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A Molecular Perspective on the Phylogeny of the Girdled Lizards (Cordylidae, Squamata)

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ABSTRACT

Mitochondrial DNA sequences were obtained for 16 species representing all nominal genera of Cordylidae (*Platysaurus*, *Chamaesaura*, *Cordylus*, and *Pseudocordylus*). Gerrhosauridae and Teiidae were used as first and second outgroups. Results indicate that the oviparous *Platysaurus* is the sister taxon of the remaining cordylids (all of which are ovoviviparous). Within the ovoviviparous group *Cordylus* is paraphyletic with respect to *Chamaesaura* and *Pseudocordylus*. No evidence of *Pseudocordylus* monophyly was discovered. The three species of *Chamaesaura* and the seven species of *Pseudocordylus* are transferred to *Cordylus* to render a monophyletic taxonomy.

INTRODUCTION

The Cordylidae, girdled lizards, form a monophyletic group composed of more than 50 species arranged in four nominal genera (Loveridge, 1944; Branch, 1998): *Platysaurus*, a group of 15 species most notable for their extremely flattened shape; *Pseudocordylus*, a group of seven species most notable for their apparent intermediacy in flatness between *Platysaurus* and deeper-bodied *Cor-*

dylus; *Cordylus*, a group of 31 species of diverse morphology from extremely spiny to relatively smooth scaled; and finally, *Chamaesaura*, a group of three serpentiform species with reduced limbs. The Cordylidae are restricted to the southern subcontinent of Africa, except for a few species that extend into central and East Africa.

Knowledge of cordylid phylogeny is in its infancy, even though its near outgroups and

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its monophyly have been known for a considerable length of time (McDowell and Bogert, 1954; Estes et al., 1988). Most of the systematic work on cordylid lizards has been focused primarily on species limits and geographic variation (e.g., FitzSimons, 1943; Loveridge, 1944), although recent work has also attempted to place species within a phylogenetic context (e.g., Mouton, 1990; Mouton and Van Wyk, 1989, 1994, 1995; Harvey and Gutberlet, 1995). The most comprehensive attempt at phylogeny reconstruction within the Cordylidae is that of Lang (1991), and although that paper provides considerable information on morphological variation within the Cordylidae and its first taxonomic outgroup, Gerrhosauridae, it only provides a beginning to our understanding of phylogenetics within the group. Genera of unsubstantiated monophyly were used as terminal taxa in Lang's revision, even though evidence to the contrary existed then in the form of considerable interspecific variation in many characters showing coherent distributions across generic boundaries. Unfortunately, a data matrix for species was not provided even though the apomorphy and change lists provided in appendices suggest that such a matrix exists. Many of the characters discussed by Lang provide tantalizing evidence for the paraphyly of such nominal genera as *Cordylus* and *Pseudocordylus*, even though his evidence, reified through the assumption of generic monophyly, necessarily appears to support monophyly of those taxa.

Our initial intentions in this project were (1) to use the morphological data provided by Lang (1991) and Harvey and Gutberlet (1995) as external criteria to evaluate differences in phylogenetic results due to sensitivity to alignment parameters (i.e., to search for topologies that minimize incongruence among data sets due to alignment parameters—sensu Wheeler, 1995) and (2) to perform a character congruence study to enhance our understanding of cordylid relationships. With respect to Lang's (1991) study, this has proven impossible for reasons of incompatible taxon sampling as well as our inability to extract species-level information from that revision with confidence. Coupled with our doubt regarding the monophyly of at least two of the four genera (i.e., *Pseu-*

docordylus and *Cordylus*) assumed to be monophyletic by Lang, these difficulties have proven insurmountable. With respect to the scale surface ultrastructure study of Harvey and Gutberlet (1995) there were very few characters addressed (and, to foreshadow our results, only three characters in conflict with the molecular evidence). The third author of this paper, P. Mouton, has for some time been engaged in a survey of the taxonomy, morphology, and anatomy of the group (e.g., Mouton, 1985, Mouton et al., 1992; Brody et al., 1993; Mouton and Van Wyk, 1994, 1997). A more inclusive publication of the morphological data will be provided by Mouton at a later date. Therefore, the purpose of this contribution is not to report on the morphology of the cordylids, but to report on an analysis of DNA sequences with comments on what these data suggest with respect to reported morphological diversification within the Cordylidae. We will mention the morphological evidence of Lang (1991) and Harvey and Gutberlet (1995), but only within a narrative context.

METHODS

SAMPLING

Mitochondrial DNA sequences were obtained for 16 terminals representing all nominal genera as well as the putative first and second functional outgroup. (The first taxonomic outgroup, the Gerrhosauridae, is represented by *Gerrhosaurus typicus* and *Tetradactylus seps*; the second taxonomic outgroup is represented by a teiid, *Cnemidophorus sexlineatus*.) See appendix 1 for voucher numbers and associated GenBank accession numbers.

DNA AMPLIFICATION AND SEQUENCING

Mitochondrial genes encoding the 12S rDNA, valine tDNA, and the 5' end of the 16S rDNA were amplified using the polymerase chain reaction. Genomic DNA extraction, primers, and amplification protocols were identical to those in Titus and Frost (1996). Amplified DNA was electrophoresed on 1% agarose gels and purified for sequencing using the GeneClean II kit (Bio 101, Inc.). Thermal cycle sequencing was done follow-

ing Titus and Frost (1996). Automated sequencing was performed in the University of Oregon Molecular Biology Sequencing Facility utilizing the Big Dye Terminator Cycle Sequencing Kit with AmpliTaq FS (Perkin-Elmer) and an ABI PRISM 377 DNA Sequencer (Perkin-Elmer) following manufacturer's specifications.

ALIGNMENT AND ANALYSIS

Sequences were not aligned prior to a parsimony analysis, which is the usual procedure for most sequence analyses. Instead, in this case we employed direct optimization (Wheeler, 1996) as implemented by the computer program POY (Gladstein and Wheeler, 1996–1999). (For summaries of this method and access to the program and command scripts see ftp.ammh.org.) Although there is obviously considerable structure in the data as suggested in Results and Discussion by the Bremer values, the *g*₁ statistic as a significance test was not employed for reasons detailed by Källersjö et al. (1992). A maximum likelihood approach has also not been employed because these have been demonstrated to converge on parsimony estimates when there is no assumption of a common model of evolution across sites (Tuffley and Steel, 1997).

Gap, transversion, and transition costs to the analysis procedure were applied in ratios of 1:2:1 and 1:1:1, for reasons detailed by Titus and Frost (1996) and to provide a conservative estimate of cladistic structure. Although we reject the notion that support statistics are measures of accuracy, there is no doubt that they are quite useful as practical indicators of whether we are making progress toward efficient and consistent description. For this reason, Bremer indices (Bremer, 1994) are provided.

RESULTS AND DISCUSSION

Topologies for gap, transversion, and transition costs of 1:1:1 and 1:2:1 are provided in figures 1 and 2, respectively. Length for the two trees was, respectively, 1501 and 2257, with consistency and retention indices of, respectively, 0.22 and 0.80, and 0.25 and 0.80. Bremer (= decay) indices (Bremer, 1994) are provided with the relevant stems

on figures 1 and 2. A strict consensus of these two topologies is provided in figure 3. The genus-terminal tree of cordylids provided by Lang (1991, his fig. 17) and a summary of our results are provided in figure 4 for comparison.

Cost parameters showed some effect with a difference of 2× in the weighting of transversions over transitions and gaps. Only one stem with a Bremer index of 10 was affected by this change (affecting the relative position of *Cordylus coeruleopunctatus*), and monophyly of the putative sister-taxon, Gerrhosauridae, is not supported by the molecular data. This is a surprising result and may have been caused by our limited sampling. However, the Bremer indices are high enough that reevaluation of the previously published evidence for gerrhosaurid monophyly would be prudent.

Monophyly of the Cordylidae sensu Lang (1991), always noncontroversial, is affirmed. Lang (1991) summarized a number of morphological synapomorphies for the group and it must be one of the most highly corroborated taxa in lizard systematics.

Only one species of *Chamaesaura* is included in the analysis, but the extraordinary morphology of this taxon strongly suggests monophyly of the three included species. The suggestion of the molecular data—that *Chamaesaura* rests within nominal *Cordylus*—is a novel hypothesis and at variance with the previous suggestion by Lang (1991) that *Chamaesaura* is the sister taxon of other cordylids. Beyond a large number of generic apomorphies, Lang (1991) suggested five characters for which *Chamaesaura* was plesiomorphic with respect to the remaining cordylids, but he also mentioned several other features of *Chamaesaura* that were found in some *Cordylus* (e.g., his S1-position of nasal scales, S3-condition of postnasal scale). Further, *Chamaesaura* is so highly apomorphic, correlated with its serpentine lifestyle, that our molecular data suggest that careful evaluation of the morphological data is required. Note, however, that the molecular data placing *Chamaesaura* within “*Cordylus*” are corroborated by at least one anatomical-ecophysiological characteristic. Mouton and Van Wyk (1997) noted that outgroup comparison would

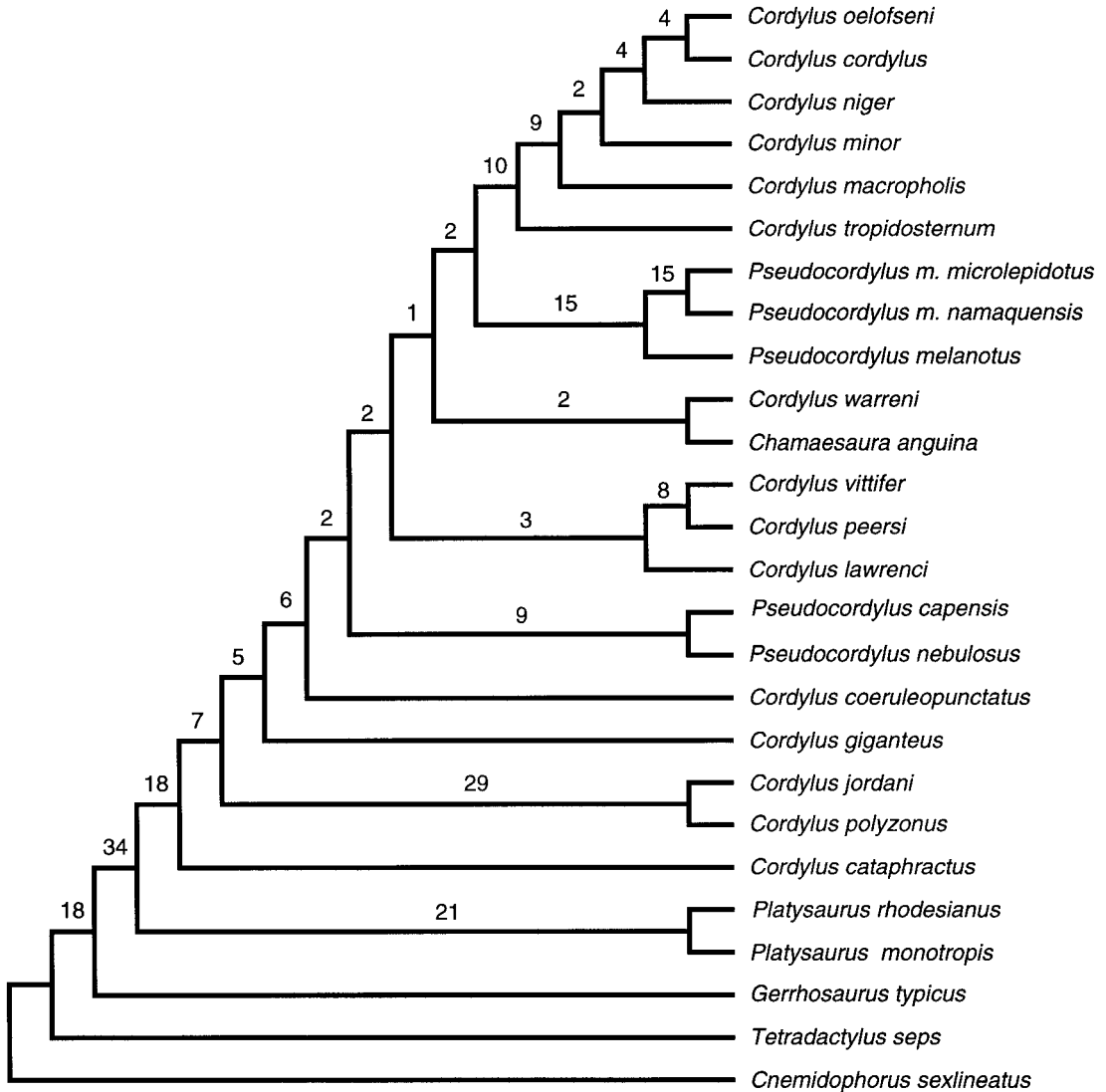


Fig. 1. Tree based on relative cost parameters (transitions: transversions: gaps) at 1:1:1. Numbers on internal stems are Bremer values.

judge ovoviviparity within *Chamaesaura*, *Cordylus*, and *Pseudocordylus* as a synapomorphy and that oviparity in *Platysaurus* (the only ovoviparous cordylid) would be counted as a plesiomorphy, a hypothesis congruent with our molecular results.

Although we have only two representatives of *Platysaurus* (*P. monotropis* and *P. intermedius rhodesianus*) we have no reason to doubt its monophyly. In Lang's (1991) cladogram, *Platysaurus* is diagnosed by sev-

en apomorphies. The molecular data place *Platysaurus* as the sister-taxon of the remaining cordylids, including *Chamaesaura*. This, like the placement of *Chamaesaura* within *Cordylus* based on molecular evidence, is surprising inasmuch as what appears to be a morphocline—from the smooth-scaled, flattened crevice-dwellers (*Platysaurus*) through the somewhat less flattened *Pseudocordylus* to variably spiny *Cordylus*—had initially appeared to us as a phylogenetic transition

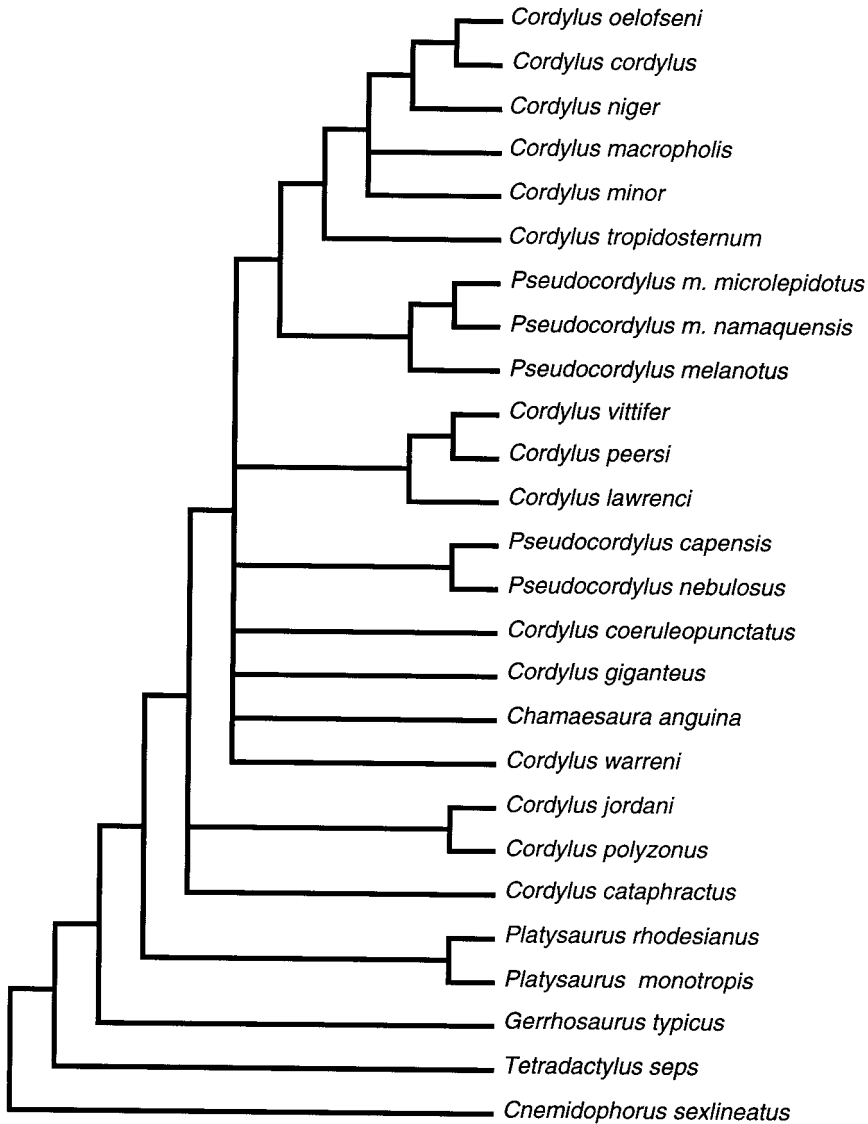


Fig. 3. Strict consensus of trees shown in figures 1 and 2.

docordylus with three characters: (a) nasal scales in contact (his S11.1; even though he suggested that *Platysaurus*, the putative sister taxon, was highly variable in this characteristic), (b) anterior parietal scales in contact (his S11.1; although he suggested that *Platysaurus*, the putative sister taxon in his analysis, showed contact in most species and variability in *Cordylus* was high), and (c) lingual exposure of the angular (his C24.0, although he noted on p. 185 that it is variable

among unspecified species of *Pseudocordylus*). In summary, we view the evidence for monophyly of *Pseudocordylus* to be equivocal and consider *Pseudocordylus* a candidate for being polyphyletic. Further, at least in this study, evidence tying either group of *Pseudocordylus* to *Platysaurus* appears to be lacking.

No evidence of monophyly of *Cordylus* is suggested by the molecular evidence. Indeed, there is very strong evidence of paraphyly

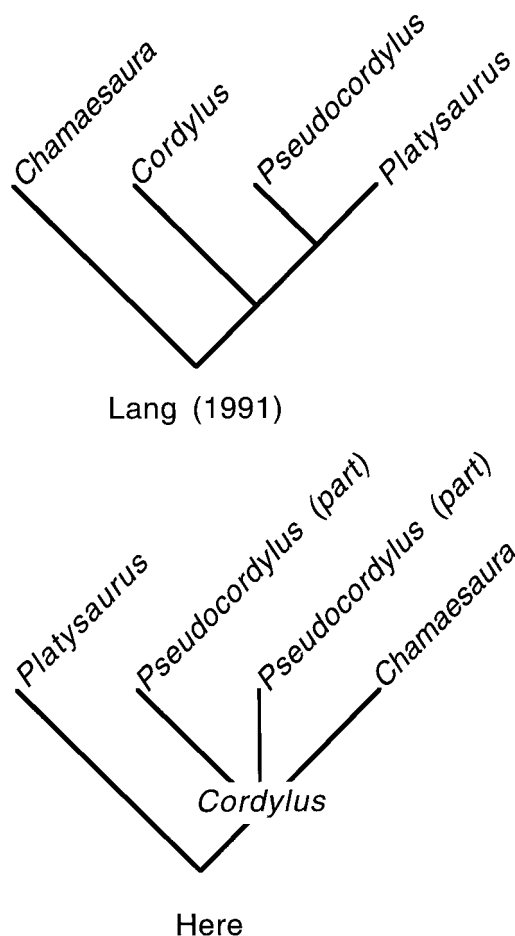


Fig. 4. Summary trees of relationships suggested by Lang (1991) and here.

with respect to *Pseudocordylus* and *Chamaesaura*. That *Cordylus* is paraphyletic with respect to *Pseudocordylus* is not surprising. Lang (1991) suggested five synapomorphies of *Cordylus*: S21.1 (keeled scales on bottom of foot; not scorable in *Chamaesaura* and variable in *Platysaurus* and *Pseudocordylus*), S24.1 (keeled subdigital lamella; not scorable in *Chamaesaura* and variable in *Pseudocordylus*), C28.0 (posteriorly tapered retroarticular process [a character influenced by degree of skull flattening]), P1.2 (intercentra fused to posterior vertebrae [sutured in *Chamaesaura*]); and M3.0 (osteoderm distribution, osteoderms apparently always reduced in flattened crevice dwellers). Nevertheless, even though *Cordylus* was assumed

to be monophyletic on the basis of these characters (and for purposes of his intergeneric analysis), Lang (1991) noted at least nine features that are variable among species of *Cordylus* and that extend to other cordylid taxa.

Differences due to transversion weighting are evident, particularly in the placement of the *Cordylus vittifer* group, *C. tropidosternum*, *C. warreni*, *C. coeruleopunctatus*, and the relative placement of *Chamaesaura* within “*Cordylus*” clade.

Clades that remain the same among our analyses within nominal *Cordylus* are: (a) a clade of *Cordylus oelofseni*, *C. cordylus*, *C. niger*, *C. macropholis*, and *C. minor*; (b) a *Cordylus vittifer*, *C. peersi*, *C. lawrenci* clade; (c) a clade composed of *C. jordani* and *C. polyzonus*. These associations are hardly surprising. Mouton (1990) first noted the association of *C. oelofseni*, *C. cordylus*, and *C. niger*. Loveridge (1944) considered these three species to be parts of a single larger species, *C. cordylus*, but he had also included a number of other populations as well, including *C. lawrenci*, judged to be distant from this group on the basis of our data. The close association of *C. jordani* and *C. polyzonus* has also been long recognized (Loveridge, 1944). Nevertheless, the scale ultrastructure data presented by Harvey and Gutberlet (1995), and discussed below, suggest that continued careful evaluation of relationships is warranted.

The scale surface ultrastructure data of cordylids were cast by Harvey and Gutberlet (1995) into 10 characters of transformation and mapped upon the cladogram of Lang (1991), although Harvey and Gutberlet discussed some additional refinement within *Cordylus*. When the accuracy of Lang’s (1991) generic arrangement is not assumed, very little discordance with Harvey and Gutberlet’s data and our results is discovered and what is found has no impact on our hypothesis of *Cordylus* paraphyly with respect to *Pseudocordylus* and *Chamaesaura*. Indeed, when their data are analyzed independently they show considerable concordance with our molecular results (Stems A and B, fig. 5; strict consensus of a sample of 16383 trees; length = 11, CI = 0.9091; without uninformative characters 0.8750). What discordance

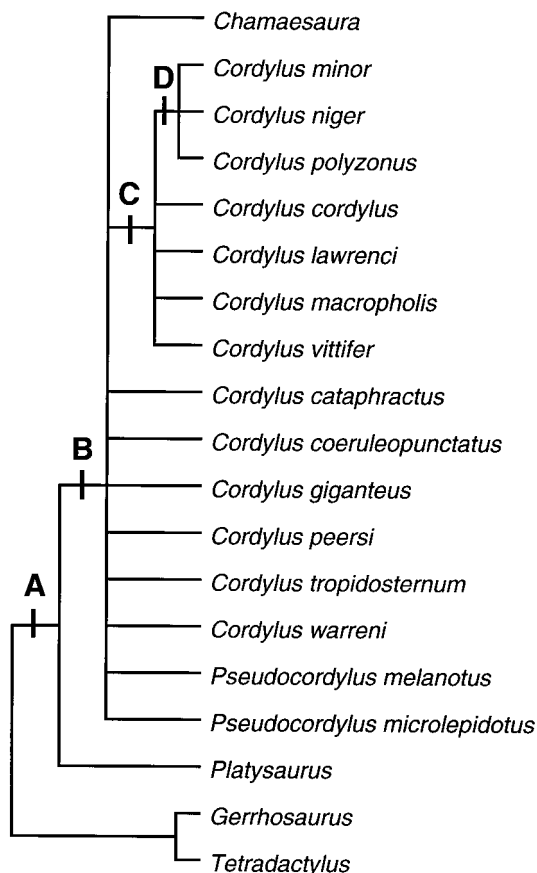


Fig. 5. Tree based on scale structure evidence of Harvey and Gutberlet (1995). A: macrohoneycomb present on venter; B: macrohoneycomb present on dorsal scales; C: flaplike free margins associated with the cell-ridge system; D: short ridgelike projections in the center of the oberhautchen cells.

exists is solely in regard to the relative placement of various species of *Cordylus* (fig. 5, stems C and D). The presence on dorsal scales of the macrohoneycomb oberhautchen (character #1 of Harvey and Gutberlet), thought to be lost in *Platysaurus* when mapped on Lang's arrangement is plesiomorphically absent in our cladogram in *Platysaurus* and its presence is a synapomorphy of the remaining cordylids. (In other words, this character corresponds in placement with the transition from oviparity to ovoviviparity.) Where their evidence does show discordance with the molecular evidence is in their character #6 (flaplike free margins associated

with the cell-ridge system; fig. 5, Stem C), and their character #7 (ridges in the center of oberhautchen cells in *C. polyzonus*, *C. niger*, and *C. minor*; fig. 5, stem D). Although discordance between ultrastructural character evidence and the molecular evidence within *Cordylus* bears further scrutiny, in this case the molecular evidence is rather strong, suggesting the existence of more homoplasy in scale surface ultrastructure than previously suspected.

Clearly a study of character congruence of both morphology and molecules of species (not of higher taxa as was done by Lang, 1991) would provide a stronger basis on which to optimize transversion, transition, and gap cost parameters and would further elucidate the relative relationships within cordylids. Nevertheless, on the basis of the molecular evidence alone, it is evident that "*Cordylus*" is paraphyletic with respect to *Chamaesaura* and *Pseudocordylus*, that *Pseudocordylus* is dubiously monophyletic, and that the oviparous *Platysaurus* is the sister taxon of the other, ovoviviparous, cordylids. On this basis we regard *Pseudocordylus* and *Chamaesaura* to be junior synonyms of *Cordylus*. This affects only 10 species names and formulates a conservative taxonomy consistent with the state of knowledge of cordylid relationships.

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APPENDIX 1 SPECIMEN VOUCHERS

All specimens deposited in the Ellermann Museum, University of Stellenbosch, Stellenbosch, Rep. South Africa, except for the single specimen of *Cnemidophorus sexlineatus* that is unnumbered in the Department of Herpetology, University of Kansas.

Cordylidae: *Cordylus anguinus* (formerly *Chamaesaura anguina*) (USEC-3503; GenBank BankIt321210-AF236042); *C. capensis* (formerly *Pseudocordylus capensis*) (USEC-H2486; GenBank BankIt319965-AF236022); *C. cataphractus* (USEC-H3490; GenBank BankIt320915-AF236034); *C. coeruleopunctatus* (USEC-H3497; GenBank BankIt321214-AF236043); *C. cordylus* (USEC-H3488; GenBank BankIt320904-AF236027); *C. giganteus* (USEC-H3494; GenBank BankIt321163-AF236036); *C. jordani* (USEC-H3492; GenBank BankIt320897-AF236024); *C. lawrenci* (USEC-H3506; GenBank BankIt321218-AF236046); *C. macropholis* (USEC-H3500; GenBank BankIt321217-AF236045); *C. melanotus* (formerly *Pseudocordylus melanotus*) (USEC-H3483; GenBank BankIt320905-AF236028); *C. m. microlepidotus* (formerly *Pseudocordylus m. microlepidotus*) (USEC-H3484; GenBank BankIt-

320910-AF236029); *C. m. namaquensis* (formerly *Pseudocordylus m. namaquensis*) (USEC-H3485; GenBank BankIt321208-AF236041); *C. minor* (USEC-H3498; GenBank BankIt320912-AF236032); *C. niger* (USEC-3489; GenBank BankIt320899-AF236025); *C. nebulosus* (formerly *Pseudocordylus nebulosus*) (USEC-H3487; GenBank BankIt320913-AF236033); *C. oelofseni* (USEC-H3499; GenBank BankIt321215-AF236044); *C. peersi* (USEC-H3495; GenBank BankIt320911-AF236031); *C. polyzonus* (USEC-H3491; GenBank BankIt320908-AF236030);

C. tropidosternum (USEC-H3496; GenBank BankIt-320859-AF236023); *C. vittifer* (USEC-H3507; GenBank BankIt320902-AF236026); *C. warreni* (USEC-H3493; GenBank BankIt320914-AF236035); *Platysaurus intermedius rhodesianus* (USEC-H3501; GenBank BankIt321165-AF236037); *P. monotropis* (USEC-H3502; GenBank BankIt321169-AF236038); **Gerrhosauridae:** *Gerrhosaurs typicus* (USEC-H3504; GenBank BankIt321222-AF236039); *Tetradactylus seps* (USEC-H3505; GenBank BankIt-321207-AF236040). **Teiidae:** *Cnemidophorus sexlineatus* (BankIt321219-AF236047).

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