (e.g. Scanlon, 1998). *Glyphodon tristis* has a different defensive display (thrashing wildly in a horizontal coil, head- and tail-hiding), while *G. dunmalli* is described as inoffensive (Wilson & Knowles, 1988; Ehmann, 1992; Greer, 1997: 162). I know of no published descriptions of defensive or foraging behaviour in *Aspidomorphus*; *A. muelleri* strikes (and bites) from a more-or-less upright defensive posture when prevented from escaping (S. Richards, *pers. comm.* 2001), but on present evidence this cannot be identified with either of the states in *Cacophis*. Although a number of other (mostly large) Australian elapids have high lateral S-bends in the defensive display (e.g. some *Pseudonaja* spp., *Oxyuranus microlepidotus* and *Hoplocephalus* spp.), the behaviour in *squamulosus* is considered unequivocally derived since it is not paralleled in the outgroups; however, this apomorphy is not cladistically informative.

PHYLOGENETIC ANALYSIS

PARSIMONY WITH ORDERED CHARACTERS

Because of the small number of taxa, it is not considered useful to construct a 'hypothetical ancestor': the outgroup genera can be included explicitly along with ingroup species, and still allow exhaustive search of tree topologies.

Characters A1-7 and B1-10 are invariant within *Cacophis*, hence uninformative for intrageneric relationships. However, A1-7 (five binary, and two three-state ordered characters) and B10 (three-state ordered) are parsimony-informative for more inclusive analyses and hence retained. Of characters C1-19 which vary among *Cacophis* spp., 14 characters are binary; characters C9 and C17 are assumed to form ordered three-state morphoclines, and C5, C10 and C11 have four ordered states (Table 2). However, characters C9, C15, C16 and C19 are cladistically uninformative (derived states, or combinations of states for those coded as polymorphism, occur in single terminal taxa). When uninformative characters are excluded, the effective size of the data set is therefore 16 binary, 5 three-state ordered, and 2 four-state ordered characters.

There are two equally most parsimonious trees, one with the topology (*Aspidomorphus* ((*Demansia* (*Glyphodon*, *Furina*)), (*C. squamulosus* (*harriettae* (*churchilli*, *krefftii*))))), and the other differing only in the interchange of *harriettae* and *churchilli*. For both, tree length = 46 steps, consistency index (CI) = 0.652, homoplasy index (HI) = 0.348, retention index (RI) = 0.686, rescaled consistency index (RC) = 0.448. However, these trees conflict with the outgroup assumptions (Fig. 1), and because the characters analysed are chosen for informativeness relative to *Cacophis* rather than the outgroups, their basal nodes are considered unreliable.

Therefore, a constraint tree was used to enforce the (*Furina*, *Glyphodon*) and (*Aspidomorphus*, *Demansia*) clades. These topological constraints reduce the space of unrooted trees for eight taxa from 10 395 to 105 distinct alternatives. With the constraints, the single most parsimonious tree has total length 48 steps (Fig. 5); CI = 0.625, HI = 0.375, RI = 0.647, RC = 0.404. Cladistic relationships among the four ingroup species are identical to one of those found in the unconstrained analysis, where *Cacophis* also emerged as monophyletic.

The degree of support for each grouping was measured by the support index (Bremer, 1988), calculated in PAUP using a command file generated by TreeRot (Sorenson, 1999). These commands were modified to use branch-and-bound rather than a heuristic search algorithm. Nonparametric bootstrapping (10 000 replicates, employing branch-and-bound search) was also used to assess the robustness of each clade (apart from the outgroup clades where monophyly was enforced). Support for monophyly of *Cacophis* with respect to the outgroups (support index 4, boostrap frequency 94%) and of the (*churchilli, harriettae, krefftii*) clade (5, 94%) are strong, but that for a clade comprising *churchilli* and *krefftii* is weak (1, 58%). The alternative grouping of *harriettae* with *krefftii* was found in 30% of bootstrap replicates, but was less parsimonious (by one step) under the assumed constraints on outgroup relationships.

On the preferred phylogenetic hypothesis (Fig. 5), characters **C1**, C2, **C4**, **C5**, **C7**, **C8**, C11, **C12**, C17, C18, A5, and B10 are homoplasious (CI = 0.5 in each case except C4, C7, C8 [0.33], and C11 [0.60]). Of these, six are convergences between ingroup and outgroup taxa (not discussed further here), while those

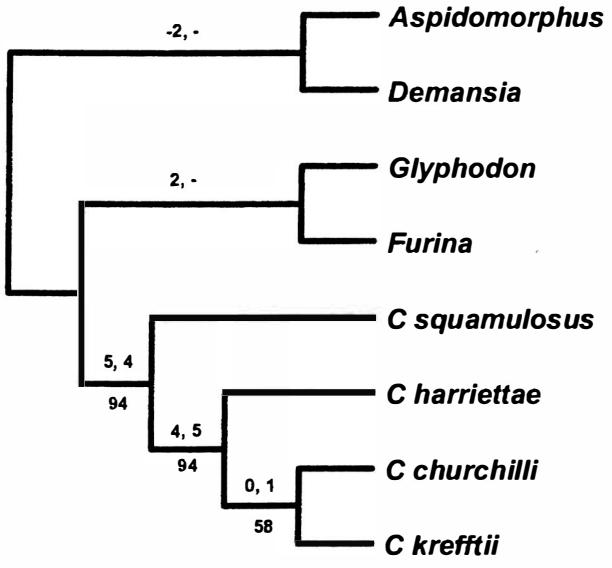

FIG. 5. Cladogram showing most parsimonious hypothesis of phylogenetic relationships among *Cacophis* spp., with multistate characters treated as ordered where applicable (analyses described in text). Numbers above branches show Bremer support for unconstrained analysis and when monophyly of both outgroups is enforced; numbers below branches bootstrap frequency (percent) in constrained analysis. Bootstrap values not applicable for the two outgroup clades (i.e. fixed at 100% by constraint tree).

shown in bold involve convergence or reversal within *Cacophis*. Characters C1 and C4 (tooth numbers on the maxilla and dentary) appear as putative synapomorphies of *harriettae* and *krefftii*, but they could easily have undergone convergence or reversal together due to common genetic basis (pleiotropy) or selective factors (i.e. they are probably not independent, cf. Lee, 1998). The frequency-based head-scale characters C5, C7 and C8 conflict with each other as well as the optimum topology, implying their independence but also the lability of such variables, consistent with 'neutral drift' or fluctuating selection on the equilibrium frequencies in each species. C12 (contrasting 'barred' ventral pattern), linking *squamulosus* and *krefftii*, is likely to be convergent as the patterns in these species differ in detail and may thus be considered to fail the similarity test of homology.

ALL CHARACTERS UNORDERED

The definitions of multi-state characters used above rely on the ordering implicit in topological and numerical relationships, i.e. on abstract properties of number rather than independent assumptions about evolutionary processes. It is nevertheless possible to analyse the 'same' matrix while disregarding this trivially available information about order, but character C10 (based entirely on rank order of meristic values) then becomes parsimony-uninformative, in addition to those excluded above. The shortest tree overall then has the topology (*Aspidomorphus* ((*Demansia* (*Glyphodon*, *Furina*)), (*squamulosus* (*churchilli* (*harriettae*, *krefftii*))))), one of the two found in the previous analysis; tree length = 38 steps, CI = 0.711, HI = 0.290, RI = 0.732, RC = 0.520. As before, this tree conflicts with the outgroup assumptions (Fig. 1).

When the unordered data are reanalysed with the two outgroups constrained to be monophyletic, there is a single most parsimonious tree with total length 41 steps; CI = 0.659, HI = 0.342, RI = 0.659, RC = 0.434. This tree differs from that found in the 'ordered' analysis (Fig. 5) in the interchange of *churchilli* and *harriettae*, i.e. the sister group of *krefftii* is found to be *harriettae*. In a bootstrap analysis under the same constraints, monophyly of *Cacophis* is found in 98% of replicates, and a clade comprising *churchilli*, *harriettae* and *krefftii* in 90% (cf. values in Fig. 5). The sister taxon to *krefftii* is found to be *harriettae* in 52% and *churchilli* in 22.4% of bootstrap replicates.

REVISED DIAGNOSES

The previous diagnosis of *Cacophis* is that of Cogger (2000; little modified from Cogger, 1975; see also Hutchinson, 1990; Greer, 1997). The revised diagnoses below list autapomorphies of *Cacophis*, included clades, and species, and to facilitate comparison with other taxa, I also list many plesiomorphic conditions for the genus, including both widespread characters and those shared with only a few outgroup taxa. Character states discussed in the text are identified by their labels (A1(0) etc.). Some of the characters listed but not mentioned elsewhere in the text, including features of the skull, dentition and vertebrae, will be discussed elsewhere (in prep.). Diagnoses of clades and species within *Cacophis*, based on the most parsimonious cladogram discovered in this work, list apomorphies according to the delayed transformation (deltran) optimization assumption; those invariant under acctran and deltran assumptions (unambiguous synapomorphies) are marked with an asterisk.

CACOPHIS GÜNTHER, 1863

Autapomorphies. Loss of paired dorsal foramina of parietal bone (A1(1)*; one member of pair may occur); palatine choanal process relatively tall, i.e. higher than long (A2(2)*); ectopterygoid does not extend forward past pterygoid-palatine joint (A3(2)*); supra-anal keels frequently present (A4(1)*; often extensive in adult males, occasionally developed in females and juveniles); parietal scale may contact lower postocular (A5(1); see also C7); temporal formula reduced from 2+2+3 to 1+3 by fusion of two anterior rows (1=1+3; A6(1)*, see also C6); iris pale (A7(1)*, melanin pigmentation reduced to faint speckling; C17(1), carotenoid yellow rather than reddish).

Features shared with outgroups. Small (C9(0/1); less than 75 cm snout-vent, 1 m total length), terrestrial, oviparous hydrophiine elapid snakes, dark brown or greyish above with pale centres on many of the dorsal scales tending to form a longitudinal pattern on the flanks and neck; no trace of transverse bands on the

body or tail. A yellowish band, including dark spots and variegations, across the snout (including most or all of the internasal scales), over and through the eyes and temporal region, and expanding towards the midline at the rear of the head, beginning one to several scale-rows behind the parietals (C13(0), not forming a continuous transverse band; C14(0), extending four or more scale rows back on the neck); dark variegations absent or faint in the nuchal portion. Pale facial band broken by a distinct dark 'bridle' marking joining the eye to the lip. Eyes equal or smaller in diameter than their distance from the lip, pupil vertically elliptical (C18(0)). Venter with diffuse dark melanin pigment (C12(0)); yellowish carotenoid pigment also present ventrally (C15(0)). Snout short and rounded, no canthus rostralis; nasal usually divided (C8(0)), usually in contact with preocular (C5(0)); preocular contacts second supralabial; six supralabials, third and fourth entering eye; seven infralabials. Internasal and prefrontal scales usually overlapping left over right (*krefftii*, Greer, 1993; remaining species, pers. obs.). 17 to 23 longitudinal rows of dorsal scales at the first ventral (pers. obs.), reducing to 15 on neck and rarely reducing again before vent (B9(3), B10(1/2)). Anal and usually all subcaudals divided (sometimes a few anterior or scattered subcaudals single). Dorsal scales matt to slightly glossy, lacking keels (except in the cloacal region, see A4). Skin between dorsal scales pale (light brown or grey). Tongue with dark pigment only on middle portion, so base reddish and tips pale pink or white in life. Superficial venom-gland constrictor muscle (m. adductor externus superficialis) without separate quadrate head from rear of gland, and m. adductor externus medialis exposed posterior to superficialis (i.e. '*Glyphodon* type' of venom-gland musculature; McDowell, 1967). Hemipenis forked, apical lobes with terminal awns; basal portion nude, bounded distally by a row of weakly enlarged spines (Keogh, 1999).

Frontal bones together oval or diamond-shaped, prefrontal articulated to anterolateral border but not usually reaching parietal or postorbital (so frontal nearly always narrowly enters orbital margin) (B1(1)); interolfactory pillars of frontals as wide as the frontal-septomaxilla contact (B2(1)). Postorbitals in edge-to-edge contact with parietal (allowing mediolateral but not anteroposterior kinesis), not or barely reaching frontals, so parietal may enter orbital margin. Parietal relatively long, narrow and slightly bulbous (not constricted), with triangular supraorbital processes clasping frontals, distinct and elongate but narrow postorbital processes, and weak adductor crests either separated or just meeting posteriorly (not forming a sagittal crest except in the largest individuals). Parasphenoid in ventral view triangular, not narrow and awl-like. Fenestra ovalis in opisthotic-exoccipital open laterally for its full width (from border of prootic), so shaft of stapes not enclosed laterally by bony crista circumfenestralis. Maxilla extends to or beyond posterior limit of orbit (B4(1)); suborbital portion smoothly concave dorsally and convex laterally; two anterior canaliculate fangs with ridged or striated surfaces, followed after a diastema by 6-8 small solid or grooved teeth (C1(0)) extending onto a rod-like posterior process defined by concavities laterally and medially. Palatine with 11-17 teeth (C2(0)), extends approximately as far anteriorly as maxilla; posterior end with lateral and medial processes clasping anterior end of pterygoid; without lateral (maxillary) process or sphenopalatine foramen, but with distinct dorsomedial 'choanal' process on the posterior part of the shaft (A2(1/2)). Ectopterygoid not extending anteriorly beyond pterygoid-palatine joint (A3(1/2)); lateral edges of ectopterygoid parallel anteriorly, angling posteromedially at a slight knob-like prominence level with the rear of the maxilla. Pterygoid with 19-24 teeth (C3(0)); lateral edge with an angular inflection or triangular process for ectopterygoid attachment; posteromedial edge usually convex, posterior tip blunt. Dentary with 14-28 teeth (C4(0/1)), increasing steeply in size from inflected anterior tip to two subequally large, robust teeth (B7(1)) with anterolateral grooves; large 6th, 7th or 8th tooth usually followed by a gap (diastema; B8(1)) and shorter, more recumbent, posterior teeth.

Zygosphenes of vertebrae in dorsal view trilobate, with rounded median lobe; prezygapophyseal processes prominent, acuminate (terms of Auffenberg, 1963) and angled anterolaterally; hypapophyses of posterior trunk vertebrae in lateral view with angle separating oblique from horizontal portions of ventral edge (B6).

Habitat is wet sclerophyll or rainforest; nocturnal, sheltering by day under rocks, logs, leaf litter, or in cavities associated with ant or termite nests; diet mainly of diurnal skinks captured at night under cover, also frogs, small snakes, and reptile eggs (Shine, 1980*a*). In defensive threat display, the anterior part of the body is raised stiffly and the head turned downward but not markedly flattened (C19(0)).

CACOPHIS SQUAMULOSUS (DUMÉRIL, BIBRON & DUMÉRIL, 1854)

Parietal contacts lower postocular in majority of specimens (C7(1)*); snout-vent length may exceed 70 cm (C9(0)*); dark ventral pigment usually forming distinct blotches or bars across base of each ventral scale, and a zig-zag median line on the subcaudals (C12(1)*); carotenoid pigment suffusing ventral and lateral scales reddish (pink or orange to deep red; C15(1)*); in defensive threat display, neck held in lateral S-shaped coils, and rear end of jaws spread laterally to widen and flatten the head (C19(1)*).

(*CACOPHIS HARRIETTAE*, *C. CHURCHILLI*, *C. KREFFTII*)

Palatine with 11 or fewer teeth (C2(1)*); pterygoid with fewer than 19 teeth (C3(1)*); usually only two scales in posterior temporal row (C6(1)*); subcaudal count may be lower than 30 (C11(1)*); pale band continuous across dorsal midline at back of head (C13(1)*).

CACOPHIS HARRIETTAE KREFFT, 1869

Maxilla with fewer than six teeth behind diastema (C1(1)); dentary with fewer than 20 teeth (C4(1)); nasal and preocular usually separated (C5(1)); nasal usually undivided (C8(1)); ventral count not less than 170 and may exceed 175 (C10(0)*); collar (and longitudinal pale stripes on body) usually white, not yellowish (C16(1)*); iris mainly white (C17(2)).

(*CACOPHIS CHURCHILLI*, *C. KREFFTII*)

Ventral count less than 176 and may be less than 165 (C10(2)*); subcaudal count not exceeding 40 (C11(2)*); pale collar only one or two scales wide (C14(1)*); pupil round or only slightly elliptical (C18(1)*).

CACOPHIS CHURCHILLI WELLS & WELLINGTON, 1985

Nasal and preocular usually separated (C5(1)); parietal usually contacts lower postocular (C7(1)*, see also A5); subcaudal counts less than 40 (C11(3)*); iris partly white (C17(2)).

CACOPHIS KREFFTII GÜNTHER, 1863

Maxilla with fewer than six teeth behind diastema (C1(1)); dentary with fewer than 20 teeth (C4(1)); nasal scale undivided (C8(1)); snout-vent length less than 35 cm (C9(2)*); ventral count does not exceed 160 (C10(3)); melanin pigment on ventral scales concentrated at posterolateral corners forming double saw-tooth pattern, or also extending medially as a continuous dark border on each ventral (leaving base of each scale white or pale yellow), and usually forming a median zig-zag stripe under tail (C12(1)*); iris red (C17(0)*).

DISCUSSION

The evidence for a sister-group relationship between *Cacophis squamulosus* and the remaining members of the genus allows us the option of resurrecting *Petrodymon* Krefft, 1866 (cf. Wallach, 1985). However, this would result in a monotypic (i.e. redundant) genus unless populations currently assigned to *C. squamulosus* prove to belong to more than one species. Variation in ventral colour patterns within this species has been noted above, and northern specimens tend to be slightly larger (Shine, 1980*a*), but no detailed investigation of geographic variation has been made. The autapomorphic modifications of defensive display and ventral pigmentation in *C. squamulosus* could be regarded as adaptive mimicry of the sympatric *Pseudechis porphyriacus*. The difference in relative height of the display, and flattening of the head rather than the neck, do not contradict this hypothesis, since *C. squamulosus* thereby both displays its black-barred red belly to advantage, and reaches the height and head-width of a 'Red-bellied Black' larger than itself.

Within the (*churchilli*, *harriettae*, *krefftii*) clade, the characters used here indicate that the sister taxon to the most derived species, *krefftii*, is either *churchilli* or *harriettae*, and less likely to be a clade comprising both. The alternative preferred here is the tree obtained in the 'ordered' analysis with outgroup constraints (Fig. 5). This hypothesis is also the only one in which the two most recently separated species have disjunct geographic distributions, consistent with a vicariance process: *krefftii* occurs from Gosford, NSW, to Mackay (e.g. QM J14287) on the Queensland coast, while *churchilli* is found further north, from Townsville (J3640) to Mossman (J5193). The greater divergence of *krefftii* (autapomorphy in features such as head shape, eye colour, and small body size) could be interpreted as character displacement due to selection, since it is broadly sympatric with both *harriettae* and *squamulosus*, whereas the distribution of *churchilli* overlaps little, if at all, with either species (see distribution maps in Wilson & Knowles, 1988, and Ehmann, 1992; those in Cogger, 1992, 2000 are less accurate, and in Longmore, 1986 the maps for *harriettae*, *krefftii*, and *squamulosus* include misidentified records of *churchilli*).

Interpretation of the cladogram in terms of species-level historical biogeography (phylogeography) is complicated by the broad sympatry between species; vicariance alone is not a sufficient explanation for their present distribution. However, even non-vicariance hypotheses are testable in terms of congruence with phylogenetic and distributional patterns in other lineages, and in this case we are favoured by the strong habitat-fidelity of *Cacophis* spp. We may assume several cycles of interruption and reconnection of the eastern wet forest corridor, which have been frequent during Plio-Pleistocene times (e.g. Bowler, 1982). One possibility would involve a persistent northern population expanding southward during three successive periods of forest continuity (cool or moist periods, perhaps the three major glaciations), and the southern populations then differentiating after interruption of the forest corridor, giving rise to the three more divergent, sympatric, southern species (*squamulosus*, *harriettae* and *krefftii* sequentially; Fig. 6).

This model is simple in several senses: it invokes only passive allopatric speciation (the preferred null hypothesis in, e.g., Brooks & McLennan, 1991), no extinctions, and a known process of historical environmental change in (at this scale) an essentially one-dimensional geographic space. The spatial asymmetry in the model (only southward range expansions) provides a uniform explanation for the observed distributions based on the most parsimonious cladogram.

Some other Australian elapids (*Hemiaspis signata*, *Cryptophis nigrescens*, *Hoplocephalus bitorquatus* and *Tropidechis carinatus*) have distributions comparable to that of *Cacophis* as a whole, interrupted by drier belts along the Queensland coast, but without evidence of speciation. In *H. signata*, a northern form *vagrans* Garman, 1901 is sometimes recognized as a subspecies or species, but no such distinction has been demon-

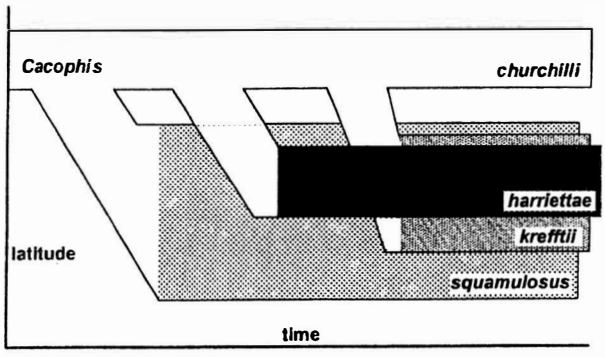

FIG. 6. Schematic distribution of *Cacophis* in space and time, according to phylogenetic hypothesis in Fig. 5 and speciation model proposed in text. Vertical axis represents latitude; present distribution of the genus is between 15° and 35°S, with *C. churchilli* north of 20° and other species mainly or entirely further south. Horizontal axis represents time, from before beginning of Pleistocene climatic fluctuations (left) to the present day (right). Connectivity of 'overlapped' regions of branches is indicated by shading.

strated. The genera *Hypsilurus* (Agamidae) and *Coeranoscincus* (Scincidae) each have two well-differentiated allopatric species with similar distributions to *Cacophis churchilli* and *krefftii* respectively; *Litoria xanthomera* and *L. chloris* (Anura, Hylidae) form a similar vicariant pair, while patterns which may be comparable to *Cacophis* as a whole are seen in *Calyptotis*, *Lampropholis*, *Saproscincus* (Scincidae) and *Mixophyes* (Myobatrachidae), each with five or more species (Cogger, 1992, 2000; Barker, Grigg & Tyler, 1995). Among small, terrestrial forest mammals, *Dasyurus maculatus*, *Antechinus flavipes* and *A. stuartii* (Marsupialia, Dasyuridae), *Rattus fuscipes* and *R. lutreolus* (Rodentia, Muridae) also have breaks between southern and north-Queensland populations (subspecies; Strahan, 1983). As relevant sequence data become available, the estimation of divergence dates using molecular 'clocks' may indicate whether *Cacophis* and other such lineages have been affected by the same sequence of environmental changes (undergoing evolution as a community), or have followed independent timetables. Based on differences in the DNA sequences of two genes, Keogh *et al.* (1998) infer a split between *Cacophis squamulosus* and *krefftii* 'of considerable antiquity', but do not give a quantitative age estimate, and comparable sequences have not yet been reported for the other species.

The monophyly of *Cacophis* is now well supported, and the results of this analysis will allow it to be treated as a unit in future work. For example, the evidence presented here may help to determine the relationships of *Demansia* with other lineages, which seems to be one of the central problems of elapid phylogeny. Morphological analyses have associated *Demansia* with *Aspidomorphus* and members of the viviparous lineage (McDowell, 1967, 1969*b*, 1985), or with *Pseudechis* (Wallach, 1985), while most genetic studies have failed to show close relationships with any Australian lineages (Cadle & Gorman, 1981; Mao, Chen, Yin & Guo, 1983; Schwaner *et al.*, 1985; Mengden, 1985). A view has developed that *Demansia* is only distantly related to other Australian taxa (Mengden, 1985; Shine, 1991; Greer, 1997), although this does not necessarily follow from large genetic distances (phenetic data). Keogh *et al.* (1998) report DNA sequence evidence for a clade comprising *Cacophis*, *Aspidomorphus*, *Demansia*, *Furina* and *Glyphodon*, and some of the skeletal characters referred to above may also support such a group. These results suggest that the earlier genetic studies were affected by accelerated genetic change in the whipsnake lineage (as first suggested by Cadle & Gorman, 1981), and that other methods may have more success.

Further studies will continue to improve understanding of the adaptive radiation of Australasian elapids, and external morphology, internal soft anatomy, cranial and axial skeletal morphology (including the fossil record), genetic and molecular methods, and behavioural and ecological data can all contribute to this end.

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APPENDIX 1

Sources of data for ingroup and outgroup species. Skeletal material examined marked with asterisk (*); specimens examined only for external characters omitted for taxa other than *Cacophis* spp. and *Demansia simplex* (available from author on request). Useful sources of further data and illustrations for Australian species include Cogger (1975, 1992, 2000), Storr *et al.* (1986), Wilson & Knowles (1988), Gow (1989), Hoser (1989), Ehmann (1992), and Greer (1997).

Aspidomorphus lineaticollis: AMS R125021*, AMNH 42376*; *A. muelleri*: AMS R16614*, R19013*; *A. schlegelii* : MCZ 7311* (AMNH and MCZ specimens examined by M. Lee); Brongersma (1934), McDowell (1967), Shine and Keogh (1996), O'Shea (1996).

Cacophis churchilli: AMS R6489, R10732, R11340, R11362, R11506, R11512, R12480, R12482, R12914, R17035, R20207, R53726, R63163, R63836, QMJ3640, J4295, J5193, J5292, J5296, J5720, J5722, J5723, J5724, J5725, J5954, J7339, J13674, J21206, J24539, J53282*; SAM R22392, JS63*.

Cacophis harriettae: AMS R648, R5844, R6182, R8507, R9399, R11687, R12734, R12854, R13027, R13701, R13716, R18277, R47545, R86763, R88468, QM J4443*, J20278*, J26544*, J46288*, J47658*, J47982*, J50600*, SAM R26989*; McDowell (1967), Shine (1980a).

Cacophis krefftii: AMS R877 (x2), R1502, R2303, R2411, R4118, R6545, R7492, R8026, R8768, R9198, R10013, R11812, R12356, R12738, R12919, R12995, R13000, R13001, R13064, R13662 (x2), R13743, R13799, R13823, R13824, R13869, R13982, R14344, R14359 (x2), R14422, R14815, R15690, R17917, R18482, R47474, R58486, R75961, R74466, R77370, R81158, R81159, R81160, R81161, R86762, R86797, R90609, R97275, R106956, R110341, R114956, R125410, AMS unreg.*, QM J966, J14287, J32725, J34031, J46583*, SAM R26974*; McDowell (1967), Shine (1980a), Wells (1980).

Cacophis squamulosus: AMS R28232, R29733, R30336, R37187, R41801, R47471, R47544, R47546, R47779, R48108, R50220, R52964, R62710, R64975, AMS unreg. (x4)*, QMJ47659*, J47976*, J47983*, SAM R2263A*, JS3*, JS14*; Shine (1980a).

Demansia psammophis: QMJ7134*, J26907*, J46291*, J47978*, AMS R-13-672*, JS 44*; *D. vestigiata* : AMS R-13-667*; *D.* sp. cf. *olivacea* : JS169*; *D.* sp. cf. *torquata* : QMJ46289*, SAM R20483*; *D. simplex* : AMS R13045, R13046, R13702, R14030, R14029, R 128403, R128404, NTM R18625*; Storr (1978), Shine (1980b), Shea and Scanlon (unpublished data).

Furina barnardi: SAM R27022*, AMS unreg.*; *F. diadema* : AMS R98165*, SAM R6075*, R6703*, JS32*; *F. ornata* : WAM R15088*; Shine (1981), Storr (1981), Scanlon (1985, unpublished data).

Glyphodon dunmalli: QM J23178*; *G. tristis* : SAM R13998*, MV unreg.*; Boulenger (1896), Worrell (1955), Shine (1981) , Scanlon (unpublished data).

APPENDIX 2

List of characters used in the phylogenetic analysis.

A. Autapomorphies of *Cacophis*. See Table 2 for distribution of states in outgroup genera.

1. Paired parietal foramina: present, at least in small specimens (0); normally absent (1).
2. Palatine choanal process: absent (0); present but low, i.e. 'short' (1); higher than long, i.e. 'tall' (2).
3. Ectopterygoid anterior extent: anterior to palatine-pterygoid joint (0); approximately level with (lateral to) joint (1); entirely posterior to palatine (2).
4. Supra-anal keels: lateral scales of cloacal region similar in gross morphology to those of rest of body (0); patch of keeled lateral scales present in adult males (1).
5. Parietal and lower postocular: separated by contact of upper postocular with anterior temporal (0); sometimes in contact, separating upper postocular from temporal (1).
6. Temporals: 2+2+3, three distinct rows (0); 1=1+3 (or 1=1+2), single large anterior temporal incorporating temporolabial (1).
7. Iris colour in preservative (melanin): entirely or mainly dark (0); pale, with at most dark flecks or faint variegation (1).

B. Characters possibly derived within Australasian elapids but shared by *Cacophis* with both outgroup clades. See Table 2 for distribution of states in outgroup genera.

1. Prefrontal and postorbital bones: widely separated and frontal broadly entering orbital margin (0); prefrontal and postorbital approach or meet, effectively excluding frontal from margin (1).
2. Interolfactory pillars of frontals: distinctly constricted (0); as wide as the septomaxillary-frontal contact, widely separating olfactory openings of frontal (1).
3. Maxilla anterior process: short and blunt (0); prominent or acute in ventral view (1).
4. Maxilla posterior extent, relative to postorbital in lateral view: short, not beyond orbit (0); long, beyond posterior margin of orbit as defined by the postorbital (1).
5. Coronoid eminence of mandible: absent, dorsal margin of compound smoothly curved (0); eminence present as slight to strong convex angulation of dorsal edge of surangular (1).
6. Hypapophysis shape in posterior trunk vertebrae: smoothly sigmoid in lateral view (0); some vertebrae with a distinct horizontal portion defined by an anteroventral angle (1).

7. Dentary teeth: uniform or with smooth gradient of size (0); distinctly larger anteriorly (1).
8. Dentary tooth row: lacks a diastema (0); diastema commonly present behind enlarged teeth (1).
9. Number of midbody scale rows: 19 or more (0); 17 (1); 15-17, intraspecifically variable (2); 15 (3). Polarity follows Wallach (1985), but state 0 is almost certainly derived within *Glyphodon* (21 rows in *G. dunmalli*).
10. Posterior scale-row reduction: one or more reductions always present (0); variable, reduction sometimes present (1); reduction rare or absent (2).

C. Characters varying within *Cacophis*. See Table 3 for distribution of states in *Cacophis* species and outgroup genera.

1. Maxillary teeth posterior to fangs: 6-8 alveoli (0); 2-5 (1).
2. Palatine teeth: usually more than 11 alveoli (0); 11 or fewer (1).
3. Pterygoid teeth: 19-24 alveoli (0): 11-18 (1).
4. Dentary teeth: 21 or more (0); less than 20 (1).
5. Nasal and preocular: usually in contact (0); usually separated, contact rare (1); normally widely separated, contact not observed (2). Ordered 0-1-2.
6. Posterior temporals: nearly always three in final row (2+2+3 or 1=1+3) (0); reduced to two in most individuals (1=1+2) (1).
7. Parietal-postocular contact, frequency: minority (0); majority (1).
8. Nasal scale: divided in majority (0); single in majority (1).
9. Maximum snout-vent length: greater than 70 cm (0); 40-65 cm (1); less than 35 cm (2). Ordered 0-1-2.
10. Ventral scale number (range): 176-200 (0); 170-175 (1); 165-169 (2); 161-164 (3); 140-160 (4). Ordered.
11. Subcaudal scale number: 41-50 (0); 38-40 (1); 30-37 (2); 25-29 (3). Ordered 0-1-2-3.
12. Ventral melanin pigment: uniformly distributed, 'peppered' or generally dark grey (0); strongly contrasting pattern, usually transverse dark and light bands on each ventral scale (1).
13. Upper light line or nape band: pale lines on neck longitudinal, separated across midline (0); transverse and connected across midline, forming a complete collar (1).
14. Upper light line or nape band: occupies at least 4 transverse scale rows (0), or 1-2 only (1).
15. Carotenoid on body: pale yellow or absent (0); orange to red (1).
16. Carotenoid on face and nape: yellow (0); very pale yellow or white (1).
17. Iris colour in life (non-melanin): red (0); yellow (1); partly or mainly white (2). Ordered 0-1-2.
18. Pupil shape: vertically elliptical (0); weakly oval or round (1).
19. Forebody in high defensive display: held straight (0); lateral curves (1).

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A NEW SPECIES OF *PSEUDOEURYCEA* (CAUDATA: PLETHODONTIDAE) FROM NORTHERN OAXACA, MÉXICO

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We describe a new species of *Pseudoeurycea* from the northern-most high peak of the Sierra de Juárez, Oaxaca, México. This species belongs to the *P. juarezi* group, a monophyletic assemblage restricted to northern Oaxaca and comprising three species: *P. juarezi, P. saltator,* and *P. aurantia* sp. nov. *Pseudoeurycea aurantia* is the sister taxon to the clade formed by *P. juarezi* and *P. saltator*. The new species is diagnosed by a distinctive coloration and by divergent mitochondrial DNA sequences.

Key words: mitochondrial DNA, new species, phylogeny, salamander, systematics

INTRODUCTION

Pseudoeurycea is a large clade of neotropical salamanders that displays extensive morphological diversity and genetic differentiation (Parra-Olea & Wake, 2001; Parra-Olea, 2002). It ranges from northern México across the Isthmus of Tehuantepec into Guatemala and – with few exceptions – its species occur at elevations above 1200 m.

The systematics of *Pseudoeurycea* have changed recently, with several species newly described or in the process of description (Parra-Olea *et al.*, 2001; Wake & Campbell, 2001) and several more identified as new (Parra-Olea, 1999, 2002). A recent phylogenetic analysis (Parra-Olea, 2002) based on mtDNA showed the paraphyly of *Pseudoeurycea* with respect to *Lineatriton* and *Ixalotriton*. This paraphyly forced the transfer of *P. parva* to *Ixalotriton*, but the taxonomic problem involving *Lineatriton* is still unsolved (Parra-Olea & Wake, 2001).

The analyses of Parra-Olea (2002) found phylogenetic support for the recognition of three species groups within *Pseudoeurycea* (*P. bellii*, *P. gadovii* and *P. leprosa* species groups). Additionally, three taxa (*P. juarezi*, *P. saltator* and *P. unguidentis*) are not grouped with any of these and form part of the basal polytomy. *P. juarezi* and *P. saltator* are sister taxa, and their relationships to the rest of *Pseudoeurycea* are not resolved. *P. unguidentis* forms part of the basal polytomy on its own.

The group formed by *P. juarezi* and *P. saltator* (here termed the *P. juarezi* group), includes species characterized by morphology associated with semiarborial life, with long limbs and toes and slender bodies. The species inhabit terrestrial habits, under rocks, logs and under the bark of logs on the ground, or bromeliads. Their range is from high altitude, unforested habitats dominated by grasses to mid-elevation cloud forest in the Sierra de Juárez of northern Oaxaca, México.

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The Sierra de Juárez, where all known species of the *P. juarezi* group occur, is a moderately high mountain chain (2900 m maximum altitude) which runs from the highlands of Cuicatlán Valley (Cerro Peña Verde) to the east of Ciudad de Oaxaca, with southern limits in the area of Cerro San Felipe and Cuajimoloyas. At Cerro San Felipe, the Sierra Aloapaneca intersects the Sierra de Juárez, and runs west and north of Cerro San Felipe. The vegetation changes according to altitudinal gradients from the nearly treeless summits of Cerro Pelón covered by low shrubs to the mesic cloud forest. The herpetofauna of the region, one of the richest in montane México (Casas-Andreu *et al.*, 1996) keeps providing many new species despite past intensive search. During the last eight years, six new salamanders (Hanken & Wake, 1994, 2001) and 10 anurans (Campbell & Duellman, 2000; Mendelson, 1997; Mendelson & Campbell, 1999; Toal, 1994; Toal & Mendelson, 1995; Ustach *et al.*, 2000) have been described for the Sierra de Juárez.

A visit to the Sierra Peña Verde – the northern-most high peak of the Sierra de Juárez in the highlands above the Cuicatlán Valley – uncovered a morphologically distinct new salamander that resembles species of the *P. juarezi* group. The analysis of mitochondrial DNA (mtDNA) partial sequences of the Cytochrome *b* (Cyt *b*) and 16S genes confirmed its close relationship with members of the *P. juarezi* group, but it is not the sister taxon of any of the known species. We present here a new phylogenetic hypothesis for the group together with the description of this new taxon. We provide some ecological data and describe the eggs of the new species.

MATERIALS AND METHODS

MORPHOLOGICAL DESCRIPTION

The descriptions follow the format used by Lynch & Wake (1989) for other species in the genus *Pseudoeurycea*, and include the same basic characters and measurements. Larger measurements were taken using dial calipers (to the nearest 0.1 mm), but measurements of feet, toes and some head dimensions

(e.g. additional measurements of the holotype), as well as tooth counts were taken using a stereoscopic microscope equipped with an eyepiece graticule. All measurements are in mm. The distance from the tip of the snout to the posterior end of the vent is treated as standard length (SL). Colour notes are based on field notes taken from living specimens, and on preserved specimens. Osteological descriptions are based on two cleared and bone-cartilage differentially stained specimens (IBH 13797, 13798).

Material examined: Nine specimens from 4 km W Peña Verde, Oaxaca, México, 2805 m elevation, 17° 50.20' N, 96° 47.05' W, (EBUAP2052-2058; IBH13793-13794). Material for coloration and mtDNA comparisons consisted of specimens of *P. juarezi*, and *P. saltator* recently collected (Parra-Olea *et al.*, 1999, Parra-Olea, unpubl. data). Comparative measurements between *P. juarezi* and *P. saltator* were taken from Lynch & Wake (1989). Institutional abbreviations: EBUAP: Escuela de Biología, Universidad Autónoma de Puebla, México; IBH: Colección Nacional de Anfibios y Reptiles, Instituto de Biología, UNAM, México.

MTDNA SEQUENCES

We obtained partial sequences of 16S and Cyt *b* for the single specimen from which tissue was available (IBH13793). Whole genomic DNA was extracted from small amounts of ethanol-preserved tissues, using the Quiagen DNA extraction kit. We sequenced 554 base pairs of the large 16S subunit ribosomal mtDNA gene corresponding to positions 2510-3059 in the human mitochondrial genome (Anderson *et al.*, 1981), and 570 base pairs of the Cytochrome *b* gene, expanding from codon 7 of the *Xenopus* Cyt *b* gene (Roe *et al.*, 1985). Amplification was done via the polymerase chain reaction (PCR) (Saiki *et al.*, 1988), using the primers "MVZ15", "MVZ18" (Moritz *et al.*, 1992) for Cyt *b*, and the primers "16Sar" and "16Sbr" (Palumbi *et al.*, 1991) for 16S. PCR reactions consisted of 38 cycles with a denaturing temperature of 92°C (1 min), annealing at 48-50°C (1 min), and extension at 72°C (1 min) in a Techne PHC-1 thermocycler. PCR reactions were run in a total volume of 25 µl, using 0.5 pmol of each primer. Double strand templates were cleaned using a QIAquick PCR purification kit (QIAgen). We used 1 µl of PCR product as the template for cycle sequencing reactions in a 10 µl total volume with the Perkin-Elmer Ready Reaction Kit to incorporate dye-labeled dideoxy terminators. Thermal cycling was performed using standard conditions. Cycle sequencing products were purified using ethanol precipitation and run in an ABI 310 capillary sequencer.

The sequences were compiled using Sequence Navigator™ version 1.0.1 (Applied Biosystems), and aligned to the previously published data set for species of *Pseudoeurycea* (Parra-Olea, 2002). Pairwise comparisons of corrected sequence divergence (Kimura 2-parameter; Kimura, 1980), were obtained using the computer program PAUP*4.0b8a (Swofford, 2002).

Phylogenetic inference was based primarily on maximum parsimony analyses (MP: Swofford, 2002). MP phylogenies were estimated using the heuristic search algorithm for each tree-building methodology. We used 10 repeated randomized input orders for taxa in all MP analysis to minimize the effect of entry sequence on the topology of the resulting cladograms. MP analyses were conducted without the steepest descent option, and with accelerated character transformation (ACCTRAN) optimization, tree bisection-reconnection (TBR) branch swapping, and zero-length branches collapsed to yield polytomies. We used nonparametric bootstrapping (1000 pseudoreplicates) and decay indices to assess the stability of internal branches in the resulting topologies (Felsenstein, 1985; Felsenstein & Kishino, 1993). Each base position was treated as an unordered character with four alternative states. Gaps were treated as missing data.

DESCRIPTION OF NEW SPECIES

PSEUDOEURYCEA AURANTIA, NEW SPECIES
PEÑA VERDE SALAMANDER
SALAMANDRA DE PEÑA VERDE

Holotype. EBUAP2051, an adult male collected 4 km W Peña Verde, Oaxaca, México; 2805 m elevation; 17° 50.20' N, 96° 47.05' W, on 7 May 2000 by Luis Canseco Márquez (Fig. 1a).

Paratypes. All from Peña Verde, Oaxaca, México: IBH13793-13794 (two specimens), and EBUAP2052-2058 (seven specimens), same data as the holotype.

Diagnosis. We include this species in the genus *Pseudoeurycea* based on the following osteological characters: distal tarsal five separated from tarsal four, smaller than four, and not articulated with central; premaxillary single; middle digits markedly larger than the outer; and presence of sublingual fold. It is distinguished from all other *Pseudoeurycea* by its orange coloration. This species is closely related to members of the *P. juarezi* group based on morphology and mtDNA (Figs. 1, 4). It is distinguished from *P. juarezi* in having fewer maxillary and premaxillary teeth (63-81 vs. 74-100 for *P. juarezi*), and a shorter tail (relative tail length=0.88 vs. 0.96 in *P. juarezi*); and from *P. saltator* in having a larger size (maximum SL = 51 mm vs. 48 in *P. saltator*), and a shorter tail (relative tail length =0.88 vs. 1.05 in *P. saltator*).

Description. A medium-sized salamander; SL in two adult males 43--43.4 (mean=43.2) and in eight females 38.5--51.7 (mean=44.6), with relatively robust habitus; head relatively broad, (15-16% SL in both males, and females); snout broadly rounded, more truncate in males than in females; neck region ill-defined, only slightly narrower than head; eyes moderate in size, only slightly protuberant. Adult males present mental gland well developed. Parotoid glands not evident. Costal folds 13, counting one each in axilla and groin. Limbs are rela-

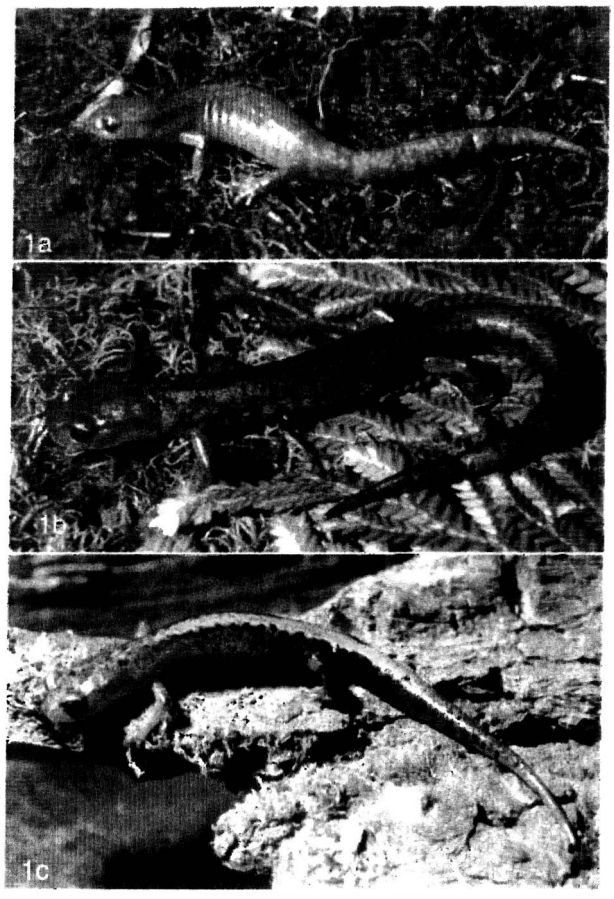

FIG. 1. Live specimens of members of the *P. juarezi* group – a, *P. aurantia*, EBUAP 2052 (paratype), b, *P. juarezi* (Cerro Pelón, Oaxaca, México); c, *P. saltator* (La Esperanza, Oaxaca, México).

tively long; digits typically meet when limbs are pressed to the side of trunk in males, or separated by no more than one costal interspace; in females, appressed limbs fail to meet by one to one and one half costal interspaces. Hands and feet relatively well developed, digits relatively long and slender for *Pseudoeurycea*, without subterminal pads; fifth toe well developed but

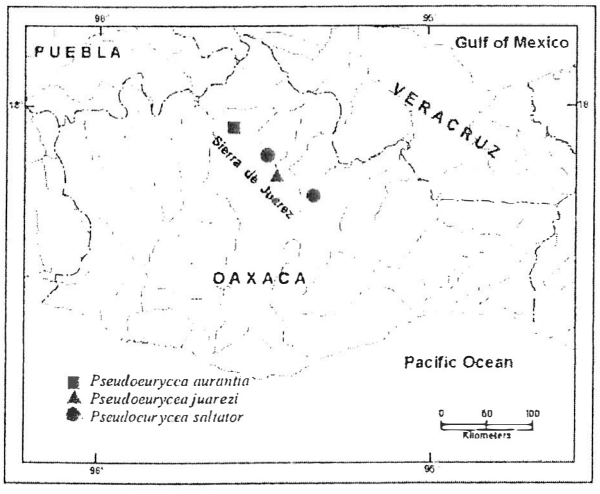

FIG. 2. Geographic distribution of species of the *P. juarezi* group in the Sierra de Juárez, Oaxaca, México. Shaded areas, > 2000 m.

much shorter than the fourth. Digits in order of decreasing length: fingers 3-2-4-1; toes 3-4-2-5-1. Tail relatively stout, and short – 72-100% SL; Maxillary teeth 66 (mean=66) in males, 55–67 (mean=62.5) in females; premaxillary teeth 2-4 (mean=3) and enlarged in adult males, 8–19 (mean=13.5) and smaller in females; vomerine teeth in long rows, 17-20 (mean=18.5) in males, 17–29 (mean=21.5) in females.

Coloration in life. The ground colour is reddish-brown dorsally grading to pale yellow ventrally. A broad and conspicuous orange mid-dorsal stripe extends from the scapular region to the tip of the tail. In the head region the dorsal stripe is broken into darker flecks. Bright yellow spots are present all over the dorsum and are more concentrated on the tail. A dark line runs from the nostril, posteriorly to the neck region. Some specimens present a few dark spots along the sides of the body and tail. The limbs generally are the same reddish-brown as the dorsum. The underside of the body and tail is uniform pale yellow with no spots. There is a bright yellow stripe beneath the lip area. The iris is coppery gold.

Coloration in alcohol. The coloration in alcohol contrasts with that of the living animals, only in the degree of brightness. The dorsum is dark brown, with a dark orange stripe running from the scapular region to the tip of the tail. The venter is pale yellow.

Measurements of holotype (in mm). Head width 6.8; head depth 3.6; eyelid length 3.0; eyelid width 2.1; anterior rim of orbit to snout 2.4; interorbital distance 2.7; distance between corners of eyes 5.6; snout to forelimb 15.1; nostril diameter 0.2; distance between external nares 2.4; projection of snout beyond mandible 0.7; snout to gular fold 11.2; width across shoulders 6.3; snout to posterior angle of vent 45.0; snout to anterior angle of vent 41.6; axilla to groin 23.2; tail length 47.0; tail depth at base 4.7; tail width at base 5.3; forelimb length 10.0; width of hand 3.0; hind limb length 12.7; width of foot 4.7; length of longest (third) toe 1.8; length of fifth toe 1.0. Numbers of teeth: premaxillary 4; maxillary 29/37; vomerine 9/8.

Variation. About 75% of females reach a larger size than males and have a more robust body. The basic colour pattern is the same, but some individuals display black spots along the sides of the body and tail. The dark line that runs from the nostril to the neck area across the eye is less evident in adult females.

Distribution. Pseudoeurycea aurantia has been collected only at the type locality – Peña Verde, Oaxaca, México, at 2805 m (17° 50.20' N, 96° 47.05' W) (Fig. 2).

Natural history. This species occurs in cloud forest where it is found under rocks, the bark of logs, and in or under decaying wood. The coexisting species of salamanders at Peña Verde include an undescribed species of *Pseudoeurycea* (D. Wake, pers. comm.), *Thorius papaloae* and *Cryptotriton adelos* (Canseco-Márquez, in press).

One female (EBUAP2057), collected on 7 May, 2000, 49.3 SL, was guarding a clutch of 22 eggs under

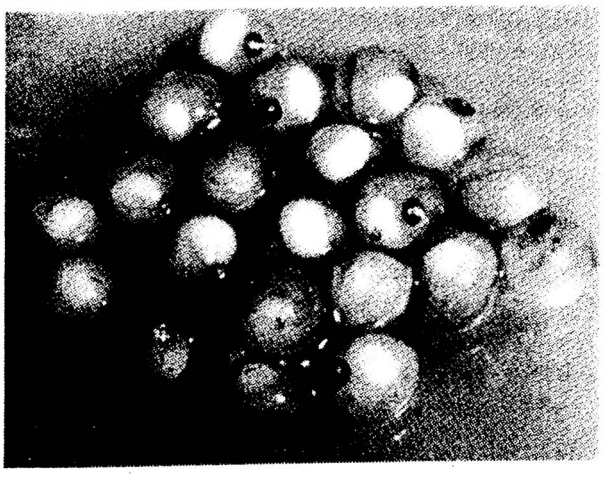

FIG. 3. Egg clutch of *P. aurantia*, at stage 31 of Marks and Collazo (1998). Egg diameter, including surrounding capsules ranges from 4.2 to 5.2 mm (mean=4.89) after preservation.

the bark of a log. The eggs were attached in a bead like fashion. The eggs were preserved in 10% neutral buffered formalin. Egg diameter, including surrounding capsules ranges from 4.2 to 5.2 mm (mean=4.89) after preservation. All embryos were preserved at approximately the same stage of development (Fig. 3), which corresponds to stage 31 of Marks & Collazo (1998). Dorsal pigmentation is extensive and extends onto proximal portions of each limb. The retina is black in all

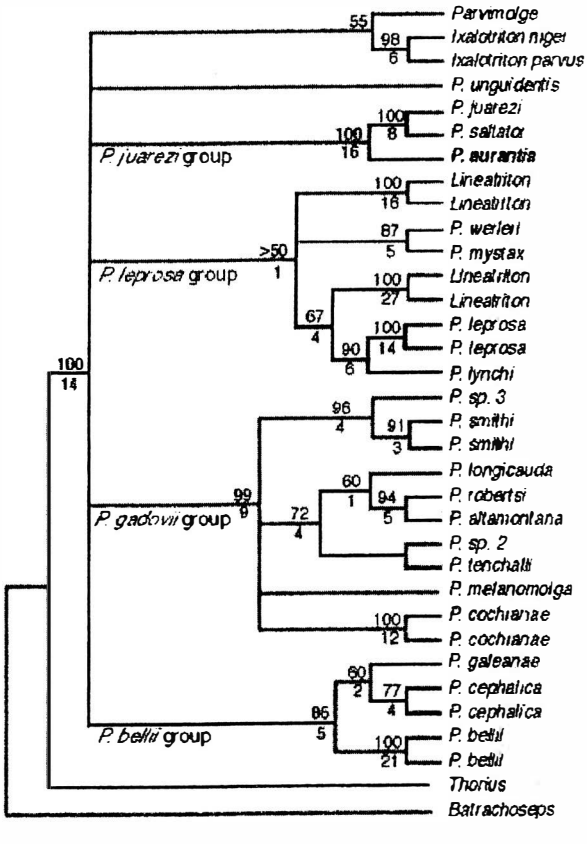

FIG. 4. Maximum Parsimony strict consensus tree of the 16S and Cyt *b* combined data set (1092 bp, TL=1467 steps, CI=0.406, RI=0.564). Numbers above branches are nonparametric bootstrap values (1000 replicates), numbers below branches are decay index values.

of the embryos. The lens is white. Rudiments of all five digits are visible on the hind limbs and rudiments of all four digits are visible in the forelimbs. Gills are tribranchiate.

Etymology. The specific epithet is derived from the Latin *aurantium*, and is used as an adjective in reference to the distinctive orange coloration of this species.

Molecular characters. Parra-Olea (2002) recently reported the results of an extensive molecular analysis of phylogenetic relationships of members of the genus *Pseudoeurycea* (*sensu lato*). Three species groups were recognized (*P. bellii*, *P. gadovii* and *P. leprosa*). The relationships of three additional species (*P. juarezi*, *P. saltator* and *P. unguidentis*) were uncertain. The samples used by Parra-Olea (2002) and in the present study under the designation of *P. unguidentis* were collected in an area relatively distant from the type locality of the species (Cerro San Felipe, Oaxaca, México) and based on preliminary morphological data they might represent a different species (D. B. Wake, pers. comm.).

We have now added sequences of 16S and Cyt *b* for the new species, *P. aurantia*, to the data set. The smallest divergences of *P. aurantia* from any other species are for *P. juarezi* (3.9% Cyt *b*, 0.3% 16S) and *P. saltator* (3.8% Cyt *b*, 0.3% 16S). Divergences between *P. aurantia* and sequences from all other *Pseudoeurycea* species groups are large, including *P. aurantia* to *P. gadovii* group (10.7–14.6% Cyt *b*, 3.8–4.8% 16S); *P. aurantia* to *P. bellii* group (15.0–16.6% Cyt *b*, 5.4–6.4% 16S); *P. aurantia* to *P. leprosa* group (11.7–17.0% Cyt *b*, 3.5–4.9%16S).

A maximum parsimony analysis produced nine equally parsimonious trees (L=1467 steps, CI=0.406, RI=0.564, 318 characters were parsimony informative). The strict consensus topology (Fig. 4) does not differ considerably from the published topology for the genus *Pseudoeurycea* (Parra-Olea, 2002). There is a high level of support for recognition of the *P. gadovii* (decay 9, bs 99%) and *P. bellii* (decay 5, bs 86%) species groups, and low support for the *P. leprosa* group (bs <50%). *Pseudoeurycea aurantia* is a sister taxon to the clade formed by *P. juarezi* and *P. saltator* (*P. juarezi* group, decay 16, bs 100%). The *P. juarezi* clade forms part of the basal polytomy.

DISCUSSION

The salamander fauna of the Sierra de Juárez in Oaxaca, México is highly diversified, including 22 described species of the genera *Pseudoeurycea, Thorius, Lineatriton, Chiropterotriton* and *Cryptotriton*. Systematic studies of the salamander fauna of the region started during the 1930's and 40's with descriptions of *P. smithi*, *P. cochranae* and *P. unguidentis* (Taylor, 1939, 1941, 1943) and species of *Thorius* (Taylor, 1940). Several more species of salamander have been described recently (Hanken & Wake, 1994, 2001; Papenfuss & Wake, 1987; Wake & Campbell, 2001), and there are still a few others yet undescribed (Wake, pers. com.). This is one of the regions of México most frequented by herpetologists, but *P. aurantia* occurs in a

remote area of Sierra de Juárez (Fig. 2). Peña Verde is the northern-most peak, and is of difficult access even now, probably the reason this species remained unknown despite field work in the general area. This species seems to be another montane specialist with restricted distribution, as is true of closely related species.

Based on morphology and mtDNA *P. aurantia* is related to the other two members of the *P. juarezi* group: *P. juarezi* and *P. saltator*, both of which also occur in the Sierra de Juárez. These three species share a general common morphology (Fig. 1) and their differences include size (*P. saltator* is the smallest of the three) number of teeth and tail length. *Pseudoeurycea juarezi* and *P. saltator* live in the same general area and differ in the altitudinal segregation of their habitats. *Pseudoeurycea saltator* is an arboreal dweller of tropical mesic cloud forest found at mid-elevations (1500–1800 m). *P. aurantia* is the most distant geographically, and occurs at elevations similar to *P. juarezi* (2200–2900 m); both occupy terrestrial habitats in the pine forest and barren alpine lands at higher elevations.

The addition of mtDNA sequences of *P. aurantia* to the previously published data set for *Pseudoeurycea*, did not alter the resulting topology (Parra-Olea, 2002). There is strong support for the *P. gadovii*, and *P. bellii* groups, and lower support for the *P. leprosa* group. There is strong bootstrap support for the new *P. juarezi* group that now includes *P. aurantia*. The basal relationships are unresolved, so the affinities of the *P. juarezi* group to the other clades of *Pseudoeurycea* and allied genera are undefined. The geographic and genetic distances between *P. juarezi* and *P. saltator* are smaller than those from *P. aurantia* to either, but morphological differentiation is larger between *P. saltator* and *P. juarezi*. Compared with *P. juarezi*, *P. saltator* is smaller in size, has a longer tail, more vomerine teeth, and a unique coloration (Lynch & Wake, 1989).

Speciation within the *P. juarezi* group happened in a limited geographic area relative to the general pattern found in *Pseudoeurycea*. This group likely represents another example of the influence of sharp ecological zonation across elevational gradients for species formation in tropical salamanders (García-París *et al.*, 2000). In these situations combined localized ecological gradients trigger selection which acts to generate phenotypic divergence and diversity, overcoming the potential homogenizing effects of gene flow, which in combination with high geological complexity promote species formation.

The Sierra de Juárez must be regarded as an important biodiversity centre not only for the high number of taxa inhabiting the mountain chain but also as a focus of species formation from ancient to relatively recent times. The small mtDNA divergence found between *P. saltator* and *P. juarezi* suggests that speciation between them occurred recently, possibly during the Pleistocene. This recent divergence will probably result in non-congruent single gene phylogenies given the short time provided for gene coalescence and the possibility of introgression along the ecotones between these geographically close taxa. The recency of the lineage split among species of the *P. juarezi* group is an unusual case within tropical salamanders, which generally display large mtDNA divergences among species (Parra-Olea, 2002). Salamanders of the *P. juarezi* species group represent an ideal study case for species formation in tropical regions since different stages of the speciation process are represented by different taxa within the group in a reduced geographic area.

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A REVIEW OF THE BIOLOGY OF THE LOGGERHEAD TURTLE, *CARETTA CARETTA*, AT FIVE MAJOR NESTING BEACHES ON THE SOUTH-WESTERN MEDITERRANEAN COAST OF TURKEY

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Most nesting by loggerhead turtles in Turkey has been recorded at 20 sites along the Mediterranean coast. In addition, sites primarily used by green turtles are also used by loggerheads. The annual number of loggerhead nests recorded on these 20 beaches ranges from 663 to 1991, with a mean of 1267 nests per season. We review the biology of nesting and predation at five of the most important and more regularly investigated loggerhead nesting sites (Dalyan, Fethiye, Patara, Belek and Kizilot). These five beaches may host up to 920 nests per season. With approximately 307 adults per season, the Dalyan beach has the highest capacity in terms of numbers of nests and of nesting females. Hatching success at the five beaches was negatively affected by fox predation (93% of the predated eggs on the beaches), crab predation (29.5% of the predated hatchlings), and light-pollution (42% of the hatchlings). In addition, predation by beetle larvae has been observed on the eggs at Fethiye beach (17.6% of the predated eggs at this site).

Key words: Chelonia, egg, hatchling, nesting, predation

INTRODUCTION

In the Mediterranean, the major nesting grounds for loggerhead turtles, *Caretta caretta* (Linnaeus, 1758) are in Turkey and Greece (Baran & Kasparek, 1989; Margaritoulis, 2000), with smaller numbers recorded in Cyprus (Broderick & Godley, 1996), Egypt (Kasparek, 1993; Clarke *et al.*, 2000), Libya (Laurent *et al.*, 1995), Tunisia (Laurent *et al.*, 1990), Israel (Kuller, 1999) and Syria (Kasparek, 1995). According to the previously substantiated records (Baran & Kasparek, 1989; Baran *et al.*, 1998; Taskavak *et al.*, 1998; Oruç *et al.*, 1997), three species of marine turtle – *Caretta caretta, Chelonia mydas* and *Dermochelys coricea* – are included in the chelonian fauna of Turkey. Only the first two are known to nest on the Turkish coast of the Mediterranean. The first nesting records of *Caretta caretta* and *Chelonia mydas* from the Turkish coasts were by Hathaway (1972). Basoglu (1973) and Basoglu & Baran (1982) gave information on the carapace plates of *C. caretta* found at Izmir, Köycegiz and Fethiye. Geldiay & Koray (1982), Geldiay *et al.* (1982) and Geldiay (1983, 1984) described marine turtle populations and their protection on the Mediterranean coasts of Turkey. Baran & Kasparek (1989) described the first comprehensive survey of the Turkish Mediterranean coast for turtle nesting sites. Its primary objective was to locate nesting sites and to allow assessment of their relative importance. More recently, various population studies have been carried out on certain beaches, and problems affecting the turtles on the nesting beaches have been determined (Canbolat, 1991; Erk'akan, 1993; Baran *et al.*, 1992; Baran, 1993*a,b*; Baran *et al.*, 1994; Baran *et al.*, 1996; Türkozan & Baran, 1996; Baran & Türkozan, 1996).

Almost half the recorded nesting sites of the Mediterranean loggerhead – and a large proportion of those for green turtles – are found on Turkish beaches (Groombridge, 1988). Although 17 important nesting sites in Turkey were given by Yerli & Demirayak (1996), only 15 of them were marked on the map given. A total of 13 beaches was considered as constituting the main nesting areas for marine turtles in Turkey (Baran & Kasparek, 1989; Baran *et al.*, 1992; Groombridge, 1994). From west to east, these beaches include: Dalyan, Dalaman, Fethiye, Patara, Kumluca, Belek, Kizilot, Demirtas Gazipasa, Göksu Deltasi, Kazanli, Akyatan and Samandag (Fig. 1). Apart from these main nesting beaches, there are others which do not hold such large numbers, but which are still of vital importance for sea turtles (Baran & Kasparek, 1989). These are Ekincik, Kale, Tekirova and Anamur (Fig. 1). These four secondarily important nesting beaches were also listed by Groombridge (1994). The beach at Demirtas (Fig. 1) given as the main nesting beach by Baran & Kasparek (1989) was not given in the updated list given by Yerli & Demirayak (1996). Additionally, the nesting beach at Çirali (Fig. 1), which was not given by Baran & Kasparek (1989), appeared for the first time as a main nesting beach in the map of Yerli & Demirayak (1996). In addition to the localities given above, two additional sites with less nesting (Agyatan and Yumurtalik) were given by Yerli & Canbolat (1998). All these localities are marked in Fig. 1. According to fieldwork carried out over the last 25

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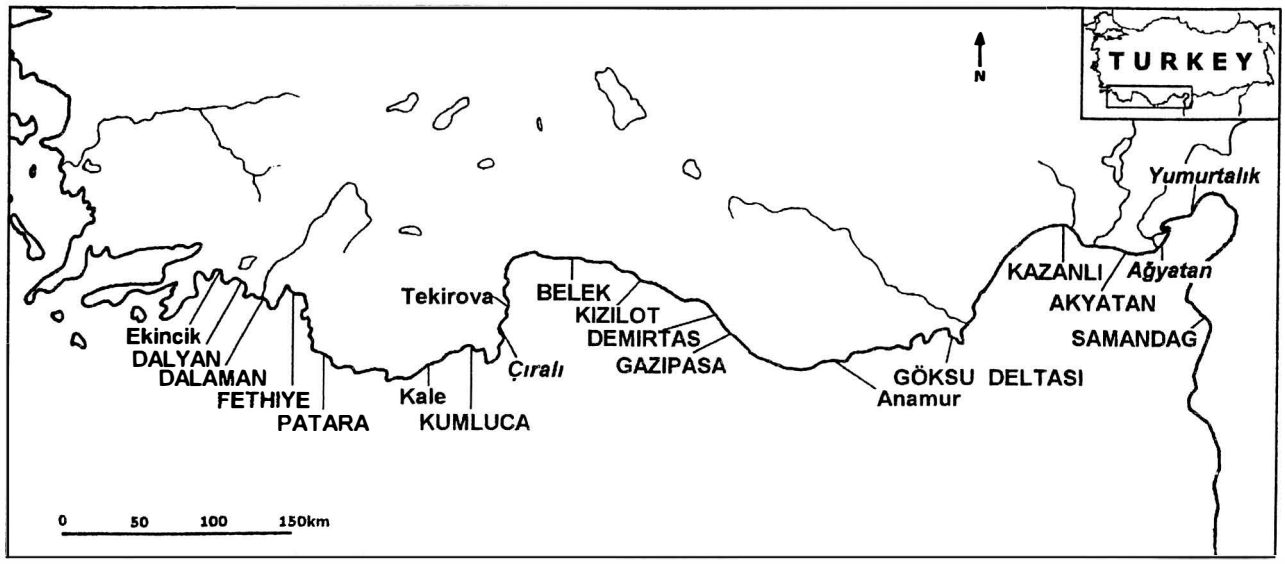

FIG. 1. The localities where the loggerhead nesting beaches are recorded (Upper case indicates main nesting beaches; lower case, secondary nesting beaches; italics, additional unsubstantiated records).

years, green turtle nesting is mostly limited to a few eastern beaches (Kazanli, Akyatan and Samandagi (Fig. 1). This study aims to provide information on the population and nesting status of the loggerhead turtle, *Caretta caretta*, on the south-western beaches (Dalyan, Fethiye, Patara, Belek and Kizilot, which appear to be the most important loggerhead beaches in Turkey. As such, the first four have been designated as Specially Protected Areas.

MATERIALS AND METHODS

Data were compiled from previously published sources and unpublished research reports. All records were scanned and the numbers of loggerhead nests recorded were collated (Table 1). At the five most important beaches, increased observer effort over the years allowed for a comparative review to be undertaken. It is likely that some nesting will have occurred undetected at sites along the Aegean and Mediterranean coasts not subject to monitoring, leading to underestimates of population size and hatchling recruitment

The values for total emergence, total number of nests, number of eggs, hatchlings reaching the sea and nest densities are presented in Table 2 for five beaches that have been monitored since 1982. It should be noted that not all of the studies listed in Table 2 were conducted throughout the entire nesting season each year. Geldiay *et al.* (1982), and Groombridge (1994) extrapolated the information from short surveys. Observations on the Dalyan beach were carried out at intervals of one or two weeks, and counts of tracks and nests were made during the day (Geldiay *et al.*, 1982). The total number of nests recorded at Dalyan for 1987 was estimated from the data collected between 4 June and 5 July (Groombridge, 1994). Except for the Fethiye beach, Yerli & Demirayak (1996) started the regular observations on four beaches after 20 June 1994. Baran *et al.* (1996) and Sak (1998) started regular studies on Dalyan and Patara, and Belek, respectively, after 20 July 1996. Yerli & Demirayak (1996), Kaska (1993) and Turkozan (2000) studied 8.5 km, 5 km and 4.5 km, respectively, of the Kizilot beach, which is 16.2 km in total length; observations by Kaska (1993) and Turkozan (2000) were made daily and included whole breeding seasons. The remaining data given in Table 2 are regular and include overall breeding seasons.

RESULTS

DALYAN BEACH

This site is in the transitional zone between the Aegean and Mediterranean regions and consists of a beach approximately 4.2 km in length. The values for Dalyan were compiled from the studies carried out by various researchers in different years (Table 2). A total of 2119 nests was recorded, with a mean of 193 nests per season, over 11 breeding seasons. The number of nests per season varies from 57 to 330. Using the assumption that each female nests an average of three times in a season (Groombridge, 1994), between 19 and 110 loggerhead turtles nest annually on the beach.

Information on predation was compiled over six years (1991 to 1997). During these six seasons, a total of 17 584 eggs was destroyed (Table 3). Of these, 17 385 eggs (98.9%) were destroyed by foxes and 199 (1.1%) by crabs. On the other hand, a total of 2833 hatchlings was destroyed over the seasons of 1991-1993 and 1997. Of these, 908 (32.1 %) were killed by foxes and 1703 (60.1%) by crabs. Strong sunlight and dehydration caused 199 hatchlings (7.0%) to die. Birds destroyed 23 hatchlings (0.8%).

FETHIYE BEACH

Approximately 8.3 km of the Fethiye beach, Specially Protected Area, situated within the boundaries of Vilayet Mugla, was examined for five nesting seasons from 1993 to 1997 (Table 2). This region was also desig-

TABLE 1. Nesting efforts of the loggerhead turtle in Turkey (Ekincik ref: Baran *et al.*, 1994. Dalyan ref: Erk'akan, 1993; Geldiay *et al.*, 1982; Groombridge, 1994; Baran *et al.*, 1996; Erk'akan, 1993; Baran *et al.*, 1992; Canbolat, 1996; Yerli & Demirayak, 1996; Baran *et al.*, 1996; Ilgaz, 1998. Dalaman ref: Yerli & Demirayak, 1996; Yerli *et al.*, 1998. Fethiye ref: Türkozan & Baran, 1996; Baran & Türkozan, 1996; Türkozan 2000. Patara ref: Baran *et al.*, 1992; Canbolat, 1996; Yerli & Demirayak, 1996; Baran, 1993*a*; Taskin, 1998. Kale ref: Yerli & Demirayak, 1996; Yerli *et al.*, 1998. Kumluca ref: Yerli & Demirayak, 1996, Yerli *et al.*, 1998; Baran & Kasparek, 1989; Baran *et al.*, 1992. Çirali ref: Yerli & Demirayak, 1996; Yerli *et al.*, 1998. Tekirova ref: Yerli, *et al.*, 1998 Belek ref: Yerli & Demirayak, 1996; Sak, 1998; Yerli *et al.*, 1998. Kizilot ref: Kaska, 1993; Yerli & Demirayak, 1996; Yerli *et al.*, 1998; Türkozan, 2000. Demirtas ref: Baran & Kasparek, 1989; Yerli & Canbolat, 1998. Gazipasa ref: Yerli & Demirayak, 1996; Yerli & Canbolat, 1998. Anamur ref: Baran *et al.*, 1992; Yerli & Demirayak, 1996; Yerli & Canbolat, 1998. Göksu Deltasi: Peters & Verhoeven, 1992. Akyatan ref: Brown & Mcdonald, 1995; Yerli & Demirayak, 1996; Yerli & Canbolat, 1998. Aureggi *et al.* 1999. Agyatan ref: Yerli & Canbolat, 1998. Kazanli ref: Baran *et al.*, 1992; Yerli & Canbolat, 1998; Durmus, 1998. Yumurtalik ref: Yerli & Canbolat, 1998. Samandag ref: Yerli & Canbolat, 1998).

	No. seasons	Average no. nests	Range
Ekincik	1	8	8-8
Dalyan	11	193	57-330
Dalaman	2	71	69-73
Fethiye	8	122	88-191
Patara	6	53	33-85
Kale	2	74	39-109
Kumluca	4	141	35-305
Çirali (Olimpos)	2	23	12-34
Tekirova	1	4	4-4
Belek	4	122	68-168
Kizilot	5	139	50-270
Demirtas	2	62	44-80
Gazipasa	2	14	14-14
Anamur	3	159	96-195
Göksu Deltasi	2	63	36-89
Akyatan	4	10	3-23
Agyatan	1	2	2-2
Kazanli	4	3	1-7
Yumurtalik	1	1	1-1
Samandag	1	3	3-3
TOTAL		1267	663-1991

nated as a feeding ground for juvenile green turtles (Türkozan & Durmus, 2000). A total of 650 nests was recorded, with a mean of 130 per season over five breeding seasons. The number of nests per season varied from 88 to 191 during the years 1993 to 1997. This means that approximately 29-64 loggerhead turtles nest annually on the beach.

Between the years 1994 and 1997, a total of 2091 eggs was destroyed. Of these, 1515 were predated by foxes (72.4%), 370 by coleopteran larvae (17.6%) and 83 by dogs (3.9%). Meanwhile, 36 (1.7%) were accidentally destroyed by researchers whilst using a metal rod to search for and locate the clutches. A plant root destroyed one egg (0.04 %) and human activities (e.g. sand extraction, beach utilization for tourism, light pollution, cattle trampling) caused the loss of 86 eggs (3.9%). A total of 743 hatchlings was destroyed from 1994 to 1997. Of these, 405 were destroyed by foxes (54.5 %), 52 by dogs (6.9%) and 14 by ghost crabs (1.8 %). Birds destroyed 93 hatchlings (12.5%). Strong sunlight and dehydration caused 173 hatchlings (23.2%) to die. Cars ran over six hatchlings (0.8%) on the beach.

Nesting success (the proportion of adult emergences resulting in egg laying) ranged from 21.5% to 49.2% between the years 1993 and 1997. The hatching success of the eggs ranged from 58.1% to 68.4 %. The total number of hatchlings reaching the sea as a percentage of the eggs hatching varied from 67.2% to 85.5%.

PATARA BEACH

Data on an 11.8 km-long sandy strip were compiled for six breeding seasons between 1990 and 1997 (not 1991 or 1995). A total of 315 nests was recorded, with a mean of 53 (Table 2). The number of nests varied from 33 to 85 for the years 1990 to 1997. It is estimated that approximately 11-28 loggerhead turtles nest annually at this site. Predation of eggs was recorded for the years 1992, 1993, 1996 and 1997. During these periods, a total of 2547 eggs was destroyed. Of these, 1783 eggs were killed solely by foxes (69.2%) and 207 by crabs (8.0%). Birds destroyed one egg (0.04%). Foxes and crabs in combination destroyed 586 eggs (22.7%).

During the 1992 and 1993 breeding seasons, a total of 460 hatchlings was lost. Of these, 60 were killed by foxes (13.0%) and 378 by crabs (82.2%). Birds destroyed one hatchling (0.2%). Strong sunlight and dehydration caused 21 hatchlings (4.6%) to die.

BELEK BEACH

For three breeding seasons, we compiled data on Belek beach, approximately 25 km in length and situated 40 km west of Antalya (Table 2). A total of 389 nests was recorded, with a mean of 130. The number of nests per season varied from 68 to 168. It is estimated that 23-56 loggerhead turtles nest annually on the beach. Some 616 eggs were destroyed either by foxes or dogs during the 1996 and 1997 breeding seasons. Of 3276 hatchlings destroyed on the beach, 89 were killed by ghost crabs (2.7%). Light-pollution, causing hatchling disorientation, and strong sunlight and dehydration caused the loss of 1263 hatchlings (38.5%) and 52 hatchlings (1.6%) respectively. Furthermore, 1872 hatchlings (57.1%) were disoriented.

KIZILOT BEACH

The Kizilot beach, 16.2 km in length and situated within the boundaries of Vilayet Antalya, was examined

TABLE 2. The data on the loggerhead turtles recorded annually at five different beaches of south-western Turkey. Letters indicate the references considered. A, Geldiay *et al.*, 1982; B, Groombridge, 1994; C, Baran *et al.*, 1997; D, Erk'akan, 1993; E, Baran *et al.*, 1992; F, Canbolat, 1996; G, Yerli & Demirayak, 1996; H, Baran *et al.*, 1996; I, Ilgaz, 1998; J, Türkozan & Baran, 1996; K, Baran & Türkozan, 1996; L, Türkozan, 2000; M, Baran, 1993*a*; N, Taskin, 1998; O, Sak, 1998; P, Kaska, 1993.

	1982 A	1987 B	1988 C	1989 D	1990 E,P	1991 F	1992 F&J	1993 G,K	1994 L	1995 H	1996 H,L, O	1997 I,L, N,O
Dalyan												
Total emergence	?	?	?	912	257	798	824	713	178	-	?	371
Total number of nests	330	300	146	235	57	271	217	235	86	-	107	135
Number of eggs	?	?	?	17254	5244	21187	18855	19595	3896	-	6450	10903
Hatchlings reaching the sea	?	?	3109	1611	3036	7539	5155	7397	?	-	3473	5439
Nest density (nest/km)	?	?	?	50	14	58.02	46.46	50.31	?	-	22.7	28.7
Fethiye												
Total emergence	-	-	-	-	-	-	-	240	439	888	235	291
Total number of nests	-	-	-	-	-	-	-	118	158	191	88	95
Number of eggs	-	-	-	-	-	-	-	8772	12926	15853	7656	6679
Hatchlings reaching the sea	-	-	-	-	-	-	-	3337	5953	6991	3488	3671
Nest density (nest/km)	-	-	-	-	-	-	-	14.75	19.75	23.9	11	11.9
Patara												
Total emergence	-	-	-	-	128	-	163	294	75	-	?	205
Total number of nests	-	-	-	-	58	-	52	85	33	-	35	52
Number of eggs	-	-	-	-	5150	-	2920	7315	1293	-	2629	3769
Hatchlings reaching the sea	-	-	-	-	?	-	1086	2030	?	-	1068	1638
Nest density (nest/km)	-	-	-	-	9.7	-	7.28	12.69	?	-	?	7.42
Belek												
Total emergence	-	-	-	-	-	-	-	-	259	-	?	389
Total number of nests	-	-	-	-	-	-	-	-	68	-	153	168
Number of eggs	-	-	-	-	-	-	-	-	1065	-	10486	10988
Hatchlings reaching the sea	-	-	-	-	-	-	-	-	?	-	6295	7082
Nest density (nest/km)	-	-	-	-	-	-	-	-	?	-	?	?
Kizilot												
Total emergence	-	-	-	-	299	-	-	-	195	-	427	303
Total number of nests	-	-	-	-	146	-	-	-	50	-	125	108
Number of eggs	-	-	-	-	11680	-	-	-	3029	-	9625	6243
Hatchlings reaching the sea	-	-	-	-	?	-	-	-	?	-	5406	3966
Nest density (nest/km)	-	-	-	-	?	-	-	-	?	-	27.7	24

for four breeding seasons: 1990, 1994, 1996 and 1997 (Table 2). A total of 429 nests was recorded, with a mean of 107. The number of nests per season ranged from 50 to 146. Approximately 17-49 loggerhead turtles nest annually on the beach. During the years 1994 to 1997, a total of 1129 eggs was destroyed. Of these, 657 were predated by foxes (58.2%), 209 by coleopteran larvae (18.5%) and 193 by dogs (17.1%). Thirty-six eggs (3.2%) were taken by a researcher for a sex determination study. Thirty-four eggs (3.0%) were accidentally destroyed by workers whilst using a metal rod to detect the nest sites.

Sixty-seven hatchlings were destroyed on the beach: fox and bird predation accounted for 37 (55.2%) and three (4.5%), respectively. Strong sunlight and dehydration caused 19 hatchlings (28.4%) to die. Dogs destroyed eight hatchlings (11.9%).

The percentage of nesting success ranged from 29.7% to 32.4 %, whereas hatching success varied from 62.4 % to 63.5%. The percentage of hatchlings reaching the sea ranged from 90% to 97.1%.

DISCUSSION

Taking into consideration all loggerhead turtle nesting activity (Table 1), the five major nesting areas described in this study account for 44%-46% of all loggerhead nesting activity in Turkey. The overall nesting activity on the 20 beaches used by loggerheads revealed the fact that approximately 221-664 loggerhead females visit the Turkish coasts. Groombridge (1994) estimated a minimum of 1650 nests for the1988 season, assuming 550 females nested. Data in Geldiay *et al.* (1982) and Geldiay (1984) suggest that around 1000

TABLE 3. The effects of various predators on eggs and hatchlings of loggerhead turtles at five beaches considered (* metal rod, ** 34 by metal rod and 36 taken for sex determination study).

	Dalyan	Fethiye	Patara	Belek	Kizilot
Eggs					
Total number	17584	2091	2547	616	1129
Fox	17385	1515	1783	-	657
Crab	119	-	207	-	-
Fox & Crab	-	-	586	-	-
Fox or dog	-	-	-	616	-
Feral dog	-	83	-	-	193
Bird	-	-	1	-	-
Coleopteran larva	-	370	-	-	209
Human activity	-	86	-	-	-
Plant root	-	1	-	-	-
Other	-	36*	-	-	70**
Hatchlings					
Total number	2833	743	460	3276	67
Fox	908	405	60	-	37
Crab	1703	14	378	89	-
Feral dog	-	52	-	-	8
Bird	23	93	1	-	3
Car	-	6	-	-	-
Strong sunlight	199	173	21	52	19
Light pollution	-	-	-	3135	-
Other	-	-	-	-	-

Caretta caretta nested per season. Yerli and Demirayak (1996) recorded a total of 884 loggerhead turtle nests for the beaches of Turkey. The five major nesting areas considered here may hold 296-920 nests per season. This means that approximately 99-307 loggerhead turtles nest annually on these beaches

The results show that Dalyan beach has the highest number of nests. According to Groombridge (1994), it is unclear to what extent the eastern turtle beaches are used by loggerheads. It is seen here that the green turtle nesting sites are also used by loggerheads, with 1 to 23 nests per season. The mean number of nests varied between 53 and 193 on the south-western beaches of Turkey. We are of the opinion that these values do not fully reflect the capacity of the beaches. However, if we consider the study periods, lengths and sections of the beaches, these numbers reflect at least the minimum capacity of the five beaches. These data also highlight the importance of the Turkish nesting sites, with Margaritoulis (2000) estimating the overall number of loggerhead nests in Greece as 2355-5287 per year. Broderick & Godley (1996) recorded a total of 1347 loggerhead turtle nests in Northern Cyprus between 1992 and 1995, estimating that 22-173 loggerhead turtles nest on these beaches per season.

Of the 23 997 eggs destroyed on the beaches, it is obvious that canid predation was the main problem, with some 22 232 eggs (93% of eggs predated) destroyed either by foxes or dogs. It is well known that land-based predators, including mammals, have less impact on hatchlings than on eggs (Hopkins *et al.*, 1979, Fowler, 1978). A total of 7399 hatchlings was destroyed on the beaches. Light pollution caused the disorientation and loss of 3135 hatchlings (42%). Crabs destroyed 2184 hatchlings (29.5%), whereas foxes destroyed 1462 (19.7%). These results represent only the general pattern of the fate of loggerhead hatchlings. If we take the beaches separately into consideration, crab predation had the most effect on the hatchlings of the Dalyan and Patara beaches, whereas fox predation was most harmful on the Fethiye and Kizilot beaches. Light-pollution was the main problem for the Belek beach, resulting in disorientation.

It is worth comparing these results with those for other loggerhead nesting sites in the Mediterranean: terrestrial predators such as red foxes (*Vulpes vulpes*), feral and domestic dogs, ghost crabs (*Ocypoda cursor*), and scavenging birds (hooded crows, *Corvus corone cornix*, and magpies, *Pica pica*) were recorded in Northern Cyprus (Broderick & Godley, 1996). Of the 48.4% predation given by Margaritoulis (1988), red fox (*Vulpes vulpes*) and stray dogs were the primary predators in Kiparissia Bay, Greece. He claimed that exposed eggs attracted other mammals (rats and martens) and birds. A total of 34 loggerhead turtle nests was recorded on the northern Mediterranean coast of Israel (Silberstein & Dmi'el, 1991). They stated that a sharp decline in the number and density of loggerhead sea turtle nests in

Israel was attributable to both regional and local processes.

A few loggerhead turtles still lay eggs at Canigli beach, which is much visited by tourists at Lampedusa, Italy (Gramentz, 1989). He stated that the black rat, *Rattus rattus,* was found to be the main predator, besides humans, on the beach. The number of loggerheads killed annually was estimated at 150-300 in Lampedusa and 500-600 in Malta.

Kasparek (1995) surveyed the entire Syrian Mediterranean coast (193 km) for marine turtle nesting in 1991 and found significant nesting sites between Latakia and Jablah. He quoted 10 tracks/km theoretically in Syria and attributed most of them to the loggerhead turtle.

Although we have described patterns of abundance and threats at these five sites, monitoring of marine turtle nesting on the south-west Mediterranean coast of Turkey has not been consistent and uninterrupted. Thus, before setting up predation management programs (hunting, trapping, transplantation, offshore-releasing hatcheries etc.), longer-term studies are recommended. Of the five beaches, Dalyan is the site on which studies and observations were most frequent. Despite the insufficient data from the five beaches considered in this survey, it is obvious that natural predation greatly reduces hatchling production of the loggerhead turtle.

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MAJOR PATTERNS OF POPULATION DIFFERENTIATION IN THE IBERIAN SCHREIBER'S GREEN LIZARD (*LACERTA SCHREIBERI*) INFERRED FROM PROTEIN POLYMORPHISM

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The genetic characteristics of the Iberian Schreiber's green lizard (*Lacerta schreiberi*) remain largely unknown. We investigated the population structure of this species using conventional electrophoresis and isoelectric focusing to screen 24 protein loci from 11 representative populations of the Iberian Peninsula. Thirteen polymorphic loci displaying a total of 30 alleles revealed significant partitioning of genetic variation among populations (F_{ST}=0.448). Analysis of standard genetic variability measures and allelic distribution profiles indicated that the most variable populations are located in the main distribution area of the species: the north-western corner of the Iberian Peninsula and the Spanish Central System. In contrast, southern isolated populations showed depleted levels of genetic diversity, indicating that severe restrictions to gene flow together with small population sizes are promoting genetic uniformity. We suggest that present-day patterns of genetic diversity in *L. schreiberi* populations are concordant with the biogeographical hypothesis of a recent expansion to the south followed by a history of contraction and fragmentation resulting in today's isolated southern populations.

Key words: electrophoresis, isoelectric focusing, Lacertidae, population genetics

INTRODUCTION

Schreiber's green lizard, *Lacerta schreiberi* (Bedriaga, 1878) is endemic to the Iberian Peninsula. Its distribution is generally continuous in north-western Iberia down to the Tejo river, with several isolated populations occurring in central and southern regions such as the mountains of Sintra, Monchique, Cercal and S. Mamede in Portugal, and Las Villuercas, Guadalupe, Toledo and Morena (San Andrés) in Spain (Marco & Pollo, 1993; Barbadillo *et al.*, 1997; Brito *et al.*, 1996) (Fig. 1). *L. schreiberi* is a medium-sized lizard (maximum adult snout-vent length of 125 mm (Galán, 1984)), inhabiting stream and river margins, in areas characterised by the rainy winters and mild summers typical for the Atlantic climate. The isolated southern populations are restricted to regions that constitute "Atlantic islands" surrounded by areas strongly influenced by Mediterranean climate.

Based on biogeographical data, Salvador (1974) suggested that *L. schreiberi* originated in north-west Iberia after splitting from the common ancestor of the green lizard group. Lutz & Mayer (1985) provided an estimate of this event as 3 to 4 million years ago, based on an immunological albumin clock. De la Riva (1987) followed Salvador (1974), adding that the species may have dispersed to the south and to the east from a north-western speciation centre, as a consequence of Pleistocenic climatic fluctuations. Later, Marco & Pollo (1993) further suggested that *L. schreiberi* might once have been distributed throughout the Iberian Peninsula. In this case, central and southern isolated populations represent the remnants of that wider distribution range that are now restricted to mountains where environmental conditions are still suitable.

Allozyme electrophoresis has been extensively used to investigate the genetic structure of natural populations in a wide range of plant and animal studies (e.g. Nevo *et al.*, 1984; Ward *et al.*, 1992). It is a particularly powerful method for solving taxonomic problems, and it has been used in several studies on lizards to quantify the divergence among populations or species (e.g. Blanc & Cariou, 1987; Busack & Maxson, 1987; Hutchinson & Schwaner, 1991; MacCulloch *et al.*, 1995; Martins, 1995; Bobyn *et al.*, 1996). In this study, both conventional electrophoresis and isoelectric focusing techniques were used for the first time to investigate the patterns of genetic diversity and the degree of genetic differentiation among populations of *L. schreiberi*.

MATERIAL AND METHODS

Samples were collected in summer (1994 and 1995) from 11 populations throughout the distribution area of the species (Fig. 1). Two hundred and forty-six individuals were caught, and the tail-tip of each was taken together with the blood that emerges from the cut. Both tail and blood were snap-frozen in liquid nitrogen and stored at -20°C. Animals were immediately released at the capture site.

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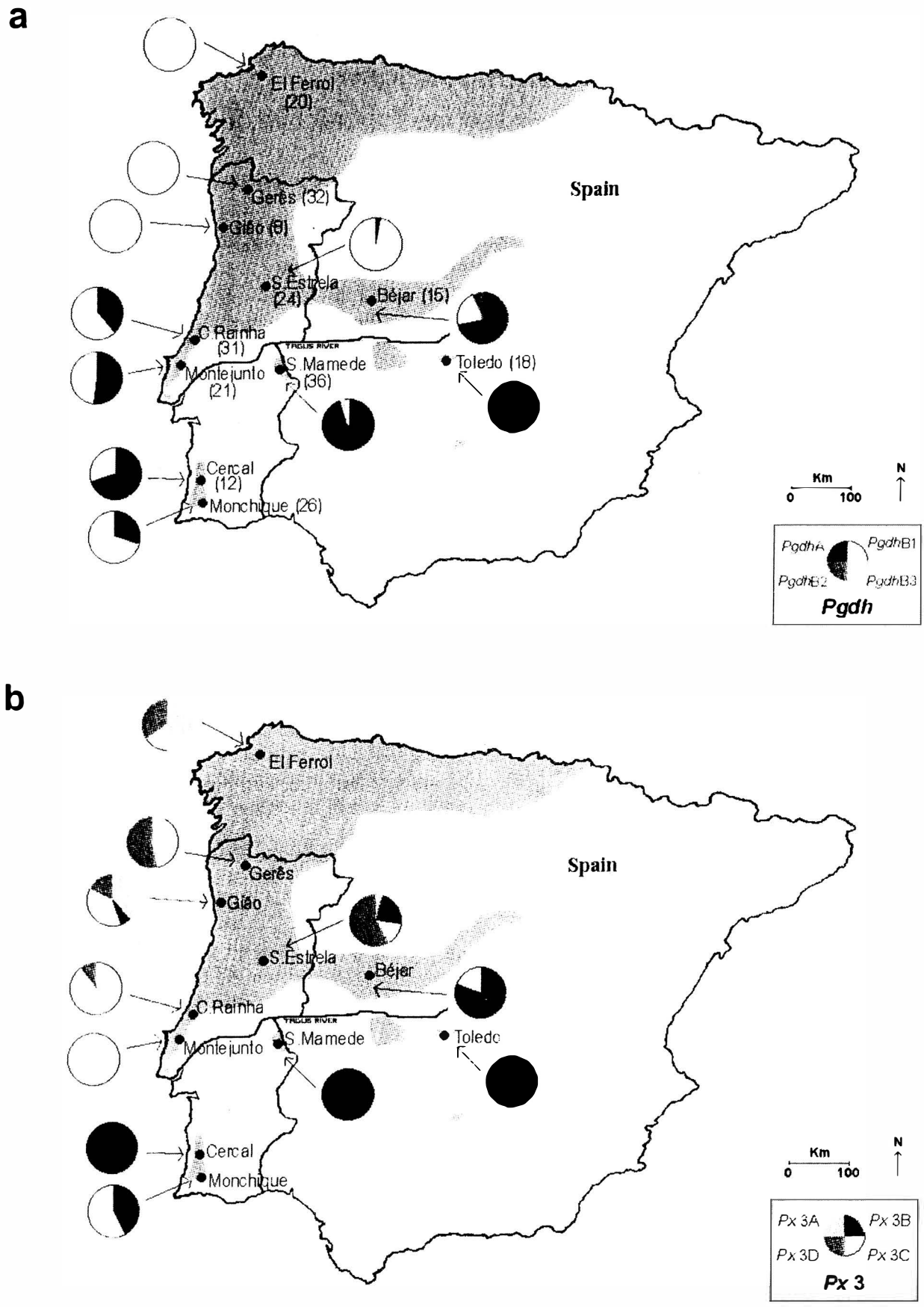

FIG 1. Distribution area of *Lacerta schreiberi*, location of sampling sites and the number of individuals sampled from each site (in parentheses). The pie charts represent the allele frequencies at the (a) *Pgdh* locus, (b) *Px*3 locus.

Tail samples were mechanically homogenized in an equal volume of Tris-EDTA-HCl buffer (pH 6.8) and centrifuged at 30 000 g for 30 min at 4°C. Supernatant fractions were subjected to horizontal electrophoresis using 13% starch gels, following the methods of Pasteur *et al.* (1987). Blood samples were diluted in equal volumes of distilled water and used directly in cellulose acetate gels (Gelman). Both tail and blood samples were used in isoelectric focusing. Twenty-one enzyme systems were consistently scored using electrophoresis and/or isoelectric focusing for each individual. Protein names, EC numbers and the technique with which the protein was screened are presented in Table 1. Enzymes are referred to in the text by their abbreviations in block letters, while enzyme loci are referred to by the italicised abbreviations in lower case.

Alleles of *Pep*B, *Pgdh*, *Px*2 and *Px*3 were resolved by isoelectric focusing in pH gradients established by the following mixtures of (1) 5-6 ampholyte, (2) 1:3 of 7-9 and 8-9.5 ampholytes, (3) 1:1 of 4.5-5.4 and 5-6 ampholytes, and (4) 1:1 of 3.5-5 and 4.5-5.4 ampholytes, respectively. All ampholytes were used at a final concentration of 6% (v/v). Anodal and cathodal electrode solutions were, respectively, (1) aspartic acid/glutamic acid 0.05M and sodium hydroxide 1M for PEPB and PGDH, (2) aspartic acid 0.04M and sodium hydroxide 0.6M for *Px2,* and (3) aspartic acid 0.04M and sodium hydroxide 0.2M for *Px*3. Gels were prefocused for 1 hr at constant power setting limits at 1500 V, 25 mA, and 1W (30 min), 2W (15 min) and 3 W

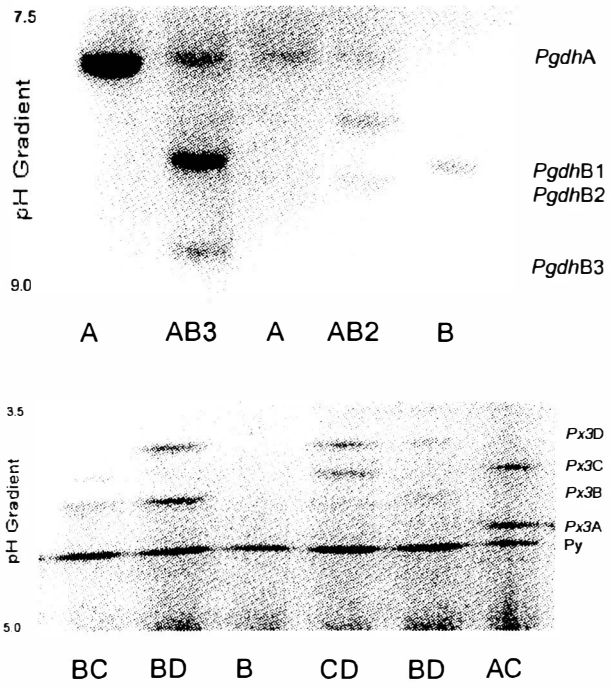

FIG 2. Electrophoretic patterns of *Pgdh* (top) and *Px*3 (bottom) revealed by isoelectric focusing. In the bottom figure appears another locus which is represented by *Py*.

(15 min). After prefocusing, 8 mL of sample were applied to the gels using a silicone strip (Serva). Focusing was then performed for 3 hr at constant power settings limits at 1500 V, 25 mA, and 4 W (1 h), 5 W (1 hr) and

TABLE 1. Enzyme systems, E.C. numbers and scoring techniques for each system. SGE - Starch gel electrophoresis: (1) Tris-citrate, pH 8.0; (2) Tris-citrate, pH 6.7; (3) LiOH-borate, pH 8.3; (4) Tris-NaH_2PO_4, pH 7.4. CAE - Cellulose acetate electrophoresis: Tris-borate, pH 7.6. AGE - Agarose gel electrophoresis: Tris-glycine, pH 8.6. IEF - Isoelectric focusing.

Enzyme system and locus	EC	Technique	System
Adenylate Kinase (*Ak*)	2.7.4.3	SGE	2
Albumine (*Alb*)	–	CAE	–
α-Amylase (*Amy*)	3.2.1.1	AGE	–
Aspartate Aminotransferase (*Aat*1; *Aat*2)	2.6.1.1	SGE	1
Creatine Kinase (*Ck*1; *Ck*2)	2.7.3.2	SGE	2
Esterase (*Est*, non specific)	3.1.1.-	SGE	3
Glucose-6-Phosphate Isomerase (*Gpi*)	5.3.1.9	SGE	1
Glycerol-3-Phosphate Dehydrogenase (*G3pdh*)	1.1.1.8	SGE	1
Lactate Dehydrogenase (*Ldh*1; *Ldh*2)	1.1.1.27	SGE	1
Malate Dehydrogenase (*Mdh*)	1.1.1.37	SGE	1
Malate Dehydrogenase-$NADP^+$ (*Me*)	1.1.1.40	SGE	2
Mannose-6-Phosphate Isomerase (*Mpi*)	5.3.1.8	SGE	1
Non specific protein (*Px*1)	–	SGE	2/3
Non specific protein (*Px*2)	–	IEF	–
Non specific protein (*Px*3)	–	IEF	–
Peptidase B (*Pep*B)	3.4.13.-	IEF	–
Peptidase D (*Pep*D)	3.4.13.9	SGE	4
Phosphoglucomutase (*Pgm*)	5.4.2.2	SGE	2
Phosphogluconate Dehydrogenase (*Pgdh*)	1.1.1.44	SGE/IEF	2/-
Nucleoside Phosphorylase (*Np*)	2.4.2.1	IEF	–
Superoxide Dismutase (*Sod*)	1.15.1.1	SGE	1

TABLE 2. Standard genetic variability measures for the 24 loci screened in this study: mean number of individuals per sample (*n*), mean number of alleles per locus (*a*), percentage of polymorphic loci ($P_{0.95}$), average observed heterozygosity (H_O) and average expected heterozygosity (H_E).

Population	*n*	*a*	$P_{0.95}$	H_O	H_E
Main distribution area					
El Ferrol	19.2	1.2	17.4	0.09	0.08
Gerês	25.8	1.3	21.7	0.07	0.08
Gião	13.0	1.2	8.7	0.04	0.03
Estrela	21.8	1.4	17.4	0.09	0.09
C.Rainha	27.2	1.3	30.4	0.09	0.09
Montejunto	20.5	1.3	26.1	0.07	0.08
Béjar	13.7	1.5	34.8	0.14	0.12
Isolated populations					
Toledo	15.3	1	0	0.00	0.00
S.Mamede	30.8	1.3	17.4	0.06	0.06
Monchique	25.6	1.2	17.4	0.05	0.05
Cercal	12.4	1.1	13.0	0.05	0.04
Average	20	1.3	18.6	0.07	0.07

6 W (1 hr). Allelic variation was visualized using histochemical techniques (Harris & Hopkinson, 1976).

Genetic variability of each population across 24 presumptive loci (*Np* was excluded from the analysis because it was considered a dominant/recessive locus) was characterized by the mean number of alleles per locus (*a*), the percentage of polymorphic loci (*P*) and the observed (H_O) and expected (H_E) average heterozygosity. For polymorphic loci, a chi-square test was used to check whether genotypic frequencies were in Hardy-Weinberg equilibrium. The magnitude of genetic differentiation was investigated with Weir & Cockerham (1984) estimators of *F*-statistics.

Cavalli-Sforza chord distances (Cavalli-Sforza & Edwards, 1967) were used to evaluate patterns of genetic differentiation and are presented in the form of a Neighbour Joining (NJ) tree (Saitou & Nei, 1987). Support for nodes was generated with bootstrap replicates (1000 trees). All calculations were performed using FSTAT version 2.9.1 (Goudet, 2000) and PHYLIP version 3.5 (Felsenstein, 1993) program packages.

RESULTS AND DISCUSSION

Thirteen out of the 24 presumptive structural loci scored were found to be polymorphic (frequency of most common allele was less than 99%) in at least one population, providing a total of 30 alleles. Although no progeny testing was performed to confirm the mode of inheritance of protein variants, zymograms conformed with simple patterns of codominant inheritance. The electrophoretic bands corresponding to the alleles identified at each locus were numbered according to their order of discovery.

Allelic frequencies of polymorphic loci are given in the Appendix 1. The two most informative loci corresponded to phosphogluconate dehydrogenase (*Pgdh*) and to a plasmatic protein (*Px*3), which received a detailed analysis (Fig. 1). While starch gel electrophoresis revealed the presence of only two alleles at the *Pgdh* locus (*Pgdh*A and *Pgdh*B), isoelectric focusing systems allowed the identification of three subtypes of *Pgdh*B (*Pgdh*B1, *Pgdh*B2 and *Pgdh*B3) (Fig. 2a). *Pgdh*B1 was fixed or almost fixed in the northern populations of El Ferrol, Gerês, Gião and S.Estrela, and showed high frequencies in central and southern Atlantic populations (Caldas da Rainha – 0.61, Montejunto – 0.48, Cercal – 0.30, and Monchique – 0.71). In contrast, this allele was absent in isolated inland populations (S. Mamede and Toledo) and occurred at low frequency (0.23) in a population from the Spanish Central System (Béjar) along the eastern edge of the species' main distribution area.

TABLE 3. *F*-statistics analysis for the polymorphic loci analysed in this study, using the Weir & Cockerham (1984) estimation of F_{IT} (F), F_{ST} (θ) and F_{IS} (f) and statistical significance of the values (NS, not significant; * $P<0.05$;** $P<0.01$; *** $P<0.001$).

Locus	*f*	*F*	θ
*Aat*1	0.003 NS	-0.000 NS	-0.003 NS
Amy	0.235*	0.449***	0.279***
*Ck*1	0.082 NS	0.525***	0.482***
Gpi	-0.017 NS	-0.001 NS	0.016*
*Ldh*1	-0.036 NS	-0.003*	0.032***
Me	0.054 NS	0.500***	0.472***
Mpi	-0.008 NS	-0.006 NS	0.001 NS
*Pep*B	-0.018 NS	0.106*	0.122***
*Pep*D	-0.076 NS	0.352***	0.397***
Pgdh	-0.106 NS	0.515***	0.562***
Pgm	-0.084 NS	-0.001*	0.076***
*Px*2	-0.158 NS	0.326***	0.418***
*Px*3	0.052 NS	0.527***	0.501***
All loci	0.025 NS	0.462***	0.448***

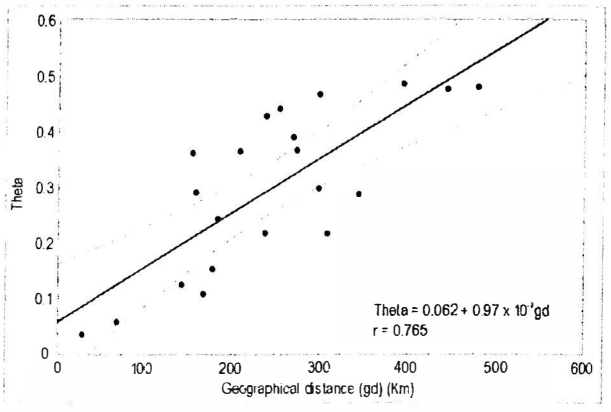

FIG 3. Regression of theta (θ) against geographical distance between pairs of populations of the main distribution area. The interrupted lines represent the bounds of the 95% confidence limits of the regression line.

The other major allele at this locus, *Pgdh*A, occurred at high frequencies in all southern populations including the isolate of S. Mamede, and reached fixation in Toledo. *Pgdh*B2 and *Pgdh*B3 were low-frequency private alleles from the populations of Béjar and S. Mamede, respectively. A second group of allelic frequencies corresponds to *Px*3, a non-specific plasmatic protein of acidic isoelectric point, showing four distinct alleles (Figs. 1 and 2b) with a well-established geographic distribution. Alleles *Px*3A and *Px*3D were present only in northern populations, where *Pgdh*A was fixed or highly predominant. *Px*3D was also present in Caldas da Rainha, although at a low frequency. *Px*3B was predominant in southern isolated populations, being the only allele present in Cercal, S. Mamede and Toledo. This allele was also present in the main distribution area, namely in Béjar (0.81) and S. Estrela (0.25). *Px*3C was almost present throughout the species' distribution, being absent only in the Cercal, S. Mamede and Toledo isolated populations.

Ten rare alleles ($P<0.10$) were found in six sampling regions, all of them included in the main distribution area of the species (central and northern Iberia). Of those, nine were private alleles (present in a single population) and only one (*Mpi*A) was shared at low frequencies by three different localities (Gerês, Béjar and S. Mamede). The population of Béjar harboured most of this diversity (five rare alleles), while two other populations (S. Estrela and S. Mamede) exhibited two rare alleles and three others (Gerês, Caldas da Rainha and Montejunto) a single one. These results are consistent with the biogeographical hypothesis described by Marco & Pollo (1993), suggesting an older age for *L. schreiberi* populations in the north-western corner of the Iberian Peninsula, and a recent post-glacial expansion to the south during periods of higher precipitation, followed by a population contraction and the persistence of isolated populations in meridional mountainous areas. Thus, the combination of population age with genetic drift phenomena during expansions may explain the present-day patterns of genetic diversity in this species.

Values of average H_E (7%) are consistent with average values reported generally for reptiles and other species of non-insular lizards (Nevo *et al.*, 1984; Ward *et al.*, 1992) (Table 2). When these measures are compared between *L. schreiberi* populations, it is remarkable that the two extreme values correspond to two geographically close locations: Béjar and Toledo. The first population showed the highest values for all the parameters ($P_{0.95}$=34.8%, a=1.5 and H_E=0.12), a fact that is in agreement with the long persistence of the species, in the Spanish Central System. On the other hand, the second population is characterized by a total lack of genetic variability, indicating that this isolate may have resulted from only a few founder individuals and/or that small population size has promoted genetic drift.

L. schreiberi exhibited a high degree of genetic substructure (F_{ST}=0.448) with most of the genetic variation existing among populations (Table 3). It is especially noteworthy that isoelectric focusing was responsible for revealing the two loci that most contribute to high population differentiation (F_{ST} of 0.562 and 0.501 for *Pgdh* and *Px*3, respectively). This is not surprising, because the high resolving power of this technique is well known (Righetti, 1990) and the effective separation of electromorphs into their constituent alleles necessarily generate higher F_{ST} values. Alternatively, selection at these loci or at closely linked loci may have increased differentiation, but only future work will clarify this issue. When populations from the main distribution area (El Ferrol, Gerês, Gião, S. Estrela, Caldas da Rainha, Montejunto and Béjar) and the southern isolates (Toledo, S. Mamede, Monchique and Cercal) are analysed separately, F_{ST} values change significantly, being much lower in the former set of populations (F_{ST} = 0.315) than in the second (F_{ST} = 0.582) (results not shown). This clearly suggests that populations in the southern isolates are more differentiated due to severe restrictions to gene flow coupled with small population numbers (Brito *et al.*, 1998). A more detailed analysis of F_{ST} values among populations is presented in Fig. 3 and Appendix 2. Consistent low levels of differentiation are found when populations included in the north-western main distribution area are compared (El Ferrol, Gerês, Gião and Estrela), probably indicating a more homogeneous population structure and fewer constraints to gene flow. This situation is also detected in the pair of marginal populations – C.Rainha/Montejunto – that are separated from the main distribution area by less than 150 km and are poorly differentiated. The relation between pairwise F_{ST} values and geographical distances for the populations on the main distribution area is depicted in Fig. 3 and fits a model for genetic structure of isolation-by-distance.

The Cavalli-Sforza distance-based NJ tree presents two main clusters corresponding to northern and southern samples (Fig. 4 and Appendix 2). The first group includes all northern populations from the main distribution area of *L. schreiberi* (93% bootstrap) that are known as large and continuous populations and have

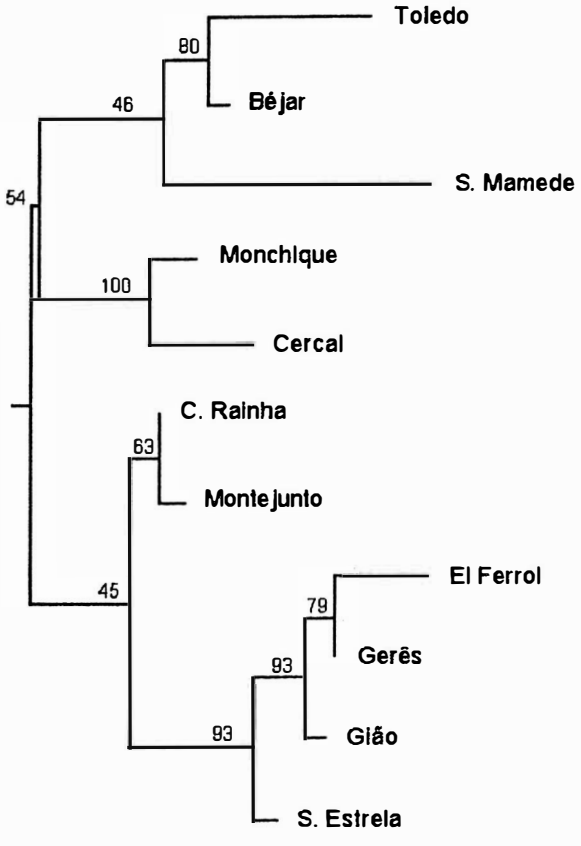

FIG 4. A neighbour-joining tree based on Cavalli-Sforza & Edwards (1967) chord genetic distance for 11 populations of *L. schreiberi*, with percent bootstrap replication scores indicated. The root was determined by estimating the midpoint between the two most divergent populations.

probably been connected by favourable habitat since at least the last glaciation. This set of populations cluster with the western Caldas da Rainha and Montejunto samples that correspond to two small isolated coastal populations. These populations have probably been isolated through human activities that fragmented habitats. These results are thus compatible with recent gene flow between Caldas da Rainha and Montejunto and the group of northern populations. The second group includes a central set of populations (Béjar, Toledo and S. Mamede), as well as the south-western isolates of Monchique and Cercal, and probably represents a history of fragmentation by a large ancestral southern population. This is clearly the case for Monchique and Cercal that are geographically distant from all other populations and still show similar allelic distribution profiles, thus reflecting a recent separation. In contrast, S. Mamede and Toledo probably result from two different expansion events originating in the Spanish Central System, resulting in very different genetic characteristics. In fact, while S. Mamede still exhibits moderate levels of H_E (0.06) and two rare alleles (*Pgdh*B3 and *Mpi*A), suggesting a relatively long history for this population, Toledo completely lacks genetic variability, indicating the occurrence of strong genetic drift.

Recent advances in molecular techniques and their application to a variety of organisms in the Iberian Peninsula have shown a complex combination of population histories, including fragmentations, contractions to glacial refugia, post-glacial expansions, and the formation of hybrid zones (Cooper *et al.*, 1995; Hewitt, 1996, 1999; Comes & Abbott, 1998; Alexandrino *et al.*, 2000; Branco *et al.*, 2000). In the future, a more detailed study comprising a considerable extension of the numbers of both genetic markers and sampled populations, as well as the use of hypervariable microsatellite and mtDNA markers, may certainly contribute to a better understanding of the recent evolution of *L. schreiberi* in the Iberian Peninsula.

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APPENDIX 1. Allelic frequencies of polymorphic loci in 11 populations of *L. schreiberi*.

Locus	Allele	El Ferrol	Gerês	Gião	Estrela	Toledo	Béjar	S. Mamede	Caldas Rainha	Monte-junto	Mon-chique	Cercal
*Aat*1	A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
	B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00
Amy	F	0.39	0.77	0.81	0.75	1.00	1.00	1.00	0.89	1.00	1.00	1.00
	S	0.61	0.23	0.19	0.25	0.00	0.00	0.00	0.11	0.00	0.00	0.00
*Ck*1	A	0.00	0.16	0.00	0.47	0.00	0.25	0.76	0.72	0.85	0.87	0.92
	B	1.00	0.84	1.00	0.53	1.00	0.75	0.24	0.28	0.15	0.13	0.08
Gpi	A	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
	B	1.00	1.00	1.00	0.96	1.00	0.97	1.00	1.00	1.00	1.00	1.00
	C	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
*Ldh*1	A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00
	B	1.00	1.00	1.00	1.00	1.00	0.93	1.00	0.95	1.00	1.00	1.00
	C	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00
Me	A	0.22	0.06	0.00	0.20	0.00	0.73	0.85	0.71	0.53	0.98	0.83
	B	0.78	0.94	1.00	0.75	1.00	0.27	0.15	0.29	0.47	0.02	0.17
	C	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mpi	A	0.00	0.04	0.00	0.00	0.00	0.03	0.02	0.00	0.00	0.00	0.00
	B	1.00	0.96	1.00	1.00	1.00	0.97	0.98	1.00	1.00	1.00	1.00
Pep B	1	1.00	1.00	1.00	1.00	1.00	0.77	0.94	0.89	0.77	1.00	1.00
	2	0.00	0.00	0.00	0.00	0.00	0.23	0.06	0.11	0.23	0.00	0.00
Pep D	F	0.00	0.00	0.00	0.00	0.00	0.41	0.52	0.10	0.00	0.00	0.00
	S	1.00	1.00	1.00	1.00	1.00	0.59	0.48	0.90	1.00	1.00	1.00
Pgdh	A	0.00	0.00	0.00	0.04	1.00	0.70	0.97	0.39	0.52	0.29	0.70
	B1	1.00	1.00	1.00	0.96	0.00	0.23	0.02	0.61	0.48	0.71	0.30
	B2	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00
	B3	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Pgm	A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00
	B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.90	1.00	1.00
*Px*2	A	0.41	0.70	1.00	1.00	1.00	0.93	1.00	1.00	1.00	1.00	1.00
	B	0.59	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	C	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00
*Px*3	A	0.50	0.09	0.37	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	B	0.00	0.00	0.06	0.25	1.00	0.81	1.00	0.00	0.00	0.40	1.00
	C	0.14	0.38	0.38	0.13	0.00	0.19	0.00	0.92	1.00	0.60	0.00
	D	0.36	0.53	0.19	0.58	0.00	0.00	0.00	0.08	0.00	0.00	0.00

APPENDIX 2. Below diagonal: Cavalli-Sforza & Edwards (1967) chord distance between populations; above diagonal: F_{ST} values per pair of population, using the Weir & Cockerham (1984) estimator θ.

	El Ferrol	Gerês	Gião	Estrela	Toledo	Béjar	S. Mamede	Caldas Rainha	Monte junto	Monc-hique	Cercal
El Ferrol	–	0.154	0.218	0.288	0.652	0.482	0.723	0.474	0.478	0.634	0.589
Gerês	0.071	–	0.060	0.110	0.575	0.440	0.675	0.366	0.466	0.556	0.550
Gião	0.124	0.080	–	0.125	0.717	0.390	0.690	0.363	0.426	0.621	0.603
Estrela	0.198	0.095	0.125	–	0.564	0.361	0.580	0.290	0.243	0.418	0.423
Toledo	0.543	0.460	0.352	0.353	–	0.400	0.647	0.625	0.530	0.756	0.812
Béjar	0.535	0.433	0.419	0.296	0.255	–	0.176	0.297	0.217	0.361	0.238
S. Mamede	0.703	0.593	0.608	0.374	0.282	0.094	–	0.465	0.275	0.472	0.231
C. Rainha	0.367	0.228	0.301	0.171	0.477	0.205	0.289	–	0.037	0.119	0.356
Montejunto	0.477	0.306	0.360	0.249	0.453	0.238	0.322	0.055	–	0.187	0.092
Monchique	0.504	0.363	0.403	0.218	0.442	0.189	0.213	0.107	0.119	–	0.277
Cercal	0.592	0.476	0.496	0.249	0.279	0.166	0.091	0.244	0.241	0.094	–

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TRACING ALIENS: IDENTIFICATION OF INTRODUCED WATER FROGS IN BRITAIN BY MALE ADVERTISEMENT CALL CHARACTERISTICS

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We have used sound analysis of male advertisement calls in a study of seven introduced populations of water frogs in Britain. Discriminant analysis of call characters identified five types of water frog, notably *Rana lessonae, R. esculenta, R. ridibunda, R. bergeri* and *R. perezi*. *Rana epeirotica* and *R. shqiperica* were not detected. Typical LE (*lessonae-esculenta*) systems were found at two sites, *R. ridibunda* occurred alone at two sites and *R. esculenta* occurred alone at one site. The remaining two sites were more complex. One had *R. ridibunda, R. perezi* and *R. esculenta* while the seventh site had four taxa of water frog (*R. lessonae, R. bergeri, R. esculenta* and *R. perezi*). The value of call analysis for the identification of water frog populations is discussed.

Key words: alien species, call frequency, green frogs, oscillograms, *Rana*

INTRODUCTION

Until recently it was generally accepted that six species of amphibian are postglacial natives of the British Isles (Smith, 1951). None of the European water frogs were included on this native list, although these amphibians are common and widespread in mainland Europe. Water frogs are a complex group and their taxonomy has been widely studied during recent decades (Berger, 1973; Wijnands, 1977; Balletto *et al.*, 1986; Nevo & Filipucci, 1988; Sjögren, 1991; Sinsch & Eblenkamp, 1994; Santucci *et al.*, 1996). It is now clear that three forms of water frog occur over much of northern and central Europe. These are the pool frog (*Rana lessonae*), the marsh frog (*R. ridibunda*) and the edible frog (*R. esculenta*). Edible frogs are fertile hybrids of pool and marsh frogs and are maintained in mixed populations of one or both parent species by the process of hybridogenesis (Berger, 1977, 1983). Water frogs commonly occur as mixed populations of *R. lessonae* and *R. esculenta* ("LE systems"), or of *R. ridibunda* and *R. esculenta* ("RE systems"), because *R. esculenta* usually requires one of its parent species to be available for back crossing (Graf & Polls-Pelaz, 1989). Other water frogs occur in southern Europe, including *R. perezi* in Iberia, *R. bergeri* in Italy, and *R. epeirotica* and *R. shqiperica* in the Balkans. Some of these southern species also hybridize with *R. ridibunda*, and another hybridogenetic cross (*R. ridibunda* x *R. perezi*) has produced *R. grafi* in southern France. Because some water frogs are true species whereas others are fertile hybrids, we have used the term 'taxon' for each of the main forms listed above (Graf & Polls-Pelaz, 1989).

Although not previously considered native, there have been many records of water frogs in Britain since the early nineteenth century (Smith, 1951). Recently, evidence has accumulated that some populations of *R. lessonae* were probably true natives of eastern England (Gleed-Owen, 2000; Zeisset & Beebee, 2001; Wycherley *et al.*, 2002*a*), though these are now extinct. However, it is clear that the great majority of water frog populations in Britain were introduced by humans in recent times. These introductions started at least as early as 1837 (Wolley, 1847) and some are well documented. Thus there are detailed reports on the introduction of the edible frog into East Anglia (Boulenger, 1884*a*, 1884*b*; Dutt, 1906; Buckley, 1986) and an introduction of the marsh frog into Kent (Smith, 1939; Menzies, 1962; Lever, 1980).

Most introductions, however, have uncertain origins and even the species present are often unknown. Populations of introduced water frogs in this category certainly occur in Greater London, and in the counties of Kent, Surrey, Sussex, Essex, Norfolk, Hampshire, Herefordshire, Worcestershire, Somerset and Yorkshire. There may well be others. Snell (1983, 1984) reported water frogs on the Isle of Sheppey in Kent and at Birdbrook, London. Elsewhere, populations have become established west of London around Heathrow airport and Staines Reservoirs (K. Morgan, *pers. com.),* the River Longford, Barnes Nature Reserve (K. Morgan *pers. com.*), and to the east of London in the Lee Valley Navigation. Surrey has had many water frog introductions. Since 1903 Beambrook Nursery near Dorking has received repeated imports of water frogs from Belgium, Germany, France and Italy, and possibly elsewhere (Gillett, 1988). Another company near Redhill also imported many specimens for commercial purposes. Frogs escaped into local ponds and streams and have subsequently spread extensively. Other Surrey sites with water frog populations include Pyrford, Old Woking, the river Wey, the Royal Horticultural Society Gardens at Wisley, Burgh Heath, Ewell, Horley, Gatwick, Capel and Ewhurst (Wycherley & Anstis, 2001). *Rana ridibunda* has extended its range in both Kent and Sus-

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sex (Beebee & Griffiths, 2000), and there are certainly other water frog populations in both of these counties.

In East Anglia there are water frogs in Essex near Hadleigh Castle and Two Tree Island, at Witham, at West Mersea (J. Cranfield, *pers. com.*) and at Ardleigh near Colchester. In Norfolk there are two recent reports of water frog populations, one at Wolterton Hall near Itteringham, and a second, first mentioned by Buckley (1986), adjacent to the Steam Museum at Forncett St Mary. Isolated water frog populations occur near Bramshill in Hampshire, Bodenham Moor in Herefordshire, Holt in Worcestershire (W. Watson, *pers. com.*), and at Shapwick Heath and West Sedgemoor in Somerset (D. Westbrook, *pers. com.* 1996). The most northerly reports of waterfrog populations are from Swinemoor Common and Hedon in Yorkshire (R. Atkinson, *pers. com.*).

Identification of the frogs in these introduced populations is important if we are to understand the origins of the invaders and their likely future spread in the UK. Although genetic analysis is possible (Zeisset & Beebee, 1998), this is time-consuming, expensive and, by necessity, laboratory-based. It is highly desirable to develop a quick, inexpensive and relatively easy methodology that can identify all types of frog occurring in mixed populations. This, in turn, should enable examination of numerous populations within a relatively short time-scale. In this paper, we demonstrate the application of sound frequency analysis to male advertisement calls and its value in determining the species composition of introduced water frog populations. With this approach it is not necessary to handle or even catch the frogs, and identification can be carried out using only a tape recorder and a personal computer. The use of this technique for identifying *R. lessonae, R. esculenta* and *R. ridibunda* has been demonstrated previously (Wycherley *et al.*, 2001; Wycherley *et al.*, 2002*a*).

MATERIALS AND METHODS

Data Collection

We obtained recordings of water frog advertisement calls from a selection of British sites during May and June 1999-2001 (Fig. 1). Several calls from each individual frog were recorded for analysis. The following list gives the site name, site location (as national grid reference), the numbers of frogs and calls (*x, y*) analysed: Somerset Levels ST 424412 (5, 22); Bramshill, Hampshire SU 759614 (13, 34); Newdigate, Surrey TQ 226421 (14, 31); Romney, Kent TR 08 21 (5, 10); Sheppey, North Kent TQ 933697 and TQ 906685 (14, 34); Wisley, Surrey TQ 064585 (5, 29); Wolterton, Norfolk TG 166317 (9, 26). Recordings were made using a Sony Electronic Condenser Microphone ECM-BMS-957 and a Sony Professional Walkman WM-D6D. The PC program Cool Edit 96™ (Syntrillium Inc., Phoenix, AZ) was used in "record mode" to transfer short samples of sound from the original audiocassettes to a PC via a 16-bit sound card (Addonics, Freemont, CA). Individual sub-units (Wycherley *et al.*, 2001; Wycherley *et al.*, 2002*a*) were selected using the editing features of Cool Edit 96™ and transferred to the program IDL® (Interactive Data Language, Research Systems Inc., Boulder, CO), where they were analysed using a set of custom-written procedures. These procedures are available free of charge, together with instructions for use, from J. Wycherley (julia.wycherley@virgin.net).

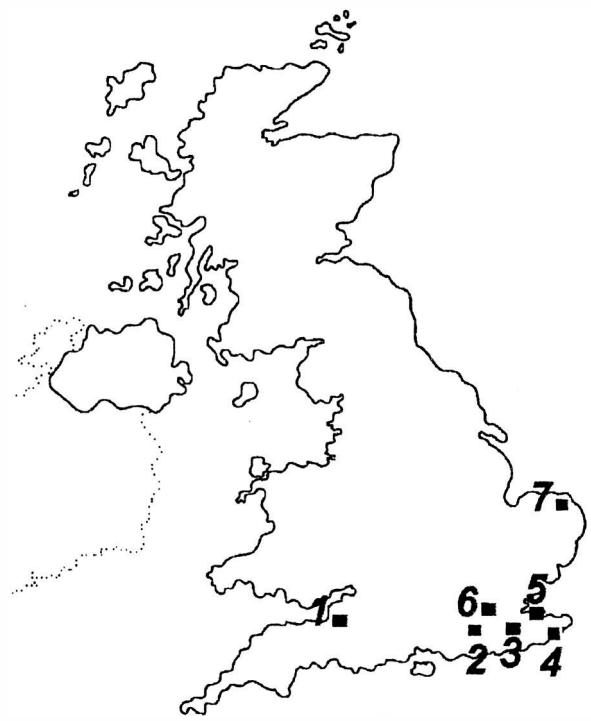

FIG 1. Distribution of British water frog sampling sites used in call frequency analyses. 1: Shapwick, Somerset; 2: Bramshill, Hampshire; 3: Newdigate, Surrey; 4: Romney, Kent; 5: Sheppey, North Kent; 6: Wisley, Surrey; 7: Wolterton Hall, Norfolk

We previously established a reference set of calls from *R. lessonae* (Germany), *R. esculenta* (France) and *R. ridibunda* (France). For each of these three taxa in turn we obtained several advertisement call repetitions from a number of individual frogs. (Wycherley *et al.*, 2002*b*) The calls of additional European water frog species were obtained for reference comparison from a CD by Jean C. Roché: *Au pays des Grenouilles, Sittelle*™ 1997. These additional calls were from *Rana bergeri* (northern Corsica), *Rana epeirotica* (southern Albania), *Rana perezi* (central Spain) and *Rana shqiperica* (Durrés, Albania).

Data Analysis

We selected several sub-units from each frog call and for each one obtained the Fourier transform (Wycherley *et al.*, 2002*b*). For each principal peak in the Fourier transform we measured the peak frequency, peak width at half the maximum height, and relative amplitude as independent variables. Data from the multiple subunits of each call were averaged and these averages then used as data points (i.e. one datum point per variable per call) in subsequent analyses. The data set for each call was considered collectively as a 'case' (Wycherley *et al.*,

TABLE 1. Differentiation among seven taxa of water frog assessed by discriminant analyses. Calls used in the analysis were from *R. bergeri, R. epeirotica, R. perezi, R. shqiperica, R. ridibunda, R. lessonae* and *R. esculenta.*

	Eigenvalue	Canonical correlation	Wilks' lambda	χ^2	df	*P*
Discriminant Function 1	725.688	0.999	0.000	2522.36	70	<0.0001
Discriminant Function 2	456.289	0.999	0.000	1916.22	54	<0.0001
Discriminant Function 3	80.971	0.996	0.000	1352.69	40	<0.0001

2001, 2002*a,b*). All data were tested for normality of distribution using the Shapiro-Wilks test in the Statistix7™ Analytical Software package. No transformations were necessary and we carried out further analyses as described below using the statistical program SPSS, Chicago.

We analysed each British population using discriminant analysis, initially with all the independent variables. However, the peak-width variable did not improve discrimination significantly and we therefore excluded it in the full analyses. There was therefore a maximum of 10 variables, each having up to five peaks and with a frequency and amplitude relative to the largest peak (Wycherley *et al.*, 2002*b*). However, the number of peaks varied between species and where peaks were absent, zero values were entered for the associated variables. For each British site we included data from all frogs recorded using the classification methods in discriminatory analysis to determine how well call samples separated and could be assigned to a particular species. We compared the call frequency characteristics from each selected British water frog population with those of our reference populations of *R. ridibunda, R. lessonae* and *R. esculenta.* When we were unable to identify all the calls from a population by comparison with these three taxa, we also made comparisons with the further four water frogs on the Roché CD (see above). Seven taxa were therefore available as standards for comparison with British frogs. The "leave-one-out" method of classification (Wycherley *et al.*, 2002*b*), which provided cross-validation of the success of the classifications, gave a further measure of the effectiveness of the analyses in differentiating water frogs.

In order to ensure that the unknown populations were assigned to the nearest reference taxon we subjected each data set, comprising data from the standards and the selected unknown population, to discriminant analysis using SPSS but only selected the standards for discrimination. The unknown cases are then marked as 'ungrouped' but their nearest group membership is predicted. In this way the probable composition of each British water frog population was determined.

RESULTS

Separation of Reference Varieties

The call-data sets from seven taxa of water frog (*R. bergeri, R. epeirotica, R. perezi, R. shqiperica, R. ridibunda, R. lessonae* and *R. esculenta*) were pooled and subjected to discriminant analysis as described above. Canonical discriminant functions showed very significant separations and results from the first three functions are shown in Table 1. The classification success of the discriminant functions for each of these seven taxa was 100% in both the original grouped cases and the cross-validated grouped cases. The extent of separation among these reference frog calls is demonstrated in Fig. 2a. Clear separations were achieved using only the first three discriminant functions although seven functions were derived in the analysis. *Rana perezi* and *R. ridibunda* aligned particularly closely, but were nevertheless fully resolved. These standards were then used as references for the identification of British water frog populations.

Identification of British Water Frogs

The advertisement calls obtained from each British site were analysed as previously described and the resulting data were compared with those from the seven standards. By this means we allocated British frog calls to one or more of the reference taxa. In every analysis the standards showed 100% classification success for grouped cases and cross validations. The nearest group membership (to a standard) of each of the unknown British cases was predicted. This indicated the range of species present at each British site. We then examined the distribution of the 'cases' in each British population to see how well they clustered with the standards. The first three discriminant functions for each British population analysis were plotted and the results from each site are shown in Fig. 2b-h. A classification summary is provided in Table 2.

Discriminant analysis of calls recorded from the frogs at Bramshill (Fig. 2b) indicated the presence of a mixed *R. lessonae* and *R. esculenta* population (i.e. a LE system). Close affinity to both *R. lessonae* (23.5% of calls) and *R. esculenta* (70.4% of calls) was observed in the two dominant clusters, with a few samples more typical of *R. perezi.* At Newdigate (Fig. 2c), the calls separated into four main clusters, including *R. perezi* (13.5%), *R. bergeri* (16.3%), *R. lessonae* (35.5%) and *R. esculenta* (34.8%). This combination could never occur without human intervention, because two of these frogs (*R. perezi* and *R. bergeri*) have widely separated natural distributions in Europe. Frogs on Sheppey were discriminated into three types (Fig. 2d), allocated to *R. perezi* (36.6%), *R. ridibunda* (19.5%) and *R. esculenta*

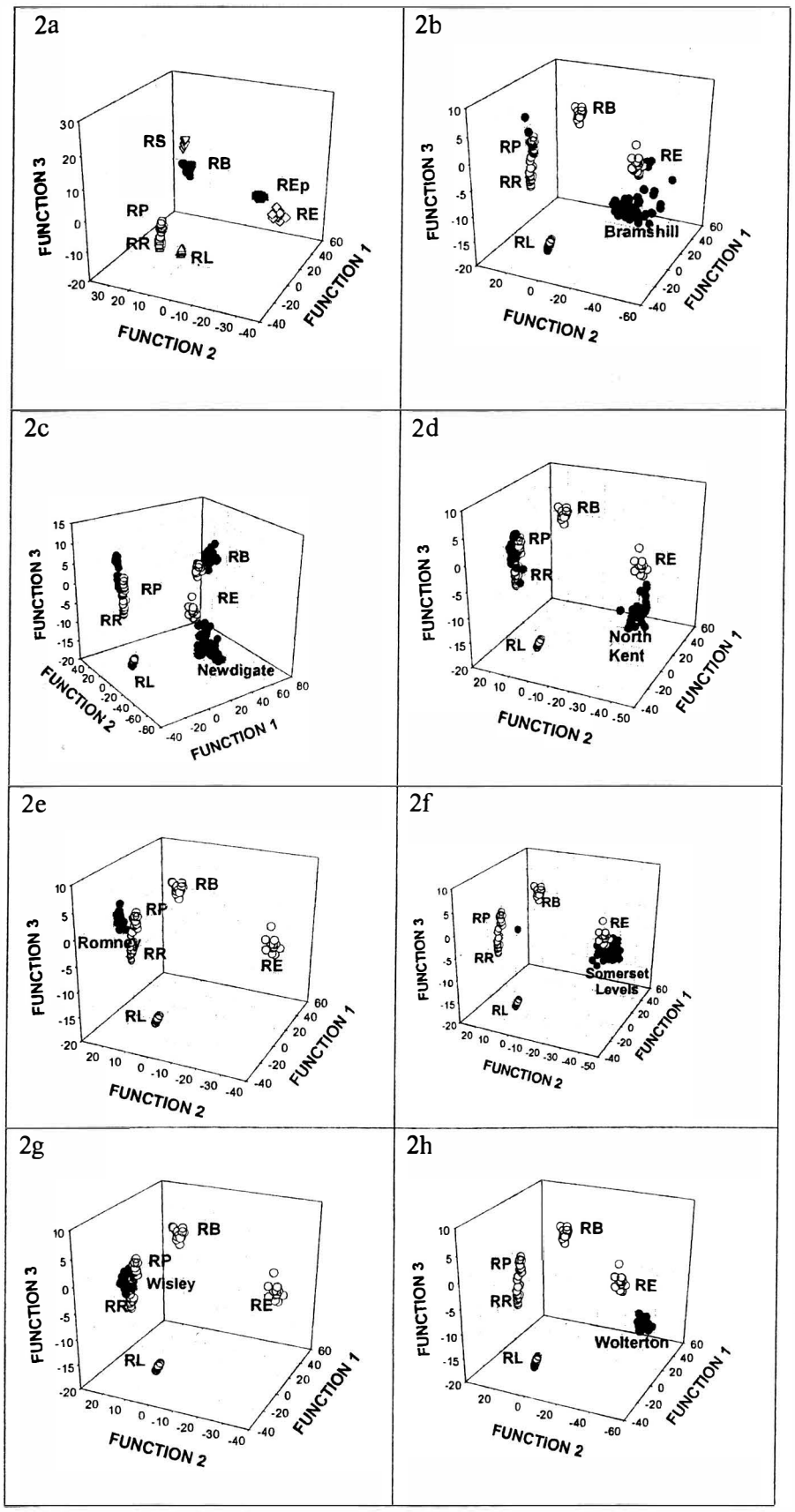

FIG 2. (a) Separation of frog taxa by call frequency analysis. RB (solid circles), *R. bergeri*; Rep (solid squares), *R. epeirotica*; RP, (circles) *R. perezi*; RS (inverted triangles), *R. shqiperica*; RR (squares), *R. ridibunda*; RL (triangles), *R. lessonae*; RE (diamonds), *R. esculenta*. (b-h) Scatter plots of discriminant functions 1, 2 and 3 derived from British water frog calls. Species eliminated by initial analyses [*R. epeirotica* and *R. shqiperica*] were not included in these scatter plots. References (open circles): *R. bergeri*, *R. perezi*, *R. ridibunda*, *R. lessonae*, *R. esculenta*; sample population (filled circles): b, Bramshill, Hampshire; c, Newdigate, Surrey; d, North Kent (Sheppey); e, Romney, Kent; f, Somerset Levels (Shapwick); g, Wisley, Surrey; h, Wolterton, Norfolk.

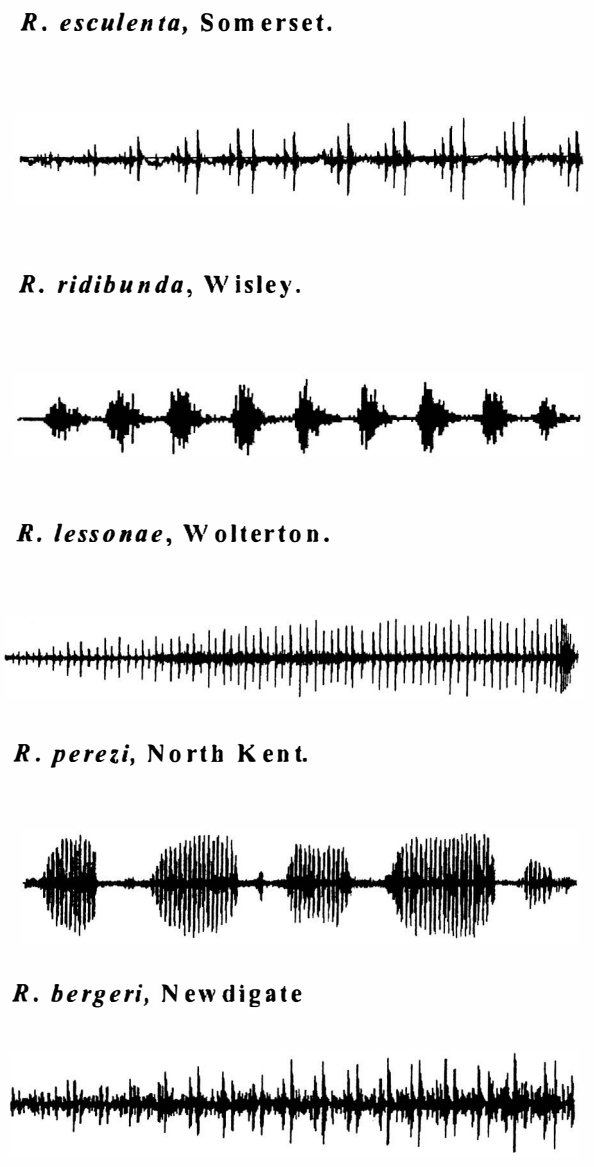

FIG 3. Advertisement call oscillograms of the five water frog taxa identified in Britain.

(43.9%). This is similar to an RE system, since *R. perezi* and *R. ridibunda* are closely related, and is a combination that could occur naturally in parts of south-eastern France. The introduction of *R. ridibunda* to the Romney Marsh area in 1935 was well documented. Discriminant analysis (Fig. 2e) confirmed that probably only *R. ridibunda* was still present at this site, although one individual was statistically assigned to *R. perezi*. By contrast there is no record of the source of the frogs introduced to the Somerset Levels, where they were first reported as recently as 1996. Canonical discriminant functions (Fig. 2f) placed all these call samples as *R. esculenta* in keeping with the morphology of individuals seen there. It is, however, very unusual to find *R. esculenta* by itself and this site clearly warrants further study. *Rana ridibunda* was first reported from Wisley about five years ago. The scatter-plot (Fig. 2g) of discriminant functions confirmed that this is indeed the species present in the Royal Horticultural Society gardens. Finally, call analysis showed that two distinct taxa of frog were present at Wolterton Hall in north-east Norfolk (Fig. 2h). These were *R. lessonae* (56.8%) and *R. esculenta* (43.2%), evidently constituting another LE system.

DISCUSSION

This study extends our previous analyses (Wycherley *et al.*, 2002*a,b*) by successfully distinguishing seven rather than three taxa of European water frog on the basis of male advertisement calls. Earlier studies on the identification of water frog populations used other techniques including genetics (Graf *et al.*, 1977; Spolsky & Uzzell, 1986), morphology (Juszczyk, 1971; Ogielska *et al.*, 1998; Sinsch & Schneider, 1999), and sound analyses based on the call pulses and repetition rate (Schneider, 1997; Gerhardt *et al.*, 2000). We have also shown that identification of unknown populations is possible by using call frequency analysis followed by the 'predicted group membership' and 'display casewise results' facilities of discriminant analysis. Frogs previously identified on morphological grounds or by knowledge of the introduction history (Wisley and Romney) were classified in the expected way, giving independent support to the accuracy of call analysis. However, at both these sites a single sample was classified as *R. perezi*. Further examination of the call data showed that both of these samples had lower peak frequencies that were well above the population average, and close to the lower range of *R. perezi*. Visual inspection of the call oscillograms indicated that these frogs

TABLE 2. Statistical assignment of British water frog populations. In this analysis the cases from each population were assigned to the nearest taxon.

Population	Taxon allocation at individual UK sites (%)						
	R. bergeri	*R. epeirotica*	*R.perezi*	*R. shqiperica*	*R. ridibunda*	*R.lessonae*	*R. esculenta*
Bramshill, Hampshire	0	0	6.1	0	0	23.5	70.4
Newdigate, Surrey	16.3	0	13.5	0	0	35.5	34.8
Sheppey, North Kent	0	0	36.6	0	19.5	0	43.9
Romney, Kent	0	0	7.1	0	92.9	0	0
Shapwick, Somerset	0	0	0	0	1.9	0	98.1
Wisley, Surrey	0	0	9.6	0	90.4	0	0

were probably *R. ridibunda*, but it will be important to make further corroborative tests of our call analysis method at other sites in future.

Two LE systems were identified by our analysis, as well as one population with a mixture of four taxa (*R. lessonae, R. bergeri, R. esculenta* and *R. perezi*) probably not found together anywhere else in the world. Of particular interest was the Somerset site where only *R. esculenta* was detected. This hybrid normally requires sympatric populations of one or other parent species and can only survive in isolation as a triploid (Graf & Polls-Pelaz, 1989). Such triploid populations are known in parts of northern Europe, and it will interesting to discover whether the Somerset frogs are triploid or whether other water frogs occur as yet undiscovered in the area.

Call analysis by our method is sufficiently sensitive to resolve local dialects in *R. lessonae* (Wycherley *et al.*, 2002*a*) and *R. ridibunda* (Wycherley *et al.*, 2002*b*). This means that classifications at the species level might vary slightly in their concordance with reference samples, according to the population source of the reference. This probably explains why clustering (Fig 2) did not always show unknown samples precisely superimposed on the references. However, the sensitivity of our analysis also means that with more comprehensive reference material it might be possible to ascribe introduced populations not just to taxon but to likely areas of origin.

It is particularly interesting to note the variable distribution of the hybrid *R. esculenta* populations in the 3-D scatter plots of discriminant functions when compared to the standard for this taxon obtained from a population in France. We have previously shown that both *R. lessonae* and *R. ridibunda* demonstrate phylogeographic variation in call characteristics across Europe (Wycherley *et al.*, 2002*a,b*). This may also be true of *R. esculenta*, since British populations were probably founded by frogs from various origins across Europe. These may well include a variety of different *ridibunda* hemiclones in the hybrids (Semlitsch *et al.*, 1997).

The use of call analysis should enable further sites to be examined relatively easily, and therefore extend our knowledge of water frogs present in Britain. However, the number of frog taxa identified at any site is obviously limited by the range of advertisement calls recorded. Where mixtures are suspected, successful identification of all the frogs present requires repeated site visits to ensure that sampling is comprehensive. Our sample sizes were rather small and therefore these results may not reflect the total range of frogs present at every site. Nevertheless, the range of European water frogs identified in Britain now includes *R. ridibunda, R. lessonae, R. esculenta, R. bergeri* and *R. perezi*. Oscillograms of the advertisement calls of these five taxa are shown in Fig. 3. This study has sampled only a very small proportion, probably less than 5%, of the water frog introduction sites in Britain. It has become apparent that many more populations exist and are widely distributed across England, since new sightings are a regular occurrence.

The future prospects of water frogs in Britain will no doubt depend on many factors. Perhaps the most significant of these are the recent climate changes that have been reported due to global warming effects (IPCC, 2001). These changes have already impacted upon many ecosystems (Walther *et al.*, 2002). Amphibians, including water frogs, can be susceptible to changes in spring temperatures (Beebee, 1995) because higher temperatures advance the breeding season. This in turn improves reproductive success and may enable some species to expand their ranges. This has already occurred in the case of the British water frog populations. Records from localities radiating from the introduction sites at Romney and Newdigate have shown steady outward advance (Menzies, 1962; Beebee & Griffiths, 2000; Wycherley & Anstis, 2001). This has been particularly apparent at Newdigate during the 1980s and 1990s. Prior to this there are few records of outward expansion since the first introductions began in the earliest decades of the last century. Opinions differ on the risks, if any, that these expansions might pose to native fauna. It seems likely, though, that introduced water frogs will continue to spread in Britain for the foreseeable future.

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BOOK REVIEWS

Amphibians and Reptiles of North-west Europe: Their Natural History, Ecology and Conservation. Ian F. Spellerberg. (2002). 216 pp. Science Publishers, Enfield, New Hampshire. £27.50 (paper).

If you were to decide to purchase this book, it would almost certainly be for the 28 colour plates. Medical and wildlife artist Peter Jack has done an excellent job of illustrating the creatures discussed in the text, his paintings accurately recording the physical characteristics of the animals whilst at the same time capturing their beauty. It is unfortunate that, in adjusting their images to fill the pages, the male crested and larval alpine newt (*Triturus cristatus* and *T. alpestris*, respectively) appear to have been stretched horizontally but not vertically, distorting their appearances. Also, the separate sexes of several species have not been enlarged by equal factors, so that a misleading impression is given as to their relative sizes. The use on one plate of the caption "*Rana lessonae/esculenta*" is unsatisfactory: is it a pool frog, or an edible frog? These two taxa are similar, but not identical, in appearance.

Ian Spellerberg, Professor of Nature Conservation at Lincoln University, New Zealand, and former Director of the Centre for Environmental Sciences at Southampton University, has here chosen to consider the Atlantic Climatic Region of Europe: this area extends from northern Spain to the southern Baltic Sea and the coastal parts of southern Norway. There is some logic to this choice. His selection of species, though, is rather more idiosyncratic: the marbled newt (*Triturus marmoratus*) and parsley frog (*Pelodytes punctatus*) might have been thought strong candidates for inclusion, with the omitted dark green, Aesculapian and viperine snakes (*Coluber viridiflavus*, *Elaphe longissima* and *Natrix maura*) – as well as the asp (*Vipera aspis*) – all being as least as well qualified as several species that are featured.

There are six figures, two of which are in colour, and 24 distribution maps. The latter mostly give an appropriate impression, but there are significant errors. For example, the range indicated for *T. cristatus* actually includes that of several related species, contradicting the text. Likewise, the Corsican fire salamander (*Salamandra corsica*) and Italian yellow-bellied toad (*Bombina pachypus*) have been included within the distributions of the European fire salamander (*S. salamandra*) and common yellow-bellied toad (*B. variegata*), respectively. The western green lizard (*Lacerta bilineata*) has been widely regarded as distinct from the eastern green lizard (*L. viridis*) for some years, but the two are not differentiated here. Equally, there is no mention of the fact that the viviparous lizard is often now referred to as *Zootoca* (rather than *Lacerta*) *vivipara*. Of course, Professor Spellerberg may not agree with some recent taxonomic changes, but this does not excuse inconsistencies within the text.

The bulk of the book is made up by the species accounts. Once again, there is an unacceptable number of errors. For example, the same word is often spelt in different ways (e.g. recognise/recognize; niche, both with and without two different – and incorrect – accents); this has, in turn, led to duplicate entries in the three-page index (e.g. Crocodilia/Crocodylia and nuptial/nuptual). Words are misspelt and punctuation misused throughout, contradictions are not uncommon (e.g. *T. cristatus* can live for twenty years on one page, twenty-seven years on the next), necessary italics are frequently omitted, and so on. These and other deficiencies do not help to make reading the text a pleasure. The ten-page "ecology" chapter that is a loose assemblage of several briefly-discussed topics, and the eleven-page chapter on conservation, represent steps in the right direction, but do not go far enough.

The species-by-species treatment tends to generate repetition, especially when the species themselves are so similar in many respects, and this leads to inefficiency in delivery. The 164 pages devoted to the accounts are described as "brief synopses of ecological information", and are broken down under the headings: *Introduction*; *Taxonomy*; *Protection*; *Description*; *Distribution and habitat*; *Seasonal movements and behaviour*; *Vagility and population ecology*; *Feeding ecology*; *Thermal ecology*; *Reproduction, growth and development*; *General comments*; and *Major references*. Most of these are dealt with briefly, often with as few as three lines of text. I therefore have to agree with the author when he admits that the accounts are selective and not definitive. English, French, German, Dutch, Spanish and Swedish vernacular names are given, but there are several errors in the accenting, some presumably resulting from confusion between the languages; there are also typographical errors such as ">" appearing in place of a full stop.

The intentions set out in the introduction (four pages) and suggested by the title of this volume are admirable; the target readers appear to be amateur herpetologists and conservationists. Errors in the text and a lack of up-to-date information, though, contribute to my disappointment with the final product. A three-page glossary explains most – but not all – of the more technical terms used, and four pages of references contain little more than a handful of publications from the mid-1990's onwards. Splendid illustrations, then, but a text that will not take a reasonably well-read herpetologist very far forward. However, having over thirty years experience with the alpine newt, I was nonetheless intrigued to learn that it is in the habit of "inflating itself and whistling when handled"!

Leigh Gillett
British Herpetological Society

Die Äskulapnatter (Elaphe longissima). *Verbreitung und Lebensweise in Mitteleuropa.* Axel Gomille. (2002). 160 pp. Edition Chimaira, Frankfurt am Main, Germany (cloth).

This book is based on the author's Master's thesis and – as he points out – is not intended to be a monograph. Axel Gomille primarily wants to present the results of his research on the Aesculapian Snake in the Odenwald area in south-west Germany – a few kilometers east of Heidelberg. Consequently, the introduction is followed by more than 50 pages on the species in that area, but then a chapter of more than 40 pages deals with what is promised in the title: its situation in central Europe. An English summary of 4½ pages is given, and a bibliography of approximately 200 titles concludes the book. The numerous photographs in colour and black and white are of high qualitiy, as are the drawings. All illustrations are well chosen and have legends in both German and English. The author presents many interesting facts and data on the distribution, habits, life cyle and many other aspects of *Elaphe longissima* from his own field work and from literature. Contrary to his own statement, he does not limit this strictly to central Europe, but also brings in some information from outside this region, e.g. Bulgaria. It is a little puzzling, then, why no information is included from, for example, Italy – a country which is, in several respects, of central importance for the Aesculapian snake and where much has been published on issues of direct relevance to the questions dealt with by the author.

In the last chapter (*The Aesculapian Snake in a new light*) Axel Gomille draws from the findings of his field research a truly new picture of the species in several areas. Interpreting climatic and habitat features together with other factors, the author gives a new and very convincing explanation of the ecological requirement of this snake in the northern part of its range. Based on the sites where the species occurs today, where it has occured in historic times and on 41 (!) subfossil and fossil findings, the author comes to the conclusion that the scattered sites of occurence in the northern part of the range of *Elaphe longissima* are definitely not the result of introduction by the Romans 2000 years ago, but rather relics within the natural range of the Aesculapian snake. This conclusion is especially remarkable as it puts an end to the "Roman theory" which has been used so often to explain the fragmented distribution patterns north of the Alps. A major portion of the English summary is dedicated to this "new light" in which the species will from now on have to be seen. The author's findings will also have to be taken into account for future conservation measures.

This is a thorough and well written study on what is perhaps the most charismatic snake species in Europe. I am sure that even those who do not read German will find this a very informative and enjoyable publication. I recommend it to anybody interested in European reptiles.

Manfred Niekisch
Ernst-Moritz-Arndt-Universität

THE HERPETOLOGICAL JOURNAL

INSTRUCTIONS TO AUTHORS
(revised July 2002)

1. The *Herpetological Journal* publishes a range of features concerned with reptile and amphibian biology. These include: *Full Papers* (no length limit); *Reviews* and *Mini-reviews* (generally solicited by a member of the editorial board); *Short Notes*; controversies, under *Forum* (details available from the Editor); and *Book Reviews*. Faunistic lists, letters and results of general surveys are not published unless they shed light on herpetological problems of wider significance. Authors should bear in mind that the *Herpetological Journal* is read by a wide range of herpetologists from different scientific disciplines. The work should therefore appeal to a general herpetological audience and have a solid grounding in natural history.

2. *Two* copies of all submissions, and illustrations, should be sent to the Scientific Editor, together with a computer diskette (IBM formatted) containing the text and, if possible, figures. Alternatively, submission by e-mail is possible - please contact the Scientific Editor for information. All papers will be subject to peer review by at least two referees. Authors are invited to suggest the names of up to three referees, although the editor may choose alternative referees to those suggested. Papers will be judged on the basis of the reports supplied by referees, scientific rigor, and the degree of general interest in the subject matter. The Editor's decision will be final.

3. Authors should consult a recent issue of the Journal regarding style. Papers should be concise with the minimum number of tables and illustrations. They should be written in English and spelling should be that of the *Oxford English Dictionary*. Papers should be typed or produced on a good-quality printer, and double-spaced with wide margins all round. The journal is typeset direct from the author's electronic text, so all manuscripts should be prepared using a word processor (preferably on a PC-compatible microcomputer). If figures are prepared using computer graphics, they should be supplied separately and NOT embedded in the text of the word processor file. Preferred formats are MS Word for Windows (text) and MS Excel, Bitmap, TIFF, Windows Metafiles (.wmf, .emf) or JPEG files (graphics).

4. For all papers the title page should contain only the following: title of paper; name(s) of the author(s); address of the Institution where the work was done; a running title of five words or less, and the name and address of the corresponding author with (if available) an email address. The text of the paper should begin on page 2 and be produced in the following order: Abstract, Keywords, Text, Acknowledgements, References, Appendices. Full papers and reviews should have the main text divided into sections. The first subhead will be centred in capitals, the second shouldered in lower case, and the third run on in italics. Footnotes are not permitted. *Short Notes* (generally less than six manuscript pages and accompanied by a single data set) should be produced as continuous text, preceded by an abstract of no more than 100 words. A *sans serif* font (e.g. Universe or Helvetica) is preferred.

5. The usual rules of zoological nomenclature apply.

6. Tables are numbered in arabic numerals, e.g. TABLE 1; they should be typed double spaced on separate sheets with a title/short explanatory paragraph above the table. Horizontal and vertical lines should be avoided.

7. Line drawings and photographs are numbered in sequence in arabic numerals, e.g. FIG. 1. Colour photographs can only be included at cost to the author (quotes can be obtained from the Managing Editor). If an illustration has more than one part, each should be identified as (a), (b), etc. The orientation and name of the first author should be indicated on the back. They should be supplied camera-ready for uniform reduction of one-half on A4 size paper. Line drawings should be drawn and fully labelled in Indian ink, dry-print lettering or laser printed. Illustrations produced using other types of computer printer are not usually of suitable quality. A metric scale must be inserted in micrographs etc. Legends for illustrations should be typed on a separate sheet.

8. References in the text should be given as in the following examples: "Smith (1964) stated —"; "—as observed by Smith & Jones (1963)." "—as previously observed (Smith, 1963; Jones, 1964; Smith & Jones, 1965)". For three or more authors, the first author's surname followed by *et al.* should be used (Smith *et al.*, 1972). In the list of references the full title of the journal should be given. Articles 'submitted' or 'in prep' may not be cited in the text or reference list. The following examples will serve to illustrate the style and presentation used by the Journal:

   Bellairs, A. d'A. (1957). *Reptiles*. London: Hutchinson.

   Boycott, B. B. & Robins, M. W. (1961). The care of young red-eared terrapins (*Pseudemys scripta elegans*) in the laboratory. *British Journal of Herpetology* **2**, 206–210.

   Dunson, W. A. (1969*a*). Reptilian salt glands. In *Exocrine glands*, 83–101. Botelho, S. Y., Brooks, F. P. and Shelley, W. B. (Eds). Philadelphia: University of Pennsylvania Press.

   Dunson, W. A. (1969*b*). Electrolyte excretion by the salt gland of the Galapagos marine iguana. *American Journal of Physiology* **216**, 995–1002.

9. Final acceptance of a paper will depend upon the production by the author of a typescript, illustrations and computer file(s) ready for the press. However, every assistance will be given to amateur herpetologists to prepare papers for publication.

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CONTENTS

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