Zootaxa 1646: 17-30 (2007)
www.mapress.com/zootaxa/

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

The pitfalls of exaggeration: molecular and morphological evidence suggests *Kaliana* is a synonym of *Mesabolivar* (Araneae: Pholcidae)

JONAS J. ASTRIN[1], BERNHARD MISOF & BERNHARD A. HUBER[2]
Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, D-53113 Bonn, Germany.
Corresponding author. E-mail: [1]j.astrin.zfmk@uni-bonn.de; [2]b.huber.zfmk@uni-bonn.de

Abstract

When the Venezuelan genus *Kaliana* Huber, 2000 was described, it was based on a single male specimen that was morphologically unique among pholcid spiders, especially in its extremely exaggerated male genitalia. The morphology of the recently discovered female suggests a close relationship with *Mesabolivar* González-Sponga, 1998. Using molecular sequences (mitochondrial CO1, 16S, and nuclear 28S) of *Kaliana yuruani* Huber, 2000 and 53 other pholcid taxa (152 sequences, 19 of them sequenced in this study) in a Bayesian and a maximum parsimony approach, we show that *Kaliana* is not sister group of, but nested within the species-rich South American genus *Mesabolivar*. Therefore, we argue that *Kaliana* is a junior synonym of *Mesabolivar* (*Mesabolivar yuruani*, n. comb.). Complementing previous studies on pholcid phylogeny, we also present evidence for a close relationship between *Mesabolivar* and *Carapoia*, support the synonymy of *Anomalaia* and *Metagonia* with molecular data, support the monophyly of 'ninetines' and question the recently postulated position of *Priscula* as nested within the New World clade.

Key words: pholcid spiders, subfamily-level groups, *Metagonia*, *Carapoia*, *Priscula*, beta-taxonomy, phylogeny

Introduction

There seems to be a tendency for taxonomists to create new genera for highly 'aberrant' species. For example, when the first spider species with directionally asymmetric male genitalia was discovered, a new genus was erected for it (*Anomalaia* González-Sponga, 1998). Subsequent morphological studies strongly suggested that *Anomalaia mariguitarensis* is just an unusual representative of the widespread and species-rich Neotropical genus *Metagonia* Simon, 1893 (Huber 2000, 2004). Our focus here is on a similar case. The genus *Kaliana* Huber, 2000 was established for a single and extremely unusual male specimen from Venezuela. The procursus, a male genital structure of pholcid spiders, is in this species about six times as long as in other representatives of the family. Other autapomorphic characters include the shape of the eye turret, the modifications of the clypeus, and the armature of the chelicerae.

During an expedition to Venezuela in 2004, further specimens of *Kaliana yuruani* Huber, 2000 were collected, and a simple but unique female character—the median pocket (Fig. 4; see also Huber 2006)—suggested an affinity to the widespread and species-rich South American genus *Mesabolivar* González-Sponga, 1998. This median pocket, located ventrally on the female genital plate (epigynum), has in fact been the only morphological synapomorphy of *Mesabolivar* (Huber 2000). Morphology thus suggested *Kaliana* to be either the sister group of *Mesabolivar* or to be nested within (and thereby a synonym of) *Mesabolivar*—without being able to falsify one of these two hypotheses. Here we use molecular evidence to test these phylogenetic hypotheses.

We also use molecular data to highlight close evolutionary ties between *Mesabolivar* and *Carapoia*

Accepted by C. Kropf: 8 Oct. 2007; published: 26 Nov. 2007

González-Sponga, to confirm the synonymy of *Anomalaia* and *Metagonia*, and to question the recently postulated position of *Priscula* Simon as nested within the New World clade. As for the subfamily-level clades proposed by Huber (2000), our data strongly corroborates monophyly of the New World clade (a large group of genera endemic to the New World); it further suggests inclusion of certain 'holocnemines' in the 'pholcines' (a suggestion that is strongly opposed by morphological evidence), and coincides with Bruvo-Mađarić *et al.* (2005) in rejecting 'holocnemines' as a para- or polyphyletic group (both 'holocnemines' and 'pholcines' have a world-wide distribution; the former contain many unusually large forms while the species-rich 'pholcines' are best known for the synanthropic spider *Pholcus phalangioides*). This is the first molecular phylogenetic study to include more than one representative of the 'ninetines' (mostly tiny, ground-living pholcids) and thus the first one to test ninetine monophyly using genetic characters.

A large part of the taxa and sequences used in this study has been analyzed before; our sampling mostly emerges as a synthesis of the studies of Astrin *et al.* (2006) and Bruvo-Mađarić *et al.* (2005). However, the first study (providing about two thirds of the sequences) was concerned with molecular alpha-taxonomy rather than with phylogeny, and the second differs in the analytical methods used (see below).

Material and methods

Genetic markers and taxon sampling

Our principal focus lay on mitochondrial genes, cytochrome *c* oxidase subunit 1 (CO1) and the ribosomal large subunit (16S) (treated together for their provenance from a single linkage unit), thus complementing the data of Bruvo-Mađarić *et al.* (2005), who present results for these genes almost exclusively in combination with nuclear DNA (nDNA). The CO1 and 16S fragments used here are short due to the fact that most of them stem from a taxonomic study (Astrin *et al.* 2006). However, the combination of both markers produced some interesting and robust results.

We used CO1 and 16S sequences from 60 specimens: 48 were taken from Astrin *et al.* (2006), nine from Bruvo-Mađarić *et al.* (2005) and three (cf. Table 1; see Table 2 for voucher, collecting data) were added for this study. The 42 represented pholcid species belong to 18 genera and all four currently recognized subfamily-level taxa (Huber 2000). Two non-pholcid outgroup species were used: a filistatid (Filistatidae being the putative sister family to all Haplogynae; Coddington and Levi 1991), and a representative of Diguetidae (together with Plectreuridae, the putative sister to Pholcidae). We included multiple specimens per species in cases in which the conspecific haplotypes were not identical. Some genera (especially the Neotropical endemics *Mesabolivar* González-Sponga, and *Metagonia*) are here represented by several species. Table 1 lists taxon names and GenBank accession numbers along with the country of origin. It is important to stress the fact that for the mitochondrial partition, we only chose specimens for which CO1 **and** 16S sequences were both available since we rejected the option to enlarge the sampling at the cost of having to code entire partitions as missing (see below).

In order to consider focal taxa through another, independent and more conserved marker, we also included a number of nuclear 28S sequences. Therefore, we selected and sequenced 13 taxa (see Tables 1 and 2). We incorporated these into a 28S dataset with 22 pholcid and two outgroup taxa (plus one dubious sequence, see Discussion) that we obtained from Bruvo-Mađarić *et al.* (2005; cf. Table 1). Of these 38 sequences, 24 belong to taxa also present in the mtDNA dataset (i.e. 14 taxa were new, since one sequence per species was used for 28S).

Finally, we concatenated the mitochondrial and the nuclear partitions—but again, only considering the (24) species in which both were available. For this combined approach, we had to use sequences obtained from different specimens in some species.

TABLE 1. List of analyzed specimens and GenBank (www.ncbi.nih.gov) accession numbers. Accession numbers starting with "AY": Bruvo-Mađarić *et al.* (2005); starting with "DQ": Astrin *et al.* (2006); accession numbers in boldface: specimens sequenced in this study (cf. Table 2). Informal higher level taxonomy: hol = holocnemines, nin = ninetines, NWC = New World clade, phol = pholcines.

Taxon	group	CO1	16S	28S
Artema atlanta Walckenaer, 1837	hol	AY560771	AY560663	--
Artema atlanta Walckenaer, 1837	hol	DQ667854	DQ667748	--
Carapoia paraguaensis González-Sponga, 1998	NWC	DQ667855	DQ667749	**DQ667839**
Carapoia ubatuba Huber, 2005	NWC	DQ667856	DQ667750	**DQ667840**
Ciboneya antraia Huber & Pérez, 2001	NWC	AY560794	AY560665	AY560732
Coryssocnemis simla Huber, 2000	NWC	DQ667858	DQ667753	--
Coryssocnemis simla Huber, 2000	NWC	DQ667859	DQ667752	--
Crossopriza lyoni (Blackwall, 1867)	hol	AY560775	AY560667	**DQ667841**
Holocnemus pluchei (Scopoli, 1763)	hol	--	--	**DQ667842**
Ibotyporanga naideae Mello-Leitão, 1944	nin	**DQ667852**	**DQ667837**	**DQ667843**
"Kaliana yuruani" = Mesabolivar yuruani (Huber, 2000)	NWC	DQ667860	DQ667754	**DQ667844**
Mecolaesthus longissimus Simon, 1893	NWC	DQ667861	DQ667756	AY560736
Mesabolivar aurantiacus (Mello-Leitão, 1930)	NWC	AY560779	AY560670	AY560735
Mesabolivar aurantiacus (Mello-Leitão, 1930)	NWC	AY560778	AY560669	--
Mesabolivar aurantiacus (Mello-Leitão, 1930)	NWC	DQ667862	DQ667757	--
Mesabolivar brasiliensis (Moenkhaus, 1898)	NWC	--	--	AY560738
Mesabolivar cyaneotaeniatus (Keyserling, 1891)	NWC	AY560781	AY560671	AY560739
Mesabolivar cyaneotaeniatus (Keyserling, 1891)	NWC	DQ667866	DQ667760	--
Mesabolivar eberhardi Huber, 2000	NWC	DQ667870	DQ667763	**DQ667845**
Mesabolivar eberhardi Huber, 2000	NWC	DQ667872	DQ667764	--
Mesabolivar luteus (Keyserling, 1891)	NWC	DQ667873	DQ667766	**DQ667846**
Mesabolivar sp. 1	NWC	DQ667874	DQ667767	--
Mesabolivar sp. 2	NWC	DQ667875	DQ667769	--
Mesabolivar sp. 3	NWC	DQ667877	DQ667771	--
Mesabolivar sp. 3	NWC	DQ667876	DQ667770	--
Mesabolivar sp. 4 (*M.* sp. 6 in Astrin *et al.* 2006)	NWC	DQ667882	DQ667775	--
Mesabolivar sp. 5	NWC	DQ667881	DQ667773	--
Mesabolivar sp. 5	NWC	DQ667880	DQ667772	--
Metagonia mariguitarensis (González-Sponga, 1998)	phol	DQ667887	DQ667781	--
Metagonia paranapiacaba Huber, Rheims & Brescovit, 2005	phol	DQ667889	DQ667783	--
Metagonia sp. 1	phol	DQ667890	DQ667784	--
Metagonia sp. 2	phol	DQ667891	DQ667785	--
Metagonia sp. 3	phol	DQ667892	DQ667786	AY560741
Metagonia sp. 4	phol	DQ667894	DQ667787	--
Metagonia sp. 5 (*M.* sp. BB-2004b in Bruvo *et al.* 2005; *M.* sp. 7 in Astrin *et al.* 2006)	phol	AY560784	AY560673	AY560742
Metagonia sp. 6	phol	DQ667898	DQ667791	--
Metagonia sp. 6	phol	DQ667897	DQ667789	--

to be continued.

TABLE 1. (continued)

Taxon	group	CO1	16S	28S
Metagonia sp. 6	phol	DQ667899	DQ667792	--
Metagonia sp. 6	phol	DQ667900	DQ667793	--
Metagonia sp. 6	phol	DQ667901	DQ667794	--
Micropholcus fauroti (Simon, 1887)	phol	DQ667902	DQ667795	AY560743
Ninetine gen. sp. indet.	nin	--	--	**DQ667847**
Ninetis subtilissima Simon, 1890	nin	--	--	AY560744
Pholcophora americana Banks, 1896	nin	DQ667905	DQ667797	**DQ667848**
Pholcophora americana Banks, 1896	nin	DQ667904	DQ667796	--
Pholcophora americana Banks, 1896	nin	DQ667906	DQ667798	--
Pholcus opilionoides (Schrank, 1781)	phol	DQ667914	DQ667804	AY560745
Pholcus phalangioides (Fuesslin, 1775)	phol	DQ667920	DQ667809	**DQ667849**
Pholcus phalangioides (Fuesslin, 1775)	phol	DQ667923	DQ667813	--
Pholcus manueli Gertsch, 1937	phol	DQ667907	DQ667801	AY560749
Physocyclus dugesi Simon, 1893	hol	--	--	AY560750
Physocyclus globosus (Taczanowski, 1874)	hol	DQ667929	DQ667822	AY560751
Physocyclus sp.	hol	DQ667930	DQ667825	--
Priscula binghamae (Chamberlin, 1916)	NWC ?	DQ667931	DQ667826	--
Priscula binghamae (Chamberlin, 1916)	NWC ?	DQ667932	DQ667827	--
Priscula sp. 1	NWC ?	DQ667933	DQ667828	**DQ667850**
AY560752 ("*Priscula* sp. ")	NWC	--	--	AY560752
Priscula sp. 2	NWC ?	**DQ667853**	**DQ667838**	--
Priscula venezuelana (Simon, 1893)	NWC ?	DQ667935	DQ667830	--
Psilochorus itaguyrussu Huber, Rheims & Brescovit, 2005	NWC	AY560782	AY560672	AY560740
Psilochorus simoni (Berland, 1911)	NWC	--	--	AY560753
Psilochorus sp.	NWC	--	--	AY560754
Quamtana bonamanzi Huber, 2003	phol	--	--	AY560757
Quamtana embuleni Huber, 2003	phol	--	--	AY560759
Quamtana vidal Huber, 2003	phol	--	--	AY560758
Smeringopus natalensis Lawrence, 1947	hol	--	--	AY560755
Smeringopus pallidus (Blackwall, 1858)	hol	--	--	**DQ667851**
Stenosfemuraia sp.	NWC	DQ667938	DQ667833	AY560760
Trichocyclus sp.	hol	--	--	AY560733
Tupigea sp. 1	NWC	DQ667941	DQ667834	--
Tupigea sp. 2	NWC	DQ667942	DQ667835	--
Tupigea sp. 3	NWC	DQ667943	DQ667836	--
Diguetidae: *Diguetia* sp.	outgroup	AY560795	AY560681	AY560766
Filistatidae: *Kukulcania hibernalis* (Hentz, 1842)	outgroup	**DQ785803**	**DQ785804**	AY560764

Extraction, amplification and sequencing

For details on the extraction of samples and 16S/CO1 amplification as well as double stranded sequencing, see Astrin *et al.* (2006). Amplification and double stranded sequencing of the 28S gene was performed

using the primers 28S-B1 and 28S-B2 (Bruvo-Mađarić *et al.* 2005). For this gene, our PCR was conducted as follows: reaction mixes of 50 µl total volume contained 125 nmol $MgCl_2$, 5 µl 10x PCR-buffer, 25pmol of forward and reverse primer each, 5 pmol dNTPs, 1.75 Units of *Taq* DNA polymerase, 5 µl total DNA template and 2.5 µl dimethyl sulfoxide (DMSO). All of the above lab chemicals were purchased from Sigma-Aldrich (Steinheim, Germany), with one exception: DMSO, which was obtained from Roth (Karlsruhe, Germany). DMSO, functioning as more than an enhancer in this case, often enabled the PCR reaction for the chosen program. We used a TGradient PCR cycler (Biometra; Goettingen, Germany) with a "Touch Down" routine (Palumbi 1996): first cycle set (5 repeats): 30 s denaturation at 94°C, 30 s annealing at 60°C (-1°C per cycle) and 50 s extension at 72°C. Second cycle set (25 repeats): 30 s denaturation at 94°C, 30 s annealing at 55°C and 50 s extension at 72°C. We used an ABI Prism 377 (Applied Biosystems; Foster City, CA, USA) for sequencing.

TABLE 2. Specimen information and voucher data for material sequenced in this work. All specimen vouchers and DNA vouchers were deposited at the ZFMK (Zoologisches Forschungsmuseum Alexander Koenig), Bonn, Germany.

Taxon	group	Collecting Data	Vouchers
Carapoia paraguaensis	NWC	VENEZUELA, km 44 from El Dorado, xii.2002, B.A. Huber	pb05-V37, DNA05-JA97
Carapoia ubatuba	NWC	BRAZIL, Fazenda Angelim, xii.2003, B.A. Huber	pb05-B2, DNA05-JA102
Crossopriza lyoni	hol	VENEZUELA, Mariguitar, Hotel, xii.2002, B.A. Huber	pb05-V47, DNA05-JA120
Holocnemus pluchei	hol	USA, Arizona, Yarnell, around houses, C. Kristenson	pb05-G94, DNA05-JA124
Ibotyporanga naideae	nin	BRAZIL, São Paulo, Campinas, iii.2004, A. dos Santos	pb05-G102, DNA05-JA123
Kaliana yuruani	NWC	VENEZUELA, km 109 (13), xii.2002, B.A. Huber	pb05-V53, DNA05-JA101
Mesabolivar eberhardi	NWC	VENEZUELA, Canaima near Salto Ara, xii.2002, B.A. Huber	pb05-V35, DNA05-JA93
Mesabolivar luteus	NWC	BRAZIL, Minas Gerais, Cotas Altas, Caraça, iv.2002, A. dos Santos	pb05-G6, DNA05-JA41
Ninetine gen. sp. indet.	nin	VENEZUELA, Canaima, Salto Sapo, xii.2002, B.A. Huber	pb05-V01, DNA05-JA83
Pholcophora americana	nin	USA, California, Mono County, Inyo Nat.Forest, vi.2003, P. Paquin	pb05-G89, DNA05-JA45
Pholcus phalangioides	phol	PORTUGAL, Madeira, São Vicente, Laranjal, ii.2003, J.J. Astrin	pb05-J17, DNA05-OJ22
Priscula sp. 1	NWC ?	VENEZUELA, Cueva Guacharo, xii.2002, B.A. Huber	pb05-V24, DNA05-JA22
Priscula sp. 2	NWC ?	PERU, Ayacucho, Wari ruins , v.2003, J.J. Astrin	pb05-J246, DNA05-JA80
Smeringopus pallidus	hol	COMOROS, Mohéli, Ikoni river, v.2003, R. Jocqué	pb05-G85, DNA05-JA127
Kukulcania hibernalis	out-group	VENEZUELA, Yacambú at houses, xii.2002, B.A. Huber	pb05-V57, DNA05-JA47

Phylogenetic analysis

Sequence alignment was performed in MUSCLE (Multiple Sequence Comparison by Log-Expectation) ver. 3.6 (Edgar 2004a, b), run with default parameters (increased iterations) and further refining the output alignment (refine option). The resulting alignments were checked by eye for obvious non-homologies. CO1 and 16S partitions and also all three, CO1, 16S and 28S partitions were concatenated for the analysis using

BioEdit ver. 7.0.4.1 (Hall 1999). Total alignment length for CO1+16S is 873 bp: 412 bp for the CO1 partition and 461 bp for 16S (16S unaligned: around 300 bp, depending on individual length variation due to indels). 28S alignment length is 943 bp, unaligned around 550 bp. The combined alignment for all three genes has a length of 1816 bp. Final alignments are available as electronic supplement and from the authors.

Modeltest ver. 3.7 (Posada and Crandall 1998), employing the Akaike Information Criterion (AIC), identified the GTR+I+Γ model of sequence evolution as the best-fit model for CO1+16S, TIM+Γ for 28S The invariable sites option as suggested by two of the Modeltest runs was not implemented, since we observed signs of overparameterization induced by this additional model complexity (i.e. unconverging, highly oscillating negative log-likelihood values). We used MrBayes ver. 3.1.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) for three separate Bayesian analyses (CO1+16S, 28S-only and CO1+16S+28S). Ambiguous positions and hence many gaps in the 16S and the 28S alignment were excluded in order to guarantee positional homology over the whole alignment (Wägele 2005). This implied exclusion of 99 characters from the analysis for 16S (for reproducibility, alignment positions are given here—cf. supplementary material; positions in the CO1+16S alignment: 441; 442; 459-462; 467-480; 574-620; 635-637; 661-664; 744-750; 766; 767; 831-835; 851-860; 871) and 482 characters for 28S (positions in the 28S-only alignment: 68-71; 91-168; 188-204; 263-280; 291-297; 305; 306; 319-384; 409-452; 458-461; 472-630; 749-792; 806-829; 850-864). The remaining gaps were treated as missing data. Bayesian Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analysis accounting for different evolutionary tendencies of rDNA (differentiating mtDNA and nDNA), 3rd codon positions and 1st plus 2nd codon positions was run for ten million generations (each run with two independent replicates, 1 cold chain and 3 chains of different temperature, sampling every 1'000th tree, 20'000 trees retained). We identified the burn-in in a separate visualization and accordingly discarded the first 96'000 generations for CO1+16S, 210'000 generations for 28S and 140'000 generations for CO1+16S+28S. Posterior probabilities are shown on the 50%-majority rule consensus tree.

We also conducted a heuristic search through maximum parsimony (MP), using the PAUP* software (ver. 4.0b10; Swofford 1998). As opposed to the Bayesian analysis, 3rd codon positions in CO1 were excluded in MP analysis since they produced too much noise in a first, discarded reconstruction (but see Björklund 1999 on their usefulness). Additionally, the same characters were excluded as in the Bayesian analysis. The remaining gaps were first treated as fifth character states, then as missing data in an additional run. The MP analyses were run with branch swapping through tree bisection and reconnection and with 10,000 replicates of random stepwise addition of taxa. Bootstrap values were determined based on 5'000 replicate matrices and added to the 50%-majority rule consensus tree.

Methodological differences to Bruvo-Mađarić *et al.* (2005)

In contrast to Bruvo-Mađarić *et al.* (2005), who coded entire partitions as missing for taxa with incomplete sampling, we reject including such taxa into the respective combined analyses. We excluded a considerable amount of characters in order to analyze only those positions of the alignment with a high probability of being homologous. This implied the exclusion of most of the indels (that can partly be considered as 'artificial'; cf. Astrin *et al*. 2006) from the 16S and 28S genes (see removed positions above). Useful as they proved to be in taxonomy (Astrin *et al.*, 2006), such indels are notoriously problematic in phylogenetics (Morrison and Ellis 1997; Wägele 2005). Thus, we did not use the GapCoder software (Young and Healy 2002, cited in Bruvo-Mađarić *et al.* 2005) employed by Bruvo-Mađarić *et al.* (2005).

One might question the objectivity in our excluding positions with dubious homology from the alignment (e.g. following Gatesy *et al*. 1993). We argue that such a removal of characters (as long as reproducible) is defensible on the grounds that it does not disturb the analysis (no systematic bias results as the excluded regions have to be considered as randomly affected). If anything, such a procedure may reduce the topological resolution, but it also minimizes the noise in the reconstruction. Thus, it can be considered a 'precautionary principle'. We chose the same approach to the use of taxa: not including those taxa for which a partition would

have to be coded as missing leads to a self-inflicted reduction of scope, but warrants a higher robustness of the presented data.

Results and discussion

Information content, base composition, and reconstruction methods

The proportion of variable characters in the analyzed CO1 fragment is 55.6%; 48.1% are parsimony informative. In 16S, variable characters make up 67.7% and informative ones 58.8%. 28S variable characters constitute 74.6% of the alignment and informative characters 54.0%. Considering the variable characters, base composition proved to be homogenous in the three markers. A+T bias existed for both mitochondrial genes, as found in other spiders (e.g. Ayoub *et al.* 2005) or insect mtDNA (e.g. Crozier and Crozier 1993). 28S featured a higher G+C content than A+T. See Table 3 for base frequencies.

The Bayesian tree is much more highly resolved and at the same time has higher nodal support values than the maximum parsimony reconstruction. It also delivered a topology that is more consistent with existing morphological and molecular hypotheses (Huber 2000, 2003a, b; Bruvo-Mađarić *et al.* 2005) than the MP tree. Consequently, results of the MP analyses are not shown graphically. For MP, both reconstructions (scoring gaps as fifth character states *versus* as missing data) delivered identical or very similar consensus trees. Where MP topologies are mentioned in the discussion, this always applies to both MP reconstructions equally.

TABLE 3. Base Frequencies (percent, with standard deviation).

	A	C	G	T	A+T
16S	29.0 (±2.5)	13.7 (±1.3)	15.8 (±2.5)	41.5 (±2.2)	70.5 (±3.6)
CO1	21.2 (±2.5)	14.7 (±1.1)	21.4 (±2.8)	42.7 (±2.0)	63.9 (±3.5)
28S	16.5 (±2.1)	31.2 (±2.2)	35.7 (±1.8)	16.6 (±2.3)	33.1 (±3.4)

The phylogenetic position of *Kaliana*

The phylogenetic trees (Fig. 1-3) suggest an inclusion of the monotypic genus *Kaliana* into the genus *Mesabolivar*. Although occupying different positions in the respective trees, *Kaliana* always appears nested with high support within *Mesabolivar* in the mitochondrial, nuclear and combined Bayesian analyses (Fig. 1-3; MP bootstrap value of combined analysis: 85). In addition, new morphological evidence suggesting a close affinity between *Kaliana* and *Mesabolivar* exists: the *K. yuruani* female, not known at the time of description, distinctly features the median pocket or groove (Fig. 4; see also Huber 2006) considered to be a synapomorphy of *Mesabolivar* (Huber 2000). The two genera share many additional morphological characters, but these have been identified as plesiomorphies (Huber 2000). Based on this new, combined evidence, we synonymize the genus *Kaliana* with *Mesabolivar*, resulting in the new combination *Mesabolivar yuruani* (Huber, 2000).

Ideally, we should have included sequences of the type species of *Mesabolivar*, *M. pseudoblechroscelis* González-Songa, 1998. However, no specimens of this species are available to us. The inclusion of several species representing two of the three operational species groups that together form the core-group of *Mesabolivar* (Huber 2000) make us feel confident about the robustness of our proposed synonymy. Even if *Kaliana yuruani* should eventually turn out to be the sister species of *Mesabolivar* rather than being nested within it, synonymizing this monotypic genus appears justified as it helps in "grouping and ordering by emphasizing affinities among groups of species" (Mayr and Ashlock 1991: 134).

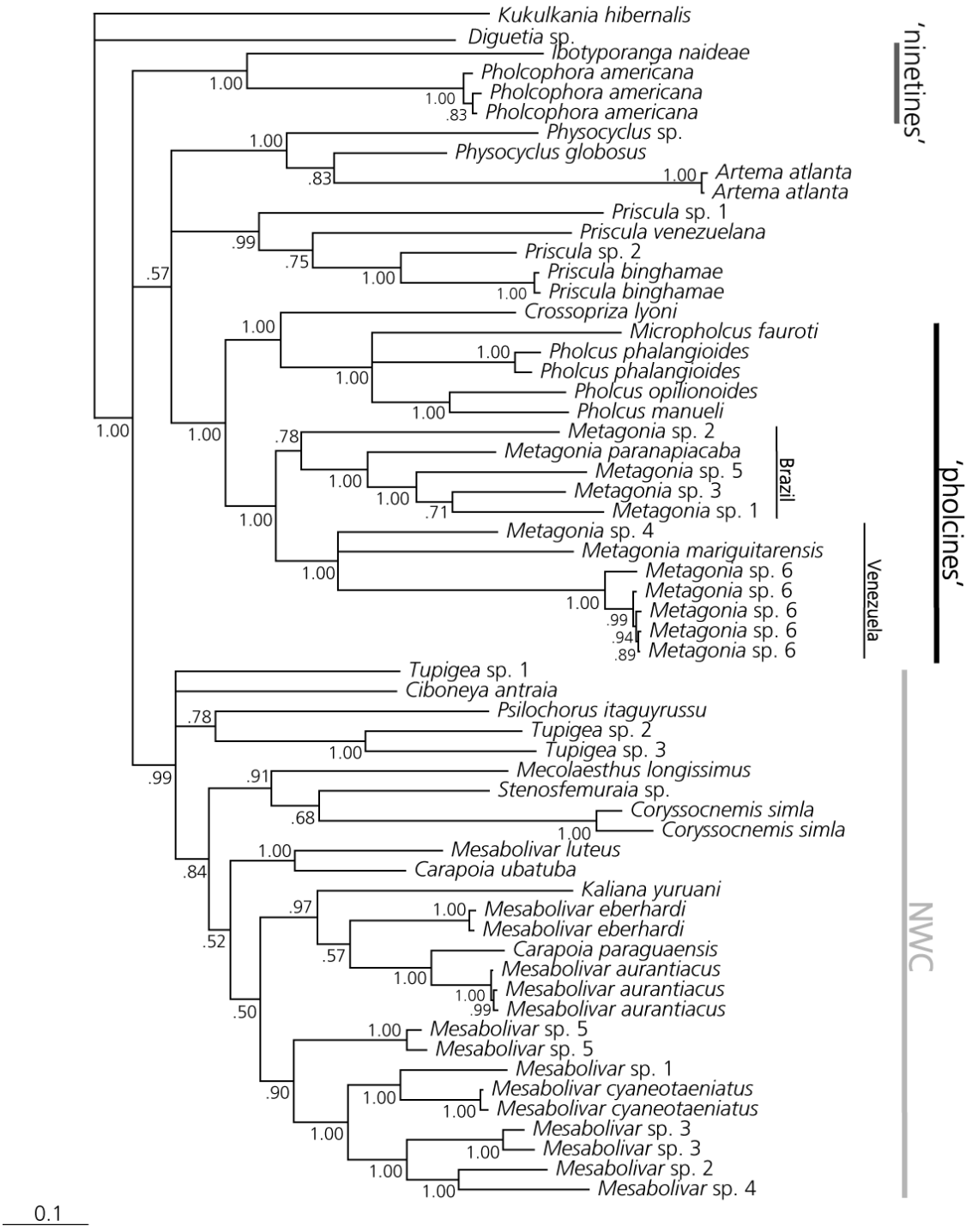

FIGURE 1. Bayesian consensus trees (after burn-in) for CO1 and 16S partitions (combined). Posterior probability values are indicated at the respective nodes; bars denote subfamily level clades sensu Huber (2000).

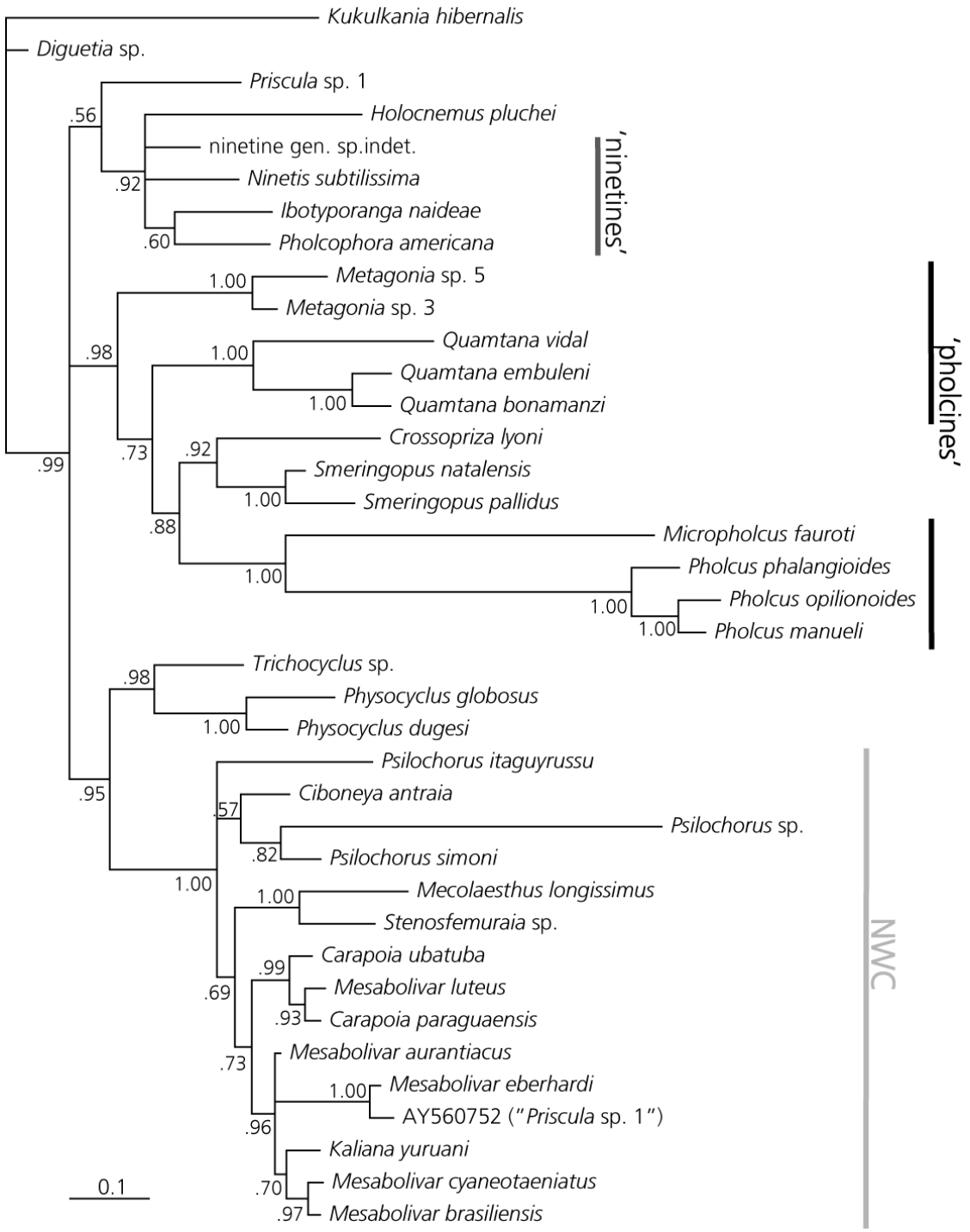

FIGURE 2. Bayesian consensus trees (after burn-in) for 28S partition. Posterior probability values are indicated at the respective nodes; bars denote subfamily level clades sensu Huber (2000).

Mesabolivar and *Carapoia*

The monophyly of *Mesabolivar* + *Carapoia* was consistently, though weakly supported (also MP individual partitions). However, our findings suggest that *Mesabolivar* might be para- or polyphyletic with respect to *Carapoia*: while the two sampled *Carapoia* species fall together in the 28S and combined analyses, *M. luteus* (Keyserling) is also nested within this group (both reconstruction methods). For mtDNA, *C. paraguaensis* González-Sponga is nested within *Mesabolivar*, and *Carapoia ubatuba* Huber + *M. luteus* appear as their sister—in MP: [*Mesabolivar*, (*Carapoia*, *M. luteus*)]. This finding is paralleled by significant homoplasy in morphological characters. On one hand, *M. luteus* shares the principal synapomorphy of *Mesabolivar*, the aforementioned epigynal median pocket (Fig. 913 in Huber 2000). Such a pocket is absent in all nine described *Carapoia* species (Huber 2005). *M. luteus* also lacks a very distinctive synapomorphy of *Carapoia*, the modified hairs frontally on the male chelicerae. On the other hand, the female genitalia of *M. luteus* show a pair of diverging sclerites (Fig. 913-915 in Huber 2000) that are otherwise unique for *Carapoia* (Huber 2005). Obviously, targeted sampling of markers with medium signal depth will be necessary to solve this problem, bearing in mind that *Mesabolivar* currently includes around 40 described and many more undescribed species.

Metagonia

The genus *Metagonia* was recovered, maximally supported, as monophyletic in all reconstructions (only two species in 28S, but eight in CO1-16S). The transfer of the unusual (asymmetric) species *Anomalaia mariguitarensis* (González-Sponga, 1998) to *Metagonia* (Huber 2000) was hence corroborated by molecular data (CO1 and 16S). The mtDNA reconstruction was able to resolve two distinct clades within *Metagonia*. One group consists of all the Brazilian (Atlantic Forest) species we used (*M. paranapiacaba* Huber, Rheims & Brescovit and four undescribed species), while the other comprises all Venezuelan species (*M. mariguitarensis* and two undescribed species).

'Ninetines'

The monophyly of ninetines is supported by several morphological characters (Huber 2000), but it has been suggested that a number of similarities might result from convergence due to miniaturization and adaptation to life in narrow spaces (Huber and Brescovit 2003).

The only previous molecular phylogenetic study (Bruvo-Mađarić *et al*. 2005) included only one representtative of ninetines (*Ninetis subtilissima* Simon from the Arabian Peninsula). Our analysis of 28S adds three further species from North and South America, and the tree in Figure 2 suggests a close relationship among these four species. Considering the fact that the position of *Holocnemus pluchei* (Scopoli) within this group is highly dubious (the genus is morphologically not distinguishable from *Crossopriza*; B. Huber, unpubl. data), we interpret this result as support of ninetine monophyly. Our mitochondrial dataset included only two species (four haplotypes), but again, these were recovered as monophyletic (Fig. 1).

Other subfamiliy-level groups

The New World clade (NWC) sensu Huber (2000) keeps standing unchallenged. It mostly achieves absolute nodal support in our analyses. Based on our findings, we oppose considering *Priscula* as part of the NWC as suggested by Bruvo-Mađarić *et al.* (2005). Their conclusion resulted from using an incorrect 28S sequence, which we proved in our nDNA reconstruction. For the 28S analysis, the *Priscula* sp. 1 sequenced by us fell into a cluster of 'ninetines' and 'holocnemines', while the "*Priscula* sp." sequenced by Bruvo-Mađarić *et al.* (2005) (GenBank accession number: AY560752) appeared as sister to *Mesabolivar eberhardi* Huber. In fact, the sequence AY560752 was much closer to *M. eberhardi* (96% sequence similarity) than to our *Priscula* sp. 1 (66%). This is the more surprising since both specimens supposedly come from an identical collecting lot. Based on the 12S tree topology in Bruvo-Mađarić *et al.* (2005) (where AY560752 appears as sister to *K. yuruani*) and extrapolating from the 28S findings, we suggest that also the 12S sequence used by Bruvo-Mađarić *et*

al. (2005) is not *Priscula*. No corresponding CO1 and 16S sequences were used in the cited study. However, in our mtDNA dataset, the *Priscula* sp. 1 sequenced by us grouped together with three congeners, thus corroborating its authenticity.

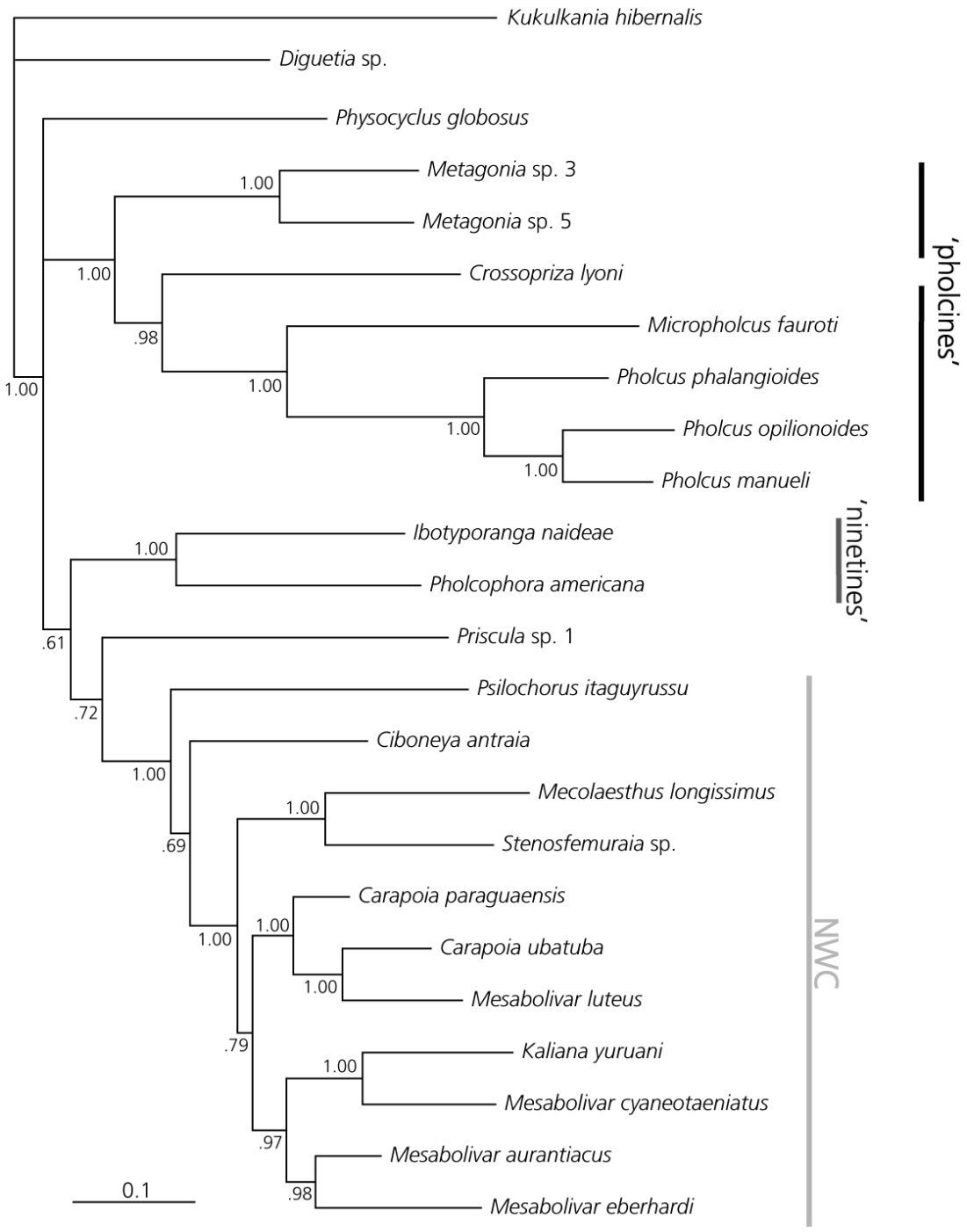

FIGURE 3. Bayesian consensus trees (after burn-in) for all concatenated partitions. Posterior probability values are indicated at the respective nodes; bars denote subfamily level clades sensu Huber (2000).

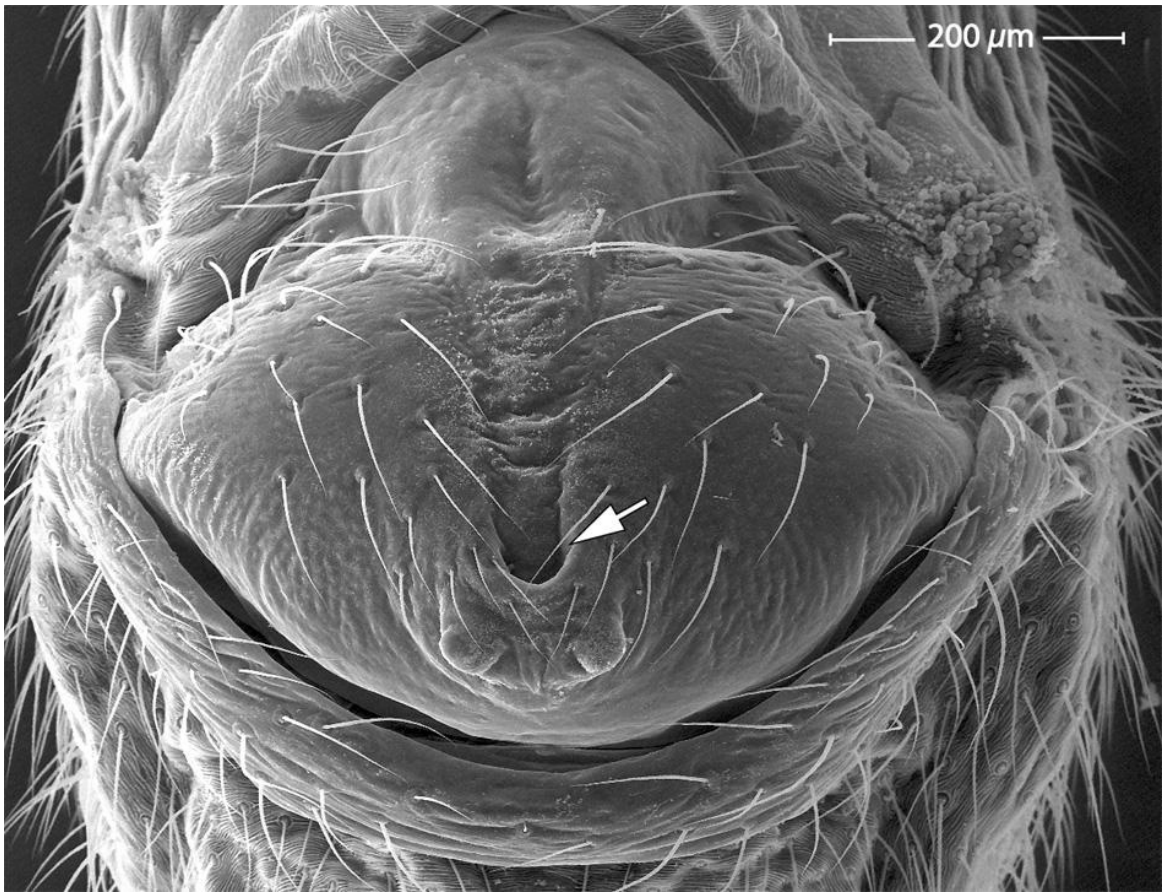

FIGURE 4. Scanning electron microscope (SEM) image of *Kaliana yuruani* female epigynum, ventral view; arrow: median pocket.

In order to judge the relationships of the 'pholcines', a denser sampling, especially at generic level, will be necessary. *Metagonia* and *Pholcus* + *Micropholcus* form a well-supported clade (along with *Quamtana*, 28S) that supports the 'pholcines'. However, *Crossopriza lyoni* (Blackwall), a 'holocnemine' sensu Huber (2000, cf. Timm 1976), appears sister to *Pholcus* + *Micropholcus* and hence nested within the 'pholcines'. Morphologically, *Crossopriza* appears closely related to *Holocnemus* (Wiehle 1933; see above) and both together are putative sister to *Smeringopus* (Huber 2000, 2001, 2003a, b). Although *Crossopriza* and *Smeringopus* group together in the 28S analysis, *Holocnemus* is allocated in a different branch of the phylogram, which we suppose to be an artifact (see above). The position of *Crossopriza* close to 'pholcines' is surprisingly robust, since the taxon is consistently allocated within the 'pholcines' by both mtDNA and nDNA reconstructions. Further research is necessary to evaluate this finding which is not supported by any known morphological data.

'Holocnemines' remain largely unresolved. For 28S, the 'holocnemine' genera *Physocyclus* + *Trichocyclus* were recovered as sister to the NWC with high nodal support, but as sister to all other Pholcidae for 28S MP and in combined analysis. *Artema* + *Physocyclus* obtain a similarly high support in mtDNA analysis as *Trichocyclus* + *Physocyclus* in 28S. Although their position in the tree cannot yet be solved (and although *Artema* appears nested within *Physocyclus*), the monophyly of the grouping *Trichocyclus* + *Physocyclus* + *Artema* (Huber 2003a, b; Bruvo-Mađarić *et al.* 2005) is therefore not questioned by our results.

In conclusion, our study provides strong support for the synonymy of *Kaliana* and *Mesabolivar*, and suggests several promising questions for future research. Beyond that, limitations result mainly from the fact that

most sequences analyzed herein were generated for a study on molecular taxonomy (Astrin *et al*. 2006) rather than to address specific phylogenetic hypotheses.

Acknowledgments

We thank Branka Bruvo who kindly answered many questions during the initial stage of our project. We are also indebted to A. dos Santos, P. Paquin, R. Jocqué and C. Kristenson for contributing material and to an anonymous reviewer for his/her comments. This study was financially supported by the Alexander Koenig Stiftung (to JJA).

References

Astrin, J. J., Huber, B. A., Misof, B. & Klütsch, C. F. (2006) Molecular taxonomy in pholcid spiders (Pholcidae, Araneae): evaluation of species identification methods using CO1 and 16S rRNA. *Zoologica Scripta*, 35, 441-457.

Ayoub, N. A., Riechert, S. E. & Small, R. L. (2005) Speciation history of the North American funnel web spiders, *Agelenopsis* (Araneae: Agelenidae): Phylogenetic inferences at the population-species interface. *Molecular Phylogenetics and Evolution*, 36, 42-57.

Björklund, M. (1999) Are third positions really that bad? A test using vertebrate cytochrome b. *Cladistics - the International Journal of the Willi Hennig Society*, 15, 191-197.

Bruvo-Mađarić, B., Huber, B. A., Steinacher, A. & Pass, G. (2005) Phylogeny of pholcid spiders (Araneae: Pholcidae): Combined analysis using morphology and molecules. *Molecular Phylogenetics and Evolution*, 37, 661-673.

Coddington, J. A. & Levi, H. W. (1991) Systematics and evolution of spiders (Araneae). *Annual Review of Ecology and Systematics*, 22, 565-592.

Crozier, R. H. & Crozier, Y. C. (1993) The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. *Genetics*, 133, 97-117.

Edgar, R. C. (2004a) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, 5, 113.

Edgar, R. C. (2004b) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792-1797.

Gatesy, J., DeSalle, R. & Wheeler, W. (1993) Alignment-ambiguous nucleotide sites and the exclusion of systematic data. *Molecular Phylogenetics and Evolution*, 2, 152-157.

González-Sponga, M. A. (1998) Arácnidos de Venezuela. Cuatro nuevos géneros y cuatro nuevas especies de la familia Pholcidae Koch, 1850 (Araneae). *Memorias de la Fundación La Salle de Ciencias Naturales*, 57, 17-31.

Hall, T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95-98.

Huber, B. A. (2000) New World pholcid spiders (Araneae: Pholcidae): a revision at generic level. *Bulletin of the American Museum of Natural History*, 254, 1-347.

Huber, B. A. (2001) The pholcids of Australia (Araneae; Pholcidae): taxonomy, biogeography, and relationships. *Bulletin of the American Museum of Natural History*, 260, 1-144.

Huber, B. A. (2003a) Cladistic analysis of Malagasy pholcid spiders reveals generic level endemism: Revision of *Zatavua* n. gen. and *Paramicromerys* Millot (Pholcidae, Araneae). *Zoological Journal of the Linnean Society*, 137, 261-318.

Huber, B. A. (2003b) High species diversity in one of the dominant groups of spiders in East African montane forests (Araneae : Pholcidae : *Buitinga* n. gen., *Spermophora* Hentz). *Zoological Journal of the Linnean Society*, 137, 555-619.

Huber, B. A. (2004) Evidence for functional segregation in the directionally asymmetric male genitalia of the spider *Metagonia mariguitarensis* (González-Sponga) (Pholcidae: Araneae). *Journal of Zoology*, 262, 317-326.

Huber, B. A. (2005) Revision and cladistic analysis of the spider genus *Carapoia* González-Sponga (Araneae: Pholcidae), with descriptions of new species from Brazil's Atlantic forest. *Invertebrate Systematics*, 19, 541-556.

Huber, B. A. (2006) Cryptic female exaggeration: the asymmetric female internal genitalia of *Kaliana yuruani* (Araneae: Pholcidae). *Journal of Morphology*, 267, 705-712.

Huber, B. A. & Brescovit, A. D. (2003) *Ibotyporanga* Mello-Leitão: tropical spiders in Brazilian semi-arid habitats (Araneae: Pholcidae). *Insect Systematics and Evolution*, 34, 15–20.

Huelsenbeck, J. P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754-

755.
Mayr, E. & Ashlock, P. 1991. *Principles of Systematic Zoology*. 2nd ed. McGraw-Hill, New York, 475 pp.
Morrison, D. A. & Ellis, J. T. (1997) Effects of nucleotide sequence alignment on phylogeny estimation: a case study of 18S rDNAs of apicomplexa. *Molecular Biology and Evolution*, 14, 428-441.
Palumbi, S. R. (1996) Nucleic Acids II: The Polymerase Chain Reaction. *In*: Hillis, D. M., Moritz, C. & Mable, B. K. (Eds), *Molecular Systematics*. Sinauer: Sunderland, MA, USA, pp. 205-246.
Posada, D. & Crandall, K. A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, 14, 817-818.
Ronquist, F. & Huelsenbeck, J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572-1574.
Simon, E. (1893) Études arachnologiques XL—Descriptions d'espèces et de genres nouveaux de l'ordre des Araneae. *Annales de la Société entomologique de France*, 62, 299-330.
Swofford, D. L. (1998) *PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods)*. Sinauer, Sunderland, MA, USA, 128 pp.
Timm, H. (1976) Die Bedeutung von Genitalstrukturen für die Klärung systematischer Fragen bei Zitterspinnen (Arachnida: Araneae: Pholcidae). *Entomologia Germanica*, 3, 69-76.
Wägele, J. W. (2005) *Foundations of Phylogenetic Systematics*. Pfeil, Munich, Germany, 365 pp.
Wiehle, H. (1933) *Holocnemus hispanicus* sp. n. und die Gattungen *Holocnemus* Simon und *Crossoprisa* Simon. *Zoologischer Anzeiger*, 104, 241-252.