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Elucidation of the relationships of spiny-footed lizards, *Acanthodactylus* spp. (Reptilia: Lacertidae) using mitochondrial DNA sequence, with comments on their biogeography and evolution

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Abstract

Mitochondrial DNA sequences consisting of 645 sites from the 12S rRNA and 16S rRNA genes were used to estimate the phylogeny of 15 of the 32 species of spiny-footed lizards *Acanthodactylus*. The resultant tree has similarities to that produced from a differentially weighted data set of 32 morphological characters but there are also significant differences. However, combined analysis of molecular and morphological data sets produces the same tree topology as DNA sequence alone. The molecular data confirm that there are distinct eastern and western clades within *Acanthodactylus*, but place *A. boskianus* in the former while the *A. scutellatus* group constitutes a third clade. Species for which only morphological information is available were integrated with the combined tree to give a provisional phylogeny for 31 species. This phylogeny indicates that the ancestor of existing *Acanthodactylus* probably originated in south-west Asia and that North Africa was invaded by more than one lineage of the genus. It also suggests that soft aeolian sand habitats may have been independently occupied more than once. Molecular data provide independent evidence that the differential weighting of morphological characters in past analyses was appropriate.

Key words: *Acanthodactylus*, Lacertidae, 12S rRNA, 16S rRNA, phylogeny, weighting

INTRODUCTION

Spiny-footed lizards *Acanthodactylus* are an Old World clade of ground-dwelling lizards found mainly on sandy ground in arid areas. They are widely distributed across Iberia, North Africa and south-west Asia, where they occur from south-east Turkey to southern Arabia and from the Mediterranean and the Red Sea to Pakistan and north-west India. Morphology indicates that the group constitutes a clade, and some 32 species are currently recognized (Salvador, 1982; Arnold, 1983, 1986*b*; Geniez & Foucart, 1995). *Acanthodactylus* is taxonomically confusing, the species often being at least superficially similar but also quite variable. However, some forms that are externally very alike are distinguishable by radical differences in the male intromittent organ, the hemipenis, and its supporting armature (Arnold, 1983, 1986*a*).

There are relatively few morphological characters potentially useful for working out phylogenetic relationships and these tend to be variable and frequently conflict with each other (Mellado & Olmedo, 1990). Because of this they have been differentially weighted in at least one analysis (Arnold, 1983), lower weight being assigned to derived states that seem likely to be labile because of such features as their parallel occurrence in outgroups, apparent ease of development and perceived probability of multiple adaptations within *Acanthodactylus* to similar environments that produce strong selection pressures for them. General arguments for the validity of such weighting are given by Arnold (1981, 1996) and Wheeler (1985).

When estimated phylogenies are based on relatively few often conflicting morphological characters, it is desirable to test the hypothesized relationships by using an additional character source, should this become available. Here we provide such a test in the form of mitochondrial DNA sequence data for 15 species. Results from morphological and molecular data are then compared and integrated to produce a more robust overall phylogeny for *Acanthodactylus* than was previously available, which is used to comment on the biogeography and evolution of the genus.

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SPECIES AND SPECIES GROUPS

Species boundaries and species groups within *Acanthodactylus* have been discussed by Salvador (1982) and Arnold (1983), and the reasons why such forms as *A. iracensis*, *A. busacki*, *A. dumerili* and *A. inornatus* are not recognized are given in the latter account. In addition to the forms discussed in these papers, two completely new species have been described subsequently: *Acanthodactylus tilburyi* Arnold, 1986*b*, and *A. taghitensis* Geniez & Foucart, 1995. Three forms previously treated as subspecies are now better regarded as full species: *A. erythrurus lineomaculatus* (now *A. lineomaculatus*; for differences from *A. erythrurus* see Bons & Geniez, 1995), *A. tristami orientalis* (now *A. orientalis*; for differences from *A. tristami*, see Arnold, 1983) and *A. scutellatus hardyi* (now *A. hardyi*; for differences from *A. scutellatus*, see Arnold, 1986*c*).

Species groups recognized by Arnold (1983), and the species presently assigned to them are:
1. *A. micropholis* group: *A. micropholis*. 2. *A. cantoris* group: *A. arabicus*, *A. blanfordii*, *A. cantoris*, *A. gongrorhynchatus*, *A. haasi*, *A. schmidti*, *A. tilburyi*. 3. *A. opheodurus* group: *A. felicis*, *A. masirae*, *A. opheodurus*, *A. yemenicus*. 4. *A. boskianus* group: *A. boskianus*, *A. grandis*, *A. schreiberi*. 5. *A. tristrami* group: *A. orientalis*, *A. robustus*, *A. tristrami*. 6. *A. erythrurus* group: *A. blanci*, *A. boueti*, *A. erythrurus*, *A. guineensis*, *A. lineomaculatus*, *A. savignyi*. 7. *A. pardalis* group: *A. bedriagai*, *A. maculatus*, *A. pardalis*, *A. spinicauda*. 8. *A. scutellatus* group: *A. aureus*, *A. hardyi*, *A. longipes*, *A. scutellatus*, *A. taghitensis*.

All of these groups are each made up of quite similar forms, and in groups 4, 5 and 8, there is at least some morphological evidence for clade status in the form of synapomorphies that are unique within *Acanthodactylus*. In the present paper, all species except *A. lineomaculatus*, *A. savignyi*, *A. yemenicus* and *A. taghitensis* have been included in analyses of morphological data and at least one member of each species group, except *A. micropholis*, in the investigation of mtDNA sequence. Morphologically *A. lineomaculatus* is quite similar to *A. erythrurus*, *A. savignyi* to *A. blanci*, and *A. yemenicus* to *A. felicis*; *A. taghitensis* has not been examined by us.

MORPHOLOGICAL ANALYSIS OF RELATIONSHIPS

The phylogeny of *Acanthodactylus* was initially estimated from 35 morphological characters without the benefit of computer analysis (Arnold, 1983), using the basic cladistic precept that historical relationships are indicated by shared derived character states (Fig. 1).

Eleven of the characters used in phylogeny estimation involve the hemipenis and can be divided into two groups: (1) reduction in size and development of asymmetry in the hemipenis or both, the latter involving loss or reduction of the medial lobe and medial side of the armature, so that the hemipenis is frequently narrowed; (2) often very distinctive features of the armature not usually found in outgroups. A circumstantial case can be made that the first kind of hemipenial character arose as part of a mechanism preventing some interspecific matings, in which the width of the female genital sinus was reduced, excluding males of other species with normal hemipenes (Arnold, 1983, 1986*a*,*d*). Such a device may well sometimes be advantageous where different species come into contact, since males in *Acanthodactylus* appear undiscerning and exhibit little courtship behaviour during which premating recognition of inappropriate partners might occur (E. N. Arnold, pers. obs.).

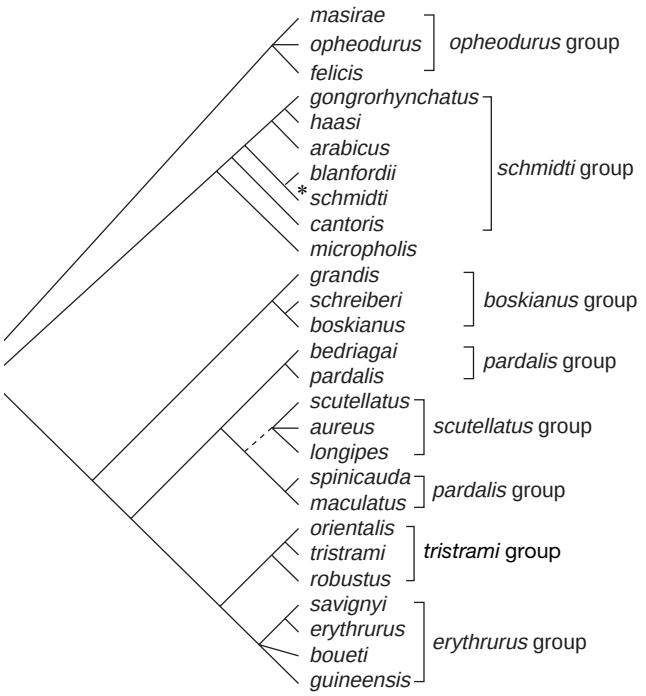

Fig. 1. Tentative hypothesis of the relationships of the species of *Acanthodactylus* based on weighted morphological characters (Arnold, 1983). *, Alternative position for the *A. scutellatus* group.

Because simple reductions in the hemipenis are probably produced easily in developmental terms and have also evolved independently several times in outgroups (Arnold, 1986*a*), the characters concerned were assigned relatively low weight in analysis, in contrast to hemipenial features in the second group. Relatively low weight was also assigned to derived features that confer performance advantage in aeolian sand habitats, such as a narrowed premaxilla that enables soft sand to be easily probed with the snout for food, and an additional lateral row of pointed scales on the fingers that enhance digging in this medium.

As part of this study, a maximum parsimony analysis was conducted on a slightly modified morphological

Table 1. Morphological characters varying within *Acanthodactylus*. Distribution among species is shown in Table 2

1. Premaxilla. Relatively broad (0); distinctly narrowed (1).
2. Number of premaxillary teeth. Six or more (0); five (1).
3. Usual number of presacral vertebrae in males. 24 or less (0); 25 or more (1).
4. Usual number of presacral vertebrae similar in males and females. No (0); yes (1).
5. Fifth sternal rib interrupted in more than 50% of individuals. No (0); yes (1).
6. One or two azygos scales often present between prefrontal scales. No (0); yes (1).
7. Supraocular scales broken up. No (0); first divided into two or three, fourth very fragmented (1); first and fourth both very fragmented (2).
8. Subocular scale usually separated from mouth. No (0); yes (1).
9. Upper labial scales in front of eye usually more than four. No (0); yes (1).
10. Ear opening reduced in size. No (0); yes (1).
11. Dorsolateral tracts of enlarged scales at least sometimes present. No (0); yes (1).
12. Ventral backwardly directed collar fold on throat. Attached at centre (0); free (1).
13. Maximum number of ventral scales across body. 10 or less (0); usually 12 (1); usually 14 or more (2).
14. Ventral scales tessellated. No (0); at sides only (1); generally (2).
15. Ventrals grade into dorsals to some extent. No (0); yes (1).
16. Number of scale rows along fingers. Three (0); partial fourth row (1); four complete rows (2).
17. Keeling present on proximal dorsal caudal scales. Yes (0); no (1).
18. Length of intact tail in terms of snout–vent distance. More than 1.5 times as long (0); less than 1.5 times as long (1).
19. Dorsal pattern of light and dark longitudinal stripes in young. Yes (0); no (1).
20. Reddish-brown spots in dorsal pattern that do not fade in alcohol sometimes present on dorsum. No (0); yes (1).
21. Two rows of large ocellar markings along back. No (0); yes (1).
22. Proximal lip of hemipenial sulcus reduced to a fold. No (0); yes (1).
23. Medial clavula narrow and pointed with a <- shaped cross-section. No (0); yes (1).
24. Lateral clavula at least sometimes with a backwardly directed pocket. No (0); yes (1).
25. Lateral clavula very narrow. No (0); yes (1).
26. Lateral clavula complexly structured with multiple lobes below. No (0); yes (1).
27. Lateral clavula complexly structured and divided at tip. No (0); yes (1).
28. Lateral clavula folded with >- shaped cross section. No (0); yes (1).
29. Most medial connector on lateral side of hemipenis thickened. No (0); yes (1).
30. Hemipenis small. No (0); yes (1).
31. Size of medial lobe of hemipenis. Equal to lateral lobe (0); somewhat reduced (1); more strongly reduced (2); very reduced or absent (3).
32. Medial side of armature reduced. No (0); somewhat reduced (1); more strongly reduced (2); reduced to a thread or absent (3).

data set of 32 characters (Tables 1 & 2), using PAUP*4.0.d51 (Swofford, 1997) with multi-state characters (numbers 7, 13, 14, 16, 31, 32) treated as ordered and all characters equally weighted. The tree was rooted using a hypothetical ancestor based on states in a range of outgroups. A heuristic search (random addition sequence with 10 replicates) produced 559 equally parsimonious trees with 89 steps. Support for nodes was estimated using 1000 bootstrap replications (Felsenstein, 1985). In this treatment all forms with asymmetrical hemipenes are assembled in a single clade (Fig. 2).

When analysis was repeated, assigning a reduced weight of 50% to characters involving hemipenial asymmetry and size reduction (Table 1, characters 30, 31 and 32), relationships changed significantly, some species with asymmetrical hemipenes being associated with forms where these features are lacking (Fig. 3). This tree has considerable resemblance to the original estimate of phylogeny produced without computer analysis (Fig. 1), the most obvious exception being that, because features conferring advantage in aeolian sand have not been downgraded in the maximum parsimony analysis, the *A. scutellatus* group forms a clade with several other species that share these features.

MOLECULAR EVIDENCE FOR RELATIONSHIPS

Materials

In the present study, portions of two mitochondrial genes, 12S rRNA and 16S rRNA, were sequenced for 15 species of *Acanthodactylus*, which are listed in Appendix 1 together with the data for the specimens used. In the very widely distributed *A. boskianus*, which shows considerable geographical variation, material from two widely separated localities was included, so both east Arabian and north-west African populations are represented. Two species of *Mesalina* were used as a closely related outgroup (Arnold, 1989), and previously published sequences of *Lacerta dugesii dugesii* (González *et al*., 1996; Harris, Arnold & Thomas, 1998*b*) provided a more distant one.

Laboratory methods

Total genomic DNA was extracted from small (1 or 2 mm^3) pieces of tail tissue. The material, which had been stored in 70% ethanol at 4 °C (Appendix 1), was finely diced and agitated overnight at 37 °C in 750 µl of extraction buffer (100 mM TRIS (pH 8), 10 mM EDTA

Table 2. Morphological characters used in the analysis. Characters are listed in Table 1. Data are adapted from Arnold (1983). 0, presumed primitive state based on outgroup comparison; 1, 2, 3, presumed derived states; V, character varies within species; –, character does not exist in species concerned; ?, no data available

	Character
	111111111122222222233
	123456789012345678901234567890 12
Hypothetical ancestor	00000VVVV00000000000000000000000
micropholis	000000??1000000100000000000000
cantoris	00000?01100010020000000000000100
blanfordii	10000001101011020000000100000012
schmidti	100V000110102102001000010000–03V
arabicus	10000001100021120000000–010000033
tilburyi	10000001100022120000000–010000033
gongrorhynchatus	10000001010021120000000–010000033
haasi	10000001110020120000000–010000033
schreiberi	00000V01000000000000011001001001
boskianus	00000V01000000000000011001001001
grandis	00000V01000122020000011001001001
tristami	00100020000000000110101000–01001
orientalis	001000200000000011?0100000101032
robustus	00100021000010011?0101000–01001
erythrurus	00100020000000000000001000101001
blanci	00100121000000000000001000101001
boueti	00100120000000000000001000101101
guineensis	00100V20000000000000000–000001033
pardalis	00V01011000010000001001000101021
bedriagai	00111011100020000000001000101021
maculatus	00001011V0001000000100–000V11133
spinicauda	00001011000001000000000–000010133
aureus	10011001000022020000000–000010033
scutellatus	11011001100122020 0V000–000010033
longipes	11011001100122020 01000–000010033
hardyi	11011001100012020 01000–000010033
opheodurus	00000001000000000000000–000000033
felicis	00000001000000000000000–000000033
masirae	1000000?00000001000000–000000033

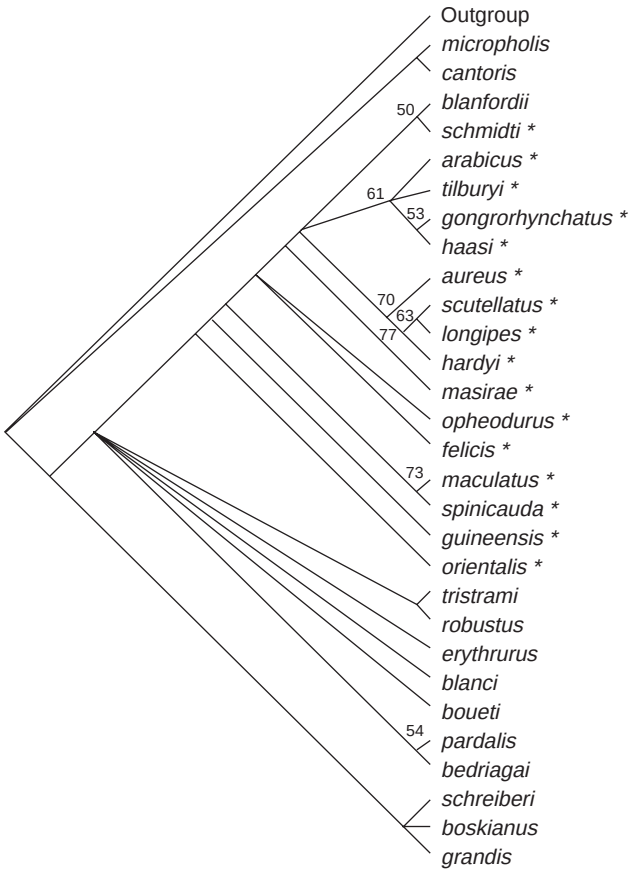

Fig. 2. Fifty per cent majority rule consensus tree derived from 559 equally parsimonious trees (89 steps) obtained using maximum parsimony based on morphological characters for most *Acanthodactylus* species. Variable characters were treated as missing, and all characters were ordered and weighted equally. Numbers, bootstrap values > 50% (1000 replicates). The tree was rooted using a hypothetical ancestor (Arnold, 1983). *, Species with strong hemipenial asymmetry.

(pH 8), 100 mM NaCl, 50 mM DTT and 0.5 μg proteinase K/ml; see Kocher *et al*., 1989). Purification was by phenol (twice) and chloroform (once) extractions (Sambrook, Fritsch & Maniatis, 1989), followed by centrifugal dialysis through a Centricon 30000 MW membrane (Amicon). Polymerase chain reaction (PCR) mix consisted of 37 μl sterile double-distilled water, 5 μl 10 × PCR buffer (2 mM $MgCl_2$ in 67 mM TRIS, pH 8), 5 μl of each 10 μM primer, 0.5 μl of dNTPs (25 mM each), 0.5 μl of *Taq* polymerase (1–2 units) with 2 μl of template DNA (*c*. 100 ng). Primers used in both the amplification and the sequencing were 12Sa and 12Sb (Kocher *et al*., 1989) and 16L1 and 16H1 (Carranza *et al*., 1991). These amplified regions of *c*. 400 bp and 450 bp, respectively. Thermocycling consisted of 30 cycles of 93 °C for 30 s, 55 °C for 1 min and 72 °C for 1 min, followed by a single cycle at 72 °C for 10 min. PCR products were checked by electrophoresis of 3 μl of the amplified mixture on a 2% agarose gel, and the DNA stained with ethidium bromide and examined under UV light. Sizes of amplified products were estimated using a molecular weight marker. Successful PCR bands were cut out and purified using a QIAEX II kit (Qiagen). The DNA was then dialysed through a Microcon 30000 MW membrane (Amicon) twice with double-distilled water and the volume made up to 10 μl; 2 μl of this was then sequenced on an Applied Biosystems Model 373A DNA Sequencing System, using a PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing kit, according to the manufacturers instructions. Centrisep spin columns (Princeton Separations Inc.) were used for excess dye extraction.

Sequence Alignment

The sequences for 12S rRNA were aligned by eye, and compared to other lacertid sequences already published (González *et al*., 1996; Harris *et al*., 1998*b*). Sequences were also checked against secondary structure models (Hickson *et al*., 1996). The sequences for 16S rRNA were similarly aligned against other lacertid sequences

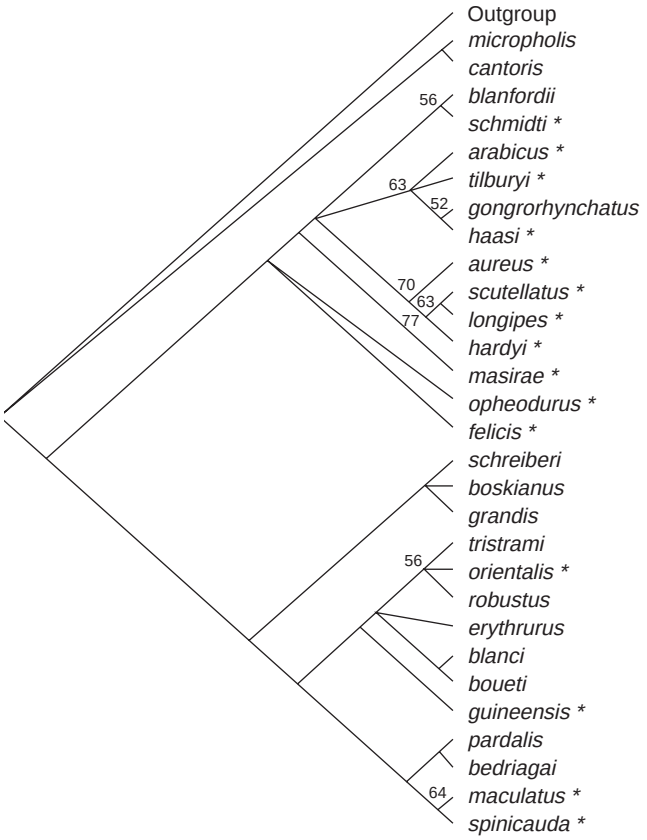

Fig. 3. Fifty per cent majority rule consensus tree derived from eight equally parsimonious trees (158 steps) obtained using maximum parsimony, based on morphological characters for most *Acanthodactylus* species. Variable characters were treated as missing, and all characters were ordered. Characters associated with hemipenial size reduction and asymmetry (characters 30–32, Table 1) were down-weighted by 50%. Numbers, bootstrap values > 50% (1000 replicates). *, Species with strong hemipenial asymmetry.

(Harris *et al*., 1998*b*). In addition they were compared to a more limited secondary structure model (Gutell, 1993). The resulting alignments contained 317 and 405 sites, respectively. Within the 12S partial sequences 11 sites were omitted from the analysis because they could not be unambiguously aligned. This was always the result of length variation within loop regions (between helices 36/38, 42/42′ and 45/45′ as given by Hickson *et al*., 1996). Within the 16S partial sequence 66 sites were omitted. This left 645 sites for all 19 individual lizards in the analysis. Alignments used are available on request from the corresponding author, and sequences have been deposited in Genbank (accession numbers AF 197481–AF 197506).

Intrapecific variation

Except for *A. boskianus*, only 1 individual from each species was sequenced. The partial gene regions used have been shown to have very low intraspecific variation in previous studies of lacertids (González *et al*., 1996; Harris *et al*., 1998*b*). Where different individuals of the same species have been sequenced independently in different laboratories (e.g. *Lacerta lepida*, González *et al*., 1996, and Harris *et al*., 1998*b*), differences have been minimal and largely confined to the hypervariable regions removed in this analysis.

Phylogenetic analysis

Phylogenetic analysis was performed using PAUP*4.0.d51 (Swofford, 1997). Base composition across the combined sequences was calculated using this program, and was found to be typical of vertebrate mtDNA, and almost identical to the proportions found across the same regions in the lacertid genus *Meroles* (average occurrences on the light strand: A 33%, C 25%. G 20%, T 22%; Harris, 1997; Harris, Arnold & Thomas, 1998*a*). Variation in base composition between species can induce systematic error if a model used assumes equilibrium base frequencies in all lineages (Hillis, Moritz & Mable, 1996). Base composition variability was tested using the approach of Rzhetsky & Nei (1995), and stationarity of base composition between all species, including outgroups, was not rejected ($I = 63.25$, $P = 18.27$). Uncorrected sequence divergence ranged from 4.25% (*A. tristami* and *A. orientalis*) to 13.95% (*A. gongrorhynchatus* and *A. maculatus*) within *Acanthodactylus*. Inclusion of *L. dugesii*, *M. guttulata* and *M. adramatana* increased the highest divergence to 16.85% (*L. dugesii* and *A. opheodurus*). Sequence divergence between the two populations of *A. boskianus* was 4.69%.

Three main methods of inferring phylogenies are widely used, namely parsimony, distance methods and maximum likelihood. Distance methods are approximations to a full likelihood approach, and are thus less desirable when a maximum likelihood method is computationally feasible (Hillis *et al*., 1996). Simulated analyses have shown that the maximum parsimony method of phylogenetic tree reconstruction is typically less effective for recovering the true tree than likelihood methods when the complexity of the process of nucleotide substitution is included in the model (Yang, 1996). Branch lengths, transition/transversion (TS/TV) ratios and among-site rate variation have all been shown to influence phylogenetic inference (Hillis, Hulsenbeck & Cunningham, 1994; Hillis *et al*., 1996), and so were taken into account in the analysis.

As divergence increases, sites may become saturated by multiple substitutions, thus obscuring phylogenetic history (Brown *et al*., 1982). Transitions are typically affected at lower divergence levels than transversions. TS/TV ratios of the combined sequences were therefore plotted against LogDet corrected (Steel, 1994) percentage sequence divergence (calculated using PAUP*4.0.d51), for all pairwise comparisons, to assess whether they demonstrated saturation (Fig. 4). Phylogenetic trees were rooted using all 3 outgroup species to

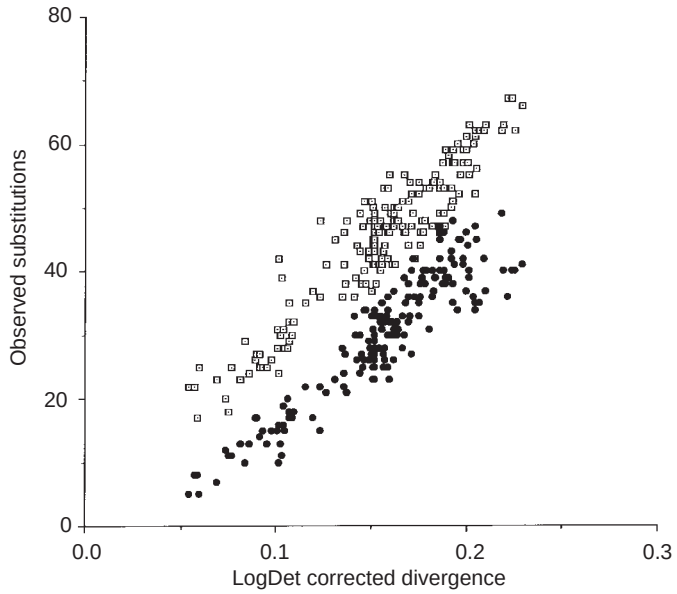

Fig. 4. Patterns of nucleotide substitution between combined 12S and 16S partial gene sequences. Transitions (squares) and transversions (circles) are plotted against LogDet corrected sequence divergence for all taxa.

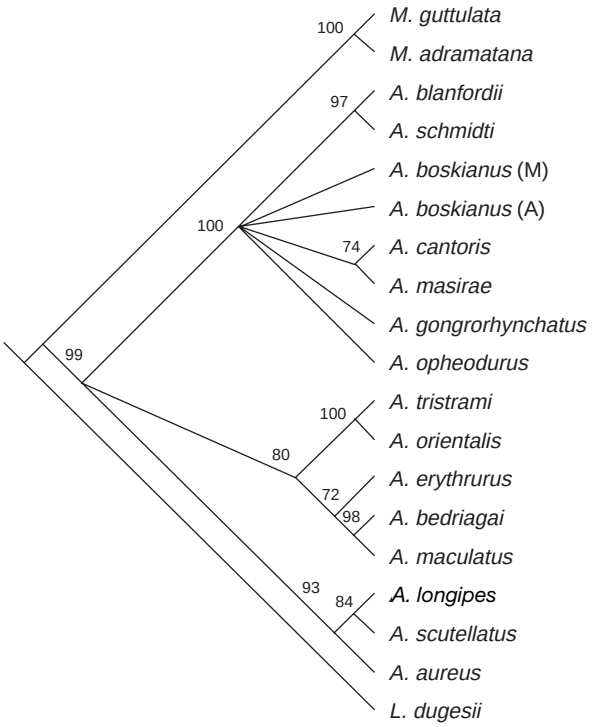

Fig. 5. Fifty per cent majority rule bootstrap consensus maximum parsimony tree based on 1000 replicates derived from the combined 12S and 16S sequence data. Numbers, bootstrap values > 50% (1000 replicates). Trees were rooted using *Lacerta dugesii*. (M), Morocco; (A), Arabia.

minimize long branches. The large number of taxa necessitated heuristic searches for trees (MULPARS option in effect, TBR branch swapping, random addition with 10 replicates). Gaps were treated as a fifth character in parsimony analyses. Confidence levels for groups were assessed with the bootstrap method (Felsenstein, 1985); 500 bootstrap replicates were performed for all analyses.

RESULTS OF MITOCHONDRIAL DNA ANALYSIS

The partial gene sequences were analysed separately using maximum parsimony. The 12S data set contained 306 characters, 74 of which were parsimony informative. When these characters were weighted equally, 12 most parsimonious trees (260 steps) were recovered. The 16S data set contained 339 characters, 93 of which were parsimony informative. When again characters were weighted equally, four most parsimonious trees (336 steps) were produced. A heuristic search carried out on the combined 12S and 16S data set resulted in three trees (610 steps). The 50% majority rule bootstrap consensus tree derived from the combined data set is shown in Fig. 5. Fifty per cent bootstrap consensus trees derived from the separate 12S and 16S data sets (not shown) differed only in the levels of resolution and in the position of *A. erythrurus*, which was sister group to (*A. orientalis*, *A. tristami*) in the analysis based only on 16S sequence data.

A maximum likelihood analysis was also performed. As a base for the analysis, PAUP*4.0.d51 was used to estimate a neighbour joining tree using LogDet corrected distances (not shown). This tree was used to simultaneously estimate the proportion of invariant sites and among-site rate variation, using a discrete approximation to the gamma distribution (α), with a general time reversible model of sequence evolution. These estimated parameters (estimated proportion of invariant sites 0.496, $\alpha = 0.633$) were included in the likelihood model and a heuristic search (10 replicates) was carried out. The estimated topology had a log likelihood of -3507.2 (Fig. 6). This tree was then used to re-estimate the parameters for a further heuristic search. The tree produced from this search was identical to the previous one (log likelihood -3507.16).

The combined sequences were further analysed using the 'split-decomposition method' (Bandelt & Dress, 1992*a*), employing the program Splitstree 1.0 (Huson & Wetzel, 1994). In a typical phylogenetic analysis, data are forced to fit a tree topology. The split-decomposition method, however, allows for conflicting alternative groupings, exhibiting networks of relationships including the more weakly supported ones that may be overridden by homoplasy in a single tree topology (Bandelt & Dress, 1992*b*). All alignable positions were used in the analysis, and the LogDet correction applied (Fig. 7).

Comparing molecular and weighted morphological trees

There are many similarities between the results of the molecular analyses and those based on weighted

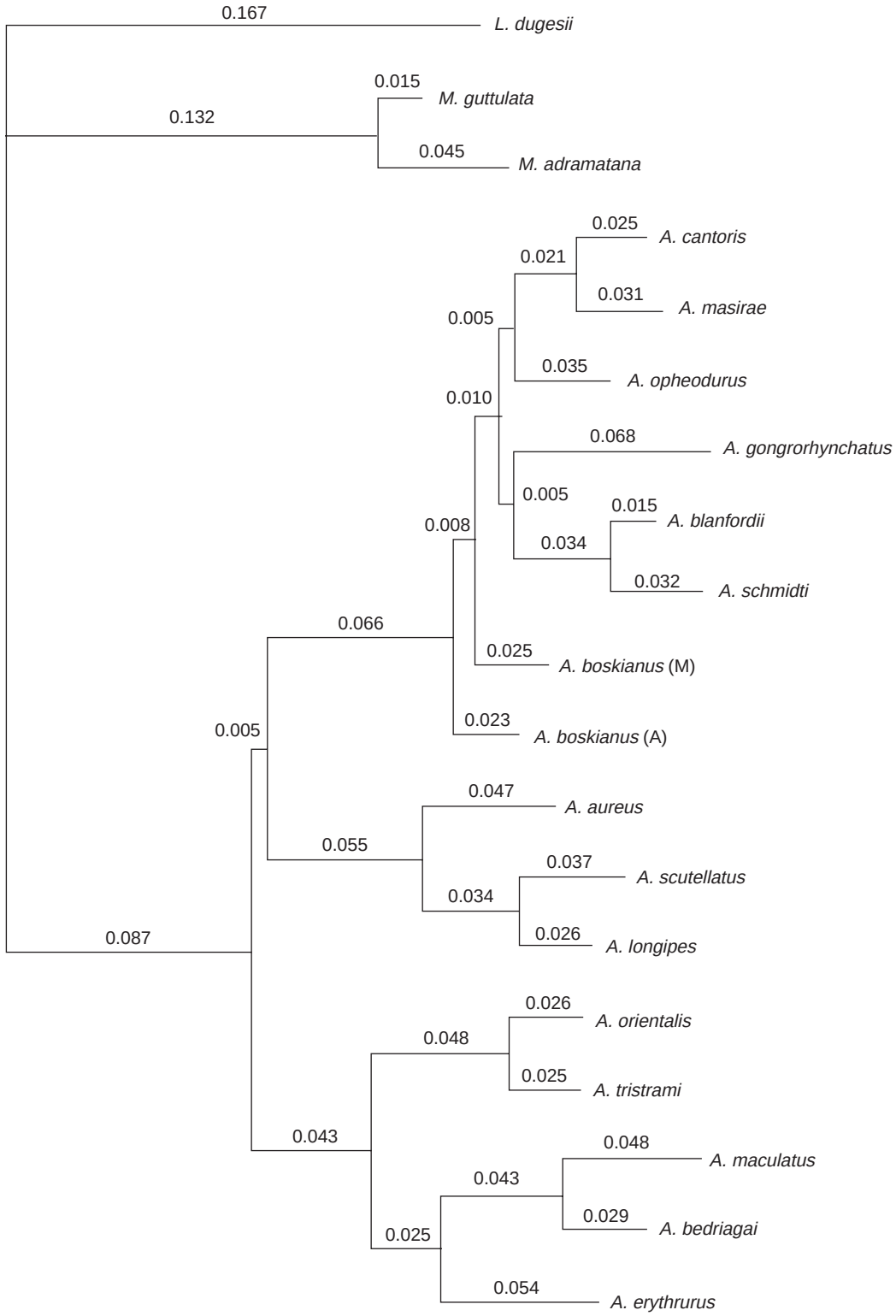

Fig. 6. Phylogeny estimated using maximum likelihood (-log likelihood 3507.2) with the general time reversible model, estimating the proportion of invariant sites (0.5) and among site rate variation using a discrete approximation of the gamma distribution (shape parameter 0.63, four rate categories). See text for further details. (M), Morocco; (A), Arabia.

morphological data. Relationships supported by both sources include the following: (1) the clade status of *Acanthodactylus*; (2) an eastern clade including *A. cantoris*, *A. masirae*, *A. gongrorhynchatus*, *A. blanfordii* and *A. schmidti*; (3) close relationship between *A. blanfordii* and *A. schmidti*; (4) close relationship between *A. tristrami* and *A. orientalis*; (5) close relationship between *A. bedriagai* and *A. maculatus* (6) representatives of the *A. tristrami*, *A. erythrurus* and *A. pardalis* groups constitute a mainly western

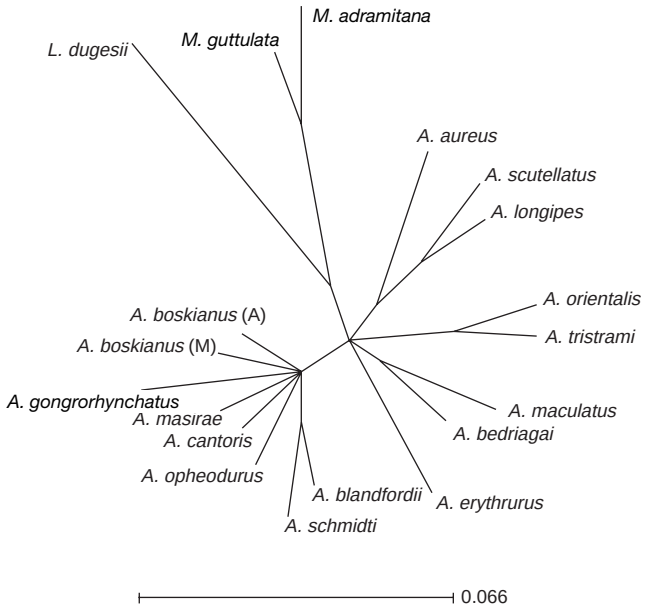

Fig. 7. Split-decomposition network of the LogDet corrected distances between species of *Acanthodactylus*, *Mesalina* and *Lacerta dugesii*. (M), Morocco; (A), Arabia.

assemblage of species that occur on relatively firm substrata; (7) members of the *A. scutellatus* group form a clade with the same pattern of species relationships, but are not associated with the eastern clade in the molecular analyses, or in the initial non-computer analysis of morphological data (Fig. 1).

There are also various ways in which the estimate of phylogeny derived from the molecular data differs from that derived from the weighted morphological analysis. (1) Close relationship between *A. cantoris* and *A. masirae* although there are no clear morphological synapomorphies supporting this. (2) *Acanthodactylus boskianus*, and presumably the rest of the *A. boskianus* group, clearly placed in the eastern clade. (3) Close relationship of *A. erythrurus* to *A. tristrami* and *A. orientalis*, rather than to *A. bedriagai* and *A. maculatus*, is not supported by the data derived from combined 12S and 16S partial sequences, although it is by 16S sequence data alone. Instead, total molecular evidence places *A. erythrurus* closer to the latter two species. This seems quite feasible as the only morphological features clearly supporting the former relationship are greater fragmentation of the supraocular scales (character 7-2) and high number of presacral vertebrae (character 3). However, while the latter does not occur in *A. maculatus* and two other members of the *A. pardalis* group, it is present in the fourth member, *A. bedriagai*. (4) The *A. scutellatus* group is not clearly associated with any other assemblage of *Acanthodactylus* and forms an independent lineage, instead of being placed with *A. schmidti* and its relatives, or with members of the *A. pardalis* group. (5) *Acanthodactylus opheodurus* and *A. masirae* are firmly placed in the eastern clade but are not clearly associated with each other within this. Their tentative association in the non-computer assessment of relationship was largely based on the grounds of overall similarity, the derived features that they share being admittedly non-exclusive and often variable (Arnold, 1983).

Status of *Acanthodactylus boskianus*

The estimate of phylogeny using maximum likelihood (Fig. 6) indicates that *A. boskianus*, as presently understood, may be paraphyletic, with the Arabian and the Moroccan populations forming successive basal branches of the eastern clade of *Acanthodactylus*. However, this pattern of relationships is not apparent in other analyses. To test whether paraphyly of *A. boskianus* is a significant possibility, the maximum likelihood was calculated, with the same parameters as in the earlier analysis, for a modified topology in which the two populations of *A. boskianus* form a subclade at the base of the eastern clade, the rest of the topology remaining unaltered. This tree had a log likelihood of −3507.76. When this was compared to the original maximum likelihood tree, using the test of Kishino & Hasegawa (1989), there was no significant difference (Δ log likelihoods = 0.6). Therefore *A. boskianus sensu lato* can provisionally be maintained as a useful taxonomic unit, but clearly needs further investigation

Combining morphological and molecular evidence

Morphological data of taxa for which mtDNA sequence is available were combined with this and a maximum parsimony analysis conducted in which multistate morphological characters were treated as ordered and all characters given equal weight. The resultant tree has exactly the same topology as the molecular one, indicating that the morphological data set had little influence on outcome. This is presumably because there are relatively few morphological characters and their areas of conflict with molecular evidence are relatively restricted.

DNA evidence indicates that the original polarities within *Acanthodactylus* assigned to morphological characters on the basis of outgroup comparison were not always correct. The complex hemipenial armature with a somewhat reduced medial side (character 32-1), a medial clavula that has a <- shaped cross-section (character 23) and a thickened connector to the medial side of the lateral clavula (character 29) may in fact be plesiomorphic. If this is so, near-symmetry of the hemipenis in *A. micropholis* and *A. cantoris* is likely to be derived, possibly indicating a relationship between these species. This may also be true of the armature pattern found in these forms and elsewhere in the eastern clade where the medial connector in the lateral clavula is not thickened and the medial clavula is not <- shaped and is simple in structure. These features give a reason for associating some members of the eastern clade, excluding the *A. boskianus* group, which retains what are likely to be primitive hemipenial states after re-polarization.

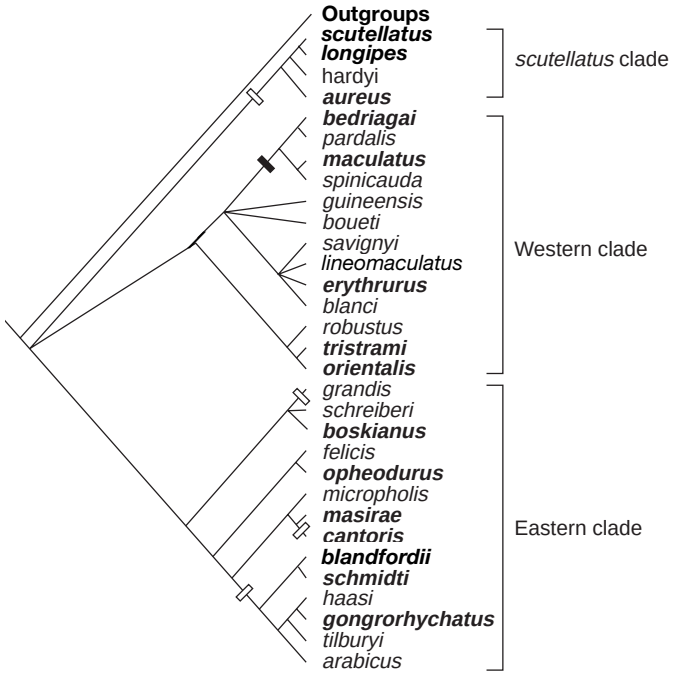

Fig. 8. Estimation of *Acanthodactylus* relationships using combined morphological and DNA sequence evidence. Molecular data are only available for species in bold and the others have been inserted on the basis of their perceived relationships to species on the molecular tree derived from morphological data. Open rectangles, ecological shifts to aeolian sand; closed rectangle, shift to loess surfaces.

Unfortunately, the second character cannot be checked in forms where the hemipenis is very asymmetrical.

This combined morphological and molecular estimate of phylogeny was used for reconstructing relationships of all the species of *Acanthodactylus* studied. Taxa for which only morphological data were available were integrated, with the combined phylogeny being placed according to their perceived relationships, based on morphology, with members of the combined tree (Fig. 8). These relationships were derived from the weighted phylogeny shown in Fig. 3, taking into account the reconsidered polarities discussed above.

COMMENTS ON THE BIOGEOGRAPHY AND EVOLUTION OF *ACANTHODACTYLUS*

Biogeography

The lacertid clade made up of *Eremias*, *Mesalina*, *Ophisops* and *Acanthodactylus* (Arnold, 1989) has most taxa in south-west Asia with the remainder in North Africa. The available phylogenetic evidence suggests that this assemblage is rooted in the former area and there has been a number of invasions of North Africa (Arnold, 1989). Three invasions by subunits of *Mesalina* may have occurred and one by *Ophisops*. If the estimate of phylogeny in Fig. 8 is accepted, African *A. boskianus* also came from the east. In the other North African *Acanthodactylus*, the western 'firm-ground' clade and the *A. scutellatus* group may possibly have shared a common ancestor which invaded Africa, or their individual ancestors may have done so separately. In either case, there would have been later secondary movement to the east by the ancestor of the *A. tristrami* group and that of *A. hardyi*. Alternative equally parsimonious possibilities are: (1) that the ancestor of the *A. erythrurus* and *A. pardalis* groups of the western firm-ground clade invaded North Africa, leaving the ancestor of the *A. tristrami* group in situ; or (2) the North African members of the *A. scutellatus* group may have resulted from two invasions from the east.

Changes in structural niche

It is most parsimonious to assume that the original habitat of *Acanthodactylus* was relatively firm sandy ground. The synthetic tree (Fig. 8) indicates there was a later shift on to loess surfaces in the ancestor of the *A. pardalis* group, and perhaps as many as four shifts on to loose aeolian sand have taken place (determined using the MacClade 3.01 program; Maddison & Maddison, 1992). If so, these occurred in the ancestor of the *A. scutellatus* group, in *A. grandis*, in *A. cantoris*, and in the ancestor of the clade made up of *A. blanfordii*, *A. schmidti*, *A. arabicus*, *A. tilburyi*, *A. haasi* and *A. gongrorhynchatus*.

Homoplasy in morphology

The estimate of phylogeny for 15 species based on molecular evidence, and that for 31 species based on both molecules and morphology, including species for which no molecular data are available, both indicate that the morphology of *Acanthodactylus* is even more homoplasious than estimates of relationships based on morphology alone suggest. Homoplasies include parallelism and reversal in fragmentation of supraocular scales (character 7), perhaps four developments of sand niche features (characters 13, 14, 16), and extra changes in some hemipenial characters (characters 23, 29, 31-1). On the basis of the phylogeny derived from molecular data, a small hemipenis (character 30) appears to have developed at least three times and the medial side of the hemipenis (characters 31, 32) has been strongly reduced three to seven times, the lower figure requiring some additional reversals.

CONCLUDING REMARKS

Molecular data corroborate parts of previous estimates of phylogeny of *Acanthodactylus* based on morphology, especially those in which some characters are down-weighted. However, it also suggests different relationships in some areas and, as the molecular evidence is

substantially more robust than that derived from morphology, these relationships are accepted.

The estimate of phylogeny derived from mtDNA sequence data provides independent evidence that the down-weighting of some characters in the analysis of morphological data is appropriate. It confirms that reduction in the size of the hemipenis, reduction and loss of its medial side and the development of features associated with loose sand are all labile features that have developed several times or been subsequently lost or both. This lability makes them poor indicators of relationships.

The synthetic tree produced from molecular and morphological data also indicates considerable morphological homoplasy. It suggests that *Acanthodactylus* arose in south-west Asia and only later invaded North Africa, which it may have done more than once while soft sand habitats may have been occupied on up to four occasions.

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Appendix 1. Locality data for specimens used to extract DNA for sequencing

Species	Locality	Source	Collection date
A. aureus	Near Agadir, Morocco	E. N. Arnold & D. J. Harris	April 1995
A. bedriagai	Near Agadir, Morocco	E. N. Arnold & D. J. Harris	April 1995
A. blanfordii	Ghalla dunes, Oman	A. S. Gardner	
A. boskianus	Ouarzazate, Morocco	E. N. Arnold & D. J. Harris	April 1995
A. boskianus	Abu Dhabi, U.A.E.	P. Osbourne & B. Tigar	August 1995
A. cantoris	Pakistan	S. Peltz	Autumn 1995
A. erythrurus belli	Algeria	E. Wade	August 1995
A. gongrorhynchatus	Abu Dhabi, U.A.E.	P. Osbourne & B. Tigar	August 1995
A. longipes	Egypt	S. Peltz	June 1995
A. masirae	Shanna, Oman	A. S. Gardner	November 1995
A. maculatus	North Algeria	E. Wade	March 1995
A. opheoduras	Harrat al Harrah, Saudi Arabia	J. Gasperetti	1992
A. orientalis	South-west of Palmyra, Syria	J. F. Schmidtler & W. Bischoff	
A. schmidti	Abu Dhabi, U.A.E.	P. Osbourne & B. Tigar	August 1995
A. scutellatus	South of Zagora, Morocco	E. N. Arnold & D. J. Harris	April 1995
A. tristrami	Syria	J. F. Schmidtler	
L. dugesii dugesii	San Miguel, Azores	P. Mordan	September 1994
M. adramitana	Al Ain, U.A.E.	E. N. Arnold & D. J. Harris	March 1996
M. guttulata	Egypt	E. Wade	