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Phylogeny of *Habronattus* jumping spiders (Araneae: Salticidae), with consideration of genitalic and courtship evolution

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Abstract. DNA sequences from the mitochondrial (including ND1, 16S) and nuclear (EF-1α) genomes of about ninety-four species were obtained to reconstruct phylogenetic relationships of *Habronattus* jumping spiders. Maximum parsimony trees were sought with both separate (mitochondrial, nuclear) and combined analyses; maximum likelihood trees were sought with both separate (ND1, 16S, EF-1α introns, EF-1α exons) and combined (mitochondrial, nuclear) analyses. All analyses agreed on some fundamental aspects of the tree, including the monophyly of the previously recognized *agilis*, *amicus*, *dorotheae* and *americanus* species groups. The deep phylogenetic structure is well resolved, placing the *agilis*, *amicus*, *tranquillus* and *dorotheae* groups basally. Several other previously unrecognized clades were well supported, including a newly formulated *decorus* group. The large group of species with modified male first and third legs was supported as monophyletic except for the surprising placement elsewhere of three species of the group. The phenotypic similarities between these three and the others are so detailed and precise that convergence in ornamentation can probably be ruled out. There are hints of phylogenetically distant genetic introgression involving the *coecatus* group. The combination *Habronattus paratus* is restored based on the species falling within *Habronattus*. Regarding patterns of character evolution, there was consistent support for the basal placement of several species groups with a long embolus, suggesting that there were more evolutionary reductions in embolus length than postulated in a previous morphological phylogeny. This is in accord with the expectation that there is a bias to an overly conservative interpretation of a character's evolution if it is interpreted on a phylogeny based in part on that same character. In contrast, the molecular phylogeny did not suggest any instances of the evolutionary transformation of one complex style of courtship into another, a possibility that could have been difficult to detect using the morphological phylogeny because of the same bias to conservativism.

Introduction

Jumping spiders (Salticidae) have a high-resolution visual system (e.g. Jackson, 1982; Blest & Sigmund, 1984), which has enabled extensive use of visual cues during courtship behaviour. Some of the most elaborate male ornamentation and courtship behaviour are found in species assigned to *Habronattus* F.O. Pickard-Cambridge (Griswold, 1987; Peckham & Peckham, 1889, 1890; Maddison, 1995), a group of about 100 species living primarily in North America. Ornamentation includes integumental processes and fringes of setae, which to human eyes range through most colours of the rainbow. Males of many species follow an elaborate sequence of motions of body and appendages during courtship (Peckham & Peckham, 1889, 1890; Griswold, 1977; Richman, 1977, 1982;

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Cutler, 1988; Maddison & Stratton, 1988; Maddison & Hedin, unpublished).

Resolution of the phylogenetic relationships of these species would be an important prerequisite to interpreting the evolution of these diverse and complex behaviours. Griswold (1987) presented phylogenetic hypotheses for the species of *Habronattus* based primarily of characters of male morphology apparently related to courtship behaviour (i.e. 'ornaments'). His phylogeny, therefore, is based on characters closely related to those whose evolution we might seek to interpret. Although we maintain that there is no danger of circularity in using this phylogeny, there is the danger that a courtship-based phylogeny will be biased toward suggesting an overly conservative view of the evolution of courtship traits (Maddison & Maddison, 1992; de Queiroz, 1996). For example, a complex 'genre' or style of courtship, with multiple ornaments and behaviours, might evolve into a different style by loss of ancestral courtship features, then rebuilding to a new complex style. There are indications that wholesale loss of multiple ornaments has occurred, for instance in *H. borealis* (Griswold, 1987). [Authors of species names are given in Appendix 2.] However, phylogenetic analysis of such data might fail to detect that one style evolved from the other, and instead mistake the complex courtship styles as independent synapomorphies for two separate monophyletic groups. To best detect such processes, we need a phylogeny reconstructed from independent data such as sequences of functionally unrelated genes.

Griswold's (1987) phylogenetic hypotheses for the species of *Habronattus* was derived from various analyses (both phenetic and cladistic). Although the different analyses resulted in different phylogenetic trees, and no single tree was presented as the definitive proposal, his cladogram (his Figs 10 and 12–16) is an informative starting point for consideration. Griswold's cladogram shows *Habronattus* divided into four basal clades. Two (the *decorus* and *dorotheae* groups) are small. The third basal clade is large, including the *viridipes*, *coecatus* and *americanus* groups. The *viridipes* and *coecatus* groups are notable for having both the first and third legs modified in the males. The fourth basal clade, which is defined by the angular distance between embolus and tegular aphophysis of the male palpus (intromittent organ), includes the *pretiosus*, *agilis*, *amicus* and *tranquillus* groups, along with other species such as *H. tarascanus*, *H. delectus*, *H. pugillus*, *H. hallani* and *H. fallax*, that appear relatively isolated and of ambiguous placement.

Using molecular data from both mitochondrial and nuclear genomes (characters presumably independent from both genitalic and courtship traits), we sought to answer several questions. Are Griswold's species groups monophyletic? What is the deeper phylogenetic structure of *Habronattus*? Can the isolated species be resolved into groups? Are there any cases in which a species group with one complex style of courtship has evolved from within a species group with another style? What does the molecular phylogeny suggest about trends in the evolution of the male genitalia?

Methods

Sampling of taxa

Our goal was to sample all known species of *Habronattus*; we sampled all major species groups and most, but not all, species. The species that we failed to sample appear to be scattered throughout the group phylogenetically. For a few species we sampled several individuals, sometimes representing phenotypically distinct populations. Although we would have preferred to sample multiple individuals and populations of each species to test for incomplete lineage sorting, we felt that it was more important at this stage to focus our efforts on sampling as many species as possible. Analyses based on more intense sampling within some species groups, e.g. as done for the *H. pugillis* complex by Masta (2000a), are planned for future papers.

Griswold (1987) used several characters, including relative leg length and genitalic structure, to support the monophyly of *Habronattus*. We used Griswold's concept of *Habronattus* to delimit this study, except that we also included *Pellenes paratus*, because of its possession of an apparent synapomorphy of *Habronattus* (an elbowed tegular apophysis). Recent study of molecular phylogeny of salticids (Hedin & Maddison, unpublished) confirms that appropriate outgroup taxa for *Habronattus* can be found among *Pellenes* Simon and its relatives. *Pellenes*, which includes several species groups in the Nearctic Region and an apparently greater diversity in the Old World (e.g. Logunov *et al*., 1999), has long been considered to be related to *Habronattus* (Peckham & Peckham, 1909; Proszynski, 1976). The molecular data suggest that, among our sampled species, those of *Pellenes* are most closely related to *Habronattus*, with the Hawaiian *Havaika* Proszynski (formerly '*Sandalodes*'; Proszynski, 2001) next closest, and *Sibianor* Logunov next. For the study reported here, we used *Havaika* (one species) and *Pellenes* (four species) as outgroup taxa. Other groups that have been suggested to be closely related to *Habronattus*, such as *Evarcha* and *Maevia*, are considerably more distantly related (Maddison, 1996; Hedin & Maddison, unpublished).

We sampled about ninety species. The precise number is difficult to gauge, given difficulties in delimiting species. The specimens analysed might be divided into three groups: (1) those apparently belonging to undescribed species; (2) those belonging to novel geographical forms that might represent new species; and (3) those conforming to currently recognized species. For those of the first category, we use code names pending a revision of the species. The code names take the form '*Habronattus* sp. (CODE)' where CODE is a distinctive combination of letters. Those of the second category are named using 'cf.' in front of the species epithet of the most similar described species to indicate that they are distinctive and may represent new species. Those of the third category appear to belong to described species (following hypotheses of Griswold, 1987), although in some cases the species limits were considered problematic due to considerable geographical variation. In at least some

taxa, such as *H. ustulatus*, *H. tarsalis* and *H. sansoni*, it appears likely that variants will eventually be recognized as separate species (as considered and discussed by Griswold, 1987). To clarify to what forms our specimens belong, brief descriptions of the phenotypic characteristics of those specimens belonging to undescribed or otherwise problematic species are given in Appendix 1.

Appendix 2 lists the taxa sampled. Species therein are grouped into species groups, not to prejudge the results but for ease of use of the table. Undescribed species are placed provisionally to species group when their phenotypic characteristics closely resemble described species of the group.

Gene sequencing and alignment

We gathered data from both mitochondrial and nuclear genomes, using considerations of prior phylogenetic utility to help guide our choice of gene region. The regions utilized included an approximately 1 kb fragment of the mitochondrial genome, spanning the 3′ end of 16S to the middle of NADH dehydrogenase subunit 1 (ND1), with an intervening $tRNA^{Leu(CUN)}$. This region (or parts thereof) has been used to resolve species-level phylogenetic problems in other spiders (Hedin, 1997), and was used to resolve phylogeographical patterns in the *H. pugillis* complex (Masta, 2000a). Molecular evolutionary properties of this gene region are presented in Masta (2000b) and Hedin & Maddison (2001a). The nuclear region sequenced included a portion of the EF-1α gene comprising two partial exons, one entire exon and two entire introns. We previously reported on the phylogenetic utility and molecular evolutionary dynamics of this region in a smaller sample of *Habronattus* species (Hedin & Maddison, 2001b). Sequence data for an additional forty-seven taxa are reported here for the first time (see Appendix 2). Sequence data are deposited in GenBank. Aligned sequences for all taxa (in NEXUS file format) are available from the first author, and can be retrieved at: http://www.salticidae.org/papers/MaddisonHedinHabro01/MaddisonHedinHabro01.zip

Extraction of genomic DNA followed protocols cited in Hedin & Maddison (2001a). Voucher specimens are preserved in alcohol at −80 °C in the personal collection of W.P.M. Alcohol-resistant labels with unique voucher numbers (see Appendix 2) are included with all voucher specimens. Mitochondrial fragments were PCR amplified, purified, sequenced, compiled and edited using strategies described in Hedin & Maddison (2001a). Mitochondrial sequences were determined only for the 'N' strand (using the 'N' primers listed in Hedin & Maddison, 2001a), with up to 60% sequence overlap. The EF-1α gene includes multiple copies in *Habronattus* (Hedin & Maddison, 2001b). We used polyacrylamide gels (see Sambrook *et al.*, 1989) to separate paralogs that differ in size (due to intron presence or absence), and focused on the 'with intron' copy (see Hedin & Maddison, 2001b). Otherwise, the EF-1α data were PCR amplified, sequenced, compiled and edited using strategies described in Hedin & Maddison (2001b). EF-1α sites including two peaks of equivalent intensity were interpreted as heterozygosity, and entered into the phylogenetic matrix using IUPAC ambiguity codes.

Variation in length indicated that alignment of sequences was needed for the non-coding portion of the mitochondrial sequences, and the introns of EF-1α. The alignment was done manually using MacClade 4 (Maddison & Maddison, 2000), and appeared straightforward.

Phylogenetic analysis

All searches to find most parsimonious and maximum likelihood trees used PAUP* (Swofford, 2000, 2001a,b) on Macintosh G4 computers. Version 4.0b4a was used for the basic maximum likelihood searches and the parsimony search with mitochondrial sequences; version 4.0b7 for the bootstrap analyses; version 4.0b8 for the parsimony search with EF-1α, the searches constraining the *viridipes* group monophyletic, and the analyses involved in parametric bootstrapping. Our descriptions of methods use the command terminology of PAUP* (Swofford, 2000, 2001a,b).

Parsimony. The unordered states assumption was used (Fitch, 1971). Gaps were treated as missing data. Sites were weighted equally. The search started with 20 000 random addition sequence replicates, each saving at most five trees in each replicate to narrow the search (D. Maddison, 1991), using TBR branch swapping (Swofford, 2000). The most parsimonious trees found were used as input trees into a second round of TBR branch swapping, unconstrained except by MAXTREES of 100 000. Replicability of clades was assessed by a non-parametric bootstrap analysis (Felsenstein, 1985) with 500 replicates, each starting with simple addition sequence, followed by TBR branch swapping holding no more than 1000 trees.

Mitochondrial and nuclear data were analysed together and separately. The separate mitochondrial analyses included data for 133 taxa; separate nuclear analyses included data for 101 taxa (see Appendix 2). The combined mitochondrial and nuclear dataset included nine composite taxa representing the combination of sequences from more than one individual (one with nuclear, one with mitochondrial data). Composite taxa included *H. ustulatus* specimen #210 plus #211, *H. geronimoi* #60 + #61, *H.* sp. (YUCUN) #635 + #636, *H. pugillis* #08 + #459, *H.* cf. *calcaratus* (Ft. Stockton) #496 + #546, *H. orbus* #27 + #101, *H. moratus* #484 + #485, *H. forticulus* #289 + #290 and *H. anepsius* #282 + #314. In all but two cases, the composite data were derived from individuals collected at the same geographical location (see Appendix 2). Remaining taxa not represented by both mitochondrial and nuclear data were excluded, resulting in a combined matrix including ninety-nine taxa. This combined analysis disregarded the possibility of processes, such as incomplete lineage sorting or differential introgression, that would yield differing genetic histories of unlinked genes (de Queiroz *et al.*, 1996).

Likelihood. Because of the excessive computational difficulty of simultaneously estimating parameters of sequence evolution and searching for maximum likelihood trees, we first estimated parameters under candidate trees then used those parameters for tree searches (Swofford *et al.*, 1996; Maddison *et al.*, 1999). A first candidate tree was obtained by neighbour joining under the assumptions of HKY85 distances, empirical base frequencies and gamma-distributed rate variation. This tree was used to estimate the six parameters of a GTR model using likelihood, and this model was used to obtain a second, refined candidate tree using neighbour joining with ML distances. This refined candidate tree was used to assess likelihood of various parameter combinations, from simpler to more complex rate matrix models, and from simpler to more complex rate variation models. This was done to choose a model of evolution for use in the full likelihood tree search. Included among the rate matrix models examined was a five-parameter rate matrix model (rclass = (a b a c d e)), because preliminary analyses suggested that this model may fit nearly as well as the full six-parameter model for several datasets. For 16S, the whole mitochondrial sequence, and EF-1α introns, the most complex site-to-site rate variation model examined involved gamma rate variation and a proportion of invariant sites: for ND1, the entire EF-1α sequence and for EF-1α exons, the most complex rate variation model examined involved the site-specific rate variation by codon position. The model chosen corresponded to the simplest model not significantly different from the most complex model. Although the HKY85 model could be rejected against the GTR model at $P = 0.05$ if its –ln likelihood were 9.49 greater (Chi-square with 3 d.f.; Goldman, 1993; Sullivan & Swofford, 1997), we erred slightly on the side of more complex models by rejecting the simpler model if the difference in –ln likelihoods exceeded 4.0. Once the model was chosen, it was used in a search starting with a random addition sequence followed by SPR branch swapping. One to several searches (random addition sequence replicates) were conducted.

For both the mitochondrial and nuclear datasets, three likelihood analyses were conducted (two subsets separate plus the entire dataset). Analyses were conducted on the entire mitochondrial sequence excluding sites 533–536 (see comments under 'Results'), on the 16S gene and on the ND1 gene. Analyses were conducted on the entire EF-1α sequence, on the introns only and on the exons only. Ten taxa were deleted from the exons-only analyses, as they had the same sequences as other included taxa.

Parametric bootstrapping. Parametric bootstrapping (see Huelsenbeck *et al.*, 1996) was used to explore an unexpected placement of three species of the *viridipes* group. For both the mitochondrial and the EF-1α data, a likelihood estimation of parameter values was conducted as described above, followed by neighbour joining using ML distances with those parameters. All of these procedures constrained the *viridipes* group monophyletic and used a gamma rate variation model. The resulting tree was used as a model tree on which datasets were simulated using the Genesis package (Maddison & Maddison, 2001b) of the Mesquite system (Maddison & Maddison, 2001a). The parameters of the simulation process were those estimated (for EF-1α, 2 parameter rate matrix, tratio = 1.795738, codon position specific rates = 2.240166:noncoding, 0.041499:pos1, 0.006119:pos2, 1.088233:pos3; empirical equilibrium and root frequencies; for mitochondrial, five-parameter rate matrix, rclass = (a b a c d e) rmatrix = (7.5062689 30.777031 7.5062689 1.7307205 130.21719), gamma shape = 0.885141, proportion invariant = 0.546136, empirical equilibrium and root frequencies). One hundred matrices of the same number of sites as in the original matrices were generated for both mtDNA and EF-1α. Parsimony searches and neighbour joining were performed on each of the simulated data files (parsimony: five random addition sequence replicates, each constrained to keep no more than 100 trees; NJ for EF-1α: NJ using ML distances based on two-parameter rate matrix, gamma shape, and proportion invariant estimated on initial HKY85 NJ tree; NJ for mtDNA: NJ using ML distances based on five-parameter rate matrix, gamma shape and proportion invariant estimated on initial HKY85 NJ tree). Neighbour joining was used in expectation that it might approximate likelihood inference but require far less processing time.

Results

Mitochondrial data

Sequences. Mitochondrial sequences include outgroup data for both *Pellenes* and *Havaika*, plus 129 *Habronattus* sequences representing at least eighty-seven species (see Appendix 2 for GenBank accession numbers). Except for *Havaika* (no ND1 data), each sequenced mitochondrial fragment includes 574–579 bp of non-coding 16S (3′-end) plus $tRNA^{Leu(CUN)}$ (entire), and 414 bp of protein data from the 5′-end of ND1. Sites 533–536 at the 16S/tRNA junction were excluded in all analyses because they represent gaps in all but two taxa. Both the 16S and tRNA data can be folded into secondary structures consistent with models proposed specifically for *Habronattus* (Masta, 2000b) and other salticids (Hedin & Maddison, 2001a). The tRNA data are further consistent with Masta (2000b) in lacking the T¥C and variable arms, and in having apparently unstable amino-acyl stems.

The non-coding data are easily aligned by eye, requiring an aligned length of 587 bp. The distribution of sites which require indels for global alignment includes nine in proposed unpaired regions of 16S, three in the 3′ tRNA amino-acyl arm, and a 4-bp insertion in a spacer region separating 16S and tRNA sequences. Observed compensatory mutations were few, including seven 16S stem sites with clear non-independence; these sites were not treated in any special manner in phylogenetic analyses.

Phylogenetic analyses. Results of phylogenetic analyses with mitochondrial data are summarized in Figs 1 and 2. The initial parsimony search of the combined mitochondrial matrix with 20 000 random addition sequence replicates resulted in 628 trees of length 2314, found on 142 different replicates. Swapping on these resulted in 828 trees of length 2314; spots in Fig. 2 show most of the clades appearing in the strict consensus. That this represents a single TBR island (D. R. Maddison (1991)) was confirmed by a separate search that found all 828 on a single swapping replicate. Many of the 'tip clades' were found in a high proportion of non-parametric bootstrap replicates, although support deeper in the tree is generally weak (Fig. 2).

The model estimated by maximum likelihood in each analysis was the five-parameter rate matrix model (a b a c d e) along with site-to-site rate variation (either gamma + pinvar or codon position specific rates) (see Table 1). Allowing rate variation increased the likelihood substantially (–ln likelihood decreased by 700–2000). Increasing the number of parameters in the rate matrix increased the likelihood, but the difference between the five-parameter and full six-parameter model (see Swofford *et al*., 1996) was small (difference in –ln likelihood less than 1.5 in all cases). The parameter estimates are for the entire mitochondrial sequence: rmatrix = (7.5175335 30.724541 7.5175335 1.7415471 131.08292), rates = gamma, shape = 0.886710, pinvar = 0.546911; for 16S alone: rmatrix = (1353.7156 2059.5561 1353.7156 358.04901 13760.371), rates = gamma, shape = 0.505327, pinvar = 0.494271; for ND1 alone: rmatrix = (2.2495679 16.058415 2.2495679 0.58976979 52.95068), rates = 0.420875 for codon position 1, 0.028546 for position 2, 2.550579, for position 3.

Likelihood searches involved a varied number of completed random addition sequence replicates, depending on the speed of the computation. Three random addition sequence replicates of likelihood searches were completed for the entire mitochondrial sequence. Two resulted in the same tree with –ln likelihood 12023.383 (Fig. 2); the third resulted in a tree with –ln likelihood 12029.133. A likelihood search using 16S data was attempted with random addition sequences and SPR swapping. No replicate completed in 366 hours of searching (224 091 rearrangements tried), but the last score of 4468.1365 persisted over the last 14 000 rearrangements, resulting in two trees summarized by the symbols in Fig. 1. Seven random addition sequence replicates of likelihood searches were completed for the ND1 sequence alone, resulting in trees with −ln likelihoods of 6806.2980 (four trees), 6806.4571 (six trees), 6808.3019 (four trees), 6809.2852 (four trees), 6810.1007 (two trees), 6814.5592 (one tree), 6815.0649 (four trees). The two best replicates (ten trees) were combined to yield a consensus tree summarized by the symbols in Fig. 1.

EF-1α data

Sequences. New EF-1α sequences were gathered for forty-seven spiders (see Appendix 2 for GenBank accession numbers), and added to those sequences already reported by Hedin & Maddison (2001b). The complete matrix included data representing at least seventy-one *Habronattus* species plus outgroup taxa. This sample lacks some *Habronattus* species included in the mitochondrial dataset, among them representatives of the *tranquillus* group. All sequences include exon data coding for 159 amino acids, and two complete intervening introns.

Phylogenetic analyses. Results of phylogenetic analyses with EF-1α data are summarized in Figs 1 and 3. The parsimony searches over the entire EF-1α found shortest trees of 547 steps. The initial search with 20 000 random addition sequence replicates resulted in 46 220 trees. Swapping on these resulted in 100 000 trees (the MAXTREES setting used) of length 547; spots in Fig. 3 show most of the clades in the strict consensus. Non-parametric bootstrap analysis supported many clades, including a deep subdivision separating *amicus* and *agilis* group members from all other *Habronattus* (Fig. 3).

The model estimated by maximum likelihood in the entire EF-1α analysis was the HKY85 model with codon-position specific rates; that in the separate exon and intron analysis was the five-parameter rate matrix model (a b a c d e) along with site-to-site rate variation (gamma + pinvar and codon position specific rates, respectively) (see Table 2). Allowing rate variation increased the likelihood substantially (–ln likelihood decreased by 15–300). Increasing the number of parameters in the rate matrix increased the likelihood, but the difference between the model chosen and the full six-parameter model was small (difference in –ln likelihood less than 2.5 in all cases).

Likelihood searches involved a varied number of completed random addition sequence replicates, depending on the speed of the computation. Eleven random addition sequence searches were completed for the entire EF-1α sequence, all yielding trees with –ln likelihood of 4166.4706. The eleven replicates found a total of sixty-two trees, but this included many duplicates, and only nineteen distinct trees were found. Figure 3 shows the first MLE tree; the consensus of all nineteen shows the exact same topology. For the exons-only analysis, no random addition sequence replicate achieved completion. The search was stopped after 100 trees were found (>2 000 000 rearrangements tried), with –ln likelihood of 1616.8166. Their strict consensus is summarized by the symbols in Fig. 1. For the introns-only analysis, five random addition sequence replicates were completed, all yielding trees with –ln likelihood of 2384.6182. Twenty-seven trees were found, but some were duplicates. The consensus tree of the twenty-four distinct topologies found is summarized by the symbols in Fig. 1.

Combined analysis

The mitochondrial plus nuclear sequence analysed together yielded 280 equally parsimonious trees of 2429 steps. Their strict consensus is shown in Fig. 4.

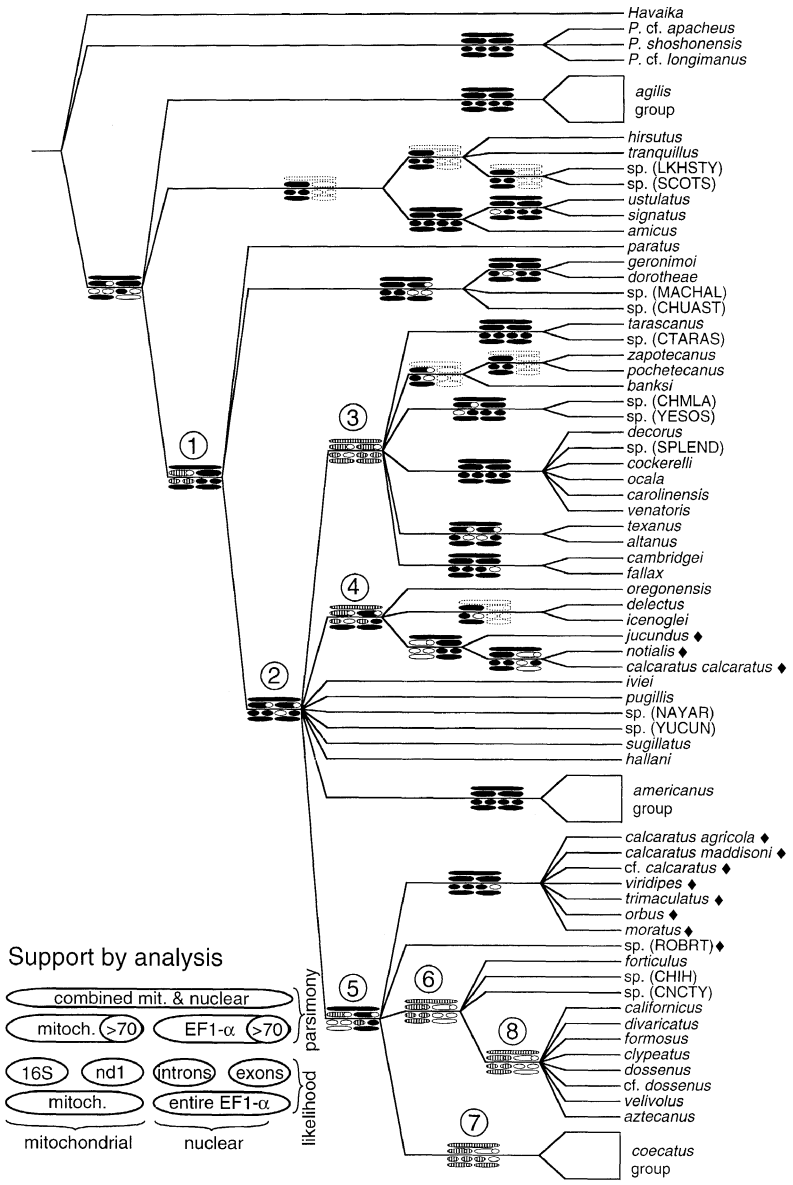

Fig. 1. Summary of results of phylogenetic analyses. On the branches are symbols indicating support by different analyses, as explained at lower left. Each analysis is represented by an oval, which if filled indicates support for that branch by that analysis. The analyses are: above the branch, parsimony; below the branch, likelihood. Left, mitochondrial sequences; right, nuclear sequences. Data are partitioned hierarchically: 16S, ND1, EF-1α intron and EF-1α exon all separate (likelihood only); entire mitochondrial sequence and entire EF-1α (parsimony and likelihood); combined mitochondrial and nuclear sequence (parsimony only). For parsimony searches with the whole mitochondrial sequence and the EF-1α sequence, the larger oval represents the presence of the branch in a basic parsimony search and the smaller oval represents recovery of the branch in more than 70% of the bootstrap replicates. Shading with black indicates support for the clade as shown; shading with grey indicates that a clade with slightly different species composition was supported. These cases with different species composition are as follows. Clade 1: *H. paratus* excluded from clade in some mitochondrial analyses. Clade 3: *fallax* group excluded in combined, mitochondria parsimony, mitochondria likelihood; *americanus* group included in combined and all mitrochondrial analyses; *H. ivei* included in mitochondria likelihood; *H. tarascanus* and *H. banksi* excluded in combined; *H. pugillis*, *H.* sp. (NAYAR), *H.* sp. (YUCUN) and clade 4 included in EF-1α analyses. Clade 4: *H.* sp. (NAYAR) included in combined and introns likelihood; *H. c. calcaratus* and *H. notialis* excluded in 16S and mitochondria likelihood; mitochondria parsimony yields clade of *H. oregonensis* plus *H. jucundus* but not the others. Clade 5: *H. oregonensis* and *H. pugillis* included in mitochondria parsimony; *H. hallani* included in introns likelihood. Clade 6: one specimen of *H. velivolus* and one of *H.* sp. (CHIH) excluded. Clade 7: one specimen of *H. velivolus* included in combined and mitochondrial analyses; *H.* sp. (ROBRT) and one specimen of *H.* sp. (CHIH) included in mitochondrial analyses; *H. zebraneus* and one specimen of *H. virgulatus* excluded in EF-1α analyses. Clade 8: one specimen of *H. velivolus* excluded. Dotted outlines of ovals indicate that the data were not available. ♦ = *viridipes* group.

Fig. 2. Maximum likelihood tree obtained for the entire mitochondrial sequence, with indications of parsimony results. The parameters estimated (see Table 1) and used in the tree search were (using the notation of PAUP*, Swofford, 2000) rmatrix = (7.5175335 30.724541 7.5175335 1.7415471 131.08292) rates = gamma shape = 0.886710 pinvar = 0.546911. Spots at nodes indicate clades recovered in the strict consensus of 828 most parsimonious trees found. Numbers on branches indicate percent of 500 parsimony bootstrap replicates recovering clade (shown only if = 70%). ◆ = *viridipes* group; ▼ = *clypeatus* group.

Table 1. –ln Likelihoods calculated for various models of sequence evolution (mitochondrial sequences) on the model tree obtained as described in the text. In italics is the –ln likelihood of the model chosen for use in tree searches using likelihood. (A) mtDNA total.

	Equal rates	Gamma + pinvar
JC	15877.86150	13655.84836
F81	15994.44563	13688.77373
HKY85	14807.04023	12246.42782
a b a c d e		*12089.89303*
GTR	14338.12771	12089.88378

Table 1B. ND1.

	Equal rates	Codon pos rates
JC	9122.45043	8245.38311
F81	9213.31215	8267.52701
HKY85	8178.83975	7045.27741
a b a c d e		*6926.23735*
GTR	7991.64684	6925.79555

Table 1C. 16S.

	Equal rates	Gamma + pinvar
JC	5634.88765	4950.22219
F81	5597.57990	4875.91723
HKY85	5412.25000	4621.14845
a b a c d e		*4514.08542*
GTR	5251.91401	4513.90928

Discussion

Habronattus *phylogeny*

The various gene regions and analyses agree on many aspects of species relationships, as summarized in Fig. 1. Separate analyses (Figs 1–3) agree substantially with the combined data analysis (Fig. 4). Some of the well supported clades match those predicted from prior work. There is strong support for the monophyly of the group of included *Pellenes* species; however, because those species represent a small part of the diversity of *Pellenes*, the results do not necessarily suggest monophyly of *Pellenes* as a whole (Griswold, 1987). The monophyly of *Habronattus* is supported by parsimony and likelihood analyses of the entire mitochondrial sequence and likelihood analysis of EF-1α introns. This result is not without question, however, as various other analyses (especially on EF-1α) suggest non-monophyly. Until a broader sample of *Pellenes* species is obtained, we will treat *Habronattus* provisionally as monophyletic.

Many aspects of Griswold's (1987) cladogram are corroborated by our data. The monophyly of the *agilis*, *amicus*, *dorotheae* and *americanus* groups (Fig. 1) are supported by both mitochondrial and nuclear data using various analyses (see also Hedin & Maddison, 2001b). The *tranquillus* group, for which we have only mitochondrial data, is also well supported, as is its relationship with the *amicus* group. The clade consisting of the *viridipes* and *coecatus* groups (those species with modified first and third legs) is supported except for a surprising placement of a few species of the *viridipes* group (discussed below).

There is good concordance among analyses regarding the basal branching of *Habronattus*. Three of Griswold's species groups, the *agilis*, *amicus* and *tranquillus* groups, are consistently placed basally, by both EF-1α and mitochondrial analyses, leaving the remainder of species as a large clade (clade 1, Fig. 1). Griswold's proposed clade including the *pretiosus*, *agilis*, *amicus* and *tranquillus* groups is therefore not supported by our data. *Habronattus zapotecanus* and *H. pochetecanus* (of Griswold's *pretiosus* group) and other species belong instead to clade 1. Within clade 1, *H. paratus* and the *dorotheae* group are basal, with the remainder of the species forming clade 2. The resolution of these deep relationships within *Habronattus* promises to be of assistance to future phylogenetic work. However, in the current analyses we were unable to resolve well the basal relationships within clade 2 (Fig. 1).

The group consisting of *H. decorus*, *H.* sp. (SPLEND), *H. carolinensis*, *H. venatoris*, *H. ocala* and *H. cockerelli* was not anticipated in Griswold's work, but is strongly supported by both mitochondrial and EF-1α sequences with various analyses. We refer to the group as the *decorus* group, recognizing that its composition is different from the *decorus* group as recognized by Griswold (who included *H. decorus*, *H. cockerelli*, *H. banksi* and *H. sugillatus*), although he did hypothesize the relationship of *H. carolinensis*, *H. venatoris* and *H. ocala*. A second unanticipated but strongly supported group is the sister species pairing of *H. fallax* and *H. cambridgei* (which we will call the *fallax* group).

Several other novel phylogenetic placements are suggested by our data, although not uniformly supported by different gene regions and analyses. One is the association of *H. delectus* and *H. icenoglei*. A second is the grouping of *H. banksi*, *H. zapotecanus* and *H. pochetecanus*. These three species, the first of which was placed by Griswold (1987) in the *decorus* group and the other two in the *pretiosus* group, may in fact be recently diverged from one another: they appear to have parapatric distributions in Mexico, occupy similar microhabitats and may even hybridize (a population from Oaxaca, 15°43.64′N 96°44.85′W, appears to show a blend of phenotypes of *H. banksi* and *H. zapotecanus*). *Habronattus zapotecanus* and *H. banksi* males share a white clypeus divided in the centre by black.

The inclusion of *Pellenes paratus* in *Habronattus* is supported by our data, and hence we return the species formally to *Habronattus*. Some analyses place *paratus* nested within *Habronattus*, in clade 1 (Fig. 1). A few other analyses place *paratus* with the *agilis*, *amicus* and *tranquillus* groups. Griswold's evidence for removing *paratus* from *Habronattus* consisted of its apparent lack of what were assumed two synapomorphies of *Habronattus*: epigynal openings hidden in atria and relatively short first leg of males. However, hidden epigynal openings appear to be plesiomorphic

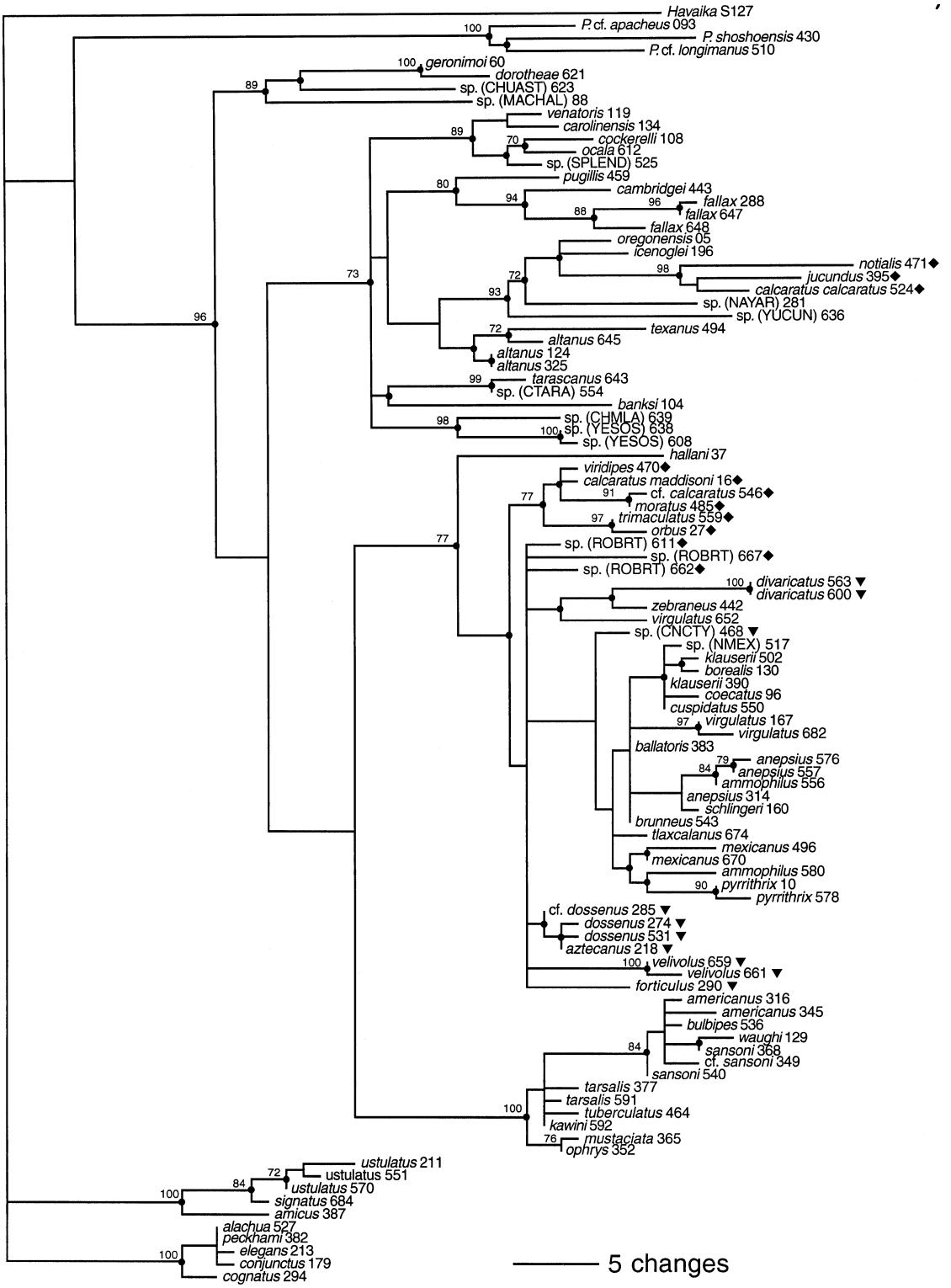

Fig. 3. Maximum likelihood tree obtained for the entire EF-1α sequence, with indications of parsimony results. Nineteen best trees were found, of which this is the first. The parameters estimated (see Table 2) and used in the tree search were tratio = 1.802262, rates = 2.233351 for noncoding, 0.043215 for position 1, 0.006373 for position 2 and 1.096507 for position 3. Spots at nodes indicate clades recovered in the strict consensus of 100 000 most parsimonious trees found. Numbers on branches indicate percent of 500 parsimony bootstrap replicates recovering clade (shown only if = 70%). ◆ = *viridipes* group; ▼ = *clypeatus* group.

Table 2. –ln Likelihoods calculated for various models of sequence evolution (nuclear EF-1α sequences) on the model tree obtained as described in the text. In italics is the −ln likelihood of the model chosen for use in tree searches using likelihood. (A) whole EF-1α.

	Equal rates	Codon pos rates
JC	4716.88754	4369.00966
F81	4665.33324	4305.95176
HKY85	4558.08192	*4185.30527*
a b a c d e		4183.26073
GTR	4551.00788	4182.87319

Table 2B. Exons.

	Equal rates	Codon pos rates
JC	1860.10038	1715.86191
F81	1848.45150	1702.16030
HKY85	1785.31414	1633.26913
a b a c d e		*1629.20730*
GTR	1773.81849	1627.56914

Table 2C. Introns.

	Equal rates	Gamma + pinvar
JC	2552.61146	2520.34796
F81	2497.08014	2474.12353
HKY85	2432.38821	2414.53736
a b a c d e		*2407.05340*
GTR	2423.65438	2406.65577

within Pelleninae, given that two of the genera that are basal according to our molecular data (*Sibianor* and *Havaika*; Hedin & Maddison, unpublished) have atria very much like those of most *Habronattus*. This would suggest that the exposed openings of *paratus* are derived from a condition like that in other *Habronattus* (and it is not clear that their exposure takes the same form as seen in *Pellenes*). That would leave the relatively long first legs of *paratus* to be the only clear reason for removing it from the clade of *Habronattus*. Discordant with this is the elbowed tegular apophysis, unique to our knowledge in salticids, that *paratus* shares with all *Habronattus* except the *coecatus* group, where it is apparently secondarily lost (Griswold, 1987). Whether or not a unique structure like this might be considered more compelling than a quantitative difference in leg length, the molecular data add to the case for inclusion of *paratus* in *Habronattus*. Thus, *Habronattus paratus* is a restored combination.

By Griswold's formulation, the species with modified first and third legs (our clade 5, Fig. 1) are placed in two species groups, the *viridipes* and *coecatus* groups. Griswold's *viridipes* group included twelve species, of which four are distributed primarily in northern and eastern North America (*viridipes*, *notialis*, *calcaratus* and *jucundus*), whereas the remaining eight are distributed primarily in southwestern North America. Griswold's cladogram has the northern/eastern species arising out of a paraphyletic southwestern group. The southwestern species are distinctive for having (usually) the basal white band of the male abdomen broken anteriorly by black, and a striped clypeus. The northern and eastern species have a V-shaped ridge of raised setae on the male carapace, and a narrow straight tip of the tibial apophysis. There are also differences in courtship behaviour. The northern/eastern species have an early stage of courtship in which the first tarsi are pointed at the female; later in courtship the first and third legs on one side of the body are vibrated synchronously. The southwestern species sidle broadly in early courtship, and have a distinctive double raise of the third legs during late courtship (Maddison & Hedin, unpublished). According to these characteristics, the undescribed species *H.* sp. (CNCTY) and *H.* sp. (CHIH) would belong with the southwestern species. *Habronattus* sp. (ROBRT) could be placed tentatively with the northern/eastern species for sharing the ridge of raised setae, but its courtship behaviour and tibial apophysis are uncharacteristic of the northern/eastern species.

Because our data cast doubt on the monophyly of the *viridipes* group, we prefer to treat these species as belonging to two separate groups: the *viridipes* group proper (including the northern/eastern species plus *H.* sp. (ROBRT)) and the *clypeatus* group (the southwestern species plus *H.* sp. (CNCTY) and *H.* sp. (CHIH)). The mitochondrial data provide some support for the monophyly of the *clypeatus* group. Included therein is *H. dossenus*, placed by Griswold nearer the *viridipes* group *s.s.* on the basis of the first leg ornamentation. Its placement with the *clypeatus* group is not entirely surprising, given that apparent hybrids between *H. dossenus* and *H. clypeatus* have been found in southern Arizona where they are microsympatric. The courtship behaviour and most ornamentation of the two species are almost identical (Maddison & Hedin, unpublished). Except for four rogue members (discussed below), the species of the *viridipes* group proper, including *H. moratus*, *H. orbus* and *H. trimaculatus*, are well supported as a clade (Fig. 1). We found no support for the monophyly of the *viridipes* group plus the *clypeatus* group together.

Our data do not provide a clear resolution of the internal relationships of the *agilis*, *americanus* and *coecatus* groups; hence, their representation in our summary (Fig. 1). For the *agilis* group, EF-1α shows little resolution, and the different regions of the mitochondrial DNA give conflicting relationships. For the *americanus* and *coecatus* groups, mitochondrial and nuclear gene trees are discordant. Whether this is due to errors in gene tree inference, incomplete lineage sorting or hybridization, we cannot say. As noted below, there are indications of hybridization involving the *coecatus* group.

Gene tree problems?

Molecular trees fail to recover the *viridipes* group as monophyletic, with four taxa (*H. notialis*, *H. calcaratus*

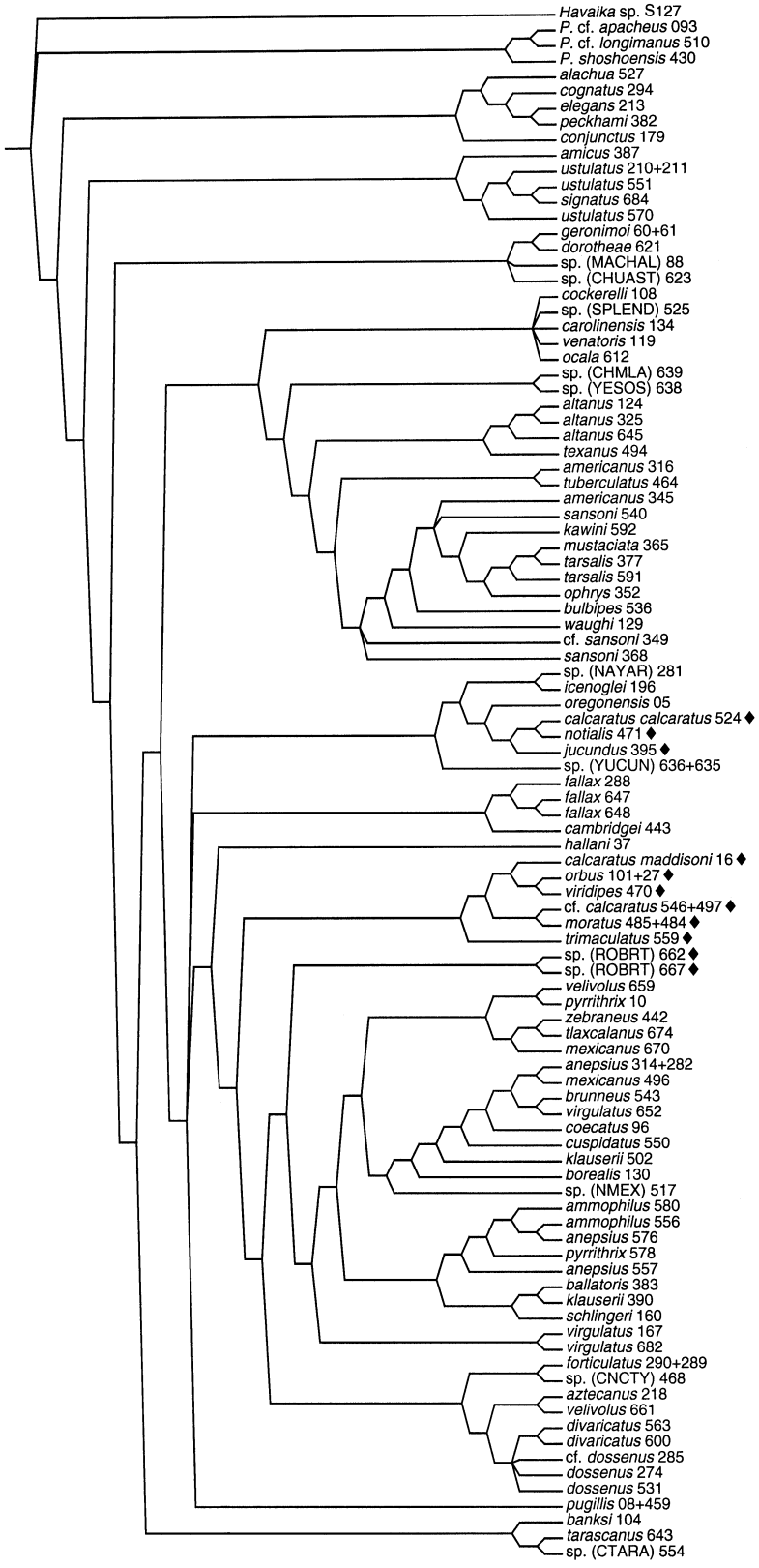

Fig. 4. Strict consensus of most parsimonious trees found combining both mitochondrial and nuclear sequence data. Some terminal taxa are composites of more than one individual, as indicated by the specimen code numbers (see also Appendix 2). ♦ = *viridipes* group.

calcaratus, *H. jucundus* and *H.* sp. (ROBRT)) consistently falling elsewhere (see diamond symbols in Figs 1–4). The failure of *H.* sp. (ROBRT) to group with the other *viridipes* group members is not surprising, given that our assignment of it to the group was tentative, based primarily on a single character (the ridge of raised setae on the male cephalic area). However, *H.* sp. (ROBRT) does fall, as expected, within the clade with first and third legs modified.

More surprising are *H. notialis*, *H. calcaratus calcaratus* and *H. jucundus* which, based on our molecular data, fall far from the remaining species, often near *H. oregonensis* (Fig. 1). This unexpected placement of the three forms is strongly supported by EF-1α, and has some support from the mitochondrial data. Parsimony searches done as described above but with the *viridipes* group proper constrained to be monophyletic yielded trees of 567 parsimony steps for EF-1α (tree length 20 steps greater than unconstrained) and 2317 parsimony steps for the entire mtDNA sequence (tree length 3 steps greater than unconstrained). The nucleotide sites supporting the rogue placement do not appear to be concentrated in any special way along the sequences (judged using the Compare 2 trees chart of MacClade (Maddison & Maddison, 2000) on one each of the constrained and unconstrained parsimony trees).

The placement of the three forms would imply that numerous phenotypic characters, including the male's fringed green forelegs with spatulate spines, third patella with spines and spots, and details of the intricate courtship behaviour (Maddison & Hedin, unpublished), are convergent between these three forms and the remaining *viridipes* group members, some of which are currently considered conspecific with *H. calcaratus calcaratus*. The details of similarity are so rich as to make convergence highly unlikely. It would seem then that both the mitochondrial and nuclear genes are misleading us about species relationships of *H. notialis*, *H. calcaratus calcaratus* and *H. jucundus*.

The parametric bootstrapping results hint that this rogue behaviour may be an artifact. With the simulated mitochondrial DNA, parsimony reconstructed the *viridipes* group monophyletic in forty-eight of the simulations and ambiguity allows the group to be monophyletic in nineteen more. Of the remaining thirty-three simulations, eighteen showed the *viridipes* group being split but remaining within a clade with first and third legs modified. Of the fifteen showing nonmonophyly of the group with first and third legs modified, in only four was the nonmonophyly generated by an external placement of *H. jucundus*, *H. notialis* or *H. calcaratus calcaratus*: twice, attaching to *H. oregonensis*, once to *H. pugillis* and once to *H. icenoglei* and *H. delectus*. Neighbour joining reconstructed the *viridipes* group monophyletic in sixty simulations; the group with modified first and third legs monophyletic in sixty-seven simulations. Of the remaining thirty-three simulations, nine have the monophyly of the *viridipes* group broken up, in part, by the attachment of *H. jucundus*, *H. notialis* or *H. calcaratus calcaratus* elsewhere, often with clades including *H. oregonensis*, *H. icenoglei* or *H. delectus*. Most of the remaining twenty-four have the group with first and third legs modified broken up by insertion of *H. oregonensis*. These results suggest that even if the *viridipes* group and the group with first and third legs modified were monophyletic, we would expect a greater than 5% chance of reconstructing trees with the monophyly of these groups broken by linkages involving *H. jucundus*, *H. notialis*, *H. calcaratus calcaratus* and *H. oregonensis*.

It surprised us that the EF-1α simulations showed none of these tendencies, despite their model tree having the rogue *viridipes* group members on a distinctly long branch. Parsimony reconstructions allowed the *viridipes* group to be monophyletic in seventy-five simulations. Of the twenty-five violating monophyly of the *viridipes* group, only eleven violate the monophyly of the group with first and third legs modified, and then by including only *H. hallani*. Neighbour joining reconstructions showed twenty-three trees violating the monophyly of the group with modified first and third legs, likewise only by the inclusion of *H. hallani*.

Although these results hint that the unexpected placement of *H. jucundus*, *H. notialis* and *H. calcaratus calcaratus* may be an analytical artifact, the case is not convincing. Other explanations for their apparent misplacement for both nuclear and mitochondrial regions include contamination of genomic DNAs, paralogy of EF-1α or genetic introgression in distantly related species. Genomic contamination is unlikely for any one of several reasons. First, the sequences derived from the genomics clearly belong within *Habronattus*, but are also novel. Second, we have two mitochondrial sequences for *H. jucundus* that always fall together. Although it is possible that both genomics are novel, but sister contaminants, a more parsimonious suggestion is that these genomics (and resulting sequences), in fact, represent *H. jucundus*. Finally, the fact that the four genomics (two *H. jucundus* + *H. notialis* + *H. c. calcaratus*) were extracted months apart (and in the case of *H. jucundus*, in different states) also makes genomic contamination unlikely. It is also possible that the three EF-1α sequences that group convincingly together represent a paralog of the copy sequenced in all other taxa. Data available for a known paralog of that analysed in this paper do not support this hypothesis (see Hedin & Maddison, 2001b), but additional studies may be needed to rule out completely the involvement of paralogy in this pattern.

Genetic introgression from distantly related species is a formal possibility. In fact, hints of genetic introgression are apparent in other species placed in the first and third legs modified groups, in particular involving the *clypeatus* group (see symbols in Figs 2 and 3). Specimen 272 of *H.* sp. (CHIH) has a mitochondrial haplotype allied with the *clypeatus* group, as expected. This species has all of the complex courtship traits typical of the *clypeatus* group. However, specimen 292 of the same morphospecies consistently falls well within the *coecatus* group for the combined mitochondrial analyses and for the 16S and ND1 analyses separately (Figs 1 and 2). A second similar example is *H. velivolus*, whose specimen 661 falls within the *clypeatus* group (as expected) but whose specimen 659 falls within the *coecatus* group (Fig. 2). In the case of *H. velivolus*, we can compare results from a nuclear gene, which places the two

specimens convincingly together (Fig. 3). Again, genomic contamination is unlikely. For example, we have multiple identical mtDNA sequences from different extractions for specimen 292. This suggests then that both *H. velivolus* and *H.* sp. (CHIH) are polymorphic for mitochondria obtained from *coecatus* group females. This result would be intriguing, because both the *coecatus* and *clypeatus* groups have complex ornamentation and behaviour that are distinctively different. Although one might expect hybridization to be rarest in groups with the most complex mating behaviour, complex and rapidly diversifying mating behaviour may in fact reflect chase-away sexual selection (Holland & Rice, 1998), which could enhance susceptibility to hybridization (Maddison & McMahon, 2000). The possibility of hybridization in the group with first and third legs modified deserves further study.

The placement of *H.* sp. (ROBRT) outside the *viridipes* group could also be explained by introgression, but we lack evidence as convincing as polymorphism supplies for *H. velivolus* and *H.* sp. (CHIH). The even more unusual placement of *H. notialis*, *H. jucundus* and *H. c. calcaratus* could also be due to introgression, but it would have been across considerably greater phylogenetic distances, outside of the group with first and third legs modified. A firm resolution of the puzzle of these three taxa awaits additional work.

Implications for courtship evolution

Our results corroborate Griswold's interpretation that the lack of courtship ornamentation in *H. orbus*, *H. trimaculatus* and *H. borealis* represent evolutionary loss, by strengthening the case that each of these is nested within the clade with modifed first and third legs in males. Additionally, *H.* cf. *dossenus* (Silver City) and *H.* cf. *calcaratus* (Ft Stockton) have apparently lost third leg ornamentation. In saying this for *H.* cf. *dossenus*, we are interpreting *H. dossenus* and *H. clypeatus* as having ornamented femur, patella and tibia of the third legs. Both have a dark and somewhat swollen femur, a red and silver stripe on the third tibia, and a third patella that is flattened and bare in front with a ridge of setae on the dorsal margin. *Habronattus* cf. *dossenus* (Silver City), however, has a third leg that appears completely unornamented, as in the female.

That wholesale loss of courtship ornamentation can occur, as seen in *H. borealis* for example, raises the question of whether one complex courtship style could evolve into another. As noted in the Introduction, use of courtship-related characters to reconstruct phylogeny could make it difficult to detect such a process, because wholesale loss could erase many of the synapomorphies that might be used to place a lineage. If that lineage subsequently evolved a new complex style of courtship, there might be little clue left that it evolved out of the previous style, and instead both styles would be interpreted as synapomorphies for independent groups. Our data provided the opportunity to detect such processes, but in fact there are no obvious cases. With the exception of possibly the *coecatus* group giving rise to the *clypeatus* group or vice versa, there are no indications that one complex style of courtship gave rise to another.

One of the unanticipated clades discovered in this work, the *decorus* group, shows remarkable diversity of courtship ornamentation and behaviour. The group includes at least three very different styles of courtship: the metallic abdomen-raising display of *H. decorus* and *H.* sp. (SPLEND) (for courtship see Peckham & Peckham, 1889), the twisted first leg with semaphorelike display of *H. carolinensis* and *H. venatoris* (courtship, Maddison & Hedin, unpublished), and the pale first leg with raised second leg pose of *H. ocala* (courtship: Maddison & Hedin, unpublished). This group is as tightly knit by the molecular data as the *agilis* or *americanus* group, and yet those other groups have relatively uniform styles of courtship.

Implications for genitalic evolution

As with study of courtship evolution, study of genitalic evolution proceeds best if interpreted with a phylogeny derived from data independent of genitalia. Griswold used characteristics of the male palpus and female epigynum (his characters 143–172) in reconstructing the phylogeny. Indeed, his characters 146 and 147 (degrees of angular separation of embolus and tegular apophysis), delimit two major clades. Here we consider one issue in genitalic evolution: the rotation of the male palpal bulb and corresponding length of the embolus.

In many salticid groups with a more-or-less circular palpal bulb, such as *Habronattus*, *Sitticus* Simon and *Amycus* C.L. Koch, there is within-clade variation in the degree of rotation of the bulb. For instance, outgroup comparisons with *Pellenes* and other genera related to *Habronattus* suggest that the ancestral condition is for a short embolus to arise from the prolateral and distal edge of the tegulum; that is, the embolus arises between 0900 and 1000, if viewing the left palpus from below as if it were a clock. Various species have evolved so as to rotate the bulb (counterclockwise in the left palpus viewed from below), thus yielding an embolus that arises at 0600, or even 0200. With these more rotated bulbs, the embolus must be considerably longer to reach the distal portion of the palpus, wrapping around the tegulum. In groups with such variation in embolus length and bulb rotation, there is correlated variation in the length of ducts of the female epigynum. For instance, there are at least five turns of spermathecae in females of *H. ustulatus*, whose male embolus wraps around 270°, in contrast to three turns versus 180° in *H. encantadas* (see Figs 150, 152, 199 and 208 of Griswold, 1987). This correlation appears strongly supported in many salticid groups but is not yet formally studied (Maddison, unpublished). If such correlated variation in male embolus and female duct lengths is related to sexual selection, either attractive (e.g. Eberhard, 1985) or antagonistic (e.g. Holland & Rice, 1998), then it would be of interest to know whether the embolus length increases and decreases within a clade, or only increases,

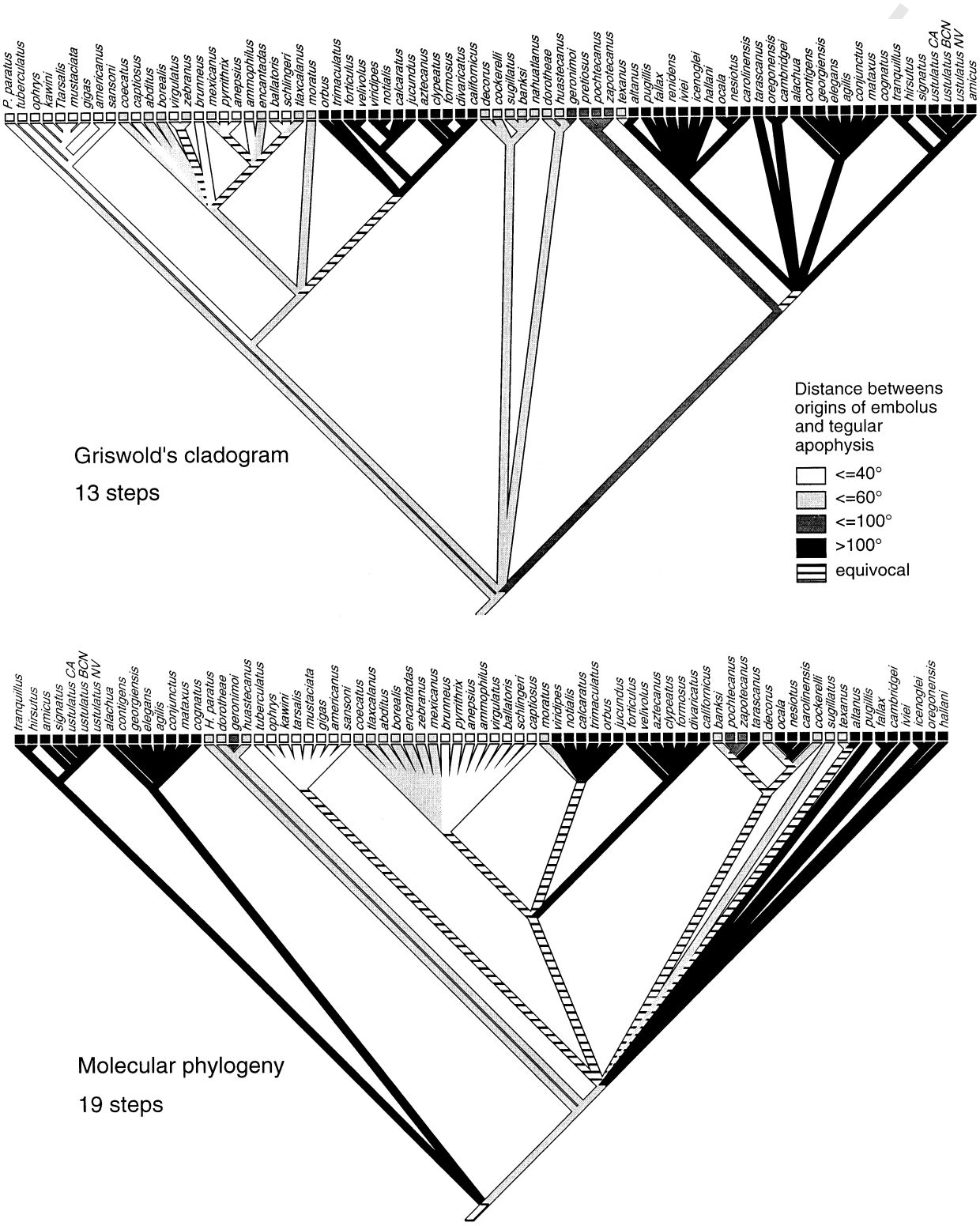

Fig. 5. Evolution of male genitalic elements as implied by (Griswold, 1987) cladogram (above, from his Figs 10 and 12–16) and our molecular phylogeny (below, corresponding to Fig. 4). The character represents Griswold's characters 144–147 recoded as a single ordered character. It refers to the distance between two elements of the male palpal bulb, and correlates with the length of the male intromittent structure (embolus).

during evolution. If only increases occur, an arms race between males and females over control of fertilization might be suggested.

Figure 5 shows Griswold's characters 148–153, recoded as a single ordered character, reconstructed on his cladogram, which requires at least thirteen evolutionary steps. If the cladogram is resolved in a way to bias toward increases in bulb rotation ('increase' defined as we have above, with 0900 being poorly rotated and 0300 being strongly rotated), then there are seven unambiguous increases in rotation and one unambiguous decrease. This is consistent with the fact that the characters (or those, such as 144–147, correlated with it) were used in reconstructing the cladogram, with poor rotation being the assumed plesiomorphic condition. If our molecular phylogeny is resolved in a way to bias similarly toward increases in bulb rotation, then there are nine unambiguous increases and four decreases (Fig. 5). Part of the difference is due to our phylogeny placing the strongly rotated *amicus*, *agilis* and *tranquillus* groups basally. Our phylogeny therefore argues against a model of increase-only of bulb rotation, a conclusion that would have been more difficult to reach with a genitalia-based phylogeny.

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References

Blest, A.D. & Sigmund, C. (1984) Retinal mosaics of the principal eyes of two primitive jumping spiders, *Yaginumanis* and *Lyssomanes*: clues to the evolution of salticid vision. *Proceedings of the Royal Society of London, Series B*, **221**, 111–125.

Cutler, B.E. (1988) Courtship behaviour in *Habronattus captiosus* (Araneae: Salticidae). *Great Lakes Entomologist*, **21**, 129–131.

Eberhard, W.G. (1985) *Sexual Selection and Animal Genitalia*. Harvard University Press, Cambridge, Massachusetts.

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.

Fitch, W.M. (1971) Toward defining the course of evolution: minimal change for a specific tree topology. *Systematic Zoology*, **20**, 406–416.

Goldman, N. (1993) Statistical tests of models of DNA substitution. *Journal of Molecular Evolution*, **36**, 182–198.

Griswold, C.E. (1977) Biosystematics of *Habronattus* in California. MSc Thesis. University of California, Berkeley, California.

Griswold, C.E. (1987) A revision of the jumping spider genus *Habronattus* F.O.P.-Cambridge (Araneae; Salticidae), with phenetic and cladistic analyses. *University of California Publications in Entomology*, **107**, 1–345.

Hedin, M.C. (1997) Speciational history in a diverse clade of habitat-specialized spiders (Araneae: Nesticidae: *Nesticus*): inferences from geographic-based sampling. *Evolution*, **51**, 1927–43.

Hedin, M.C. & Maddison, W.P. (2001a) A combined molecular approach to phylogeny of the jumping spider subfamily Dendryphantinae (Araneae, Salticidae). *Molecular Phylogenetics and Evolution*, **18**, 386–403.

Hedin, M.C. & Maddison, W.P. (2001b) Phylogenetic utility and evidence for multiple copies of elongation factor-1a in the spider genus *Habronattus* (Araneae: Salticidae). *Molecular Biology and Evolution*, **18**, 1512–1521.

Holland, B. & Rice, W.R. (1998) Perspective: chase-away sexual selection: antagonistic seduction versus resistance. *Evolution*, **52**, 1–7.

Huelsenbeck, J.P., Hillis, D.M. & Jones, R. (1996) Parametric bootstrapping in molecular phylogenetics: applications and performance. *Molecular Zoology: Advances, Strategies, and Protocols* (ed. by J. D. Ferraris and S. R. Palumbi), pp. 19–45. John Wiley and Sons, Inc, New York.

Jackson, R.R. (1982) The behaviour of communicating in jumping spiders (Salticidae). *Spider Communication: Mechanisms and Ecological Significance* (ed. by P. N. Witt and J. S. Rovner), pp. 213–247. Princeton University Press, Princeton, New Jersey.

Logunov, D.V., Marusik, Y.M. & Rakov, S.Y. (1999) A review of the genus *Pellenes* in the fauna of Central Asia and the Caucasus (Araneae, Salticidae). *Journal of Natural History*, **33**, 89–148.

Maddison, D.R. (1991) The discovery and importance of multiple islands of most-parsimonious trees. *Systematic Zoology*, **40**, 315–328.

Maddison, W.P. (1995) *Habronattus*, in Salticidae section of Tree of Life Web Project. http://spiders.arizona.edu/salticidae/habronattus/habronattus.html.

Maddison, W.P. (1996) *Pelegrina* Franganillo and other jumping spiders formely placed in the genus Metaphidippus (Araneae: Salticidae). *Bulletin of the Museum of Comparative Zoology*, **154**, 215–368.

Maddison, D.R., Baker, M.D. & Ober, K.A. (1999) Phylogeny of carabid beetles as inferred from 18S ribosomal DNA (Coleoptera: Carabidae). *Systematic Entomology*, **24**, 103–138.

Maddison, W.P. & Maddison, D.R. (1992) *MacClade: Analysis of Phylogeny and Character Evolution, Version 3.0. Sinauer Associates, Sunderland, Massachusetts.*

Maddison, D.R. & Maddison, W.P. (2000) *MacClade 4: Analysis of Phylogeny and Character Evolution, Version 4.0. Sinauer Associates, Sunderland, Massachusetts.*

Maddison, D.R. & Maddison, W.P. (2001a) *Genesis: Models of Evolution*, *Version 0.97*. A package of modules for the Mesquite system. http://mesquiteproject.org/mesquite/genesis/genesis.html. 2

Maddison, W.P. & Maddison, D.R. (2001b) *Mesquite: a Module System for Evolutionary Analyses, Version 0.97*. http://mesquiteproject.org. 3

Maddison, W.P. & McMahon, M.M. (2000) Divergence and recticulation among montane populations of the jumping spider *Habronattus pugillis* Griswold. *Systematic Biology*, **49**, 400–421.

Maddison, W.P. & Stratton, G.E. (1988) Sound production and associated morphology in male jumping spiders of the *Habronattus agilis* species group (Araneae: Saltcidae). *Journal of Arachnology*, **16**, 199–211.

Masta, S.E. (2000a) Phylogeography of the jumping spider *Habronattus pugillis* (Araneae: Salticidae): recent vicariance of sky island populations? *Evolution*, **54**, 1699–1711.

Masta, S. (2000b) Mitochondrial sequence evolution in spiders: intraspecific variation in tRNAs lacking the TYC arm. *Molecular Biology and Evolution*, **17**, 1091–1100.

Peckham, G.W. & Peckham, E.G. (1889) Observations on sexual selection in spiders of the family Attidae. *Occasional Papers of the Wisconsin Natural History Society*, **1**, 3–60.

Peckham, G.W. & Peckham, E.G. (1890) Additional observations on sexual selection in spiders of the family Attidae, with some remarks on Mr Wallace's theory of sexual ornamentation. *Occasional Papers of the Wisconsin Natural History Society*, **1**, 117–151.

Peckham, G.W. & Peckham, E.G. (1901) Spiders of the *Phidippus* group of the family Attidae. *Transactions of the Wisconsin Academy of Science, Arts and Letters*, **13**, 282–359.

Peckham, G.W. & Peckham, E.G. (1909) Revision of the Attidae of North America. *Transactions of the Wisconsin Academy of Science, Arts and Letters*, **16**, 355–646.

Proszynski, J. (1976) Studium systematyczno-zoogeograficzne nad rodzina Salticidae (Aranei) Regionow Palearktycznego i Nearktycznego. *Wyzsza Szkola Pedagogiczna w Siedlcach Rozprawy*, **6**, 1–260.

Proszynski, J. (2001) Remarks on Salticidae (Aranei) from Hawaii, with description of *Havaika* – General nov. *Arthropoda Selecta*, in press.

de Queiroz, K. (1996) Including the characters of interest during tree reconstruction and the problems of circularity and bias in studies of character evolution. *American Naturalist*, **148**, 700–708.

de Queiroz, A., Donoghue, M.J. & Kim, J. (1996) Separate versus combined analysis of phylogenetic evidence. *Annual Review of Ecology and Systematics*, **26**, 657–681.

Richman, D.B. (1977) Comparative studies on the mating behaviour and morphology of some species of *Pellenes* (Araneae – Salticidae). MSc Thesis, University of Arizona, Town?

Richman, D.B. (1982) Epigamic display in jumping spiders (Araneae, Salticidae) and its use in systematics. *Journal of Arachnology*, **10**, 47–67.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sullivan, J. & Swofford, D.L. (1997) Are guinea pigs rodents? The importance of adequate models in molecular phylogenetics. *Journal of Mammalian Evolution*, **4**, 77–86.

Swofford, D.L. (2000) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0b4a*. Sinauer Associates, Sunderland, Massachusetts.

Swofford, D.L. (2001a) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0b7*. Sinauer Associates, Sunderland, Massachusetts.

Swofford, D.L. (2001b) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0b8*. Sinauer Associates, Sunderland, Massachusetts.

Swofford, D.L., Olsen, G.J., Waddell, P.J. & Hillis, D.M. (1996) Phylogenetic inference. *Molecular Systematics*, 2nd edn (ed. by D. M. Hillis, C. Moritz and B. K. Mable), pp. 407–514. Sinauer, Sunderland, Massachusetts.

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Appendix 1. Undescribed species and geographical variants.

Among the specimens analysed are some belonging to species new to science. Others represent known or new geographical variants in species complexes that have proven difficult to separate into species. We here give brief descriptions of some of these forms, pending a formal review of the species, to clarify to what forms the names refer. In these descriptions, angle of origin of tegular apophysis refers to direction of basal straight portion (before the elbow) in left palpus viewed from below, stated as if on an analogue clock (thus, 0900 is pointing prolaterally, 0600 is pointing basally). For instance, the tegular apophysis of *H. paratus* points to 0900 (poorly rotated), and that of *H. tranquillus* to about 0430 (strongly rotated).

Species apparently undescribed

H. sp. (CHIH). Superficially resembling *H. californicus*, but with distinctive third patella (yellowish, with red swelling distally) and dense yellowish fringe on male's first leg. From the Sierra Madre Occidental of Chihuahua.

H. sp. (CHMLA). Similar to *H.* sp. (YESOS) but bulb of palpus poorly rotated with tegular apophysis pointing to 0900. Tibial apophysis as in *H. pochetecanus*. Male clypeus typically with narrow medial vertical white stripe, flanked by dark stripes, flanked by broad white stripes. Chelicera with vertical white band. Sierra Manantlan and further south-west in Jalisco.

H. sp. (CHUAST). In the *dorotheae* group, known from western Mexico (Chiapas, Jalisco, Nayarit). Resembles *H. huastecanus* except for much longer tibial apophysis of the palpus.

H. sp. (CNCTY). Superficially resembles *H. californicus*, but more robust-bodied and with different male ornamentation. Male first leg green with orange fringe; third patella a large blue-grey triangle. Known from near Cañon City, Colorado, and Davis Mountains of Texas.

H. sp. (CTARA). Known from a single male from Michoacan, similar to *H. tarascanus*, but differing in form of palpus and in having a red face.

H. sp. (LKHSTY). In the *tranquillus* group, known from eastern Colorado and southern Texas. Males with first leg slightly greenish, with black, white and red dorsal tufts of setae; second leg pale with black spot. Resembles most closely *H. tranquillus* (in dorsal tufts and pale second leg).

H. sp. (MACHAL). In the *dorotheae* group, from mainland coast of Ecuador. Palpus bulb poorly rotated, with tegular apophysis pointing to 0930. Male first leg femur, patella and tibia dark; face dark with reddish scales.

H. sp. (NAYAR). A relatively unornamented species from mountains west and south of Tepic, Nayarit. Clypeus and chelicerae dark; legs with oblique white stripes that appear as maculations; palpus resembles that of *H. oregonensis*.

H. sp. (NMEX). In the *coecatus* group, from mountains of New Mexico. Similar to *H. festus*, but male face black with a pair of thin oblique white stripes on clypeus.

H. sp. (ROBRT). Superficially similar to *H. moratus* but with tegular apophysis pointing to 0530. As in *viridipes* group, both first and third legs of males modified. Third patella in most populations with small purple protuberances dorsally. From Jalisco and Nayarit.

H. sp. (SCOTSD). In the *tranquillus* group. Of the two species currently confused under '*H. tranquillus*', *H*. sp. (SCOTSD) is that with dark brown on male first tibia and patella (Griswold, 1987: Fig. 76). The other has yellow first leg with a distinctive black stripe and spot, and is *H. tranquillus* proper judging by the original description and figure (Peckham & Peckham, 1901; the type specimen in MCZ, examined, is too faded and rubbed to be of much use). Both live in desert bushes, but differ ecologically (*H. tranquillus* a specialist on creosote bush, *Larrea*).

H. sp. (SPLEND). Considered by Griswold (1987) a synonym of *H. decorus*. We consider it a distinct species, based on strikingly different markings (black prosoma and legs with metallic scales, in contrast to yellow legs and snowy-white clothing on carapace in *H. decorus*) and different rotation of bulb of male palpus (tegular apophysis pointing to 0830). In south-eastern United States from Texas to Florida and north to Virginia. The name *H. splendens* may apply to it.

H. sp. (YESOS). Similar to *H*. sp. (CHMLA) but with bulb of palpus rotated as in *H. cockerelli*, with tegular apophysis arising at about 0700. Tibial apophysis as in *H. pocetecanus*. Male clypeus like that of *H*. sp. (CHMLA) but often lacks medial white stripe. Chelicera with vertical white band. North-east of Sierra Manantlan in Jalisco.

H. sp. (YUCUN). A little-ornamented species from Cerro Yucunuchica, Oaxaca. Males with dark brown face (sometimes with vertical white bars). Palpus resembles that of *H. icenoglei*.

Highlighted geographical variants

H. cf. *calcaratus* (Texas, Fort Stockton). A form from western Texas and Chihuahua, closely resembling *H. calcaratus agricola* but paler, and lacking an apophysis on third patella of males.

H. cf. *dossenus* (New Mexico, Silver City). Similar to *H. dossenus*, but with male third leg completely unmodified and like that of female (third patella and tibia of *H. dossenus* are modified, like those of *H. clypeatus*).

H. cf. *sansoni* (Utah, Cedar City). Similar to *H. americanus* and *H. sansoni*, but males nearly unornamented, lacking both red setae of *H. americanus* and carapace tufts of *H. sansoni*.

Notes on other geographical variants

Habronattus coecatus group. *Habronattus anepsius* shows geographical variation, especially in the markings on the faces of males. The population from Isla Magdalena, Baja California Sur, is different enough that it might be a distinct species. Similarly, what we call *H. ammophilus* from Isla Magdalena might be distinct from *H. ammophilus* elsewhere (and no other populations are known from the Pacific coast). The *H. mexicanus* sampled from Texas and Jalisco appear to be of the typical form. Those from Laguna Colorado, Oaxaca, may represent a different species: they are larger and darker than usual; males have the lateral white bar on the abdomen as in *H. carpus* but lack its dark first tarsus (Griswold, 1987). The *H. pyrrithrix* from San Carlos, Baja California Sur, has an unusually dark third patella and may be a distinct species.

Habronattus amicus group. This group shows rather confusing variation, particularly among forms called *H. ustulatus* and *H. amicus*. Specimens from three localities are here labelled as *H. ustulatus*, but these probably represent at least two species. The specimens from Maricopa Mountains, Arizona, represent a form whose males have black first femur and body covered with coppery scales. This form appears to be sympatric, or nearly so, with a grey-bodied form with white-clothed femur represented here by the specimen from Rail X, Arizona.

Habronattus americanus group. This group shows striking geographical variation that may require recognition of more species in the future (Griswold, 1987). The *H. americanus* from White Mountains is of the typical form with limited red on the first legs; that of Devil's Gate is a form found in some parts of the Sierra Nevada that shows an extensive fringe of red hairs under the first legs and prominent tufts above the front eyes, as if the result of introgression from the '*kubai*' form of *H. sansoni*. The *H. sansoni* of Bull River, British Columbia, is similar to the type specimen, with lateral bands of brick red on the face. That from Hyatt Lake, Oregon, is similar to the form described by Griswold as *H. kubai*, having a dark face and extensive fringe under the first legs. The *H. tarsalis* from Klamath River, California, is that without moustache (Griswold, 1987: 86); that from El Socorrito has a broader face and unique, cream-coloured chelicerae.

Species in other groups. The form here labelled as *H. divaricatus* from El Socorrito, Baja California Norte, was tentatively considered by Griswold a variant of *H. divaricatus*: the male third patella is more or less unornamented. The *H. dorotheae* males from Jalisco have a pale first femur. Specimens of *H. fallax* from Texas, Chiapas and Nayarit show the typical dark brown and cream markings; Arizona specimens are much paler, with a golden face.

Appendix 2. Specimens from which DNA sequences were obtained. In square brackets are names used by Hedin & Maddison (2001b) that differ from those used here. For *Havaika* sp., only the 16S portion of the mitochondrial sequence was obtained. Nuclear DNA accession numbers AF359058–AF359111 were previously reported in Hedin & Maddison (2001b).

Species	Specimen no.	Locality	mtDNA GenBank no.	nDNA GenBank no.
Outgroups				
Havaika sp. [*Sandalodes*]	S127	U.S.A. Hawaii, Island of Hawaii, Volcano	AF477249	AF359058
Pellenes cf. *apacheus* Lowrie & Gertsch	HA093	U.S.A. Arizona, Huachuca Mts, Carr Peak	AF477250	AF477202
P. cf. *longimanus* Emerton [*P. longimanus*]	HA510	U.S.A. Texas, Hidalgo Co., Bentsen-Rio Grande Valley SP	AF477251	AF359059
P. shoshonensis Gertsch	HA430	U.S.A. California, Inyo Co., White Mts	AF477252	AF359060
agilis group				
H. alachua Griswold	HA527	U.S.A. Florida, Putnam Co., Ordway Preserve	AF477253	AF359061
H. cognatus (Peckham & Peckham)	HA294	U.S.A. Texas, Travis Co., near Austin	AF477254	AF359062
H. cognatus	HA617	MEXICO: Chiapas, Puerto Arista 15°56.73′N 93°49.82′W	AF477255	—
H. conjunctus (Banks)	HA179	U.S.A. Arizona, Cochise Co., Whetstone Mts	AF477256	AF359063
H. contigens (Chamberlin)	HA620	MEXICO: Nayarit, Bahia Matachén, 21°30.02′N 105°12.16′W	AF477257	—
H. elegans (Peckham & Peckham)	HA213	U.S.A. California, Orange Co., San Juan Creek	AF477258	AF477203
H. mataxus Griswold	HA602	MEXICO: Hidalgo, Tasquillo, 20°32.46′N 99°19.08′W	AF477259	—
H. peckhami (Banks)	HA382	U.S.A. California, Monterey Co., S of Carmel City	AF477260	AF477204
amicus group				
H. amicus (Peckham & Peckham)	HA387	U.S.A. California, Shasta Co., Subway Cave	AF477261	AF359064
H. signatus (Banks)	HA684	U.S.A. California, Imperial Co., near Ocotillo	AF477262	AF359066
H. ustulatus (Griswold)	HA210	U.S.A. Arizona, Pinal Co., E of Tortolita Mts, Rail X Road	AF477263	—
H. ustulatus	HA211	U.S.A. Arizona, Pinal Co., E of Tortolita Mts, Rail X Road	—	AF477205
H. ustulatus	HA551	U.S.A. Arizona, Maricopa Co., Maricopa Wilderness	AF477264	AF359065
H. ustulatus	HA570	MEXICO: Baja California Sur, N of Agua Verde	AF477265	AF477206
tranquillus group				
H. hirsutus (Peckham & Peckham)	HA321	U.S.A. California, Siskiyou Co., near Gumboot	AF477266	—
H. hirsutus	HA565	MEXICO: Baja California Sur, Los Barriles	AF477267	—
H. tranquillus (Peckham & Peckham)	HA675/676	U.S.A. California, Imperial Co., Midway Well	AF477268	—
H. sp. (LKHSTY)	HA498	U.S.A. Texas, Starr Co., Falcon Lake SP	AF477269	—
H. sp. (SCOTSD)	HA492	U.S.A. Texas, Terrell Co., Independence Creek	AF477270	—
dorotheae group				
H. dorotheae (Gertsch & Mulaik)	HA473	U.S.A. Texas, Hidalgo Co., Bentsen-Rio Grande Valley SP	AF477271	—
H. dorotheae	HA621	MEXICO: Jalisco, Laguna Sayula, 19°59.72′N 103°32.19′W	AF477272	AF359068
H. geronimoi Griswold	HA060	U.S.A. Arizona, Huachuca Mts, Miller Canyon	—	AF359067
H. geronimoi	HA061	U.S.A. Arizona, Huachuca Mts, Miller Canyon	AF477273	—
H. sp. (CHUAST) [*H. huastecanus*]	HA623	MEXICO: Chiapas, Huamuche, 15°57.66′N 93°47.06′W	AF477274	AF359069
H. sp. (CHUAST)	HA624	MEXICO: Nayarit, N of Compostela, 21°19.39′N 104°55.27′W	AF477275	—
H. sp. (MACHAL)	HA088	ECUADOR: Manabi, Salaite	AF477276	AF477207

decorus group				
H. carolinensis (Peckham & Peckham)	HA134	U.S.A. Florida, Putnam Co., Florahome	AF477277	AF359075
H. cockerelli (Banks)	HA108	U.S.A. New Mexico, Cibola Co., Mt Taylor	AF477278	AF359070
H. decorus (Blackwall)	HA051	U.S.A. Massachusetts, Dedham	AF477279	—
H. ocala Griswold	HA612	U.S.A. Florida, Alachua Co., Ocala NF	AF477280	AF359077
H. venatoris Griswold	HA119	U.S.A. New Mexico, San Miguel Co., Pecos	AF477281	AF359076
H. sp. (SPLEND)	HA525	U.S.A. Florida, Alachua Co., junction State Routes 325 and 20	AF477282	AF359071
[*H.* cf. *decorus*]				
texanus group				
H. altanus (Gertsch)	HA124	U.S.A. Arizona, San Francisco Mts, Agassiz Peak	AF477283	AF359078
H. altanus	HA127	U.S.A. New Mexico, Coal Mine Cmpgd, 35°13.97′N 107°42.11′W	AF477284	—
H. altanus	HA325	MEXICO: Chihuahua, 40 Casas	AF477285	AF477208
H. altanus	HA645	MEXICO: Oaxaca, Xuchilquitango, 17°15.17′N 96°53.32′W	AF477286	AF477209
H. texanus (Chamberlin)	HA494	U.S.A. Texas, Hidalgo Co., Bentsen-Rio Grande Valley SP	AF477287	AF359079
fallax group				
H. cambridgei Bryant	HA443	MEXICO: Morelos, Cañada de Ajuchitlan, 18°27.98′N 98°59.61′W	AF477288	AF359084
H. cambridgei	HA651	MEXICO: Chiapas, Puerto Arista, 15°56.73′N 93°49.82′W	AF477289	—
H. cambridgei	HA649	MEXICO: Jalisco, Lo de Marcos, 20°57.53′N 105°21.34′W	AF477290	—
H. fallax (Peckham & Peckham)	HA166	U.S.A. Arizona, Pima Co., Santa Catalina Mts	AF477291	—
H. fallax	HA288	U.S.A.Texas, Travis Co., near Austin	AF477292	AF359080
H. fallax	HA647	MEXICO: Chiapas, Chahuitillo, 16°27.7′N 94°02.7′W	AF477293	AF477210
H. fallax	HA648	MEXICO: Nayarit, W of Compostela, 21°13.40′N 104°56.29′W	AF477294	AF477211
americanus group				
H. americanus (Keyserling)	HA316	U.S.A. California, Inyo Co., White Mtns	AF477295	AF359087
H. americanus	HA345	U.S.A. California, Mono Co., Devil's Gate	AF477296	AF477212
H. bulbipes (Chamberlin & Ivie)	HA536	U.S.A. California, Trinity Co., Long Canyon Trail	AF477297	AF477213
H. kawini (Griswold)	HA592	MEXICO: Baja California Norte, Laguna Hanson	AF477298	AF359089
H. mustaciata (Chamberlin & Ivie)	HA365	U.S.A. California, Mendocino Co., Big River Beach	AF477299	AF477214
H. ophyrs Griswold	HA352	U.S.A. California, Humboldt Co., Stone Lagoon	AF477300	AF477215
H. sansoni (Emerton)	HA368	CANADA: British Columbia, Bull River, 49°28.44′N 115°26.99′W	AF477301	AF359088
H. sansoni	HA540	U.S.A. Oregon, Jackson Co., Hyatt Lake	AF477302	AF477216
H. cf. *sansoni*	HA349	U.S.A. Utah, Iron Co., near Cedar City	AF477303	AF477217
H. tarsalis (Banks)	HA377	U.S.A. California, Siskiyou Co., Klamath River	AF477304	AF359090
H. tarsalis	HA591	MEXICO: Baja California Norte, El Socorrito	AF477305	AF477218
H. tuberculatus (Gertsch & Mulaik)	HA464	U.S.A. Arizona, Cochise Co., Willcox Playa	AF477306	AF477219
H. waughi (Emerton)	HA129	CANADA: Ontario, Nellie Lake, 48.75235°N 80.79332°W	AF477307	AF477220
viridipes group				
H. calcaratus calcaratus (Banks)	HA524	U.S.A. Florida, Putnam Co., Ordway Preserve	AF477308	AF359100
H. c. agricola Griswold	HA295	U.S.A. Texas, Travis Co., near Austin	AF477309	—
H. c. maddisoni Griswold	HA016	U.S.A. Tennessee, Wilson Co., Cedars of Lebanon SP	AF477310	AF359101
H. cf. *calcaratus*	HA497	U.S.A. Texas, Pecos Co., Fort Stockton	AF477311	—
H. cf. *calcaratus*	HA546	U.S.A. Texas, Pecos Co., Fort Stockton	—	AF477221
H. jucundus (Peckham & Peckham)	HA001	U.S.A. California, Siskiyou Co., Mt Shasta	AF477312	—
H. jucundus	HA395	U.S.A. Idaho, 65 km NE of Lowell	AF477313	AF359102
H. notialis Griswold	HA471	U.S.A. Florida, Nassau Co., 1 km E of Boulogne	AF477314	AF359104

Appendix 2. Continued.

Species	Specimen no.	Locality	mtDNA GenBank no.	nDNA GenBank no.
H. orbus Griswold	HA101	U.S.A. Oklahoma, Delaware Co., Spavinaw Creek	AF477315	—
H. orbus	HA027	U.S.A. Missouri, Ste. Genevieve Co., Hahn SP	—	AF359110
H. moratus (Gertsch & Mulaik)	HA484	U.S.A. Texas, Starr Co., N of Rio Grande City	AF477316	—
H. moratus	HA485	U.S.A. Texas, Starr Co., N of Rio Grande City	—	AF359111
H. trimaculatus Bryant	HA559	U.S.A. Florida, Levy Co., SW of Archer	AF477317	AF359109
H. viridipes (Hentz)	HA044	U.S.A. Massachusetts, Dedham	AF477318	—
H. viridipes	HA470	U.S.A. Texas, 30 km S of Sarita, 26.970°N 97.793°W	AF477319	AF359103
H. sp. (ROBRT)	HA611	MEXICO: Jalisco, El Tuito, 20°20.21′N 105°18.94′W	—	AF477222
H. sp. (ROBRT)	HA662	MEXICO: Jalisco, Chamela, 19°31.74′N 105°04.62′W	AF477320	AF477223
H. sp. (ROBRT)	HA667	MEXICO: Jalisco, Los Yesos, 19°45.02′N 104°04.00′W	AF477321	AF477224
H. sp. (ROBRT)	HA228	MEXICO: Nayarit, Singaita, near San Blas	AF477322	—
clypeatus group				
H. aztecanus (Banks)	HA218	MEXICO: Nayarit, San Blas	AF477323	AF359106
H. californicus (Banks)	HA380	U.S.A. California, San Luis Obispo Co., Cholame	AF477324	—
H. clypeatus (Banks)	HA221	U.S.A. Arizona, Jack's Canyon, 34.75°N 111.1°W	AF477325	—
H. clypeatus	HA039	U.S.A. Arizona, Tucson	AF477326	—
H. divaricatus (Banks)	HA563	MEXICO: Baja California Sur, Sierra de La Laguna, near San Juan del Aserradero	AF477327	AF359107
H. divaricatus	HA600	MEXICO: Baja California Norte, El Socorrito	AF477328	AF477225
H. dossenus Griswold	HA274	U.S.A. Arizona, Cochise Co., Rucker Canyon	AF477329	AF477226
H. dossenus	HA531	MEXICO: Sonora, Yecora	AF477330	AF477227
H. cf. *dossenus*	HA285	U.S.A. New Mexico, S of Tyrone. 32°42.119′N, 108°18.259′W	AF477331	AF477228
H. formosus (Banks)	HA007	U.S.A. California, Kern Co., Buttonwillow rest area, 35.42°N 119.42°W	AF477332	—
H. forticulus (Gertsch & Mulaik)	HA289	U.S.A. Texas, Travis Co., Austin	AF477333	—
H. forticulus	HA290	U.S.A. Texas, Travis Co., Austin	—	AF359105
H. velivolus Griswold	HA659	MEXICO: Jalisco, San Juanito, 19°58.15′N 103°36.93′W	AF477334	AF477229
H. velivolus	HA661	MEXICO: Jalisco, Tequila, 20°51.38′N 103°51.16′W	AF477335	AF359108
H. sp. (CHIH)	HA292	MEXICO: Chihuahua, 16 km E of Tomochic	AF477336	—
H. sp. (CHIH)	HA272	MEXICO: Chihuahua, 24 km N of Madera, near 40 Casas	AF477337	—
H. sp. (CNCTY)	HA468	U.S.A. Texas, Davis Mts, 30.593°N 103.939°W	AF477338	AF477230
coecatus group				
H. ammophilus (Chamberlin)	HA556	MEXICO: Baja California Sur, Pichilingue	AF477339	AF359092
H. ammophilus	HA580	MEXICO: Baja California Sur, Puerto Magdalena, Isla Magdalena	AF477340	AF477231
H. anepsius (Chamberlin)	HA282	U.S.A. California, Riverside Co., Indian Truck Trail	AF477341	—
H. anepsius	HA314	U.S.A. California, Riverside Co., Indian Truck Trail	—	AF477232
H. anepsius	HA557	MEXICO: Baja California Sur, Los Barriles	AF477342	AF477233
H. anepsius	HA576	MEXICO: Baja California Sur, Puerto Magdalena, Isla Magdalena	AF477343	AF359093
[*H.* cf. *anepsius*]				
H. ballatoris Griswold	HA383	U.S.A. California, Humboldt Co., Orleans	AF477344	AF359094
H. borealis (Banks)	HA130	CANADA: Ontario, Hamilton	AF477345	AF359095

H. brunneus (Peckham & Peckham)	HA543	U.S.A. Florida, Miami	AF477346	AF477234
H. coecatus (Hentz)	HA096	U.S.A. Georgia, Green Co., Oconee River	AF477347	AF359096
H. cuspidatus Griswold	HA550	U.S.A. Colorado, Rio Grande Co., Monte Vista	AF477348	AF477235
H. festus (Peckham & Peckham)	HA392	U.S.A. Utah, Washington Co., Pine Valley	AF477349	—
H. klauserii (Peckham & Peckham)	HA390	U.S.A. California, Inyo Co., Bishop	AF477350	AF477236
H. klauserii	HA502	U.S.A. Texas, El Paso Co., Hueco Tanks SP	AF477351	AF477237
H. mexicanus (Peckham & Peckham)	HA670	MEXICO: Jalisco, Laguna Sayula, 19°59.78′N 103°33.13′W	AF477352	AF477238
H. mexicanus	HA496	U.S.A. Texas, Pecos River, 29.704°N 101.363°W	AF477353	AF359098
H. mexicanus	HA654	MEXICO: Oaxaca, Laguna Colorada, 15°57.93′N 95°33.53′W	AF477354	—
H. pyrrithrix (Chamberlin)	HA010	U.S.A. Arizona, Tucson	AF477355	AF359097
H. pyrrithrix	HA578	MEXICO: Baja California Sur, San Carlos	AF477356	AF477239
H. schlingeri (Griswold)	HA160	U.S.A. California, San Diego Co., Oceanside	AF477357	AF477240
H. tlaxcalanus Griswold	HA674	MEXICO: Michoacan, Carapán, 19°35.15′N 102°05.64′W	AF477358	AF477241
H. virgulatus Griswold	HA167	U.S.A. Arizona, Pima Co., Santa Catalina Mts	AF477359	AF359099
H. virgulatus	HA652	MEXICO: Hidalgo, S of Pachuca, 19°59.98′N 98°42.48′W	AF477360	AF477242
H. virgulatus	HA682	U.S.A. Arizona, Cochise Co., Pearce	AF477361	AF477243
H. zebraneus (F.O.P.-Cambridge)	HA442	MEXICO: Morelos, Alpuyeca, near Xochicalco	AF477362	AF359091
H. sp. (NMEX)	HA517	U.S.A. New Mexico, San Juan Co., Chuska Mts	AF477363	AF477244
Miscellaneous				
H. banksi (Peckham & Peckham)	HA104	COSTA RICA: Guanacaste, Palo Verde NP	AF477364	AF359072
H. delectus (Peckham & Peckham)	HA467	U.S.A. Texas, Terrell Co., Independence Creek	AF477365	—
H. hallani (Richman)	HA037	U.S.A. Arizona, Tucson	AF477366	AF359081
H. icenoglei (Griswold)	HA196	U.S.A. Arizona, Yuma Co., Mohawk Mts and Highway I-8	AF477367	AF359085
H. iviei Griswold	HA641	MEXICO: Oaxaca, Puente las Tejas, 11 km W of Tehuantepec, 16°20.84′N 95°20.06′W	AF477368	—
H. oregonensis (Peckham & Peckham)	HA005	U.S.A. Arizona, Santa Rita Mts, Mt Hopkins	AF477369	AF359082
H. paratus (Peckham & Peckham)	HA085/086	ECUADOR: Manabi, Aqua Blanca	AF477370	—
H. pochtecanus Griswold	HA632	MEXICO: Nayarit, W of Compostela, 21°13.40′N 104°56.29′W	AF477371	—
H. pugillis Griswold	HA008	U.S.A. Arizona, Winchester Mts	AF477372	—
H. pugillis	HA009	U.S.A. Arizona, Baboquivari Mts	AF477373	—
H. pugillis	HA459	U.S.A. Arizona, Patagonia Mts	—	AF359083
H. sugillatus Griswold	HA486	U.S.A. Texas, Davis Mts, 30.593°N 103.939°W	AF477374	—
H. tarascanus Griswold	HA643	MEXICO: Michoacan, El Tzararacua, 19°20.71′N 102°04.96′W	AF477375	AF359086
H. zapotecanus Griswold	HA441	MEXICO: Morelos, Estacion CIAMISH, near Huautla, 18°27.76′N 99°02.21′W	AF477376	—
H. sp. (CHMLA)	HA639	MEXICO: Jalisco, Estacion de Biologia Chamela, 19°29.9′N 105°02.7′W	AF477377	AF477245
H. sp. (CTARA)	HA554	MEXICO: Michoacan, near Infernillo (hydroelectric plant), Rio Infernillo	AF477378	AF477246
H. sp. (NAYAR) [*H.* cf. *sugillatus*]	HA281	MEXICO: Nayarit, W of Tepic	AF477379	AF359073
H. sp. (YESOS) [*H.* cf. *pochtecanus*]	HA608	MEXICO: Jalisco, Los Yesos, 19°45.02′N 104°04.00′W	—	AF359074
H. sp. (YESOS)	HA638	MEXICO: Jalisco, Los Garcia, 19°38.72′N 103°39.36′W	AF477380	AF477247
H. sp. (YUCUN)	HA635	MEXICO: Oaxaca, Cerro Yucunuchica, 17°09.75′N 97°50.02′W	AF477381	—
H. sp. (YUCUN)	HA636	MEXICO: Oaxaca, Cerro Yucunuchica, 17°09.75′N 97°50.02′W	—	AF477248

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