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Systematics of the Californian euctenizine spider genus *Apomastus* (Araneae : Mygalomorphae : Cyrtaucheniidae): the relationship between molecular and morphological taxonomy

Jason E. Bond

East Carolina University, Department of Biology, Howell Science Complex–N211, Greenville, NC 27858, USA. Email: bondja@mail.ecu.edu

Abstract. The genus *Apomastus* Bond & Opell is a relatively small group of mygalomorph spiders with a limited geographic distribution. Restricted to the Los Angeles Basin, San Juan Mountains, and San Joaquin Hills, *Apomastus* occupies a fragile habitat rapidly succumbing to urban encroachment. Although originally described as monotypic, the genus was hypothesised to contain at least one additional species. However, females of the two reputed species are morphologically indistinguishable and the authors were unable confidently to assign specific status to populations for which they lacked male specimens. Using an approach that combines geographic, morphological and molecular data, all known populations are assigned to one of two hypothesised species. Mitochondrial DNA *cytochrome c oxidase I* sequences are used to infer population phylogeny, providing the evolutionary framework necessary to resolve population and species identity issues. Conflicts between the parsimony and Bayesian analyses raise questions about species delineation, species paraphyly, and the application of molecular taxonomy to these taxa. Issues relevant to the conservation of *Apomastus* species are discussed in light of the substantive intraspecific species divergence observed in the mtDNA data. The type species, *Apomastus schlingeri* Bond & Opell, is redescribed and a second species, *Apomastus kristenae*, sp. nov., is described.

Additional keywords: conservation genetics, cytochrome oxidase, molecular systematics, molecular taxonomy, phylogeography, species paraphyly, spider taxonomy.

Introduction

The euctenizine genus *Apomastus* Bond & Opell, 2002 (Cyrtaucheniidae) belongs to the spider infraorder Mygalomorphae (trapdoor spiders, tarantulas, and their relatives), a group that retains several traits thought to be primitive for spiders (e.g. two pairs of book lungs). *Apomastus* species have very restricted geographical distributions, occurring mainly in and around the mountains and ravines of the Los Angeles Basin in California, USA (Fig. 1). Predominantly from moist riparian habitats, the proximity of this genus to such a large growing urban area portends of a particularly insecure future and a disrupted past (see discussion of conservation issues below). Members of the genus, unlike any other known member of the subfamily, have open subterranean burrows (i.e. lacking trapdoors).

For many years, species of *Apomastus* were informally considered to be members of *Aptostichus* Simon, 1891 by Gertsch (*in litt.*) and others (Usherwood and Duce 1985; Skinner *et al*. 1992). However, in phylogenetic analyses of the Rastelloidina (Bond and Opell 2002) and *Aptostichus* (Bond 1999), *Apomastus* was recovered as a sister taxon to a monophyletic *Aptostichus*; thus, based primarily on phylogenetic position in the 'California clade', these spiders were considered sufficiently divergent to warrant generic status. *Apomastus* is easily distinguished from the species of its sister taxa, *Aptostichus* and *Promyrmekiaphila* Schenkel, 1950, on the basis of several general morphological features (e.g. males with a recurved thoracic groove, females with a straight thoracic groove and uniform abdominal colouration) and several male secondary sexual characteristics (e.g. unique mating clasper spination).

Only one species, *Apomastus schlingeri* Bond & Opell, 2002, has been described to date. Although museum material available at the time suggested that there was a second species, the authors refrained from describing it in addition to *A. schlingeri* because it was impossible to distinguish females of the two hypothesised species. That is, females appeared to lack diagnostic morphological features whereas the males of the two putative species are morphologically quite different—a problem often encountered in mygalomorph taxa (Goloboff 1995; Bond and Opell 2002). In the absence of male specimens from all known localities,

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several populations could not be confidently assigned to either species. As a consequence, I misidentified the eastern Los Angeles County populations as *Apomastus schlingeri* (see fig. 19, Bond and Opell 2002).

In this paper I seek to assess the geographic boundaries of the two putative *Apomastus* species using a coupled morphological–molecular taxonomic approach to investigate species boundaries. Using molecular data, an attempt is made to assign populations to species based on phylogenetic associations (inclusion or exclusion from molecular clades) with other populations for which males have been collected. Males have been collected from only three locations: Old Topanga Canyon (OTC), Cajalco Canyon (CJE), and the Cleveland National Forest (CLF). Old Topanga Canyon is the type locality for *A. schlingeri*. Males from the latter two localities appear to represent a second morphological species based on differences in tibial spination patterns of leg I, a common species diagnostic character in many mygalomorph taxonomic revisions (e.g. Coyle 1995; Goloboff 1995). Using a total evidence approach to taxonomy, a new species of *Apomastus* (*Apomastus kristenae*, sp. nov.) is diagnosed and described and the geographic boundaries of both species are clarified using molecular phylogenetics. Finally, I discuss the role molecular data have played in this taxonomic study, the potential paraphyly of *Apomastus schlingeri* and issues related to the conservation of *Apomastus* populations and species.

Materials and methods

Sample vouchering, georeferencing, morphological methods, abbreviations and terminology

Three to five individuals were sampled per collection site from 10 localities throughout the known distribution of both species (Table 1). Unique specimen voucher numbers were assigned to all specimens (AS_001–AS_200); a label indicating voucher number and DNA

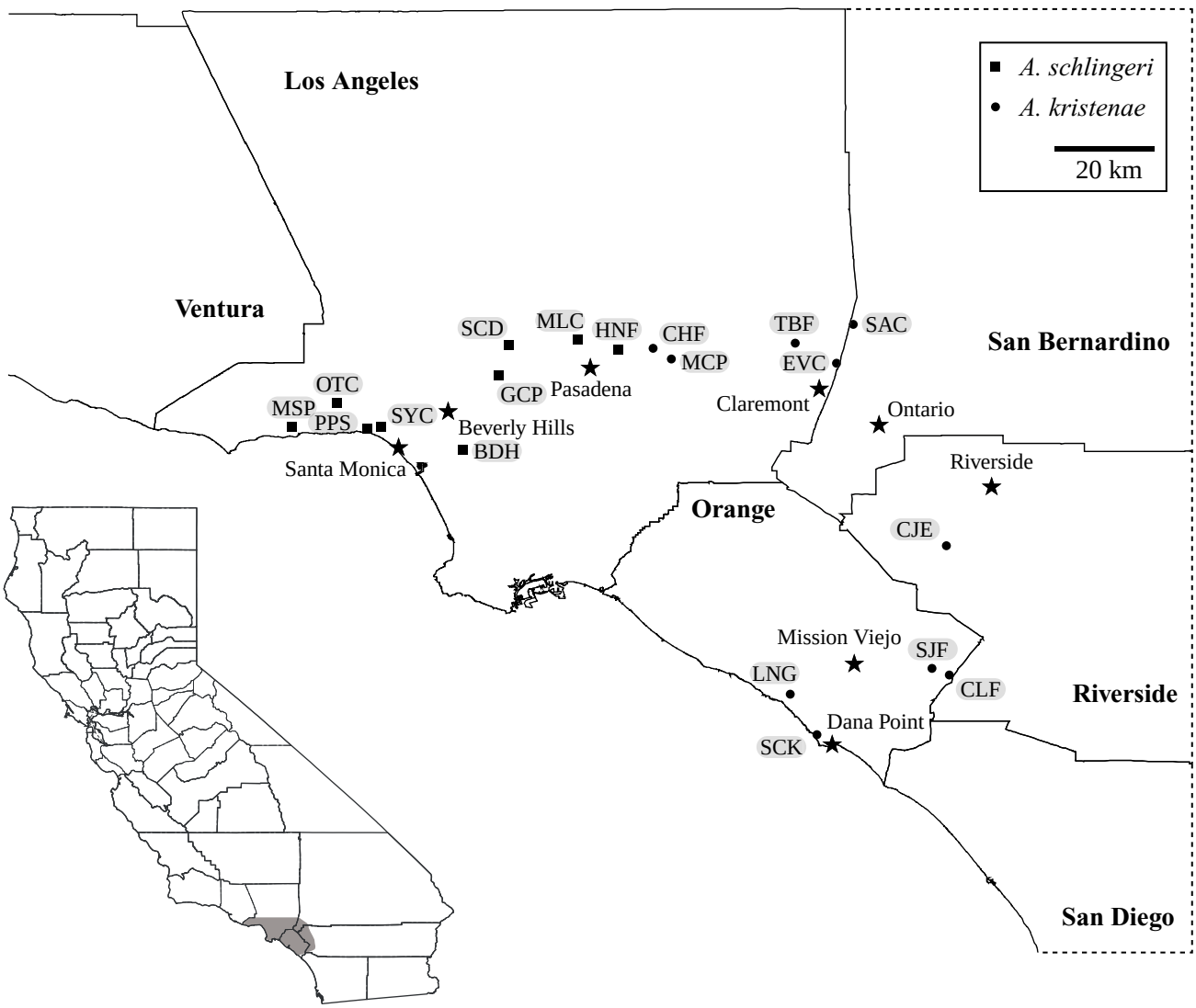

Fig. 1. Distribution map for all known *Apomastus* populations. Population acronyms are defined in Table 1.

haplotype (where applicable) was added to each vial for future reference. All collection localities were georeferenced using a global positioning system receiver or by finding the approximate locality, based on collection label data, on United States Geological Survey topographic maps. Latitude and longitude approximated in the latter manner are given in square brackets in the 'Material examined' sections (below) and in Table 1. Detailed locality data and associated GIS data can be downloaded at http://www.mygalomorphae.org and as accessory material (I) from the journal's website (www.publish.csiro.au/journals/is) as a simple tab-delimited table or as a Biota (Colwell 2003) dataset. Voucher specimens have been deposited in the California Academy of Sciences collection.

All quantitative measurements are given in millimetres and were made with a Leica MZ 9.5 stereomicroscope equipped with a 10× ocular and ocular micrometer scale. Appendage measurements, unless otherwise stated, were taken from the left side in the retrolateral view using the highest magnification possible. Lengths of leg articles were taken from the midline-proximal point of articulation to the midline-distal point of the article. Leg I, pedipalp, and habitus drawings were prepared by Nadine Dupérré (Québec, Canada). Colour images of leg I and pedipalp were prepared using a Microptics Imaging System (Microptics, Inc., Ashland, VA, USA). Spermathecae were removed from the abdominal wall and made optically clear for photographing by soaking them overnight in clove oil.

Although standard institutional and descriptive Araneae abbreviations are used whenever possible, they are defined here for the purposes of clarity.

Institutional

AMNH	American Museum of Natural History, New York, USA
CAS	California Academy of Sciences, San Francisco, USA
FMNH	The Field Museum of Natural History, Chicago, USA

Morphological

Cl/w	carapace length/width
STRl/w	sternum length/width
LBl/w	labium length/width
A/PER	anterior/posterior eye row
A/PME	anterior/posterior median eye
PL/MS	posterior lateral/median spinnerets
TSp/r & TSrd	male tibia I spines prolateral/retrolateral and retrolateral distal
PTw/l	male palpal tibia width/length
Bl	palpal bulb length
PT/TB3s	female patella/tibia 3 spines

Leg article measurements are given in the following order: femur, patella, tibia, metatarsus, and tarsus. Mean values are given in parentheses following ranges in the 'Variation' sections of the taxonomic descriptions.

Deoxyribonucleic acid amplification and sequencing

Genomic DNA was extracted from 1–2 legs using the Qiagen DNeasy Tissue Kit (Qiagen, Inc., Valencia, CA, USA). Deoxyribonucleic acid amplifications were performed using standard polymerase chain reaction (PCR) protocols. PCR primers C1J–1751SPID (5′-GAG CTC CTG ATA TAG CTT TTC C-3′) and C1N–2776 (5′–GGA TAA TCA GAA TAT CGT CGA GG-3′) (Hedin and Maddison 2001) were used to amplify an ~1000 bp region of the *cytochrome c oxidase I* (*COI*) gene of the mitochondrial genome. PCR products were column purified and sequenced directly with both PCR primers using an ABI 377 automated DNA sequencer (Applied Biosystems Inc., Foster City, CA, USA) using the ABI Big Dye Terminator version 3.2 Cycle Sequencing Ready Reaction Kit. Sequences were edited and aligned using the computer programme Sequencher (Genecodes, Inc., Madison, WI, USA). Alignment was trivial owing to the absence of both insertions and deletions. The complete dataset in NEXUS file format is available at http://www.mygalomorphae.org and as accessory material (II) on the journal's website (www.publish.csiro.au/journals/is).

Table 1. List of all known *Apomastus* collecting localities

Acronyms correspond to those used in Fig. 1; latitude and longitude taken from topographic maps given in square brackets; *n* = number of specimens sampled for DNA studies

Place name and GenBank accession no.	Acronym	Latitude/longitude	*n*
LA County, Malibu Creek State Park	MSP	[34.05130°N, 118.69060°W]	0
LA County, Old Topanga Canyon[A], AY621484	OTC	34.09860°N, 118.61630°W	5
LA County, Pacific Palisades, AY621482, AY621483	PPS	34.06220°N, 118.53040°W	
LA County, Santa Ynez Canyon	SYC	34.04470°N, 118.55210°W	0
LA County, Baldwin Hills	BDH	[34.00660°N, 118.37370°W]	0
LA County, Griffith City Park, AY621504	GCP	34.14543°N, 118.30818°W	4
LA County, Sunset Canyon, AY621487, AY621488	SCD	34.20100°N, 118.28980°W	5
LA County, Millard Canyon, AY621486	MLC	34.21030°N, 118.16240°W	3
LA County, Henninger Flats	HNF	[N34.19250°, W118.08670°]	0
LA County, Chantry Flats	CHF	[34.19470°N, 118.02330°W]	0
LA County, Monrovia Canyon, AY621489, AY621490	MCP	34.17440°N, 117.98890°W	3
LA County, Tan Bark Flats	TBF	[34.20390°N, 117.75970°W]	0
LA County, Evey Canyon, AY621503	EVC	34.16730°N, 117.68380°W	5
San Bernardino County, Santa Antonio Canyon	SAC	[34.23780°N, 117.65390°W]	0
Riverside County, Cajalco Canyon[B], AY621494–AY621497	CJE	33.82560°N, 117.49570°W	4
Orange County, Cleveland Ntl. Forest, AY621499–AY621501	CLF	3359194°N, 117.47694°W	5
Orange County, San Juan Fire Station, AY621491–AY621493	SJF	33.59070°N, 117.47500°W	5
Orange County, Salt Creek	SCK	33.48190°N, 117.72060°W	0
Orange County, Laguna Beach, AY621502	LNG	33.1770°N, 117.76783°W	1

[A]Type locality for *A. schlingeri*.
[B]Type locality for *A. kristenae*.

Phylogenetic analyses

Parsimony analyses were performed using PAUP* version 4.0b10 (Swofford 2002) using the branch and bound search algorithm. Relative branch support was evaluated using non-parametric bootstrap analysis based on 10000 pseudoreplicates using the heuristic search algorithm with TBR branch swapping. Bootstrap and posterior clade probabilities are not interpreted here as measures of accuracy but rather as measures of internal precision. Lack of internal precision is viewed as an indication that the data are equivocal for nodes lacking support. Uncorrected pairwise distances and χ^2 tests of homogeneity of base frequencies were computed in PAUP*. Two *Aptostichus* species (*A. simus* Chamberlin, 1917 (from Zuma Beach, LA County) and *Aptostichus*, sp. nov. (from San Diego County, Anza Borrego Desert State Park, CA)) and a *Promyrmekiaphila* species (from Glenn County, CA) were used to estimate the location of the phylogeny's root. Choice of these outgroup taxa was based on the phylogeny proposed by Bond and Opell (2002), which placed both genera as sister to *Apomastus* as part of the 'California clade'.

Character optimisations used to diagnose lineages were performed initially in PAUP* using ACCTRAN optimisation. Based on optimisations in PAUP*, character sets were then constructed and entered by hand into the NEXUS file format. Optimisations were visualised in the computer programme MacClade (Maddison and Maddison 2001) by excluding all characters except those optimised for the node of interest.

Modeltest version 3.1 (Posada and Crandall 1998) was used to determine the appropriate model of DNA substitution (by likelihood ratio test, lrt) for analyses that employ Bayesian inference. The computer programme MrBayes version 3.0b4 (Huelsenbeck and Ronquist 2001) was used to infer tree topology using the likelihood model indicated by lrt. Four simultaneous Markov Chain Monte Carlo (MCMC) chains (Metropolis-coupled MCMC) were run for one million generations saving the current tree to file every 100 generations. Trees before –ln likelihood stabilisation (burn-in) were discarded and clade posterior probabilities were computed from the trimmed set of trees by computing a 50% majority rule consensus tree in PAUP*. Bayesian analyses were repeated three times to compare separate MCMC runs to ensure topological convergence and homogeneity of posterior clade probabilities (Huelsenbeck *et al*. 2002). The –ln likelihood value reported for the preferred tree topology based on the results of the Bayesian analyses was computed by importing the tree with the highest –ln likelihood from the first MrBayes run into PAUP* and setting the likelihood parameter values to those indicated by Modeltest.

Results

Deoxyribonucleic acid sequence characteristics

The results presented here are based on 810 bp of the mitochondrial *COI* gene surveyed for 40 *Apomastus* specimens from 11 localities (see Table 1). Of these 810 bp, 281 were variable, 198 of which were parsimony informative; 24 unique haplotypes were recovered (GenBank accession numbers AY621482–AY612508). When these nucleotide data are translated to amino acid sequences, 17 sites contain non-synonymous substitutions. Of these 17 ingroup substitutions, only 11 are parsimony informative. The uncorrected base frequency composition across all haplotypes (outgroup and ingroup) is considered homogenous (χ^2 = 46.94, d.f. = 78, P = 0.9979) with a nucleotide base composition that is A–T rich (A: 0.25, C: 0.12, G: 0.20, T: 0.43). The average sequence divergence across *Apomastus* (the ingroup) was 0.07 (uncorrected P) with a minimum divergence of 0.001 and a maximum of 0.11.

Phylogenetic inference: parsimony

A branch and bound search conducted in PAUP* resulted in six equally parsimonious trees (613 steps, *CI* = 0.62, *RI* = 0.76). These six trees differed only with respect to the resolution of some closely related tip haplotypes (PPS, CJE1, CLF). Two basal clades were recovered, but this basal dichotomy lacks bootstrap support (Fig. 2). The Los Angeles area clade (hereafter referred to as the LA Clade) is strongly supported and unites haplotypes from western Los Angeles County (PPS, OTC), the Santa Monica Mountains, and the western portion of the San Gabriel mountains (MLC, SCD). The second, more widespread clade unites haplotypes from the central-eastern portions of the San Gabriels (MCP, EVC) with populations of the Santa Ana Mountains and its eastern and western foothills (San Joaquin Hills). Although reasonably high relative support exists for the clade that unites haplotypes from the Santa Ana Mountains (CLF and SJF) and the San Joaquin Hills (LNG), there is no relative support for the clade that unites the eastern Santa Ana foothills haplotypes (CJE) with those from the eastern extensions of

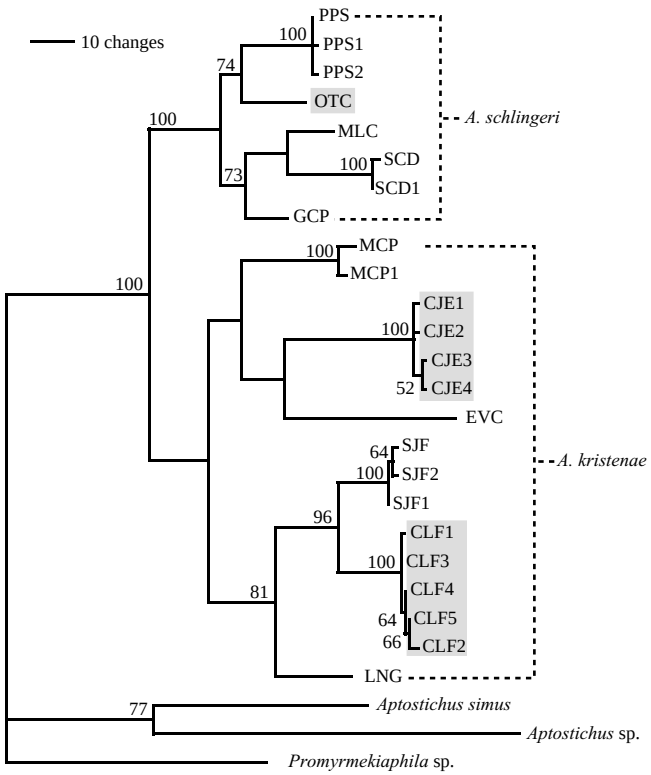

Fig. 2. One of six most parsimonious trees recovered in maximum parsimony analysis of *COI* sequences (613 steps, *CI* = 0.62, *RI* = 0.76). Bootstrap values (10 000 pseudoreplicates) are indicated above those nodes with support values >50%. Grey shaded boxes indicate haplotypes from populations from which males have been collected. Population acronyms are defined in Table 1.

the San Gabriel's (EVC + MCP); hereafter, these clades are referred to as the Riverside and Orange clade respectively.

Phylogenetic inference: Bayesian analysis

Modeltest indicated the most suitable model of DNA evolution for these sequence data to be the General Time Reversible model with a gamma distribution and invariants model of rate heterogeneity (GTR + Γ + I). Burn-in was considered (conservatively) to have occurred during the first 50000 MCMC generations in all three runs. The tree topology with the highest –ln likelihood value was identical in each of the three runs; posterior clade probabilities differed for only a few nodes by no more than ±3%.

The results of the Bayesian analysis are illustrated in Fig. 3 (–ln likelihood = 3824.59707, Γ shape parameter α = 1.044, proportion of invariable sites = 0.567). This analysis recovers three basal clades, although the terminal-most dichotomy lacks support. These three clades correspond directly to the LA, Riverside, and Orange clades reported for the parsimony analysis (see above) and have high (LA) to moderate (Orange and Riverside clades) posterior clade probabilities.

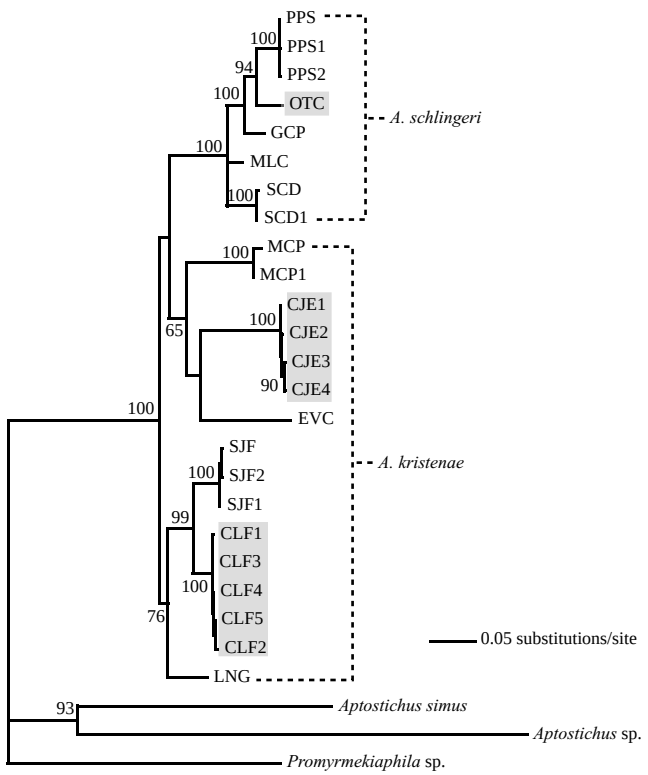

Fig. 3. Maximum likelihood tree based on analysis using Bayesian inference under a GTR + Γ + I model of DNA evolution (–ln = 3824.59707, α = 1.044, i = 0.567). Posterior clade probabilities >50% are indicated above each node. Grey shaded boxes indicate haplotypes for populations from which males have been collected. Population acronyms are defined in Table 1.

Discussion

The phylogenetic analyses of *COI* sequence data show a pattern of molecular divergence consistent with the existence of at least two or more hypothesised *Apomastus* species. Although parsimony and likelihood results differ slightly, both recover three phylogenetic groupings (LA, Riverside, Orange clades) with strong geographic associations. The degree to which these three 'major' lineages have diverged in their respective *COI* sequences is 8–11%, values comparable to what have been considered divergence 'thresholds' for species recognition in other groups (Hebert *et al*. 2003*a*). However, 'intraspecific' divergence values range as high as 9% (e.g. LNG *v.* CJE haplotypes), a value much higher than those for the majority of phylogeographic studies ('intraspecific divergences are rarely greater than 2% and most are less than 1% (Avise 2000)' Hebert *et al.* 2003*b*). Such a complex pattern of geographic divergence presents problems for resolving species boundaries in *Apomastus* and consequently raises questions about the utility of universal, molecular approaches in species identification, particularly ones that rely partly on a divergence criterion.

At the onset of this study, the primary objective seemed relatively straightforward: assign all *Apomastus* populations to either one of two species using a molecular taxonomic approach. Ideally, populations would group phylogenetically, with substantial support, with one or the other sets of male specimens. Species assignment would be a trivial matter and would demonstrate the utility of molecular taxonomy in resolving species identities when limited morphological information is available. Proponents of a strict molecular approach to taxonomy (e.g. Hebert *et al*. 2003*a*; Tautz *et al*. 2003) would anticipate such an outcome (see discussion of molecular taxonomy below). However, as with most questions concerning species delineation the data are seldom unambiguous. This particular situation is complicated by both the paucity of male specimens and the phylogenetic results. Neither parsimony nor likelihood analysis recovered, with confidence, two well-delineated population groupings associated with either of the type males from the Topanga Canyon or Cajalco Canyon type localities. The parsimony analysis did in fact recover two such groupings; unfortunately, the node uniting *A. kristenae*, sp. nov. populations lacks bootstrap support and is consequently supported by only a single non-homoplasious character change. Although it is topologically a pleasing result, I am hesitant simply to accept this result without further question or comment particularly in light of conflicting results in other analyses that implement a more explicit, statistically consistent model of DNA evolution.

The tree topology based on the likelihood analysis (Fig. 3) using Bayesian inference indicates that *A. kristenae*, sp. nov. populations are non-exclusive (i.e. 'paraphyletic') with respect to *A. schlingeri* populations. This result indicates

potential gene tree–species tree incongruence. When *A. kristenae*, sp. nov. exclusivity is forced as a topological constraint, this tree does not differ significantly from the most likely tree ($P > 0.05$, Shimodaira and Hasegawa (1999) test using RELL (resampling of the estimated log-likelihood) bootstrap resampling, 1000 replicates); alternatively *A. kristenae*, sp. nov. exclusivity occurs in 21% of the trees sampled after burn-in in the Bayesian analysis. Such ambiguities pose potentially serious problems for delineating species using molecular techniques. In this particular example, if we accept *prima facie* that mating clasper differences (aspects of male leg I morphology thought to be involved in mating) accurately reflect species boundaries then we must ask why these obvious morphological differences are not unequivocally reflected in a mitochondrial gene tree? After all, mitochondrial genes are haploid and maternally inherited and thus are expected more rapidly to reflect species boundaries than nuclear genes (Moore 1995; Wiens and Penkrot 2002).

The equivocal nature of these phylogenetic data coupled with the available morphological data suggests two potentially competing species hypotheses: H_1 = multiple *Apomastus* species *v.* H_2 = a single species that is highly structured geographically. If H_1 is considered to be the preferred species hypothesis then several causes for weak species tree–gene tree congruence must be considered (Funk and Omland 2003): (*1*) inadequate phylogenetic information, (*2*) interspecific hybridization and (*3*) incomplete lineage sorting. First, saturation of nucleotide site substitutions at the third position could obscure phylogenetic signal owing to high levels of homoplasy, thus effectively blurring the 'lines' between *A. kristenae*, sp. nov. and *A. schlingeri.* Of the 198 parsimony informative characters, 166 involve substitutions that occur at the third position (ingroup + outgroup). Second, although introgression would confuse species boundaries, the nature of mtDNA data precludes its obvious effects in this example. Here, the more likely outcome of introgression would be the incorrect species assignment of populations for which male specimens have not been collected (discussed further below); albeit a potential confounding issue for these data, it is one that cannot be evaluated or detected at present. Third, incomplete lineage sorting seems unlikely given the total amount of divergence across the dataset. Based on the shear number of nucleotide changes it would appear that a sufficient amount of time has passed to sort species to exclusivity. Alternatively, under a scenario of incomplete lineage sorting sexual selection by female choice is one plausible explanation for divergence of male *Apomastus* secondary sexual features outpacing mtDNA divergence (Masta and Maddison 2002; Pattern 1 divergence, Bond *et al*. 2003). Given the moderate nature of ambiguity in the phylogeny it is likely under this scenario that as more time passes the two hypothesised species will eventually sort to reciprocal monophyly (Avise 2000).

Because male specimens are known from so few localities, it is impossible to know how leg I spination morphology might be structured geographically. Thus, it is important to recognise the limitations of the morphological data with respect to the molecular phylogeographic pattern. Perhaps the DNA data are indicating that all populations compose one highly geographically structured species (H_2 from above) and male morphological variation is simply clinal. Such discordance between morphologically based taxonomy and the molecular phylogeographic pattern would fall into the category of 'imperfect taxonomy' described by Funk and Omland (2003) where a species is 'oversplit' when intraspecific variation is misidentified as species level variation. If populations have been sampled insufficiently, molecular divergence as a function of geographic distance would be difficult to detect. Additional sampling may very well break up long branches, consequently allowing us to differentiate between isolation by distance and actual fragmentation (Hedin and Wood 2002). One final possibility worth considering along the lines of imperfect taxonomy is that there may be multiple cryptic species of *Apomastus*. Several related studies suggest that cryptic species, species undiagnosable using traditional genitalic morphology-based approaches, may be a real problem for assessing biodiversity in some arthropod groups (Hedin 1997, 2001; Bond *et al*. 2001; Bond and Sierwald 2002). Again, in the absence of male specimens from all populations and detailed natural history data, it is impossible to draw any steadfast conclusions about species crypsis based on such a small sampling of the spider genome. Future planned phylogeographic studies of the *Apomastus* species complex will carefully explore these possibilities more thoroughly.

Molecular taxonomy

'Molecular taxonomy' is rapidly becoming the catch phrase of modern 21st century systematics; its appeal is easy to understand. Protocols and aids to PCR, automated sequencing and the ubiquity of universal PCR primers (particularly for mtDNA genes) make molecular approaches to taxonomy available to virtually every biologist. Moreover, the mastery of molecular techniques requires a fraction of the time usually needed to achieve traditionally based taxonomic expertise for most groups of organisms.

The most explicit implementation of a molecular taxonomy approach has been that proposed by Hebert *et al*. (2003*a*, 2003*b*): *COI* sequences are used in DNA profiles (neighbour-joining trees) to barcode taxa. They favour this approach over more traditional morphology-based taxonomic systems because morphological diagnoses are limited significantly by (*1*) phenotypic plasticity and genetic variability, (*2*) an inability to detect cryptic species, (*3*) keys that are designed for only a single stage in the life history of an organism, and (*4*) taxonomic keys requiring a high level of expertise. I believe this study demonstrates the limited

utility of such an explicit molecular approach to taxonomy for three major reasons. First, delineation of species boundaries requires a thorough investigation of populations spanning the species' geographic distribution. Such an approach that accounts for geographic variation is nothing new and is an approach employed by most practicing 'morphological' taxonomists. Second, strict adherence to molecular taxonomy, particularly those approaches that depend upon phylogenetically based profile criteria, overlooks the process of speciation and ultimately fails to account for the likelihood of incomplete lineage sorting. Paraphyly of species early in speciational history is a real issue (Funk and Omland 2003) that would be problematic for such an approach. Finally, a system of molecular taxonomy will only be as good, and as interesting, as the preexisting morphological framework upon which it must be initially grounded.

In an attempt at objectivity, imagine for a moment an alien being visiting Earth. It would be inconceivable to us that this visitor to our planet would approach species identification using an arbitrary standard without first carefully evaluating the biological reality of such a construct. Molecular taxonomy will succeed or fail on the back of the very system that it seeks to supercede. As aptly stated by Lipscomb *et al*. (2003: 65), 'There is no credible reason to give DNA characters greater stature than any other character type. When species descriptions are based on a broad range of data, they become interesting scientific hypotheses making explicit predictions about the distribution of attributes of organisms'. That said, molecules (not molecular taxonomy *sensu stricto*) without question add another useful dimension to species delineation and identification. Molecular data will undoubtedly facilitate the detection of cryptic species and thus help guide subsequent studies of morphology, ecology, etc. and will aid in the identification of species where morphological differences are difficult to detect or are restricted to a particular life history stage or sex. Moreover, once species boundaries are known and thoroughly investigated molecular diagnoses of taxa may in fact serve as an easy and rapid means of identification. In the example presented in this paper, *Apomastus*, and elsewhere (e.g. Bond *et al*. 2001; Puorto *et al*. 2001; Bond and Sierwald 2002, 2003) molecules serve as a set of data that compliments insights gained through the study of morphology, geography, and speciation pattern and process.

Conservation

As mentioned earlier, the proximity of members of this genus, particularly *A. schlingeri*, to a large and growing urban area is problematic for this species' future and likely was a problem in its past. The greatest concentration of euctenizine species lies within the California Floristic Province, an 'evolutionary centre' that stretches from Oregon to Baja California (Myers 1988, Myers *et al*. 2000) and is considered to be a 'high priority terrestrial ecosystem for conservation' (Cincotta *et al*. 2000) because of the large number of endemic plants and animals. As a point of fact, over one-third (conservatively excluding theraphosids) of all nominal mygalomorphs in the United States are endemic to California, a statistic that will only increase as more genera are revised (e.g. *Aptostichus* and *Promyrmekiaphila*). Unfortunately, much of this region, particularly southern California, is subject to urban encroachment and agricultural development (Cincotta *et al*. 2000) and only a small percentage (24.7%) of the primary vegetation remains (Myers *et al*. 2000). Given their fossorial life history characteristics and the fact that they are long-lived and univoltine (traits making them comparable to some terrestrial vertebrate taxa), there is little doubt that spiders like *Apomastus* are extremely sensitive to environmental perturbation (a feature that could make them useful for biomonitoring).

Understanding the geographic distribution of the genus and correct species determinations are critical first steps in addressing conservation issues relevant to this unique and fragile genus of mygalomorph spider. However, our limited understanding of what constitutes an *Apomastus* species will influence decisions with regard to what can be defined as an evolutionarily significant unit (ESU) for the purposes of conservation (Moritz 1994*a*, 1994*b*). Although significant levels of genetic divergence occur within putative *A. schlingeri* and *A. kristenae*, sp. nov., it is not clear how this divergence should be interpreted within the context of species boundary delimitation or whether divergent populations should be treated as ESUs (see Avise's (2000) summary of these issues). Regardless of ambiguities in how these divergent populations are viewed with respect to species status, it is hard to imagine that such divergent populations are no doubt important components of evolutionary diversity and thus worthy of conservation consideration (Moritz and Faith 1998). Already some *Apomastus* populations are extinct. Searches for *Apomastus kristenae*, sp. nov. at the Salt Creek–Dana Point locality, now a golf course, have proven futile. Although *Apomastus* has recently been discovered close by (near Laguna Beach), Salt Creek is a critical locality for other mygalomorph species. It is the type locality for *Aliatypus gulosus* Coyle, 1974, and is the only locality from which an undescribed species of *Aptostichus* is known (Bond 1999).

Conclusions

Although the technical aspect of naming species, nomenclature, is governed by a set of rules (i.e. The International Code of Zoological Nomenclature, ICZN 1999), nominal species are hypotheses–constructs subject to future testing and falsification. I make this distinction only because I believe that species constructs, as hypotheses, are often and incorrectly given a special status because the 'naming' of these hypotheses is done in such a formal way despite the fact that the formality relates explicitly to the nomenclatural

act, not the underlying science. Despite the equivocal nature of the molecular phylogenies reported herein I propose the following hypothesised classification scheme for the genus *Apomastus*: populations from the Henninger Flats locality westward constitute *A. schlingeri*; those populations from the Chantry Flats locality east to south-east are determined as *A. kristenae*, sp. nov. These determinations are formulated on the basis of phylogenetic and geographic position. For those populations for which I have genetic data, species placement is based upon phylogenetic associations with specimens collected from the type localities of Old Topanga Canyon (OTC, *A. schlingeri*) and Cajalco Canyon (CJE, *A. kristenae*, sp. nov.). For those populations not yet represented by *COI* sequence data, species determination is based on geographic proximity to populations defined by genetic and phylogenetic criteria. These determinations will be subject to future testing as new material becomes available from other populations. Based on character state optimisations across the haplotype phylogeny (parsimony tree assuming a two-clade structure), female specimens are diagnosed using a unique combination of derived nucleotide site substitutions (see 'Taxonomy' section below).

Taxonomy

Family **CYRTAUCHENIIDAE** Simon

Subfamily **EUCTENIZINAE** Raven

Genus ***Apomastus*** Bond & Opell

Apomastus Bond & Opell, 2002: 523–525.
Type species: *Apomastus schlingeri* Bond & Opell, 2002: 525–527, by original designation.

Diagnosis

Male of this genus can be recognised by the presence of a recurved thoracic groove and the absence of a proximo-ventral metatarsal excavation and distinctive leg I spination. Females have a straight to slightly procurved thoracic groove and uniform dark, chestnut brown, abdominal coloration. All known species build underground burrows, lacking a trapdoor, with a short flexible open turret.

Apomastus schlingeri Bond & Opell

(Figs 4–6)

Apomastus schlingeri Bond & Opell, 2002: 525–527, figs 17–19.

Material examined

Holotype. ♂, California, Los Angeles County, Topanga Canyon, 229 m, [34.09860°N 118.61630°W], coll. C. Kristensen, xii.1982 (AMNH, AS_117).

Paratype. ♀, data as for holotype (AMNH, AS_117).

Other material examined. **USA:** California: Los Angeles County: 16♀, 1♂, Old Topanga Canyon Road, 229 m; 1♀, San Antonio Canyon; 1♀, Henninger Flats, 792 m; 6♀, Pacific Palisades, 91 m; 2♀, Baldwin Hills, 91 m; 9♀, Millard Canyon; 1♀, Malibu Creek State Park, elevation 152 m; 4♀, Country Club Drive, Sunset Canyon; 5♀, Griffith City Park. All material in AMNH and CAS.

Diagnosis

This species can be distinguished from *A. kristenae*, sp. nov. on the basis of the following unique combination of mtDNA *COI* gene nucleotide site substitutions (Fig. 4): A (91), R (172), C (194), T (214), Y (229), C (353), A (442), T (571), G (592), G (619), G (685). Male leg I with unique pattern of spines on tibial surfaces (Fig. 5*c*,*d*), prolateral surface, tibia I with numerous spines (TSP >30). *Apomastus schlingeri* females cannot at this time be distinguished from *A. kristenae*, sp. nov. females using morphology.

Redescription of male holotype

Specimen preparation and condition. Specimen collected in 1982, preserved in 70% ethanol. Colouration appears faded relative to specimens collected more recently. Pedipalp, leg I left side removed, leg II right side detached at patella. *Colour*. Carapace and chelicerae dark reddish-brown, legs lighter in colour. Abdomen uniform dark chestnut brown dorsally, ventrum and spinnerets dark yellow-brown (Fig. 5*b*). *Cephalothorax*. Carapace 5.19 long, 4.25 wide, hirsute, numerous fine white setae intermingled with more stout black bristles; surface smooth, pars cephalica elevated slightly. Fringe and posterior margin with black bristles. Thoracic groove deep, moderately recurved. Eyes on very low tubercle. AER slightly procurved, PER slightly recurved. PME–AME subequal in diameter. Sternum widest at coxae II/III, moderately setose. STRl 3.85, STRw 2.92. Sternal sigilla small, oval, marginal. Chelicerae with distinct outer tooth row (eight teeth) with inner marginal denticle patch. Palpal endites and labium lack cuspules, LBw 0.75, LBl 0.51. Rastellum consists of six very stout spines, not on a mound. *Abdomen.* Setose, heavier black setae intermingled with fine white setae. Triangular patch of epiandrous fusillae distinct from surrounding setae (wide bases, stout in stature). PLS all three segments with spigots. Terminal segment 2/3 length of medial segment, two enlarged spigots visible at tip. PMS single segment, with spigots, short with rounded terminus. *Walking legs*. Slender, distal 2/3 of each tarsus pseudosegmented. Tarsus III, IV slightly curved distally. Leg I: 4.85, 2.55, 4.00, 3.50, 2.45; leg IV: 4.90, 2.50, 4.50, 4.50, 2.75. Tarsal scopulae of intermediate density, leg I, lighter on all other tarsi. Tarsus I with single, slightly staggered row of 12 trichobothria. Leg I spination pattern illustrated in Fig. 5*c*,*d*; TSp ~39, TSr 8, TSrd absent. *Pedipalp*. Articles slender, lacking distinct spines (Fig. 5*e*). PTw 0.75, PTl 2.25, Bl 0.82. Embolus with slight mid-distal curvature, moderately stout.

Variation (single topotypic male). CL 5.25, CW 4.1; STRl 2.87, STRw 2.25; LBw 0.73, LBl 0.48; leg I: 4.85, 2.40, 3.80, 3.56, 2.38; leg IV: 4.80, 2.45, 4.55, 4.48, 2.68; PTw 0.85, PTl 2.40, BL 0.87; TSp 44, TSr 7.

Redescription of female paratype

Specimen preparation and condition. Prepared with male holotype. Genital plate removed and stored in microvial with specimen. *Colour.* Carapace and chelicerae dark orange-brown, legs lighter in colour. Abdomen uniform light chestnut-brown dorsally, ventrum and spinnerets yellow-brown (Fig. 5*a*). *Cephalothorax*. Carapace 5.84 long, 5.12 wide, glabrous; generally surface smooth, pars cephalica moderately elevated with thin row of mesal black setae. Fringe lacks setae. Thoracic groove deep, slightly procurved. Eyes not on a tubercle. AER slightly procurved, PER slightly recurved. PME slightly larger in diameter than AME. Sternum widest at coxae II/III, moderately setose. STRl 3.90, STRw 3.10. Three pairs of sternal sigilla anterior pairs small, oval, marginal, posterior pair slightly larger, oval, more mesially positioned. Chelicerae with distinct outer tooth row (10 teeth) with inner margin denticle patch. Palpal endites with numerous cuspules (~65) concentrated at the inner (promargin) posterior heel; labium lacks cuspules, LBw 1.09, LBl 0.87. Rastellum consists of seven very stout anterior margin spines, not on a mound. *Abdomen.* Moderately setose. PLS all three segments with spigots. Terminal segment 2/3 length of medial segment, two enlarged spigots visible at tip. PMS single segment, with spigots, short with rounded terminus. *Walking legs*. Anterior two pairs noticeably more slender than posterior pairs. Leg I: 5.00, 2.94, 3.60, 2.75, 1.75; leg IV: 4.75, 2.90, 4.20, 3.70, 1.80. Tarsus I with zigzag row of 13 trichobothria. Legs I and II with moderately heavy tarsal and metatarsal scopulae of intermediate density, legs III and IV light scopulae tarsi only. PT3s 20, TB3s 4. Distinct preening comb on retrolateral distal surface (at tarsus–metatarsus joint) of metatarsus IV, rudimentary preening comb, same position metatarsus III. *Spermathecae*. Two simple spermathecal bulbs (Fig. 6*g*; Bond and Opell 2002, fig. 17D), arranged in a transverse row, consisting of a moderately sclerotised stalk and an enlarged apical bulb, lightly sclerotised; base of stalk slightly enlarged such that it takes on the appearance of a smaller, reduced secondary bulb.

Variation (n = 10). Colouration of freshly collected and preserved specimens much darker. Carapace dark tannish

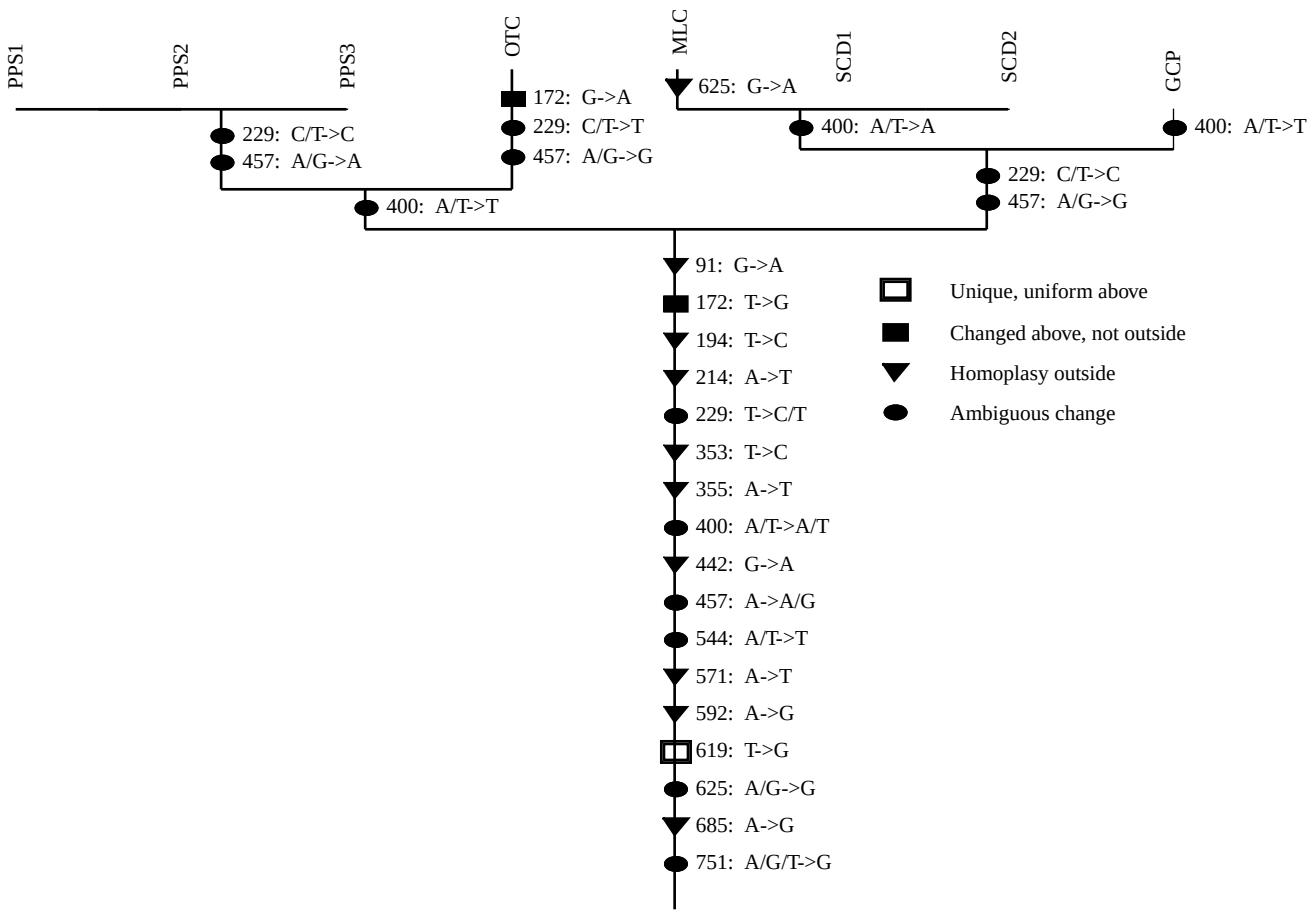

Fig. 4. Character transformations for *Apomastus schlingeri* diagnostic nucleotide site substitutions using ACCTRAN optimisation.

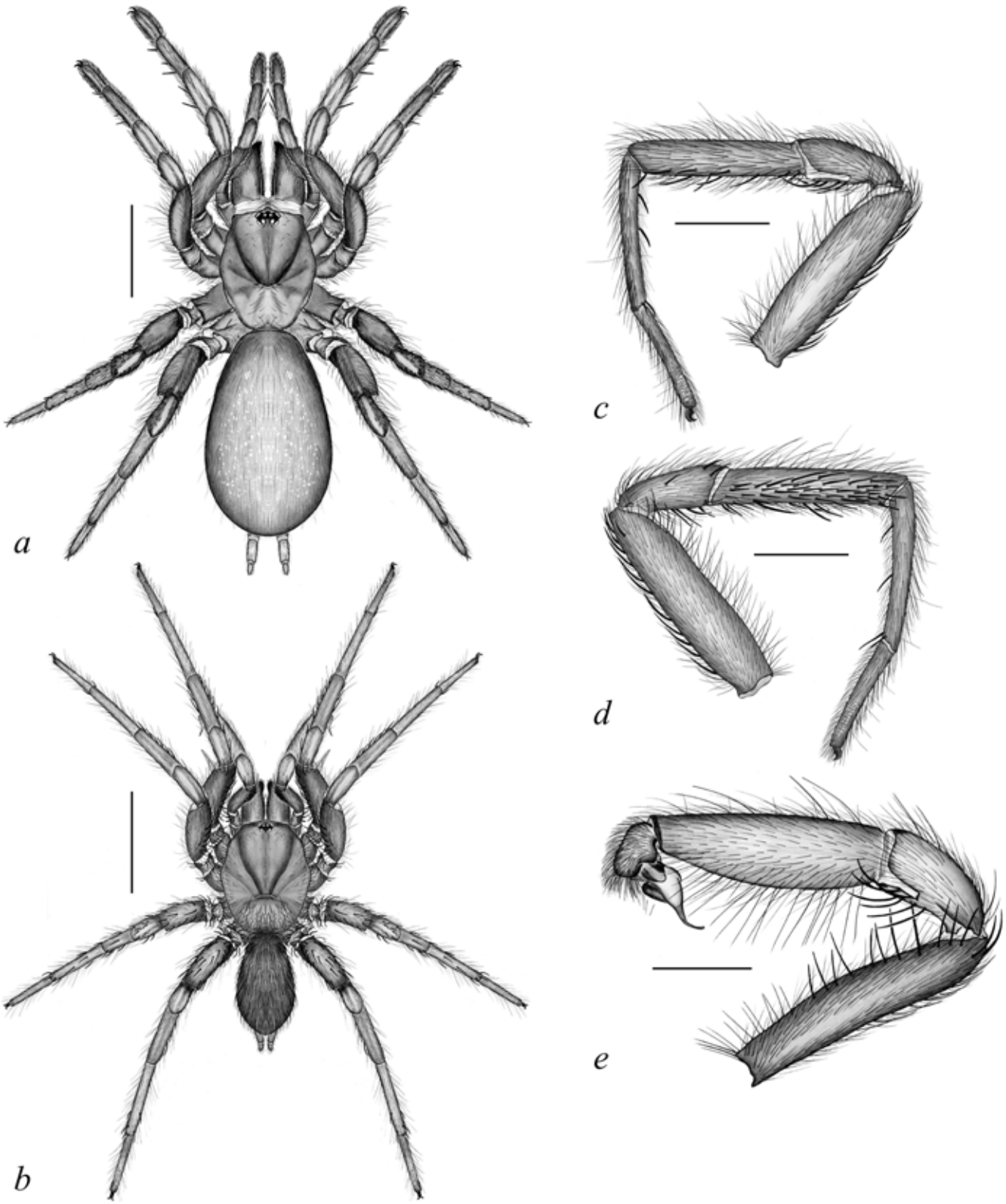

Fig. 5. *Apomastus schlingeri* Bond & Opell. *a*, Habitus of female paratype; *b–e*, male holotype: *b*, habitus; *c*, leg I retrolateral aspect; *d*, leg I prolateral aspect; *e*, pedipalp retrolateral aspect. *a,b* Scale bars = 5 mm; *c,d* scale bars = 2 mm; *e* scale bar = 1 mm.

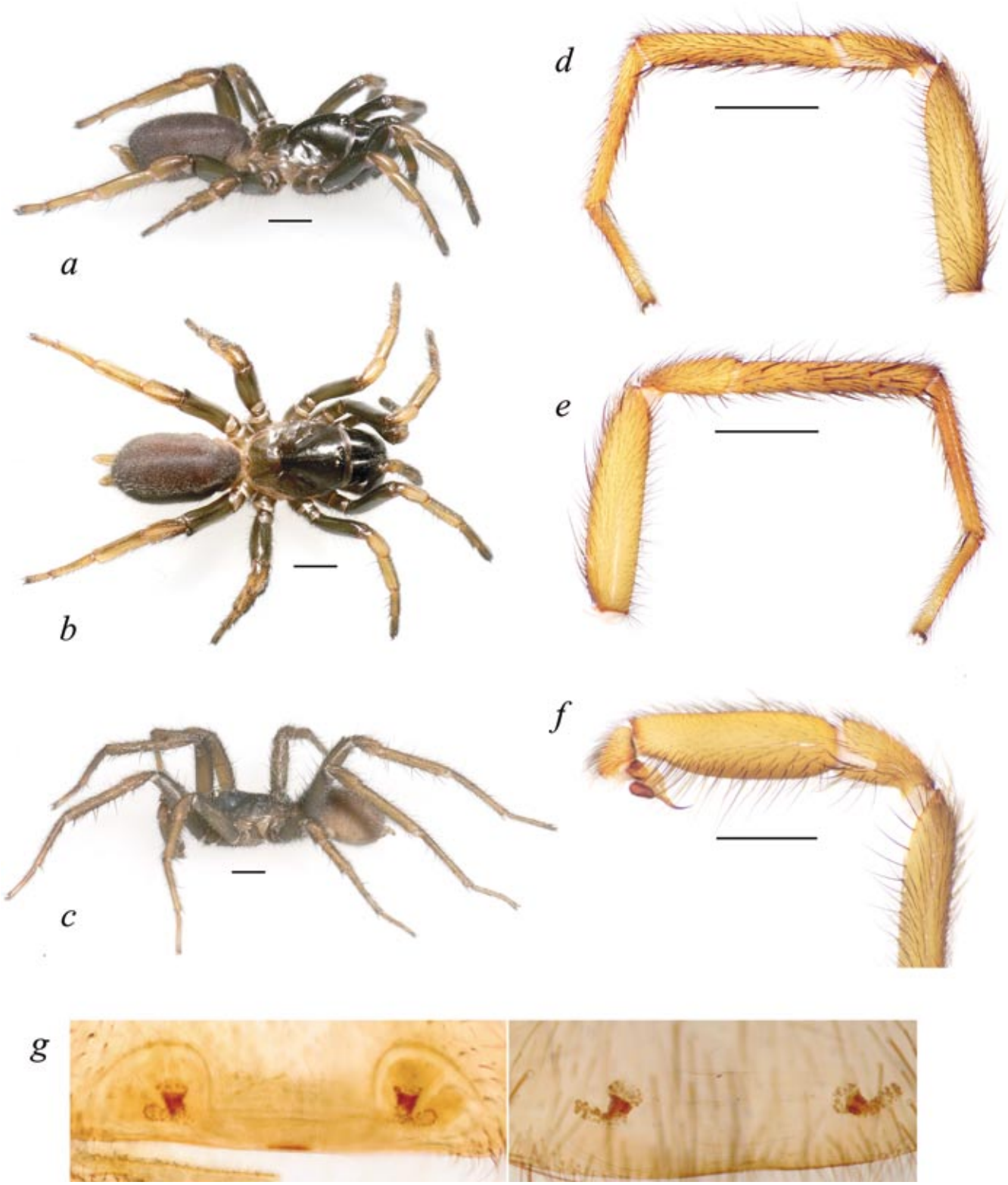

Fig. 6. *a,b*, Live *Apomastus kristenae*, sp. nov. from Laguna Beach locality (LNG); *c*, live topotypic male *A. kristenae*; *d–f*, male holotype *A. kristenae*: *d*, leg I retrolateral aspect; *e*, leg I prolateral aspect; *f*, pedipalp retrolateral aspect; *g*, *A. schlingeri* spermathecal bulbs: left panel specimen from Baldwin Hills (BDH), right panel female paratype.

brown, abdomen dark chestnut brown. CL 5.30–7.90 (6.14); STRl 3.25–4.65 (3.74), STRw 2.60–3.70 (2.98); LBw 0.93–1.28 (1.08), LBl 0.60–0.95 (0.72); anterior cheliceral teeth 6–11 (9), endite cuspules 55–100 (78), rastellar spines 7–9 (8); leg I: 4.20–5.55 (4.76), 2.33–3.30 (2.82), 2.55–4.35 (3.29), 2.00–3.15 (2.59), 1.40–2.10 (1.70); leg IV: 4.00–5.30 (4.53), 2.48–3.50 (2.84), 3.50–4.75 (3.97), 2.96–4.05 (3.53), 1.48–2.10 (1.73); PT3s 9–24 (18), TB3s 2–6 (4).

Distribution

Los Angeles County, California, USA (Fig. 1).

Apomastus kristenae, sp. nov.

(Figs 6–8)

Material examined

Holotype. ♂, USA, California, Riverside County, 1.8 miles west of Lake Mathews, just south of Cajalco Expressway, Cajalco Canyon, 305 m, 33.82990°N 117.47880°W, coll. W. Icenogle, 21. i.1999 (CAS, AS_118).

Paratypes. ♂, data as for holotype (FMNH, AS_78); 2♀, data as for holotype, coll. J. Bond, 20.i.2001 (CAS, AS_81; FMNH AS_79).

Other material examined. **USA:** California: Los Angeles County: 1♀, Chantry Flats, 823 m; 1♀, San Antonio Canyon; 2♀, Tanbark Flats; 6♀, Evey Canyon; 1♀, Santa Ynez Canyon Park; 6♀, Monrovia Canyon County Park. Orange County: 1♀, west of Laguna Beach, end of Canyon Acres Rd, 39 m; 22♀, Salt Creek 1.5 miles north of Dana Point; 1♂, 8♀, Cleveland National Forest, off Hwy 74 at San Juan Fire Station, Hot Springs Canyon; 8♀, 2♂, Ortega Hwy (Hwy 74), ~2 miles from Riverside Co. Line. Riverside County: 8♀, 11♂, 1.8 (road) miles west of Lake Mathews, just south of Cajalco Expressway and Cajalco Canyon. All material in AMNH and CAS.

Diagnosis

This species can be distinguished from *A. schlingeri* on the basis of the following unique combination of mtDNA *COI* gene nucleotide site substitutions (Fig. 7): A (152), T (178), A (262), G (610), R (769). Male leg I with unique pattern of spines on tibial surfaces (Fig. 8), prolateral surface, tibia I with few spines (TSP <10). *Apomastus kristenae*, sp. nov. females cannot at this time be distinguished from *A. schlingeri* females using morphology.

Description of male holotype

Specimen preparation and condition. Specimen collected in live pitfall trap, preserved in 85% ethanol. Colouration appears to have only faded slightly. Pedipalp, leg I left side removed and stored in vial with specimen, legs III, IV right side removed and stored in RNAlater (Qiagen) at –80°C. *Colour.* Carapace and chelicerae reddish-brown, legs lighter in colour. Abdomen uniform dark chestnut-brown dorsally, ventrum and spinnerets dark yellow-brown (Fig. 6*c*). *Cephalothorax*. Carapace 4.35 long, 3.60 wide, hirsute, only a few fine white setae intermingled with more stout black bristles; surface smooth, pars cephalica elevated slightly. Fringe and posterior margin with black bristles. Thoracic groove deep, moderately recurved. Eyes on very low tubercle. AER slightly procurved, PER slightly recurved. PME–AME subequal in diameter. Sternum widest at coxae II/III, moderately setose. STRl 2.50, STRw 1.90. Sternal sigilla small, oval, marginal. Chelicerae with distinct outer tooth row (eight teeth) with inner margin denticle patch. Palpal endites and labium lack cuspules, LBw 0.58,

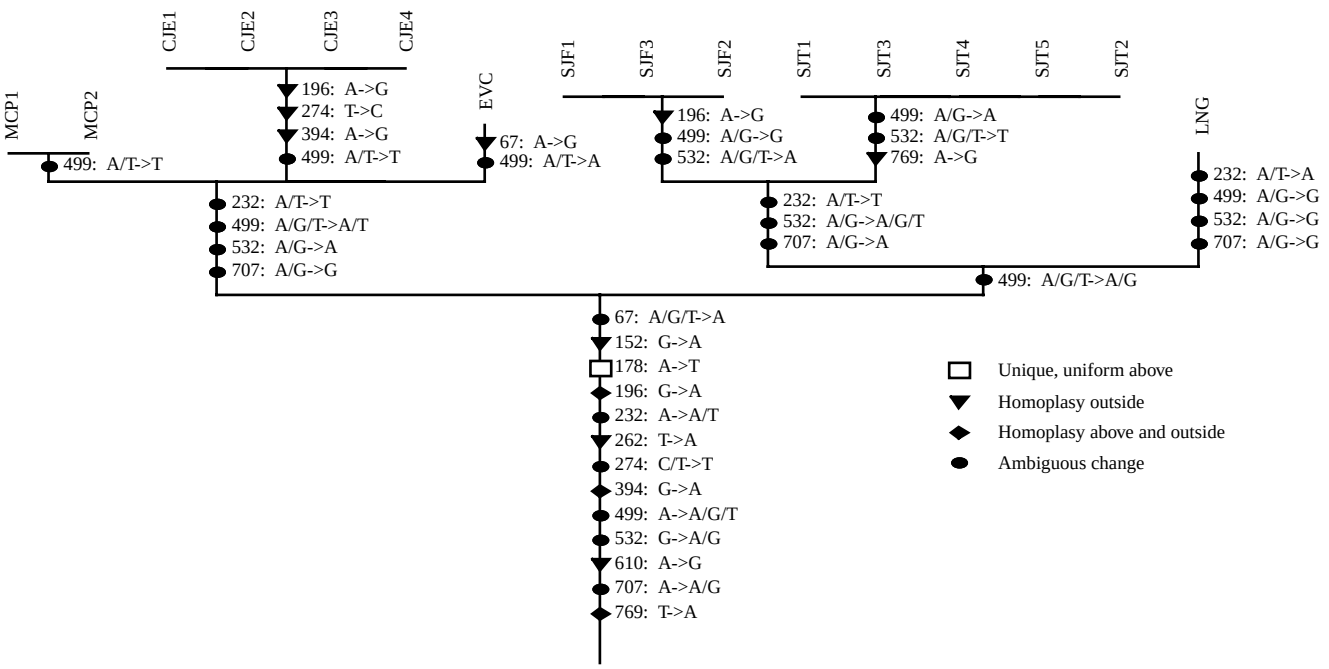

Fig. 7. Character transformations for *Apomastus kristenae*, sp. nov. diagnostic nucleotide site substitutions using ACCTRAN optimisation.

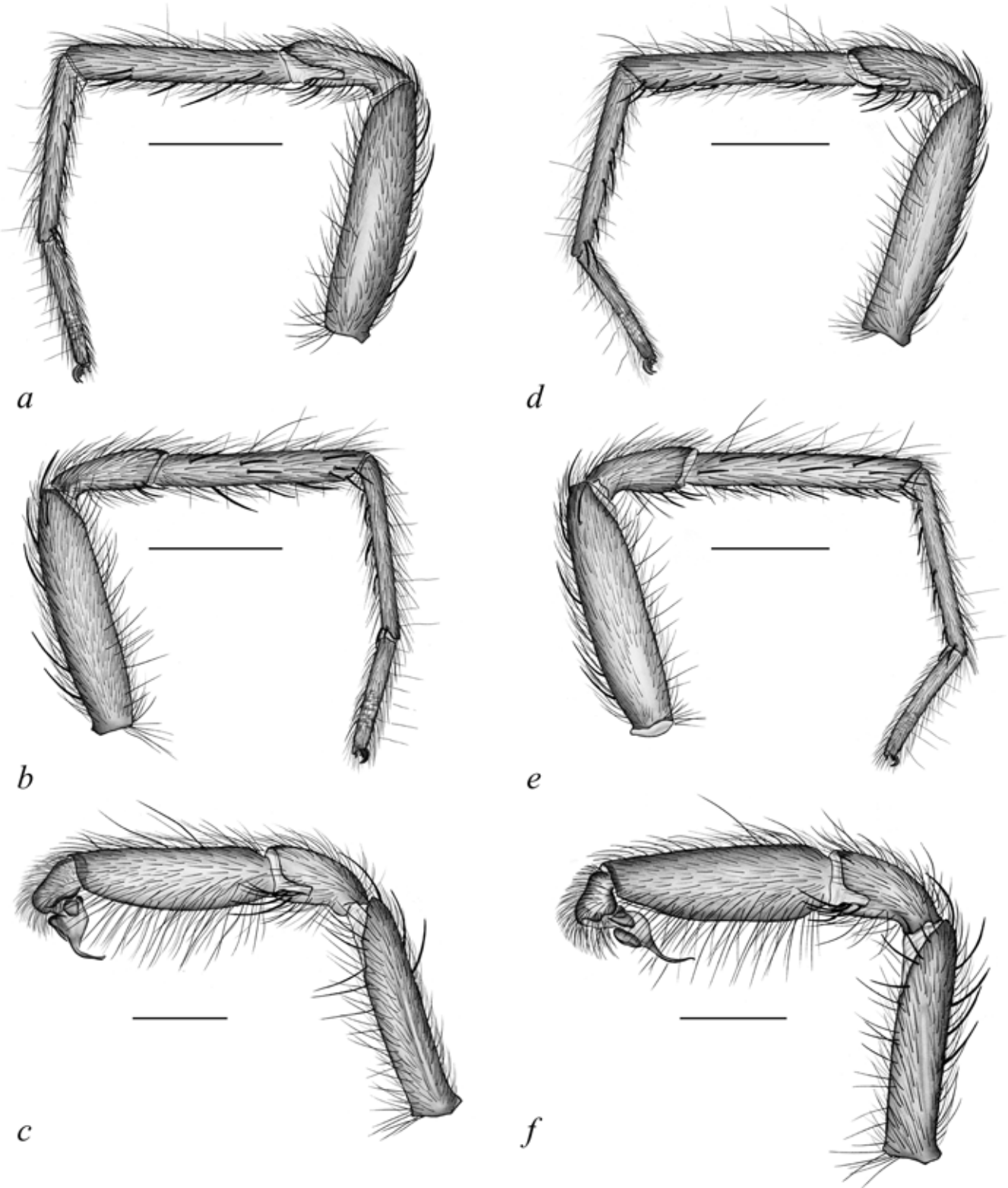

Fig. 8. *Apomastus kristenae*, sp. nov. *a–c*, Specimen AS-120 from Cleveland National Forest locality (CLF): *a*, leg I retrolateral aspect; *b*, leg I prolateral aspect; *c*, pedipalp retrolateral aspect. *d–e*, Male holotype: *d*, leg I retrolateral aspect; *e*, leg I prolateral aspect; *f*, pedipalp retrolateral aspect. *a–b*,*d–e* Scale bars = 2 mm; *c*,*f* scale bars = 1 mm.

LBl 0.39. Rastellum consists of five very stout spines, not on a mound. *Abdomen.* Setose, heavier black setae intermingled with fine white setae. Triangular patch of epiandrous fusillae distinct from surrounding setae (wide bases, stout in stature). PLS all three segments with spigots. Terminal segment 2/3 length of medial segment, two enlarged spigots visible at tip. PMS single segment, with spigots, short with rounded terminus. *Walking legs*. Slender, distal 2/3 of each tarsus pseudosegmented. Tarsus III, IV slightly curved distally. Leg I: 4.45, 2.10, 3.72, 3.20, 2.08; leg IV: 4.45, 1.98, 4.36, 4.08, 2.51. Tarsal scopulae of intermediate density, leg I, lighter on all other tarsi. Tarsus I with single, slightly staggered row of nine trichobothria. Leg I spination pattern illustrated in Figs 6*d,e* and 8*a,b,d,e*; TSp 7, TSr 8, TSrd absent. *Pedipalp*. Articles slender, lacking distinct spines (Figs 6*f* and 8*c,f*). PTw 0.73, PTl 2.10, Bl 0.80. Embolus with slight mid–distal curvature, moderately stout.

Variation (*n* = 5). CL 3.96–4.60 (4.31); STRl 2.27–2.51 (2.41), STRw 1.78–1.95 (1.87); LBw 0.51–0.65 (0.57), LBl 0.37–0.42 (0.39); leg I: 3.80–4.55 (4.24), 1.86–2.14 (2.01), 3.16–3.72 (3.43), 2.57–3.20 (2.95), 1.75–2.08 (1.95); leg IV: 3.64–4.45 (4.16), 1.85–2.01 (1.95), 3.60–4.36 (4.09), 3.60–4.08 (3.90), 1.97–2.60 (2.28); PTw 0.63–0.73 (0.68), PTl 1.84–2.12 (2.02), BL 0.70–0.83 (0.78); TSp 7–10 (8), TSr 4–8 (5).

Description of female paratype (AS_81)

Specimen preparation and condition. Female collected from burrow, prepared in same manner as male holotype. Genital plate removed and stored in microvial with specimen. Legs III, IV right side removed and stored in RNAlater (Qiagen) at –80°C, housed in the Department of Biology, East Carolina University, Greenville, NC, USA. *Colour.* Carapace and chelicerae dark tannish-brown, legs lighter in colour. Abdomen uniform light chestnut-brown dorsally, ventrum and spinnerets yellow-brown (Fig. 6*a,b*). *Cephalothorax*. Carapace 5.50 long, 4.35 wide, glabrous; generally smooth surface, pars cephalica moderately elevated with thin row of mesal black setae. Fringe lacks setae. Thoracic groove deep, slightly procurved. Eye group slightly elevated on low tubercle. AER slightly procurved, PER slightly recurved. PME slightly larger in diameter than AME. Sternum widest at coxae II/III, moderately setose. STRl 3.32, STRw 2.51. Three pairs of sternal sigilla anterior pairs small, oval, marginal, posterior pair slightly larger, oval, more mesially positioned. Chelicerae with distinct outer tooth row (eight teeth) with inner margin denticle patch. Palpal endites with numerous cuspules (~65) concentrated at the inner (promargin) posterior heel; labium lacks cuspules, LBw 0.93, LBl 0.68. Rastellum consists of eight very stout anterior margin spines, not on a mound. *Abdomen.* Moderately setose. PLS all three segments with spigots. Terminal segment 2/3 length of medial segment, two enlarged spigots visible at tip. PMS single segment, with spigots, short with rounded terminus. *Walking legs*. Anterior two pairs noticeably more slender than posterior pairs. Leg I: 4.16, 2.36, 2.80, 1.86, 1.30; leg IV: 3.75, 2.40, 3.41, 2.82, 1.50. Tarsus I with zigzag row of 12 trichobothria. Legs I and II with moderately heavy tarsal and metatarsal scopulae of intermediate density, legs III and IV light scopulae tarsi only. PT3s 17, TB3s 2. Distinct preening comb on retrolateral distal surface (at tarsus–metatarsus joint) of metatarsus IV, rudimentary preening comb, same position metatarsus III. *Spermathecae*. Identical to that illustrated for *A. schlingeri* (Fig. 6*g*; Bond and Opell 2002, fig. 17D). Two simple spermathecal bulbs, arranged in a transverse row, consisting of a moderately sclerotized stalk and an enlarged apical bulb, lightly sclerotized; base of stalk slightly enlarged such that it takes on the appearance of a smaller, reduced secondary bulb.

Variation (*n* = 10). CL 5.10–6.80 (5.66); STRl 2.95–4.65 (3.55), STRw 2.38–3.40 (2.73); LBw 0.87–1.19 (1.02), LBl 0.59–0.75 (0.67); anterior cheliceral teeth 6–11 (9), endite cuspules 55–100 (78), rastellar spines 8–10 (8); leg I: 3.76–5.05 (4.27), 2.17–3.00 (2.48), 2.48–3.36 (2.88), 1.86–2.48 (2.16), 1.30–1.66 (1.44); leg IV: 3.50–4.85 (4.05), 2.36–3.00 (2.58), 3.16–3.90 (3.62), 2.73–3.50 (3.09), 1.40–1.87 (1.54); PT3s 13–20 (16), TB3s 2–4 (3).

Distribution

Eastern Los Angeles, western Riverside, and Orange County, California, USA (Fig. 1).

Etymology

The specific epithet is a patronym named for my wife and partner Kristen DeVos Bond in expression of my gratitude for all of her love, help, and support over the past 10 years and in honour of the birth of our daughter Elisabeth Morgen Bond (21 September 2003).

Acknowledgments

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