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Molecular Phylogenetics and Evolution 45 (2007) 612–619

MOLECULAR PHYLOGENETICS AND EVOLUTION

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Phylogenetic relationships within an endemic group of Malagasy 'assassin spiders' (Araneae, Archaeidae): ancestral character reconstruction, convergent evolution and biogeography

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Received 4 February 2007; revised 5 July 2007; accepted 8 July 2007
Available online 19 July 2007

Abstract

The phylogenetic relationships in an endemic group of Malagasy 'assassin spiders' (Araneae, Archaeidae: *Eriauchenius*) called the gracilicollis group, are inferred from mitochondrial 12S, 16S and COI DNA sequence data. Archaeid spiders of Madagascar have evolved varying degrees of elongation in the cephalic area. These molecular data support the monophyly of the gracilicollis group. The evolution of the cephalic area is examined by performing an ancestral character reconstruction on this character, which reveals that the cephalic area is elongating independently. The biogeography of the gracilicollis group reveals an east–west split of the clade on Madagascar.
Published by Elsevier Inc.

Keywords: *Eriauchenius*; Madagascar; Monophyletic group; Phylogeny

1. Introduction

The island of Madagascar is known for its high rate of endemism, its species richness, and its radiations (Ganzhorn et al., 2001). Molecular studies have shown that song birds (Cibois et al., 2001), ranid frogs (Bossuyt and Milinkovitch, 2000), tortoises (Caccone et al., 1999), primates (Yoder et al., 1996), clades of Satyrinae butterflies (Torres et al., 2001), carnivores (Yoder and Flynn, 2003), and rodents (Jansa et al., 1999) are all monophyletic groups that have speciated on Madagascar. A group of Malagasy spiders may be another example of such a monophyletic clade that is endemic and has diversified on the island.

The Archaeidae (Koch and Berendt, 1854) or 'assassin spiders' are a bizarre group hunting spiders that are unique due to the extreme elongation of the cephalic area and the chelicerae ('jaws'), giving these spiders the appearance of a 'neck' and 'head' (see Figs. 1 and 2). On Madagascar, these spiders have evolved 'necks' of varying degrees of elongation, from long and constricted to short and stout. These unusual spiders exhibit morphological and ecological traits that are very deviant from all other known spiders. While most spiders are generalists, the Archaeidae are obligate araneophages, which means that they prey only on other spiders (Millot, 1948; Legendre, 1961; pers. obs. 2005). The elongated cephalic area may have evolved because of the way these spiders hunt: they reach their long chelicerae out and stab other spiders with the fangs at the tip of the chelicerae; the greater the length of the chelicerae and 'neck,' the further these spiders can reach to attack and also the greater the distance that impaled, struggling prey can be held away from the archaeid's body (see Fig. 1).

Assassin spiders were first described from three species fossilized in Baltic amber that was dated to be of Eocene

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1055-7903/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.ympev.2007.07.012

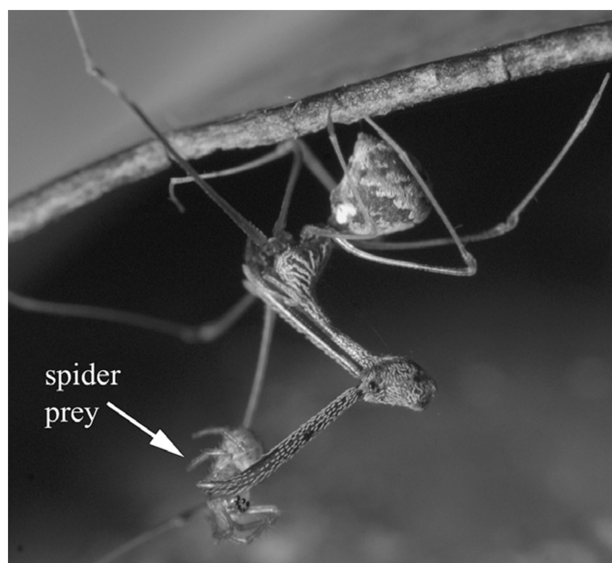

Fig. 1. ♀ *E. gracilicollis*, lateral view, immediately after capture of small spider, the right chelicera ('jaw') is extended and is holding captured prey. Photo by J. Miller.

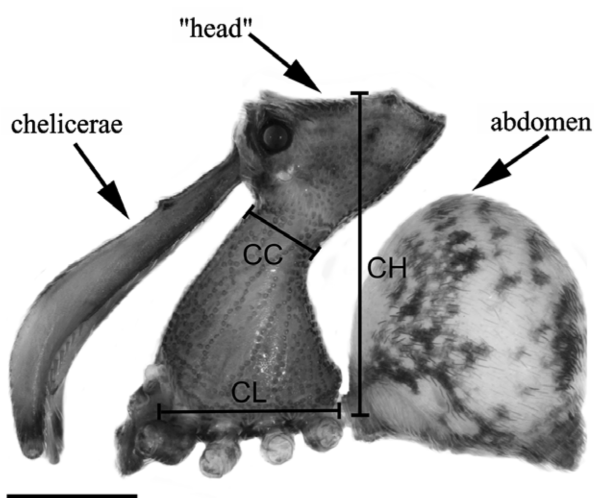

Fig. 2. ♂ *E. vadoni*, lateral, legs removed, showing carapace height (CH), carapace length (CL) and neck constriction (CC). Scale bar = 0.5 mm.

age (approximately 30–40 million years old) (Eskov, 1992; Wunderlich, 2004). Later, in 1881, O.P.-Cambridge discovered a living archaeid in Madagascar with a very long 'neck,' *Eriauchenius workmani*. Although the Baltic amber fossils suggest that archaeids were once more widespread, today these spiders occur only in the southern hemisphere: South Africa, Australia, and Madagascar. Their grotesque appearance, araneophagous predatory behavior, 'living fossil' status, and endemism have made assassin spiders the icon of Madagascar spiders (Griswold, 2003).

Regardless, archaeid spiders are still relatively unstudied. Previous research has focused mostly on species descriptions and little is known about the natural history and phylogenetic relationships of these spiders. The historical classifications of these spiders are dominated by the idea that the extremely long 'neck' has evolved only once, with species with similar neck lengths being more closely related. When Forster and Platnick revised and relimited the family in 1984, the genus *Eriauchenius* was separated from other extant genera based on the presence of a grossly extended and constricted cephalic area, the 'neck.' Platnick later proposed that *E. gracilicollis* (Millot, 1948) and *E. workmani*, which are both endemic to Madagascar, are sister taxa due to a "uniquely elongated and narrowed pars cephalica" (Platnick, 1991, p. 135), an idea previously suggested by Legendre (1970). This study focuses on a clade of archaeid spiders endemic to Madagascar, called the gracilicollis group, and is the first study ever performed on these bizarre spiders that uses molecular data to create a phylogeny. An ancestral character reconstruction on the 'neck' length character will also be performed and the biogeography and monophyly of the gracilicollis group will be examined.

2. Methods and materials

2.1. Taxon sampling

The analysis presented here includes a total of 18 taxa. Seventeen taxa are from the genus *Eriauchenius*. Fifteen of these 17 represent 13 species from the gracilicollis group, which are included for the purpose of determining phylogenetic relationships in this clade. Two individuals were sequenced per species for *E. voronakely* (Wood, in press) and *E. griswoldi* (Wood, in press), for a total of 13 species from 15 individuals in the gracilicollis group. For the species *E. vadoni* (Millot, 1948) and *E. tsingyensis* (Lotz, 2003) the COI sequence came from a different specimen than the 12S and 16S sequence. The two remaining species, *E. workmani*, and *E. bourgini* (Millot, 1948) do not belong to the gracilicollis group but are included in order to examine the monophyly of the gracilicollis group and also to better understand the evolution of the cephalic area. One *Afrarchaea* (Forster and Platnick, 1984) species is included as the outgroup to root the analyses. Table 1 contains a full list of all specimens, their taxonomic status, and their collection localities. All sequences have been deposited in GenBank under Accession Nos. DQ914564–DQ914617. The exact collection data for each specimen can be obtained from the author. Voucher specimens for all sequenced individuals have been deposited at the California Academy of Sciences, San Francisco, CA.

The new taxon names included here are based on terminology used by Wood (in press) and are disclaimed and unavailable for nomenclatural purposes (ICZN Art. 8.3). All new taxon names mentioned in this manuscript will be described and diagnosed in Wood (in press).

2.2. DNA isolation amplification and sequencing

Field collected specimens were placed in 70% EtOH and kept in the museum collection at room temperature until

Table 1
List of species used for phylogenetic analysis, museum voucher numbers, collection localities, genes sequenced, and accession numbers

Taxon	CASENT number	Province	Locality	DNA sequence	GenBank Accession No.
Afrarchaea sp.	9018940	Mpumalanga	Songimvelo nature reserve, SA	12S, 16S, COI	DQ914617, DQ914599, DQ914581
E. anabohazo	9002611	Antsiranana	Fôret d'Anabohazo, northwestern M	12S, 16S, COI	DQ914609, DQ914591, DQ914573
E. borimontsina	9015520	Toamasina	PN Masoala, northeastern M	12S, 16S, COI	DQ914607, DQ914589, DQ914571
E. bourgini	9015571	Toamasina	PN Masoala, northeastern M	12S, 16S, COI	DQ914616, DQ914598, DQ914580
E. gracilicollis	9009758	Mahajanga	PN Tsingy de Bemaraha, western M	12S, 16S, COI	DQ914605, DQ914587, DQ914569
E. griswoldi	9018899	Fianarantsoa	PN d'Isalo, western M	12S, 16S, COI	DQ914611, DQ914593, DQ914575
E. griswoldi	9018926	Mahajanga	PN de Baie de Baly, western M	12S, 16S, COI	DQ914610, DQ914592, DQ914574
E. halambohitra	9004603	Antsiranana	Montagne d'Ambre, northern M	12S, 16S, COI	DQ914602, DQ914584, DQ914566
E. jeanneli	9018921	Fianarantsoa	PN Ranomafana, eastern M	12S, 16S, COI	DQ914614, DQ914596, DQ914578
E. lavatenda	9018928	Antsiranana	Fôret d'Ampondrabe, northern M	12S, 16S, COI	DQ914606, DQ914588, DQ914570
E. legendrei	9015766	Toamasina	Ivoloina Parque Zoologique, eastern M	12S, 16S, COI	DQ914612, DQ914594, DQ914576
E. namoroka	9018916	Mahajanga	PN de Namoroka, central western M	12S, 16S, COI	DQ914603, DQ914585, DQ914567
E. spiceri	9001002	Antsiranana	Montagne des Français, northern M	12S, 16S, COI	DQ914604, DQ914586, DQ914568
E. tsingyensis	9018925	Mahajanga	PN de Namoroka, central western M	12S, 16S	DQ914608, DQ914608
E. tsingyensis	9009385	Mahajanga	PN Tsingy de Bemaraha, western M	COI	DQ914572
E. vadoni	9015209	Toamasina	PN Masoala, northeastern M	12S, 16S	DQ914613, DQ914595
E. vadoni	9018901	Toamasina	Montagne d'Anjanaharibe, northeastern M	COI	DQ914577
E. voronakely	9018897	Toliara	Fôret Classée d'Analavelona, western M	12S, 16S, COI	DQ914601, DQ914583, DQ914565
E. voronakely	9009659	Toliara	RS de cap Sainte Marie, southern M	12S, 16S, COI	DQ914600, DQ914582, DQ914564
E. workmani	9005571	Toamasina	PN Andasibe, eastern M	12S, 16S, COI	DQ914615, DQ914597, DQ914579

Abbreviations: CASENT, California Academy of Sciences Entomology Department; M, Madagascar; PN, Parc National; RS, Réserve Spéciale; SA, South Africa.

the time of DNA extraction. Total genomic DNA was isolated by grinding 1–2 legs in lysis buffer with a Teflon grinding implement. The homogenate was incubated overnight at 55 °C and then purified using the DNeasy™ Tissue Kit (Qiagen Inc., Valencia, CA) following the manufacturer's protocols.

For each specimen, three mitochondrial genes, 16S, 12S, and COI, were amplified via PCR (Mullis et al., 1987; Saiki et al., 1988). Double-stranded DNA was amplified, with some volume modifications depending on the specimen, in the following reaction: 50 μL volume reactions of 28.8 μL PCR water, 10 μL 5× buffer (Invitrogen) (300 mM Tris–HCL, 75 M $(NH_4)SO_4$, pH 8.5, 12.5 mM $MgCl_2$), 4 μL 10 mM dNTP, 2.5 μL of each 10 mM primer, and 0.2 μL AmpliTaq® DNA Polymerase (Applied Biosystems Inc., Foster City, CA). PCR amplification primers for these fragments are listed in Table 2. All reactions were initially denatured at 94 °C for 2 min in a MJ Dyad Thermal Cycler (MJ Research, Waltham, MA) or a DNA Engine Dyad, Peltier thermal cycler, then subjected to 35 cycles of 30 s denaturation at 94 °C, 30 s annealing at 45–50 °C, and 60 s extension at 72 °C. Amplified PCR products were cleaned using the UltraClean PCR clean-up Kit (MoBio, Solana Beach, CA) prior to sequencing.

Table 2
List of primers used for DNA sequencing

Primer	Sequence	Primer citation
16Sar	5′-CGCCTGTTTATCAAAAACAT-3′	Hillis et al. (1997)
16Sbr	5′-CCGGTCTGAACTCAGATCACGT-3′	Hillis et al. (1997)
12Sai	5′-AAACTAGGATTAGATACCCTAT TAT-3′	Hillis et al. (1997)
12Sbi	5′-AAGAGCGACGGGCGATGTGT-3′	Hillis et al. (1997)
HCO	5′-TAAACTTCAGGGTGACCAAAAA ATCA-3′	Folmer et al. (1994)
LCO	5′-GGTCAACAAATCATAAAGATAT TGG-3′	Folmer et al. (1994)

All sequencing was done using dye terminator cycle sequencing following the protocol specified by Applied Biosystems. Primers used for amplification served as sequencing primers (see Table 2). All samples were sequenced in both the forward and reverse directions by way of an ABI Prism® 377 DNA Sequencer (Applied Biosystems Inc., Foster City, CA) using a membrane comb (The Gel Company, San Francisco, CA) and following the manufacturers protocol.

2.3. Sequence alignment

After the mitochondrial gene sequences were collected, they were initially aligned using the computer programs ClustalX (Thompson et al., 1997) and Sequencher 3.1.1 (GeneCodes 1998). The aligned dataset was then further manually aligned using MacClade 4.03 (Maddison and Maddison, 2001) and PAUP*4.0b10 (Swofford, 2001). The conserved regions were identified and aligned, and gaps were assigned to minimize changes. Preliminary analysis of the dataset showed that gaps were irrelevant to the general conclusions of this study because the same parsimony tree topology was found regardless of whether gaps were included or excluded. The data were also initially run with and without gaps using maximum likelihood, which resulted in two trees with the same topology except for one taxon, *E. halambohitra* (Wood, in press), which moved to a different clade in the gracilicollis group.

Base composition bias was calculated (Irwin et al., 1991) for the entire fragment. A value of zero indicates no bias and a value of one indicates complete bias. An extreme overabundance of one nucleotide state can increase the

tendency for those sites to become saturated (Irwin et al., 1991). The heterogeneity chi square test in PAUP*4.0b10 (Swofford, 2001) was used to test for bias among taxa.

2.4. Phylogenetic analysis

To infer relationships among members of the gracilicollis group, phylogenetic analyses were performed using PAUP*4.0b10. Maximum likelihood and parsimony were employed to infer phylogenetic relationships. Maximum parsimony searches were performed using the random stepwise addition option of the heuristic search for 1000 replicates with tree bisection-reconnection (TBR) branch swapping, collapse of zero-length branches, and equal weighting of all 16S and 12S characters, and COI weighted for transversions. The data were also run twice more, once with equal weighting of all characters, and once with equal weighting with the COI third position codons excluded. To measure the robustness of branching patterns of the parsimony trees, bootstrap analyses (Felsenstein, 1985; Hillis and Bull, 1993) were executed by using the closest stepwise addition of the heuristic search for 1000 replicates.

In order to evaluate the fit of the data, a maximum likelihood analysis of the complete dataset was conducted using PAUP*4.0b10 (Swofford, 2001). To determine which model best fit, the dataset was subjected to Modeltest 3.06 (Posada and Crandall, 1998) and the resulting Akaika information criterion was used. After the best-fit model was found, a heuristic search was executed using the initial parameter estimates obtained from a neighbor-joining tree generated in PAUP. Once a better tree was found, the parameters were re-estimated and the search was repeated. This process was continued until a tree converged on the same maximum likelihood tree. To test the robustness of the final maximum likelihood tree, a bootstrap analysis was performed using the neighbor joining tree starting option of the heuristic search for 100 replicates.

The maximum likelihood model was used to determine whether the sequences among taxa are evolving at a constant rate and fit a molecular clock (Felsenstein, 1993). We used the likelihood ratio test (LRT) to test for a molecular clock (Felsenstein, 1993). The LRT is used to determine if there are significant differences between the likelihood scores obtained from an analysis where the branch lengths are unconstrained as compared to an analysis where the branch lengths are constrained so terminal ends are contemporaneous. The likelihood test statistics was assumed to be approximately equal to an χ^2 distribution with $n-2$ degrees of freedom, where n equals the number of taxa sampled (Felsenstein, 1981).

2.5. Ancestral character reconstruction

In order to better understand the evolution of the 'neck,' an ancestral character reconstruction was performed on this trait which was treated as a continuous character. The 'neck' length is described as a ratio (CH/CL) of the carapace height (CH) divided by the carapace length (CL). The CH/CC ratio is a numerical description that quantifies the length (CH) and narrowness (carapace constriction, CC) of the 'neck' (see Fig. 2). All measurements were taken using an Olympus SZH10 dissecting microscope. The measurements were taken from 5 males and 5 females, when possible, of each species and are reported as ranges for each sex. The mean value is calculated for both sexes. In many species, there were less than 5 individuals of each sex, or one sex was unknown, so only those numbers are reported (see Table 3). The ancestral character reconstruction was performed using squared change parsimony in Mesquite version 1.12 (Maddison and Maddison, 2006). This analysis was done using the log of the CH/CC mean value and was reconstructed onto the maximum likelihood phylogeny.

Table 3
List of 'neck' measurement values

Taxon	Numbers (♂,♀)	CH/CL (♂)	CH/CL (♀)	CH/CL (μ)	CH/CC (♂)	CH/CC (♀)	CH/CC (μ)
E. anabohazo	(3,5)	1.83–1.86	1.78–1.96	1.85	2.80–3.04	2.82–3.27	2.98
E. borimontsina	(0,2)	n/a	2.14–2.35	2.25	n/a	3.21–3.53	3.37
E. bourgini	(4,5)	1.92–2.14	2.21–2.35	2.18	4.50–5.10	4.58–5.40	4.86
E. gracilicollis	(5,5)	2.86–3.18	2.81–3.03	2.97	14.83–18.00	14.17–17.40	16.11
E. griswoldi	(5,5)	1.80–1.91	1.83–2.00	1.88	3.00–3.33	3.00–3.71	3.25
E. halambohitra	(0,2)	n/a	1.71–1.80	1.75	n/a	2.93–3.46	3.20
E. jeanneli	(5,5)	1.38–1.50	1.44–1.67	1.47	2.64–2.91	3.00–3.18	2.93
E. lavatenda	(5,5)	2.69–3.00	2.44–2.75	2.73	12.15–13.50	10.80–12.83	12.26
E. legendrei	(5,5)	1.77–2.05	1.76–1.83	1.84	3.90–4.71	3.73–4.09	4.17
E. namoroka	(1,2)	2.50	2.27–2.31	2.36	6.00	5.90–6.67	6.19
E. spiceri	(1,0)	1.88	n/a	1.88	3.33	n/a	3.33
E. tsingyensis	(5,5)	1.76–1.88	1.79–1.89	1.83	2.75–3.14	3.00–3.20	3.04
E. vadoni	(5,5)	1.52–1.71	1.73–1.83	1.69	3.47–4.00	3.46–3.82	3.71
E. voronakely	(3,3)	1.87–1.93	1.88–1.92	1.90	3.58-4.00	3.33–4.00	3.73
E. workmani	(5,5)	2.18–2.85	2.35–3.08	2.53	8.86–11.00	7.06–9.38	9.04

Table includes species measured, number of individuals measured from each sex, range of carapace height (CH) and carapace length (CL) ratio for each sex, average CH/CL, range of CH and carapace constriction (CC) ratio for each sex, and average CH/CL and CH/CC; n/a, not applicable.

3. Results

3.1. Simple sequence statistics

This study produced a final aligned 1450 base pair fragment, consisting of 488 aligned bp for 16S, 345 aligned bp for 12S, and 617 aligned bp for COI, for each taxon. The aligned fragment contained 710 sites that were variable (49%) and 551 sites that were parsimoniously informative (38%). Examinations of base composition in the entire dataset resulted in the following: A: 0.31136; C: 0.07557; G: 0.21800; T: 0.39506. The COI dataset exhibited 0.2328533 base composition bias for all characters and 0.26031333 for only variable characters; a Chi-square test for homogeneity of base frequency among taxa was 73.58 when all characters were included and 173.72 when constants were excluded, resulting in a P value of 0.0208 and <0.0001, respectively. The 16S dataset exhibited 0.25555333 base composition bias for all characters and 0.28744 for only variable characters; a Chi-square test for homogeneity of base frequency among taxa was 67.61 when all characters were included and 119.58 when constants were excluded, resulting in a P value of 0.0595 and <0.0001, respectively. The 12S dataset exhibited 0.33214 base composition bias for all characters and 0.33945333 for only variable characters; a Chi-square test for homogeneity of base frequency among taxa was 70.84 when all characters were included and 117.39 when constants were excluded, resulting in a P value of 0.0343 and <0.0001, respectively. The entire combined dataset exhibited 0.23898667 base composition bias for all characters and 0.28925333 for only variable characters; a Chi-square test for homogeneity of base frequency among taxa was 173.43 when all characters were included and 337.71 when constants were excluded, resulting in both P values of <0.0001. The degrees of freedom was 51 for all calculations. The heterogeneity test suggests that the sequence is heterogeneous. However, inspection of the dataset did not reveal any extreme bias, so this heterogeneity bias does not appear to present a problem.

The outgroup sequence from *Afrarchaea* differed from all other sequences by 20–26%. The *E. workmani* and *E. bourgini* sequences differed from all other members within the gracilicollis group by 20–27%, and differed by 15% from each other. Among members of the gracilicollis group, *E. tsingyensis*, *E. anabohazo* (Wood, in preparation), and *E. griswoldi* sequences differed by 13%; all other members of the gracilicollis group differed by 14–23%. The intraspecific variation for *E. voronakely* and for *E. griswoldi*, was 5.7% and 4.8%, respectively, which suggests that the taxa used in this study differ at a species level.

3.2. Phylogenetic analyses of 16S, 12S and COI DNA

The maximum parsimony analysis of all characters with COI weighted for transversions resulted in a single minimum length tree of 1942 steps (see Fig. 3). The data were also analyzed with equal weighting of all characters and resulted in one tree of 2509 steps (not shown). This tree differed from the tree of length 1942 in that *E. jeanneli* (Millot, 1948) is sister to the rest of the members of the gracilicollis group, although this difference has bootstrap support of less than 50%. The final parsimony analysis was done with third position codons in COI excluded, which resulted in one tree of 1662 steps (not shown). This tree was the same topology as the tree of length 1942 except that the clade containing *E. spiceri* (Wood, in press) and the clade containing *E. halambohitra* were switched, again with bootstrap support of less than 50%. Pairwise distance comparisons of codon position 1 and 2 are much smaller, by an order of magnitude, than pairwise distance comparisons of codon position 3 in the COI sequence data. The different outcomes of the three parsimony analyses do not alter the general conclusions of this study.

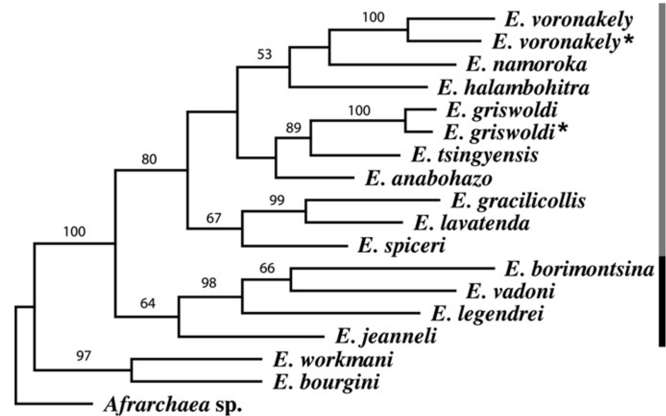

Fig. 3. Maximum parsimony phylogeny, single tree of 1942 steps. Asterisk indicates where two individuals of the same species were included in analysis. Bar indicates gracilicollis group; grey portion indicates the western clade and black portion indicates the eastern clade. Bootstrap values are given above each node.

The best fit maximum likelihood model determined using the Akaike criteria in Modeltest 3.06 (Posada and Crandall, 1998) suggested that the best model for these data was K81uf+I. The maximum likelihood search in PAUP using this model resulted in one maximum likelihood tree with a $-\ln L = 11930.41044$ (see Fig. 4). The parameter values as estimated from this tree were: $A \leftrightarrow T$ and $C \leftrightarrow G$: 1.066772, $A \leftrightarrow G$ and $C \leftrightarrow T$: 3.563180, and $A \leftrightarrow C$ and $T \leftrightarrow G$: 1.0 for the K81 model (Kimura, 1981), $I = 0.490344$ and nucleotide frequencies are $A = 0.31136$, $C = 0.07557$, $G = 0.21800$ and $T = 0.39506$. Maximum likelihood was also used to test for a clocklike evolution. The molecular clock tree produced with the same parameter estimates as above gave a likelihood score of $-\ln L = 11967.32272$, which indicates that the molecular clock should be rejected ($\chi^2 = 73.82$, df $= 16$, $P > 0.0001$).

3.3. Phylogenetic relationships

Both parsimony and maximum likelihood trees show 100% bootstrap support for the monophyly of the gracili-

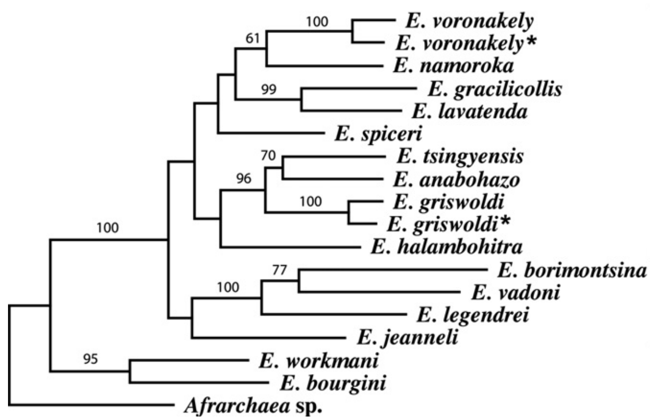

Fig. 4. Maximum likelihood phylogeny, single tree, $-\ln L = 11930.41044$. Asterisk indicates where two individuals of the same species were included in analysis. Bar indicates gracilicollis group; grey portion indicates the western clade and black portion indicates the eastern clade. Bootstrap values are given above each node.

collis group, with *E. workmani* and *E. bourgini* falling outside of the gracilicollis group. There is a distinct eastern–western biogeographic split among the gracilicollis group (see Figs. 3 and 4). The clade containing *E. borimontsina* (Wood, in press), *E. vadoni* and *E. legendrei* (Platnick, 1991) also has strong bootstrap support of 98% and 100% for the parsimony and likelihood tree, respectively. The clade containing the species from the western part of Madagascar has 80% bootstrap support in only the parsimony tree, with the eastern clade having a bootstrap value of 64%. The clade containing *E. tsingyensis*, *E. griswoldi* and *E. anabohazo* has strong bootstrap support (89% and 96%) as well as the sister taxa *E. gracilicollis* and *E. lavatenda* (Wood, in press) (99% and 100%). Individuals from the same species, *E. griswoldi* and *E. voronakely*, group together with a bootstrap value of 100% in both trees. The major difference in the topology of both trees is the

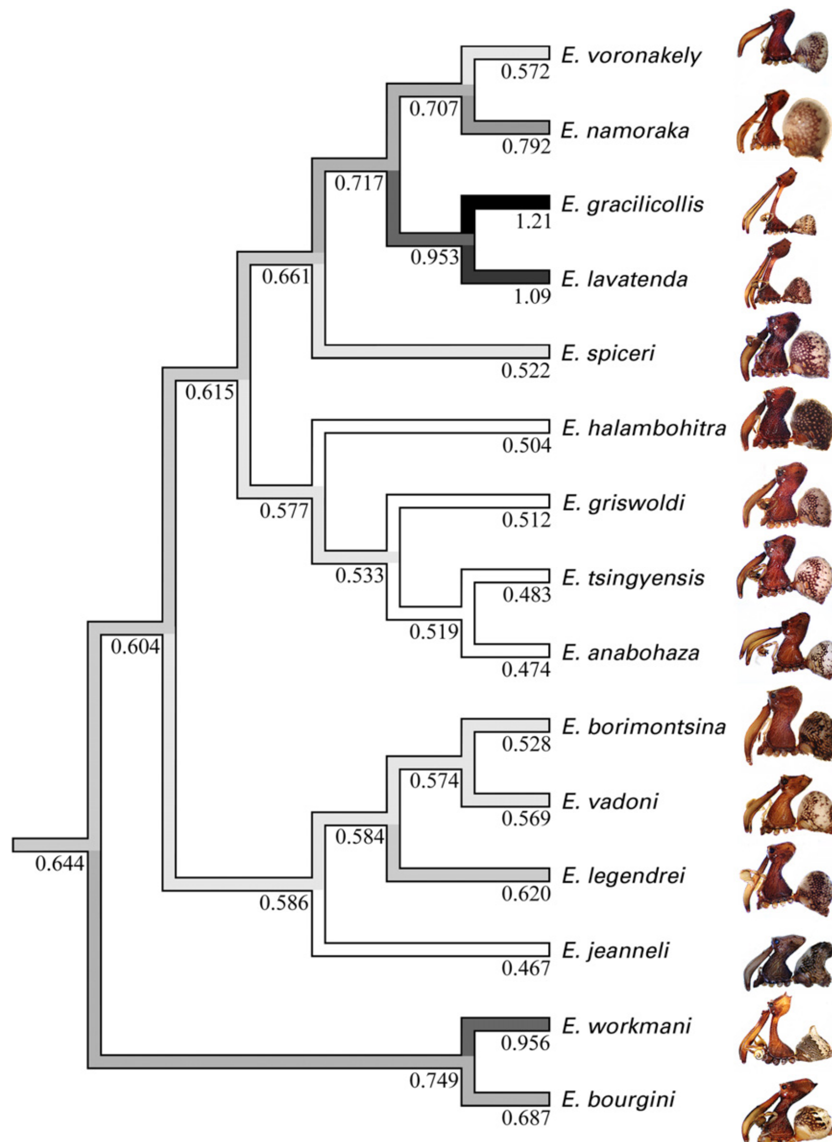

Fig. 5. Ancestral character reconstruction of the log mean CH/CC value mapped on the maximum likelihood phylogeny, with a species image next to each terminal branch (lateral view, legs removed, images not to scale).

placement of *E. halambohitra* and this placement has less than 50% bootstrap support in either tree.

3.4. Ancestral character reconstruction

The ancestral character reconstruction of the 'neck' length and narrowness character value (CH/CC) shows the morphological disparity found within this trait (Fig. 5). This analysis suggests that the long, slender 'neck' has evolved independently in *E. workmani* and in the clade containing *E. gracilicollis*.

4. Discussion

4.1. Phylogenetic relationships

This is the first molecular study to examine phylogenetic relationships among members of the family Archaeidae. From our analysis, we are able to infer that *Eriauchenius* contains at least one strongly supported monophyletic group (gracilicollis) that has diversified on Madagascar. These findings support the morphological work of Wood (in press) who defined the gracilicollis group based on several characters: homology among sclerotized structures of the male palp, the presence of an apophysis on the patella of the male palp, and the presence of six protrusions on the cephalic area. The clade containing species *E. tsingyensis*, *E. griswoldi* and *E. anabohazo* reflects the similar morphology of the genitalic structures in these species. The same can be said for the clade containing species *E. borimontsina*, *E. vadoni*, and *E. legendrei*. For a complete discussion of morphology see Wood (in press).

4.2. Biogeography

Members of the gracilicollis group are split into distinct eastern and western clades (for mapped species distributions see Wood, in preparation). This finding is not surprising since the habitats in the east and west are so different. Madagascar can be split into several vegetative types (Gautier and Goodman, 2003; Wells, 2003): eastern humid evergreen forests; central montane forests and secondary grasslands; a band of northwestern humid forests that is similar to the eastern humid forests (the Sambirano region); the western deciduous forests; and the southern arid spiny bush forests. Winds, which blow to the northwest, hit the eastern side of Madagascar and release their moisture on the steep eastern side of a range of mountains that runs north to south (Wells, 2003), making the eastern side of the island generally very wet and humid and the western side generally drier. It has traditionally been proposed that the differing eastern and western vegetative types may create east/west biogeographic patterns, an idea that seems to be supported by this study.

A previous molecular study on lemurs showed a north–south biogeographic split on Madagascar (Yoder et al., 2000). Goodman and Ganzhorn (2004) suggested that rivers may acts as barriers to dispersal and create North–South biogeographic patterns in some lemur species. Raselimanana and Rakotomalala (2003) have also suggested this for some chameleon species. In our study there are north–south patterns nested within the larger east–west biogeographic pattern. For example, *E. voronakely* and *E. namoroka* (Wood, in press) are sister taxa and *E. namoroka* is found in the central west while *E. voronakely* is found in the southwest. This is also true for the clade containing the species *E. anabohazo* (found in the northwest), *E. tsingyensis* (found in the central west), and *E. griswoldi* (found in the southern-central west). These results differ from ours, suggesting different biogeographic patterns may emerge for different organisms.

5. Conclusion

There seems to be more morphological disparity in the amount of elongation in the 'neck' and in the shape of the elongated cephalic area among the taxa found in Madagascar than among sets of assassin spider taxa found in other parts of the world (South Africa and Australia). While the araneophagous predatory behavior explains why the 'neck' initially evolved, it does not explain why there is so much variation in this character. Maybe archaeid spiders are filling different niches based on the type and size of prey that they can capture or maybe this variation is due to habitat type. While these suggestions are not resolved by this study, it helps to understand a potential motive for why this diversity exists and it emphasizes the need for further studies. It is also important for conservation planning to note that all members of the gracilicollis group are endemic to Madagascar, and that the geographical range of each species is fairly localized.

Acknowledgments

The primary funding for this project came from National Institutes of Health Grant MBRS SCORE SO6 GM52588 (G. Spicer, P.I.); a Graduate Assistantship from the Research and Education Divisions at California Academy of Sciences; the Exline–Frizzell Fund and Lindsay Travel Fund at California Academy of Sciences; the National Science Foundation Grant EAR-0228699 (Assembling the Tree of Life: Phylogeny of Spiders) (C. Griswold, senior scientist; W. Wheeler, P.I.); and the National Science Foundation Grant DEB-0072713 (Terrestrial Arthropod Inventory of Madagascar) (B. Fisher and C. Griswold, P.I.s). Specimens used for sequencing came from the California Academy of Sciences collection. Thanks to J. Miller for help with the manuscript and for the picture of *E. gracilicollis* feeding, to N. Hallinan for help with the ancestral character reconstruction, to C. Reading, R. Carson, F. Cipriano, and S. Sabatino for advice on sequencing and to D. Fenster for help assembling the color figure. Thanks to E. Routman, J. Banbury, A.

Carmichael, I. Mendez, and M. Flannery and two anonymous reviewers for reading and critiquing this manuscript.

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