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ScienceDirect
Molecular Phylogenetics and Evolution 43 (2007) 833–851
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

The phylogeny of the social *Anelosimus* spiders (Araneae: Theridiidae) inferred from six molecular loci and morphology

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Received 18 May 2006; revised 7 September 2006; accepted 15 September 2006
Available online 27 September 2006

Abstract

We use fragments of three nuclear genes (Histone 3, 18SrDNA, and 28SrDNA) and three mitochondrial genes (16SrDNA, ND1, and COI) totalling approximately 4.5 kb, in addition to morphological data, to estimate the phylogenetic relationships among *Anelosimus* spiders, well known for their sociality. The analysis includes 67 individuals representing 23 of the 53 currently recognized *Anelosimus* species and all species groups previously recognized by morphological evidence. We analyse the data using Bayesian, maximum likelihood, and parsimony methods, considering the genes individually as well as combined (mitochondrial, nuclear, and both combined) in addition to a 'total evidence' analysis including morphology. Most of the data partitions are congruent in agreeing on several fundamental aspects of the phylogeny, and the combined molecular data yield a tree broadly similar to an existing morphological hypothesis. We argue that such congruence among data partitions is an important indicator of support that may go undetected by standard robustness estimators. Our results strongly support *Anelosimus* monophyly, and the monophyly of the recently revised American '*eximius* lineage', although slightly altered by excluding *A. pacificus*. There was consistent support for the scattering of American *Anelosimus* species in three clades suggesting intercontinental dispersal. Several recently described species are reconstructed as monophyletic, supporting taxonomic decisions based on morphology and behaviour in this taxonomically difficult group. Corroborating previous results from morphology, the molecular data suggest that social species are scattered across the genus and thus that sociality has evolved multiple times, a significant finding for exploring the causes and consequences of social evolution in this group of organisms.

Keywords: Cobweb spiders; Combined analysis; Congruence; Evolutionary dead end; Evolution of sociality; Sociality; Total evidence

1. Introduction

The cosmopolitan cobweb spider (Theridiidae) genus *Anelosimus* Simon, 1891 (Fig. 1) contains 53 described species (Platnick, 2006; Agnarsson, 2006; Agnarsson and Zhang, 2006), with most species found in tropical or subtropical habitats. Among the species are several that are permanently social (quasisocial, henceforth 'social'), a trait otherwise rare in spiders (see Avilés, 1997 for review). Thus the genus plays an important role in the study of sociality and its evolution (e.g. Kullmann, 1972; Avilés, 1997; Avilés and Gelsey, 1998; Furey, 1998; Avilés et al., 2000, 2001; Saffre et al., 2000; Jones and Parker, 2002; Gonzaga and Vasconcellos-Neto, 2002; Agnarsson, 2006; Bukowski and Avilés, 2002; Powers and Avilés, 2003; Avilés and Bukowski, 2006; Agnarsson et al., 2006). An existing morphological phylogenetic hypothesis suggests phylogenetic scattering of social *Anelosimus* species, implying multiple origins of sociality in this genus (Agnarsson, 2005, 2006). This was somewhat unexpected, because social species not only share many unique derived characters associated with sociality, but they are also geographically close: only American *Anelosimus* species are social while the genus is common on all continents. Multiple origins of sociality would

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1055-7903/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2006.09.011

Fig. 1. *Anelosimus* habitus and web photographs. (A) female *A. kohi* carrying her egg sac in a typical *Anelosimus* manner—in the chelicerae; (B) male *A. kohi*; (C) social nest of *A. eximius*, the web measures over 1 m^3 and contains over 1000 individuals; (D) cooperative prey capture in *A. eximius*.

have important implications: for instance, that the inbreeding that characterizes social *Anelosimus* has originated several times independently (Smith, 1986; Riechert and Roeloffs, 1993; Smith and Hagen, 1996; Avilés, 1997; Avilés and Bukowski, 2006). This would provide an opportunity to study the causes and consequences of the association between inbreeding and sociality (e.g. Avilés, 1997; Bilde et al., 2005; Mcleish et al., 2006). Multiple origins would also imply that sociality can be advantageous over the short term, although over the long term the negative consequences of inbreeding may ultimately doom the lineage (Avilés, 1997; Agnarsson et al., 2006). The contrast between within-species advantage and among-species disadvantage is another example of multilevel selection, already studied extensively in *Anelosimus* with respect to sex ratios and population structure (Avilés, 1993; Smith and Hagen, 1996).

Resolving the number of origins of sociality is a high priority for studies of *Anelosimus* phylogeny. Lack of formal phylogenetic data, limited information from morphological data to resolve species level relationships, and difficulty of interpreting some of the morphological variation (Agnarsson, 2006) have made that task difficult. In addition, progress on this topic has long been hindered by basic confusion on the very identity and limits of the units of study—the monophyly of the genus and the taxonomic status of its individual species. *Anelosimus* has a long and tortuous taxonomic history as perhaps predicted by the etymology of its name, interpreted to mean 'very uncertain' and reflect Simon's uncertainty as to its status (Cameron, 2005). Simon (1894) himself almost immediately rejected his own genus and synonymized it with *Theridion* Walckenaer, 1805 where its species had been before. The genus was revalidated by Levi (1956), but was circumscribed based on plesiomorphic characters and soon became a polyphyletic 'waste-basket' genus for hard-to-place theridiids (Agnarsson, 2004, 2006). Recent work has clarified its borders and offered morphological support for a monophyletic *Anelosimus* restricted almost entirely to species showing some level of social behaviour, while transferring solitary species to at least seven other theridiid genera (Agnarsson, 2004, 2005, 2006). However, not only has the composition of *Anelosimus* been troublesome, but also the taxonomy of species within it. In particular, the concepts of *A. studiosus* and *A. jucundus* have historically fluctuated. While most often treated as two very variable species (e.g. Levi, 1956, 1963), Cambridge (1902) suggested the variation was so profuse it was impossible to tell (based on museum specimens) whether they represented a number of closely related species, or perhaps were even conspecific. Agnarsson (2006), based on morphology and novel behavioural data recognized 13 species within the *studiosus* and *jucundus* groups, thereof three newly described social species.

As acknowledged by Agnarsson (2006) taxonomic decisions based on morphological data were often difficult, and the morphological data lacked power to fully resolve, or

strongly support, many nodes in the *Anelosimus* phylogeny. Our data here simultaneously allow us to test the taxonomic limits of the genus and some of the newly proposed species concepts of Agnarsson (2006), and to reconstruct the phylogenetic relationship of species, an essential backbone for addressing evolutionary questions related to sociality, inbreeding, and multilevel selection.

2. Materials and methods

2.1. Taxon sampling

Field work was conducted across the Americas, in Africa and Southeast Asia during 2003–2006, attempting broad geographic and taxonomic sampling. With limited resources, however, we felt justified in focusing most our fieldwork in the Americas, where the genus is most diverse and where all the known social species occur. By especially densely sampling the clade that is best known behaviourally (the American *eximius* group) we are best able to address questions of independent origins of sociality.

Anelosimus terminals included 53 individuals representing 23 of the 53 *Anelosimus* species described to date (see Table 1 for species list and species author names). For the molecular data we included all described species of which we were able to obtain fresh material; these represent all species groups identified previously using morphology (see Agnarsson, 2006). According to the morphological data the missing species appear scattered throughout the phylogeny, but the majority belong to three main groups represented each by two species: The 'epigynal scape clade' (*A. ethicus* and *A. nigrescens*), the 'Madagascar clade' (*A. may* and an undescribed species) and the 'filiform embolus clade' ('Tanzania group' of Agnarsson, 2006) an apparently monophyletic group of East African and Asian species (*A. linda* and *A. agnar*).

To test *Anelosimus* monophyly, outgroups included the closely related *Kochiura* Archer, 1950 (previously in *Anelosimus*, see Agnarsson, 2004) and 10 species from Theridiinae whose sister relationship to *Anelosimus*, or *Anelosimus* plus *Kochiura* has been supported previously by both morphological (Agnarsson, 2004) and molecular (Arnedo et al., 2004) data. To root the phylogenies, we also include more distant outgroups (two argyrodines, one spintharine). When sufficient material was available, more than one specimen of each *Anelosimus* species were included. This allows a first test of the taxonomic limits of species. The list of the specimens sampled in the present study is shown in Table 1.

2.2. Data

Live specimens were collected in the field and fixed in 95–100% ethanol, or killed in ethanol and then immediately placed in RNAlater® (Ambion). When fresh material was not available, specimens from collections preserved in 75% ethanol were used. When sufficient material was available, entire specimens (minus excised genitalia kept as vouchers) were used for extraction, while for rare species only two to four legs were used. The vouchers will be deposited at the National Museum of Natural History, Smithsonian Institution, in Washington, DC. Genomic DNA was extracted with the QIAGEN DNeasy extraction kit.

Fragments of the nuclear genes 18S rRNA (18S), 28S rRNA (28S), and Histone H3 (H3), and the mitochondrial genes cytochrome *c* oxidase subunit I (CO1), 16SrRNA (16S), and NADH dehydrogenase subunit 1 (ND1) were amplified. For primer pairs and annealing temperatures see Table 2. For 16S, the primers LR-J-12864 (Simon et al., 1994) and LR-N-13398 (Arnedo et al., 2004) were used initially with low rate of success. The sequences obtained from these were used to develop a new primer pair (Table 2), which worked much more effectively, especially within *Anelosimus*. MJ Research PC-100 Thermal cyclers were used to perform 34 iterations of the following cycle: 30 s at 95°C, 45 s at 42–58 °C (depending on primers, see Table 2), and 45 s at 72 °C, beginning with an additional single cycle of 2 min at 95 °C and ending with another one of 10 min at 72 °C. For the 18S primer a "touchdown" strategy was applied, beginning at 58 °C and lowering proportionally the temperature in each cycle for 20 cycles down to 45 °C and keeping that annealing temperature for an additional 20 cycles. The PCR mix, per sample contained: 13.6 μl sterile water, 0.6 μl Roche AmpliTaq DNA polymerase, and 2.5 μl each of dNTPs, buffer, and each primer. To this mix 0.8 μl of genomic DNA were added. For a portion of the samples, PCR products were then purified using the Qiagen PCR Purification Kit as per manufacturer's specifications. Remaining purification of samples, and sequencing of DNA, in both directions for each PCR product, was done by the Macrogen® Inc (ABI 377 sequencer).

Sequences were inferred using Phred to read bases and assign quality scores (Green and Ewing, 2002), and Phrap to assemble the reads (Green, 1999) through the chromaseq package (D. Maddison and W. Maddison, in preparation) in the evolutionary analysis program Mesquite (Maddison and Maddison, 2005a,b,c). Phred was run with default options; phrap was used with options -qual_show 20 -vector_bound 0. Sequence ends were trimmed by chromaseq using a moving window analysis: the first window of 10 bases within which at least 6 were above quality score 20 was used as the start or end of the sequence. If a site had secondary peaks at least 0.3 the height of the primary peak, it was treated as ambiguous. Subsequently the sequences were proofread by comparing them with the chromatograms by eye.

All lines of data in the molecular matrices represent single individuals, except *Kochiura*, a chimera of *K. aulica* (COI, 16S, 18S, from Arnedo et al., 2004), and *K. rosea* (28S, H3).

2.3. Analyses

2.3.1. Alignment and matrices

For the protein coding genes (H3, COI, NDI) the alignment was trivial with no gaps implied. The other genes were

Table 1
List of specimens, collection data, sample code referring to vouchers stored at the National Museum of Natural History, Smithsonian Institution, and GenBank accession numbers

Genus	Species and Author	Country	Region	Locality	Lat. Long.	Code	COI	NDI	16S	18S	28S	H3
Anelosimus	agnar Agnarsson, 2006	Malaysia	Johor	Teluk Mahkota	N 1.9000 E 104.104000	093A	EF050279	n/a	n/a	n/a	EF050215	n/a
Anelosimus	analyticus (Chamberlin, 1924)	USA	California	La Julla	N 33.8572 W 117.8755	023A	n/a	n/a	n/a	n/a	EF050207	n/a
Anelosimus	analyticus (Chamberlin, 1924)	USA	California	La Julla	N 33.8572 W 117.8755	d083	EF050271	EF050374	EF050151	EF050184	EF050206	n/a
Anelosimus	arizona Agnarsson, 2006	USA	Arizona	Garden Canyon	N 31.55 W 110.28	060A	EF050273	EF050376	EF050161	n/a	EF050209	EF050332
Anelosimus	arizona Agnarsson, 2006	USA	Arizona	Patagonia	N 31.55 W 110.83	087A	EF050272	EF050375	EF050160	n/a	EF050208	EF050331
Anelosimus	baeza Agnarsson, 2006	Ecuador	Napo	nr. Quercos	S 0.17469 W 77.6793	004A	EF050284	EF050384	EF050146	n/a	EF050220	n/a
Anelosimus	baeza Agnarsson, 2006	Brazil	São Paulo	Serra do Japi	S 23.183 W 46.867	022A	EF050281	EF050381	EF050143	n/a	EF050217	EF050338
Anelosimus	baeza Agnarsson, 2006	Ecuador	Manabí	Puerto Lopez	S 1.5497 W 80.8104	042A	EF050282	EF050382	EF050144	n/a	EF050218	n/a
Anelosimus	baeza Agnarsson, 2006	Ecuador				216A	EF050285	EF050385	EF050147	n/a	EF050221	n/a
Anelosimus	baeza Agnarsson, 2006	Ecuador	Pichincha	nr. Mindo	S 0.00395 W 78.67722	005A	EF050283	EF050383	EF050145	EF050185	EF050219	n/a
Anelosimus	domingo Levi, 1963	Ecuador	Napo	Jatun Sacha	S 1.067 W 77.617	009A	EF050288	EF050387	EF050162	EF050186	EF050224	EF050341
Anelosimus	domingo Levi, 1963	Ecuador	Ecuador	Cuyabeno		059A	EF050287	n/a	n/a	n/a	EF050223	EF050340
Anelosimus	dubiosus (Keyserling, 1891)	Brazil	São Paulo	Serra do Japi	S 23.183 W 46.867	017A	EF050290	EF050389	EF050165	EF050187	EF050226	EF050343
Anelosimus	dubiosus (Keyserling, 1891)	Brazil				078A	EF050289	EF050388	EF050164	n/a	EF050225	EF050342
Anelosimus	elegans Agnarsson, 2006	Ecuador	Pichincha	km 20 from Limón towards Gualaceo	S 3.0044 W 78.5142	068A	EF050292	EF050391	EF050140	n/a	EF050228	EF050345
Anelosimus	elegans Agnarsson, 2006	Ecuador	Morona Santiago	km 20 from Limón towards Gualaceo	S 3.00098 W 78.51206	115A	EF050293	EF050392	EF050141	n/a	EF050229	n/a
Anelosimus	ethicus (Keyserling, 1884)	Uruguay	Montevideo	Montevideo, Melilla	S 34.90 W 56.15	021A	EF050294	n/a	n/a	EF050189	EF050230	n/a
Anelosimus	eximius (Keyserling, 1884)	Ecuador	Napo	Jatun Sacha	S 1.067 W 77.617	034A	EF050298	EF050397	EF050168	EF050191	EF050235	EF050348
Anelosimus	eximius (Keyserling, 1884)	Ecuador	Sucumbios	Guyabeno		058A	n/a	EF050396	EF050166	n/a	EF050234	EF050347
Anelosimus	eximius (Keyserling, 1884)	Ecuador				085A	EF050297	EF050395	EF050167	n/a	EF050233	n/a
Anelosimus	guacamayos Agnarsson, 2006	Ecuador	Morona Santiago	km 6.7 from Limón-Gualaceo	S 2.99368 W 78.43411	002A	EF050299	EF050398	EF050150	EF050192	EF050236	EF050349
Anelosimus	guacamayos Agnarsson, 2006	Ecuador	Napo	NE of El Chaco, Rio Salado	S 0.2025 W 77.7015	007A	EF050301	EF050400	EF050149	EF050194	EF050238	EF050351
Anelosimus	guacamayos Agnarsson, 2006	Ecuador	Napo	NE of El Chaco, Rio Salado	S 0.2025 W 77.7015	010A	EF050300	EF050399	EF050148	EF050193	EF050237	EF050350
Anelosimus	jabaquara Levi, 1956	Brazil	São Paulo	Serra do Japi	S 23.183 W 46.867	062A	EF050302	EF050401	EF050163	n/a	EF050239	EF050352
Anelosimus	jucundus (O. P.-Cambridge, 1896)	Costa Rica	Alajuela	San Ramón	N 10.24725 W 84.52365	148A	n/a	EF050402	EF050158	n/a	EF050240	n/a
Anelosimus	jucundus (O. P.-Cambridge, 1896)	Costa Rica	Cartago	Cerro de la Muerte	N 9.79596 W 83.95991	150A	EF050303	n/a	n/a	n/a	EF050241	n/a
Anelosimus	kohi Yoshida, 1993	Singapore	Palau Ubin	Chek Jawa	N 1.407 E 103.991	088A	EF050274	EF050377	EF050172	n/a	EF050210	EF050333
Anelosimus	kohi Yoshida, 1993	Singapore	Palau Ubin	Chek Jawa	N 1.407 E 103.991	089A	EF050277	EF050379	EF050171	n/a	EF050213	EF050336
Anelosimus	kohi Yoshida, 1993	Singapore	Palau Ubin	Chek Jawa	N 1.407 E 103.991	090A	EF050278	EF050380	EF050170	n/a	EF050214	EF050337
Anelosimus	kohi Yoshida, 1993	Malaysia	Johor	Teluk Mahkota	N 1.9000 E 104.104000	092A	EF050275	EF050378	EF050169	n/a	EF050211	EF050334
Anelosimus	kohi Yoshida, 1993	Malaysia	Johor	Teluk Mahkota	N 1.9000 E 104.104000	095A	EF050276	n/a	n/a	n/a	EF050212	EF050335

Anelosimus	linda Agnarsson, 2006	Malaysia	Pahang	Cameron Highlands, Arcadia	N 4.4820 E 101.3880	099A	EF050280	n/a	n/a	n/a	EF050216	n/a
Anelosimus	may Agnarsson, 2005	Madagascar	Tomasina	Andasibe N.P., E of Moramanga	S 18.943944 E 48.417583	039A	EF050305	EF050404	EF050174	n/a	EF050244	EF050355
Anelosimus	nigrescens (Keyserling, 1884)	Brazil	São Paulo	Serra do Japi	S 23.183 W 46.867	019A	EF050308	EF050406	EF050177	EF050196	EF050247	EF050357
Anelosimus	nr. sallee	Madagascar	Antananarivo	Forêt d'Ambohitantely, NE of Akazobe	S 18.171389 E 47.281944	040A	EF050304	EF050403	EF050173	n/a	EF050243	EF050354
Anelosimus	octavius Agnarsson, 2006	Costa Rica	Guanacaste	Rincón de la Vieja	N 10.78466 W 85.34877	144A	n/a	EF050407	EF050159	n/a	EF050248	n/a
Anelosimus	oritoyacu Agnarsson, 2006	Ecuador	Napo	Lago Agrio, nr Baeza	N 0.0833 W 76.883	008A	EF050311	EF050410	EF050136	EF050198	EF050251	EF050360
Anelosimus	oritoyacu Agnarsson, 2006	Ecuador	Napo	3.9 km from Baeza	S 0.466667 W 78.185833	032A	EF050310	EF050409	EF050135	EF050197	EF050250	EF050359
Anelosimus	oritoyacu Agnarsson, 2006	Ecuador				076A	EF050309	EF050408	EF050134	n/a	EF050249	EF050358
Anelosimus	pacificus Levi, 1956	Costa Rica	Puntarenas	Parrita	N 9.5167 W 84.3167	097A	EF050312	EF050411	EF050175	n/a	EF050252	n/a
Anelosimus	pacificus Levi, 1956	Costa Rica	Puntarenas	Parrita	N 9.5167 W 84.3167	098A	EF050313	EF050412	EF050176	n/a	EF050253	EF050361
Anelosimus	rupununi Levi, 1956	Ecuador	Morona Santiago	road between Limón and Patuca	S 2.82825 W 78.3582	016A	EF050316	n/a	n/a	EF050199	EF050256	EF050363
Anelosimus	rupununi Levi, 1956	Argentina	Formosa	Laishi, El Poagual Reserve	S 26.181389 W 58.949722	029A	EF050315	n/a	n/a	n/a	EF050255	EF050362
Anelosimus	studiosus Hentz, 1850	Ecuador	Azuay	S of Azogues on road to Gualaceo	S 2.8539 W 78.9141	011A	EF050317	EF050413	EF050154	EF050200	EF050257	EF050364
Anelosimus	studiosus Hentz, 1850	USA	Louisiana	Tulane University		061A	EF050321	EF050417	EF050156	n/a	EF050261	EF050367
Anelosimus	studiosus Hentz, 1850	Ecuador	Pichincha	Hacienda Collas	S 0.0884 W 78.3920	067A	EF050319	EF050415	EF050152	n/a	EF050259	EF050366
Anelosimus	studiosus Hentz, 1850	Ecuador	Pichincha	NE of Calderon	S 0.0691 W 78.3866	069A	EF050318	EF050414	EF050153	n/a	EF050258	EF050365
Anelosimus	studiosus Hentz, 1850	Costa Rica	Alajuela	Arenal	N 10.53752 W 84.99279	146A	EF050320	EF050416	EF050157	n/a	EF050260	n/a
Anelosimus	tosum (Chamberlin, 1916)	Ecuador	Chimborazo	6 km NE of Chunchi	S 2.2636 W 78.8887	003A	EF050327	EF050421	EF050138	EF050204	EF050267	EF050372
Anelosimus	tosum (Chamberlin, 1916)	Ecuador	Cañar	W of Suscal	S 2.4588 W 79.1644	013A	EF050325	EF050419	EF050137	EF050202	EF050265	EF050370
Anelosimus	tosum (Chamberlin, 1916)	Ecuador	Cañar	W of Suscal	S 2.4671 W 79.1185	014A	EF050326	EF050420	EF050139	EF050203	EF050266	EF050371
Anelosimus	tungurahua Agnarsson, 2006	Ecuador	Tungurahua	Baños	S 1.666944 W 78.700556	063A	EF050328	n/a	n/a	n/a	EF050268	n/a
Anelosimus	tungurahua Agnarsson, 2006	Ecuador	Tungurahua	Baños	S 1.666944 W 78.700556	064A	EF050329	EF050422	EF050155	n/a	EF050269	EF050373
Argyrodes	argentatus O. P.-Cambridge, 1880	USA	Hawaii	Oahu		A80	AY231032	n/a	AY230957	AY230900	AY231090	AY230992
Coleosoma	acutiventer (Keyserling, 1884)	Ecuador	Morona Santiago	km 7 from Limón towards Gualaceo	S 2.9962 W 78.4558	037A	EF050286	EF050386	EF050181	EF050188	EF050222	EF050339
Echinotheridion	otlum Levi, 1963	Ecuador	Napo	Jatun Sacha	S 1.067 W 77.617	036A	EF050291	EF050390	EF050179	n/a	EF050227	EF050344
Exalbidion	pallisterorum (Levi, 1959)	Costa Rica	Puntarenas	Monteverde, road to Las Torres	N 10.31206 W 84.81042	164A	EF050296	EF050394	EF050182	n/a	EF050232	n/a
Kochiura	rosea/aulica	Chile	Osorno	Puyehue N.P., Aguas Calientes	S 40.767 W 72.283333	073A	AY231045	n/a	AY230949	EF050195	EF050242	EF050353

(*continued on next page*)

Table 1 (*continued*)

Genus	Species and Author	Country	Region	Locality	Lat. Long.	Code	COI	NDI	16S	18S	28S	H3
Meotipa	sp.	Malaysia	Pahang	Gunung Brinchang	N 4.515 E 101.383	104A	EF050306	EF050405	EF050180	n/a	EF050245	n/a
Moneta	sp.	Malaysia	Pahang	Cameron Highllands, Arcadia	N 4.482 E 101.388	100A	EF050307	n/a	n/a	n/a	EF050246	EF050356
Rhomphaea	metaltissima Soares & Camargo, 1948	Guyana	Waiwai	South of Gunns landing	S 1.612778, W 58.637500	MS102	AY231052	n/a	AY230950	AY230921	AY231083	AY231009
Rugathodes	sp.	Costa Rica	Guanacaste	Playa Hermosa	N 10.57941 W 84.67635	149A	EF050314	n/a	n/a	n/a	EF050254	n/a
Exalbidion?	Generic placement uncertain	Malaysia	Pahang	Tanah Rata	N 4.46 E 101.40	101A	EF050295	EF050393	EF050183	EF050190	EF050231	EF050346
Ameridion?	Generic placement uncertain	Malaysia	Pahang	Gunung Brinchang	N 4.515 E 101.383	102A	EF050270	n/a	n/a	n/a	EF050205	EF050330
Theridiinae	sp.	Malaysia	Pahang	Gunung Brinchang	N 4.515 E 101.383	103A	EF050323	n/a	n/a	n/a	EF050263	n/a
Theridion	calcynatum Holmberg, 1876	Ecuador	Napo	Caucheras, Yenayacu	S 0.5907 W 77.8829	035A	EF050322	n/a	n/a	n/a	EF050262	EF050368
Theridion	nigroannulatum Keyserling, 1884	Ecuador	Morona Santiago	6.6 km N of Limón on way to Méndez	S 2.9227 W 78.4079	055A	EF050324	EF050418	EF050178	EF050201	EF050264	EF050369

aligned in Clustal W (Thompson et al., 1997), followed by minor manual editing in MacClade (Maddison and Maddison, 2005a,b,c) to correct conspicuously misaligned blocks mostly near each end of the alignments. Hedin and Maddison (2001) explored alignment space ranging from relatively "gappy" alignments (e.g. gap opening/gap extension = 8/2) to relatively "compressed" alignments (e.g. gap opening/gap extension = 24/6), generally favouring compressed alignments based on congruence with an elision matrix (see Wheeler et al., 1995). We choose gap opening and gap extension costs of 24/6, resulting in a compressed alignment, for our main analyses, and then choose alignment parameters from the other end of the spectrum (8/2) to examine if our results are sensitive to alignment parameter choice.

In our case, most gaps occur in regions where alignment appears ambiguous. Treating gaps as informative may add unwarranted weight to such ambiguous regions and for our main analyses gaps were treated as missing data. We nevertheless examined the effect of treating gaps as fifth state in the 'all data combined' analysis under parsimony.

Prior to analyses the alignments were trimmed on each end to exclude primer sequences. Mitochondrial DNA was obtained by PCR of two mtDNA segments. One segment includes partial 16S plus $tRNA^{LEU}$ totalling 577 bp (max unaligned length 529 bp, the intervening $tRNA^{LEU}$ 48–51 bp), and ND1 of 353 bp. The other segment contains a partial COI sequence totalling 1173 bp. The nuclear data included partial 28S totalling 788 bp (max unaligned 779 bp), 18S totalling 1074 bp (max unaligned 1068 bp), and Histone 3 totalling 354 bp.

In addition to each gene separately, the following combination matrices were constructed: all mitochondrial data combined, nuclear data combined, mitochondrial protein coding genes combined and translated to amino acid sequences, all genes combined, and all genes combined with morphology (from Agnarsson, 2006, and Agnarsson et al., 2006). All the matrices will be made available online at: http://theridiidae.com/cladogramsi.html and the matrices of combined molecular and molecular plus morphology will be submitted to Treebase.org.

2.3.2. Phylogenetic methods and software

Bayesian analysis was performed using MrBayes V3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Based on Modeltest 3.6 (Posada and Crandall, 1998), the best fitting model for each matrix was either the GTR + Γ + I (Rodríguez et al., 1990; Yang, 1994), or the TrN + Γ + I model (Tamura and Nei, 1993, GTR-type model where parameters controlling the rates of the different types of transversions are equal). As the TrN + Γ + I model is not available in MrBayes the GTR + Γ + I model was used throughout. For the protein coding genes, Bayesian analyses were partitioned by codon position. For the combined molecular matrix the analysis was partitioned by loci and by codon, using the same model for each partition as in the single-gene

Table 2
Primer sequences and source, and annealing temperatures (range used, and optimal in bold)

Forward	Sequence	Reference	Reverse	Sequence	Reference	Annealing temp.
18S-4F	5′-CCAGCAGCCGCGCTAATTC-3′	(Giribet et al., 1996)	9R	5′-GATCCTTCCGCAGGTTCACCTAC-3′	(Giribet et al., 1996)	See text
28SC	5′-GGTTCGATTAGTCTTTCGCC-3′	(Whiting et al., 1997)	28SO	5′-GAAACTGCTCAAAGGTAAACGG-3′	(Whiting et al., 1997)	48–50°, **50°**
H3aF	5′-ATGGCTCGTACCAAGCAGACVGC-3′	(Colgan et al., 1998)	H3aR	5′-ATATCCTTRGGCATRATRGTGAC-3′	(Colgan et al., 1998)	48–56°, **56°**
LCOI1490	5′-GGTCAACAAATCATAAAGATATTGG-3′	(Folmer et al., 1994)	C1-N-2776	5′-GGATAATCAGAATATCGTCGAGG-3′	(Hedin and Maddison, 2001)	44°
16SF	5′-CTAAGGTAGCATAATCA-3′	This study	16SR	5′-ATGATCATCCAATTGAT-3′	This study	47°

analyses, and estimating all parameters independently for each partition ('unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all)'). The model employed 6 substitution types ("nst=6"), with rates and proportion of invariable sites ("rates=invgamma"), and base frequencies, estimated from the data. For each analysis, four MCMC (Markov Chain Monte Carlo) chains (one cold and three heated) were run for 10,000,000 generations, except the combined analyses which were run for 20,000,000 generations, sampling the Markov chain every 1000 generations, and the sample points of the first 1,000,000 generations were discarded as "burn-in", after which the chain reached stationarity. Posterior probabilities were computed from a majority rule consensus tree of the post-burn in trees (consensus trees are depicted in Figs. 3–10 and 12).

Maximum likelihood analyses were performed using RAxML-VI (Stamatakis, 2005), using default settings under the GTR + Γ model, and PAUP* (Swofford, 2002), using the GTR + Γ + I model. With RaxML analyses were done by 20 separate runs with the default hill climbing, and by simulated annealing with time limit of 24 h, choosing the run with the best likelihood. The model GTRCAT was used for nucleotide matrices, JTTCAT for amino acid matrices. Under PAUP* each matrix was analyzed for 10 random addition sequences, with maximum rearrangement limit set to 10,000 each replicate.

Parsimony analyses were conducted with the programs PAUP* (Swofford, 2002) and NONA (Goloboff, 1993). All matrices were analyzed using a heuristic search with 10,000 random additions, keeping a maximum of 10 trees per iteration, in PAUP* this was followed by swapping on the best trees with a maximum limit of 50,000 trees. Branch support was estimated using nonparametric bootstrapping (Felsenstein, 1985) using 1000 replicates of a heuristic search with 10 iterations of random addition of taxa holding 10 trees per iteration.

3. Results

3.1. Sequences

All sequences are deposited in GenBank (See Table 1 specimen information and Accession Nos.). The protein coding NDI, COI, and H3fragments were readily aligned, with no gaps, whereas the tRNA^{LEU}-16S, 28S, and 18S ribosomal sequences required some gaps to account for indel events.

3.2. Phylogenetic analyses

Most of the analyses (partitioned by matrix and methods) agree on some fundamental aspects of the phylogeny. The results of the various analyses are summarized in Fig. 2 where support for major clades is indicated by data partition and method. We number 17 clades to facilitate comparison and highlight congruence and disagreement among the separate analyses. The same clade numbers are used throughout. Table 3 gives tree scores under parsimony and maximum likelihood.

In the single-gene analyses, different methods often disagree (Fig. 2), however, for the larger datasets (combined mitochondrial, combined nuclear, all molecular, and all data combined) the different methods typically yield largely identical results with respect to these 17 clades (Figs. 2, 8–10). The majority of the nodes in the combined Bayesian hypothesis are well supported by posterior probability values between 90 and 100 (Fig. 10).

The majority of the analyses, especially the more character-complete analyses, support *Anelosimus* monophyly (Fig. 2). The monophyly of *Kochiura* plus *Anelosimus*, Theridiinae, and of clade 1 (the 'lost colulus clade') are also supported by multiple lines of evidence (Figs. 2–12),

Table 3
Tree statistics for the various analyses

	Parsimony				Likelihood
	Tree length	CI	RI	# trees	In *L* score
DNA and morphology	5727	0.46	0.71	3888	n/a
DNA combined	5451	0.45	0.70	1295	31405.3003
Morphology	See Agnarsson (2006)				n/a
mtDNA	3507	0.41	0.68	504	18228.6776
nDNA	1906	0.54	0.75	>50.000	12301.8328
16S	803	0.49	0.72	1440	4087.7991
NDI	574	0.50	0.72	1541	2958.9724
COI	2095	0.36	0.66	48	10805.8267
18S	198	0.80	0.70	>50.000	2677.3440
28S	1303	0.51	0.75	874	6873.3293
H3	372	0.48	0.75	7483	2210.6019
COI-NDI amino acid	365	0.57	0.8	>50.000	3116.3634

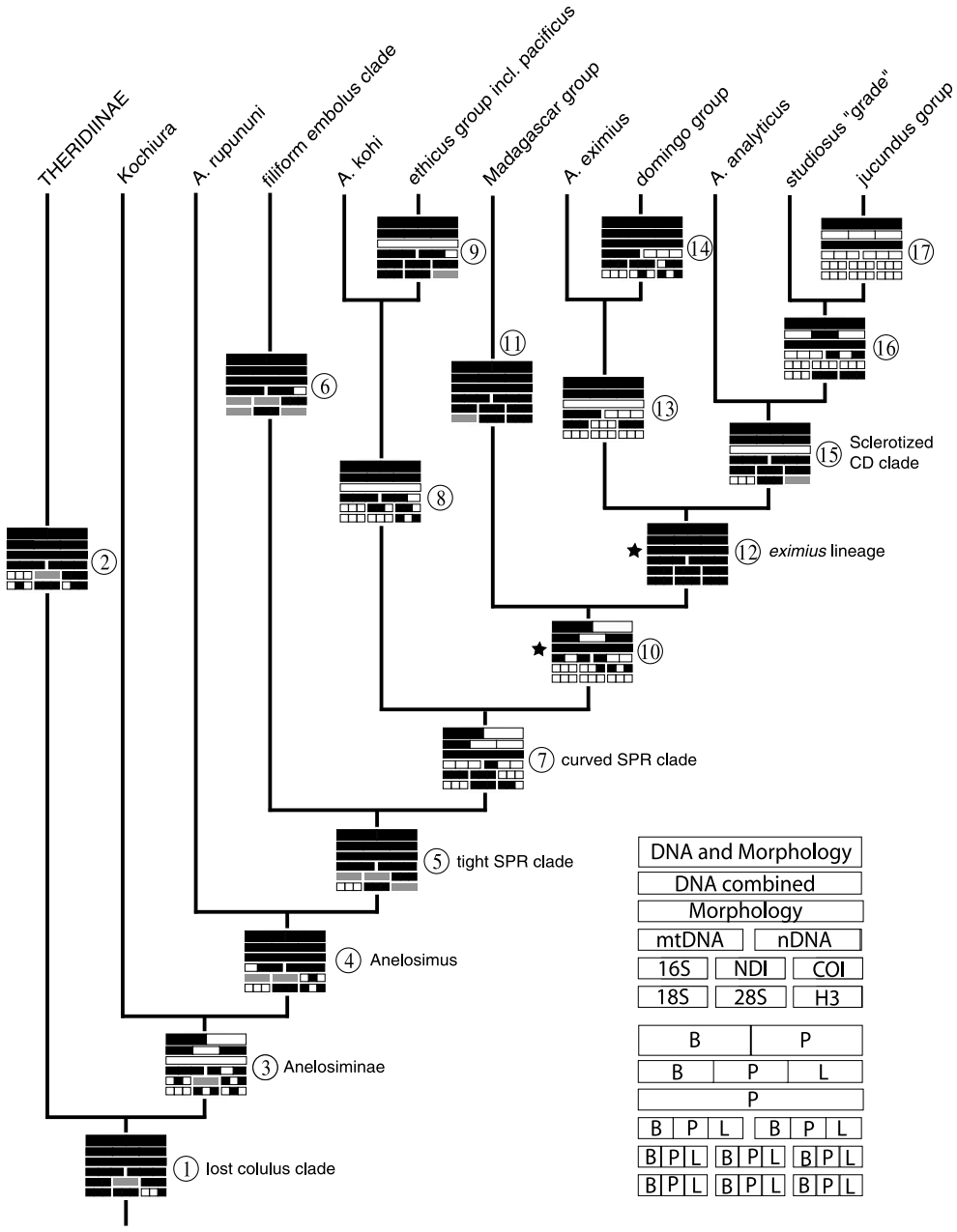

Fig. 2. Summary cladogram including major *Anelosimus* groups, and support for them from the combined and partitioned analyses. Clades are numbered (numbers in circles) to facilitate discussion, names of clades refer to previously named clades (Agnarsson, 2006) based on robust morphological support. Boxes on nodes show analyses by data partition, and the methods used to analyse each partition (B, Bayesian; P, Parsimony; L, Maximum Likelihood). Filled black boxes indicate the clade was recovered in the given analyses, empty boxes that the clade was not recovered, grey boxes indicate that the clade was not tested in the given analyses due to missing data. *Note that clades 10 and 12 were supported by morphology with the only difference that both clades include *A. pacificus*; the position of *A. pacificus* in the morphological dataset was poorly supported and considered ambiguous by Agnarsson (2006).

although *Kochiura* plus *Anelosimus* is negated by morphology alone, and by parsimony analyses of the combined datasets. Every analysis supports the monophyly of the recently revised '*eximius* lineage' (Agnarsson, 2006), slightly altered as the molecular data unequivocally excludes *A. pacificus* from that group.

The alternative, more gappy, 8/2 alignment resulted in an all data combined matrix 22 characters longer than the 24/6 alignment. Bayesian analysis of this matrix gave identical tree topology to the 24/6 analysis. Under parsimony the 8/2 matrix gave identical results to the 24/6 matrix except for slightly improved congruence with the Bayesian results, in placing *Kochiura* sister to *Anelosimus* and clade 11 sister to clade 6, together sister to clade 12. The only effect of treating gaps as fifth state in the 24/6 matrix was, similarly, to place clade 11 sister to clade 6, together sister to clade 12. None of these minor rearrangements impact the optimization of sociality.

4. Discussion

4.1. Anelosimus monophyly and outgroups

There is strong support for the monophyly of *Anelosimus*, a result the omitted species are not likely to alter as most of them are readily placed within species groups based on morphology. The results broadly corroborate the cladograms of Agnarsson (2005, 2006), while some of the supported clades differ slightly in composition. The difference surrounds mainly species whose placement in the morphological analysis was deemed particularly weakly supported, e.g. *A. pacificus*, *A. analyticus*, and *A. eximius*. The similar outgroup structure found previously by molecular (Arnedo et al., 2004) and morphological (Agnarsson, 2004, 2006) data is again supported. The monophyly of *Kochiura* plus *Anelosimus*, Theridiinae, and of clade 1 (the 'lost colulus clade') are supported by multiple lines of evidence (Figs. 2–12).

4.2. Branching patterns in the Anelosimus phylogeny

The 'basal' branching pattern is quite concordant among analyses, most agreeing that the following groups diverged successively on the line leading to the *eximius* group: the *rupununi* group, the filiform embolus clade (clade 6), *A. kohi* plus the *ethicus* group (clade 8), and the Madagascar group (clade 11). The placement of the social *rupununi* group sister to other *Anelosimus* is intriguing as all other social *Anelosimus* belong to the *eximius* lineage (clade 12), but this placement is also suggested by morphology (Agnarsson, 2006).

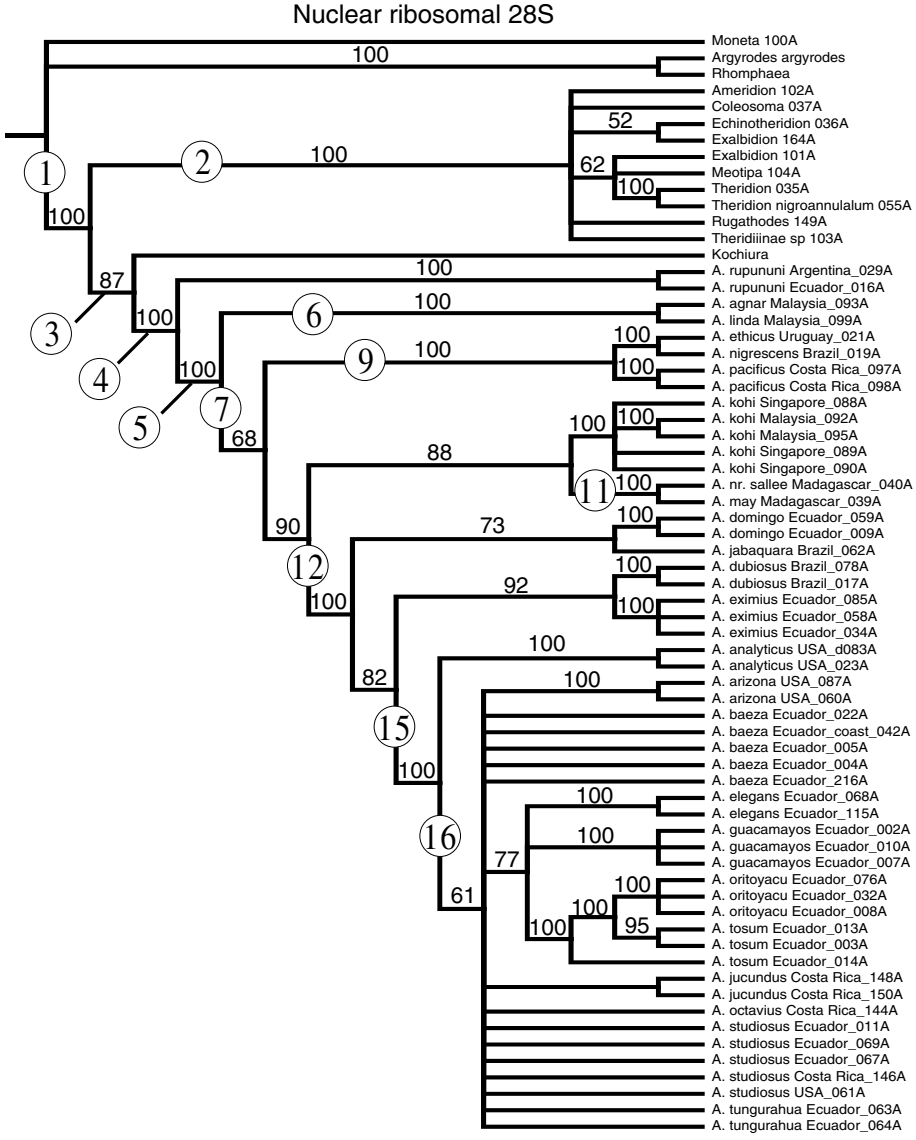

Fig. 3. Bayesian analysis of the 28S dataset. Numbers in circles refer to clades as in Fig. 1, numbers above branches are posterior probability support values. The Bayesian analysis independently recovered 12 of the 17 focal clades suggested by the combined analysis. The maximum likelihood analysis is similar, while the parsimony analysis negated clade 3, but supported clade 14.

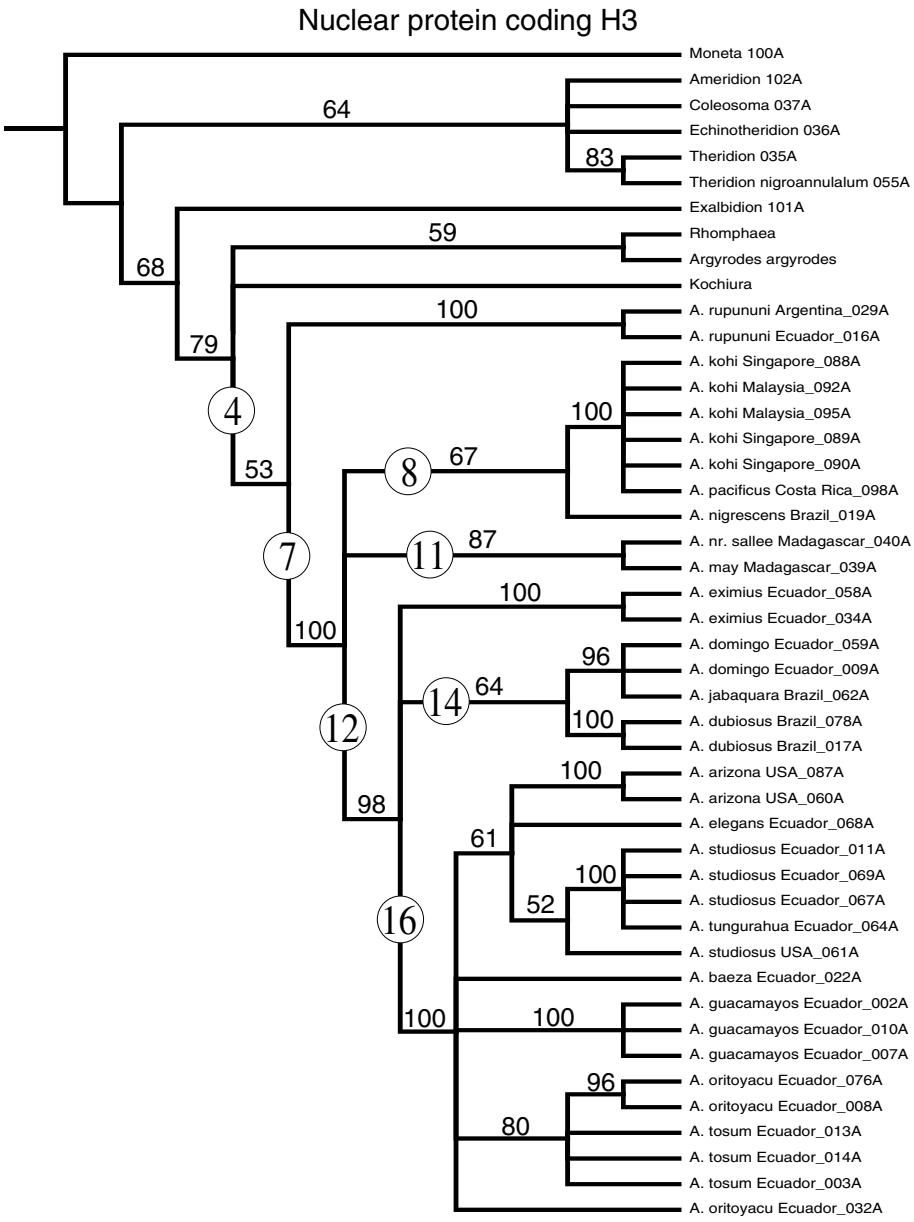

Fig. 4. Bayesian analysis of the Histone 3 dataset. Numbers in circles refer to clades as in Fig. 1, numbers above branches are posterior probability support values. Due to missing taxa clades 5, 6, and 15 are not tested.

Clade 6 is represented here by *A. linda* and *A. agnar* for both of which males are unknown. We believe these species belong to the 'filiform embolus clade' (see Agnarsson, 2006) containing numerous East African and Asian species, a notion that is supported by the identical placement of clade 6 in the combined analyses (Figs. 2 and 10) as that of the filiform embolus clade in the morphological analysis (Fig. 9). However, the discovery of males of these species and obtaining sequence data for other species of the filiform embolus clade is necessary for confirmation.

The placement of *A. pacificus* within, and of *A. kohi* sister to, the *ethicus* group (clade 9) is novel. However, both seem well supported and conflict with morphology was unsurprising (see below).

4.3. The eximius lineage

The monophyly of the American *eximius* lineage (clade 12; Agnarsson, 2006), excluding the American *rupununi* and *ethicus* groups (Agnarsson, 2005) is supported by every analysis of the molecular data. The composition of the *eximius* lineage, however, consistently differs from Agnarsson (2006) by excluding *A. pacificus*. This result is not surprising for as stated by Agnarsson (2006, 471) "the position of *A. pacificus* is particularly weakly supported..." in the morphological data. The current placement of *A. pacificus* is also congruent with behavioural information—*A. pacificus*, *A. ethicus*, and *A. nigrescens* (together forming clade 9) are the only *Anelosimus* species known, or suspected, to be solitary (L. Avilés pers. obs., Agnarsson et al. unpublished)

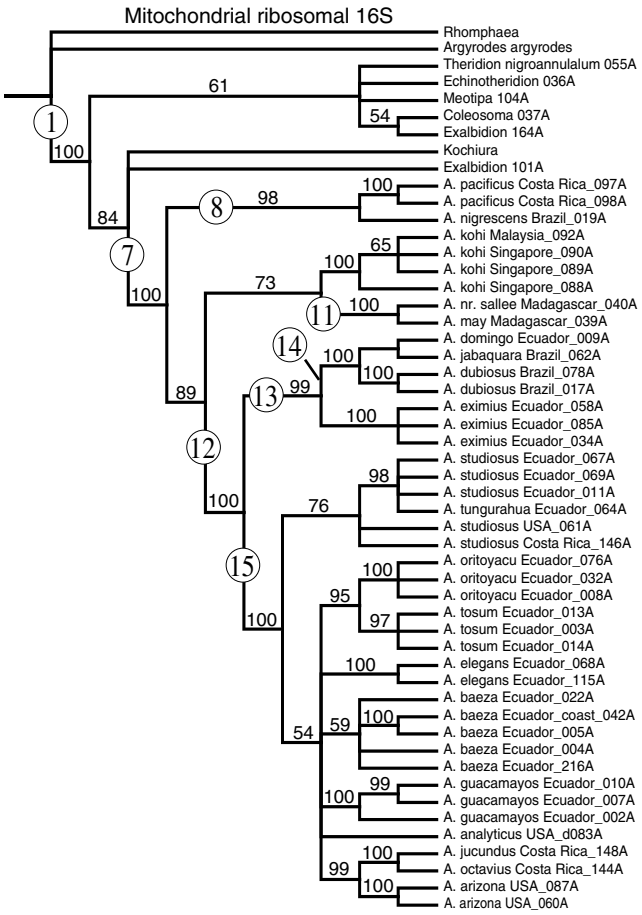

Fig. 5. Bayesian analysis of the 16S mitochondrial dataset. Numbers in circles refer to clades as in Fig. 1, numbers above branches are posterior probability support values. Due to missing data, clades 4–6 are not tested.

while the *eximius* lineage contains most of the well known social and subsocial species.

The *domingo* group (clade 14) is supported by most analyses. Clade 13, uniting the social *A. eximius* with the *domingo* group (contra morphology) is of special interest because it affects the optimization of sociality on the cladogram (Fig. 10). This relationship is supported by the combined molecular and the all data combined analyses, but in the partitioned data, only the 16S and COI mitochondrial genes support it (Fig. 11). COI seems to do so weakly, when combined with the other mitochondrial protein coding gene NDI and translated to amino acids, this arrangement is no longer supported (Fig. 12). The amino acid data support a placement of *A. eximius* sister to clade 15, as do morphology and the Bayesian and likelihood analyses of the 28S nuclear data (Fig. 11). Alternatively, parsimony analysis of the 28S data, the H3 data, and the combined nuclear data suggest placement of this taxon sister to all other members of clade 12 (Fig. 11). The precise placement of *A. eximius* has important implications for the evolution and number of origins of sociality in *Anelosimus* but more data are necessary to solve it satisfactorily.

The 28S analysis supported the 'sclerotized CD clade' (clade 15) including clade 16 (the *studiosus* and *jucundus* groups sensu Agnarsson, 2006), but excluding *A. analyticus*, while the other analyses supported that clade with *A. analyticus* included. The alternative more 'basal' placement of *A. analyticus* in the morphological analysis was poorly supported and the monophyly of the '*analyticus* group' was "… weakly supported by two convergent characters … both also present in the *studiosus* and *jucundus* groups" (Agnarsson, 2006: 471). The placement of *A. analyticus* sister to, or within, the *studiosus* and *jucundus* groups thus is also favoured by some of the morphological data.

None of the molecular analyses support the monophyly of the *studiosus* group or the *jucundus* group; in all analyses the two are intertwined. This is a surprising result given the ease of distinguishing the groups morphologically. However, almost every analysis implies different interrelationships of species within this complex, indicating that the phylogenetic structure of this portion of the *Anelosimus* tree is still much in question. Nonetheless, in agreement with morphology, all analyses place the two social species of the *studiosus* group, *A. guacamayos* and *A. oritoyacu*, apart on the tree: in most analyses *A. oritoyacu* is sister to *A. tosum*, and when resolved *A. guacamayos* is sister to *A. elegans*.

4.4. Species monophyly

The all-data Bayesian analyses (Fig. 10) support the monophyly of the *Anelosimus* species represented by more than one individual, except *A. jucundus* and *A. studiosus s.l.* For *A. jucundus*, this seems likely to be an artefact of missing data. The two *A. jucundus* individuals have no overlapping gene sequences, apart from 28S which lacks variability to be informative at this low level. Practically every analysis places a monophyletic *A. tungurahua*, only known from its type locality, within a paraphyletic *A. studiosus*, occurring from USA to Argentina (Agnarsson, 2006). This geographical and phylogenetic pattern is consistent with a 'peripheral isolates speciation' model: a "…geographically restricted daughter species whose monophyletic set of haplotypes is embedded within a widely distributed and still paraphyletic parental species" (Funk and Omland, 2003: 409). However, it is also consistent with 'bad taxonomy' i.e. that *A. tungurahua* is a junior synonym of *A. studiosus*. A more detailed study is required to evaluate these hypotheses.

Reassuringly, the combined analyses support the monophyly of the newly described, or resurrected, *A. baeza*, previously subsumed within *A. jucundus*, and *A. elegans*, *A. guacamayos*, *A. tosum*, and *A. oritoyacu*, previsously subsumed within *A. studiosus* (Agnarsson, 2006). The results therefore in general give support to recent taxonomic decisions based on morphology and behaviour, in this notoriously difficult group. However, due to limited taxon sampling at the individual level these results are preliminary, the implications of the molecular data to

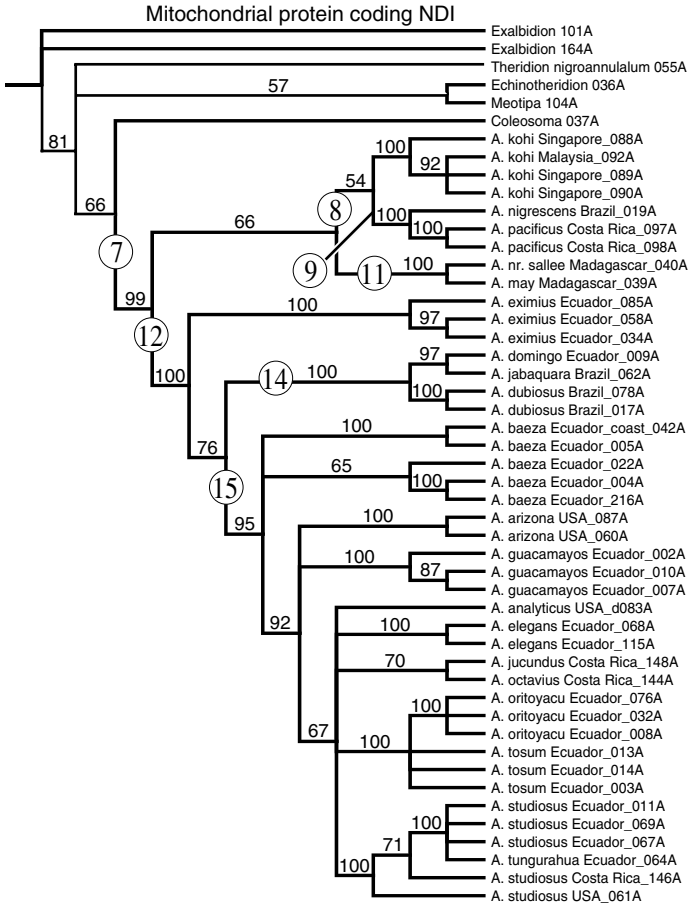

Fig. 6. Bayesian analysis of the NDI mitochondrial dataset. Numbers in circles refer to clades as in Fig. 1, numbers above branches are posterior probability support values. Due to missing data, clades 1–6 are not tested.

taxonomy will be explored in more detail elsewhere (Agnarsson et al., in preparation).

Some of the species previously subsumed within *A. studiosus* and *A. jucundus*, however, are not monophyletic in at least some the single-gene analyses, which may imply either taxonomic problems, or incomplete lineage sorting. We await the more detailed study (Agnarsson et al., in preparation) to discuss these alternatives in detail.

4.5. Implications for the evolution of sociality and inbreeding

The novel placement of *A. pacificus* suggested here brings together the three known quasi-solitary (species with extended maternal care, but dispersal of juveniles at a much earlier instar than in typical subsocial species) *Anelosimus* species (Fig. 10), suggesting a single reversal to solitary behaviour within the genus. It has been suggested that spider sociality is associated with dense (irregular, three-dimensional) webs (e.g. Shear, 1970; Buskirk, 1981; Avilés, 1997). It is thus interesting to note that while social and subsocial *Anelosimus* webs indeed differ from, and appear denser than, other theridiid webs (Agnarsson, 2004, 2005, 2006; Agnarsson and Kuntner, 2005; Agnarsson and Zhang, 2006), the quasi-solitary *Anelosimus* species, secondarily, have rather typical flimsy theridiid webs (L. Avilés pers. obs.; Agnarsson et al. unpublished). Although this observation does not establish a correlation between sociality and web density, it is at least congruent with the idea and suggests that examining the association of social behaviour and web types across spiders may be fruitful.

On the preferred phylogeny, social species are scattered, implying at least five independent origins of sociality (Fig. 10). Many of the analyses suggest a different placement of the social *A. eximius* (Figs. 11 and 12), with some implying six social origins similar to the morphological evidence alone (Agnarsson, 2006; Agnarsson et al., 2006). We do not have molecular data for the putatively social *A. puravida* (see Agnarsson, 2006), the putative sister species of the extremely similar, but subsocial, *A. baeza*; it seems likely that the molecular data, when available, will corroborate its placement.

Available evidence, such as museum collections consisting only of single specimen per sample, and implying roughly equal sex ratios, suggests that the *Anelosimus* species not included in this analyses (due to lack of specimens) are not social. Of course, we cannot rule out the possibility that some small number of behaviourally unknown African, Asian, or Australian species may be social. However, such species would only add to the count of numbers of derivations of sociality.

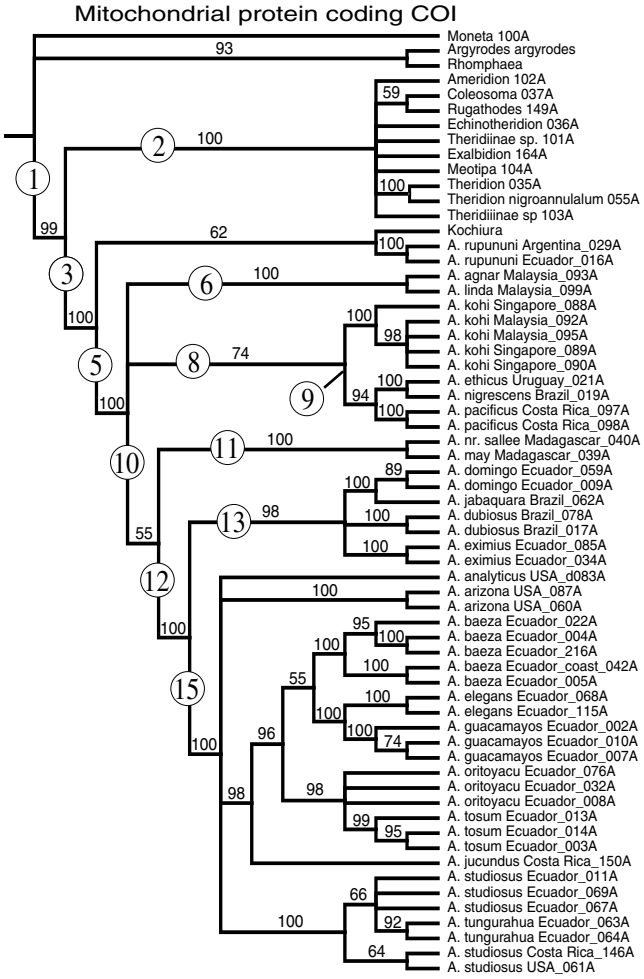

Fig. 7. Bayesian analysis of the COI mitochondrial dataset. Numbers in circles refer to clades as in Fig. 1, numbers above branches are posterior probability support values. The Bayesian analysis independently recovered 12 of the 17 focal clades suggested by the combined analysis.

As expected based on morphology, the social *Theridion nigroannulatum* belongs to Theridiinae (see Avilés et al., 2006), and most analyses agree on placing the social *rupununi* group sister to the remaining *Anelosimus* species (see Agnarsson, 2004, 2006). The social *A. guacamayos* is sister to the subsocial *A. elegans*, and the social *A. oritoyacu* sister to the subsocial *A. tosum* in most analyses. The members of each of these latter two pairs have similar palpal organs and hence these placements make intuitive morphological sense—even though this arrangement was not supported by the morphological dataset alone (see Agnarsson, 2006). The *domingo* group (clade 14), containing two social (*A. domingo* and *A. dubiosus*) and one subsocial species (*A. jabaquara*) is supported by most analyses. However, unlike morphology which suggested that the two socials were sister (Agnarsson, 2006), the molecular data all place *A. jabaquara* sister to *A. domingo*.

Each time sociality evolves in *Anelosimus*, it is accompanied by inbreeding and biased sex ratios (Avilés, 1997; Agnarsson et al., 2006); implied origins of inbreeding are thus equally numerous. Because the evolutionary experiment is repeated, the co-occurrence of these traits seems hardly coincidental. However, as sociality and the switch to inbreeding always occur at the same node our data do not allow us to determine whether this is a causal relationship and, if so, what the direction of causality is. Both traits in effect result from the 'decision' of adults not to disperse, but to stay in the natal colony. Inbreeding, however, requires that members of both sexes remain in the natal nest, while sociality does not, as is clear from the fact that most social organisms are, in fact, not inbred. In spiders, the existence of at least two outbred social species, *Tapinillus* sp. (Avilés, 1994) and *Delena cancerides* (Rowell and Avilés, 1995), shows that even in spiders inbreeding is neither a requirement for sociality nor a necessary consequence of it. Therefore, even though for some insect groups it has been argued that inbreeding may have facilitated a transition to sociality (e.g. Mcleish et al., 2006), in our case we cannot rule out the reverse direction of causality (Avilés, 1997). Ecological and life history factors, such as a need to invest in dense, expensive webs, as those characteristic of *Anelosimus*, and the opportunity to access large prey, may have played a prominent role in driving the evolution of spider sociality (Avilés, 1997; Powers and Avilés, in preparation). If benefits of remaining in the natal nest versus dispersing accrued similarly to both males and females, and if inbreeding costs were not too large (Day et al., 2003; Bilde et al., 2005; Avilés and Bukowski, 2006), suppression of the dispersal phase for both sexes and a transition to inbreeding would have followed. Under such a model nest-mate relatedness and inbreeding, rather than facilitating sociality, result from it, as also argued by Wilson (2005) and Wilson and Holldobler (2005) for social insects.

Whatever the short term role of inbreeding in social evolution, its long term consequences may be detrimental. Although sociality has evolved repeatedly, in no cases did a diverse social clade arise: each origin has yielded at most a small clade of one or two species. This "spindly" distribution suggests that sociality in spiders may be an evolutionary dead end, as we argue elsewhere (Agnarsson et al., 2006).

4.6. Biogeography

The scattering of American *Anelosimus* species in three separate clades is supported by both molecules and morphology. The limited available fossil evidence suggests a radiation of a major theridiid lineage including *Anelosimus* and Theridiinae occurred long after the split of Gondwana (Penney and Perez-Gelabert, 2002; Marusik and Penney, 2005). Hence the phylogeny suggests instances of successful intercontinental dispersal of *Anelosimus* species (see also Agnarsson, 2006). However, determining its direction or frequency is not possible without further sampling of species, especially representatives from Africa and Asia.

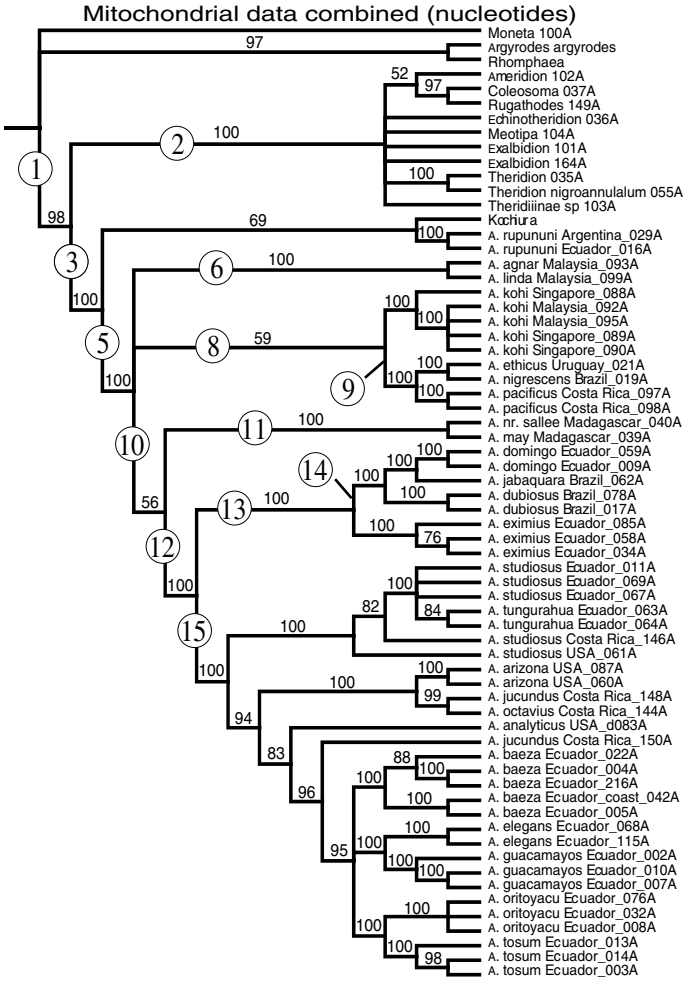

Fig. 8. Bayesian analysis of the combined mitochondrial dataset. Numbers above branches indicate posterior probability support values, numbers in circles refer to clades as in Fig. 1. The Bayesian analysis independently recovered 13 of the 17 focal clades suggested by the combined analysis.

Social species are only found in the Americas which is curious given their phylogenetic distribution. Why is it that diverse clades in the old world have not given rise to social species, while the American *eximius* group and the *rupununi* group have given rise to several? Although this is intriguing, it may be premature to speculate why this may be so, for much less is known about the biology of *Anelosimus* spiders in Africa and Australasia, than in the Americas.

4.7. Congruence

The range of analyses of the different gene regions agree on many of the fundamental aspects of the phylogeny, an agreement reflected in the combined analyses (Fig. 10). Many of the well supported clades are identical, or nearly so, to clades suggested by prior work using morphological data alone (Agnarsson, 2006). Measures of data congruence abound (e.g. Farris et al., 1995; Shimodaira and Hasegawa, 1999; Zelwer and Daubin, 2004; Struck et al., 2006), however, the merit of separate versus combined analyses, and whether lack of congruence forbids combining data partitions remains debated (e.g. Kluge, 1989; Bull et al., 1993; Miyamoto and Fitch, 1995; Huelsenbeck et al., 1996). Similarly, many methods exist to estimate clade robustness (e.g. Felsenstein, 1985; Bremer, 1988; Huelsenbeck and Ronquist, 2001), but their interpretation is often difficult. For example, quantitative measures of support for a clade can attain a maximum value with a single data partition (e.g. Bootstrap or posterior probability support of 100), and thus it would seem that support for the clade could not be any higher. But, concordance from additional independent data would lend additional support, although these measures could not reflect it. Additionally, insofar as different genes and morphology are probably evolving according to different models, concordance among them reassures us that our results are probably robust to errors in our assumptions. Therefore, independent of robustness measures, and regardless of personal preferences, or philosophical arguments in favour of partitioned or combined analyses, confidence in the combined result is increased by observing agreement from separate partitions. This is important for our study: while many clades in the combined analysis

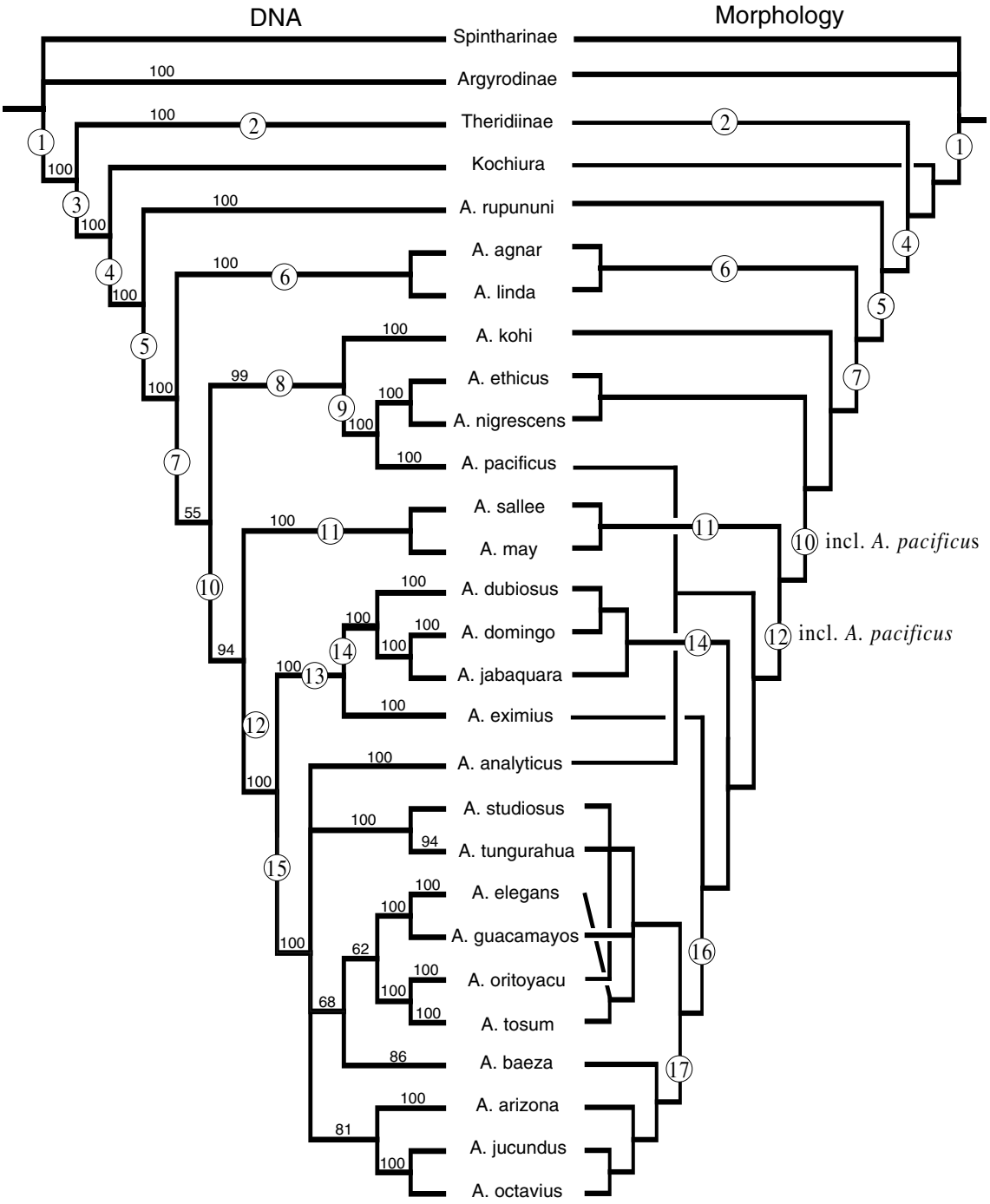

Fig. 9. Comparison of the combined molecular (Bayesian analysis) and the morphological (from Agnarsson, 2006) datasets. Numbers above branches in the Bayesian analysis indicate posterior probability support values, numbers in circles refer to clades as in Fig. 1. Eight of the focal clades are identical in the two datasets. Clades 10 and 12 are furthermore shared with the minor difference in that the morphological data weakly supports the inclusion of *A. pacificus*. Most of the disagreements between molecular and morphological data represent minor rearrangements (e.g. clades 3 and 8), and the position of *A. pacificus* (clades 8–10, 12). A notable disagreement is the placement of *A. eximius* for which different partitions of the molecular data also disagree amongst themselves (see Figs. 10, 11). In the combined analysis the molecular signal appears stronger and decides the placement of *A. eximius*.

receive 100% posterior probability support, our confidence is greatest in those clades supported by more than a single line of evidence. In the partitioned analyses two clades are supported by a single line of evidence—clade 17 by morphology and clade 13 by mitochondrial data (Fig. 2). Both clades have 100% posterior probability support in the combined Bayesian analysis (Fig. 10), yet, given incongruence among partitions we would not be surprised to find these clades refuted with additional evidence. In the remaining clades, however, we feel reasonably confident, including e.g. two component clades of clade 17: *A. baeza*, on the one hand, and *A. octavius*, *A. jucundus*, and A. *arizona*, on the other that are supported in many analyses.

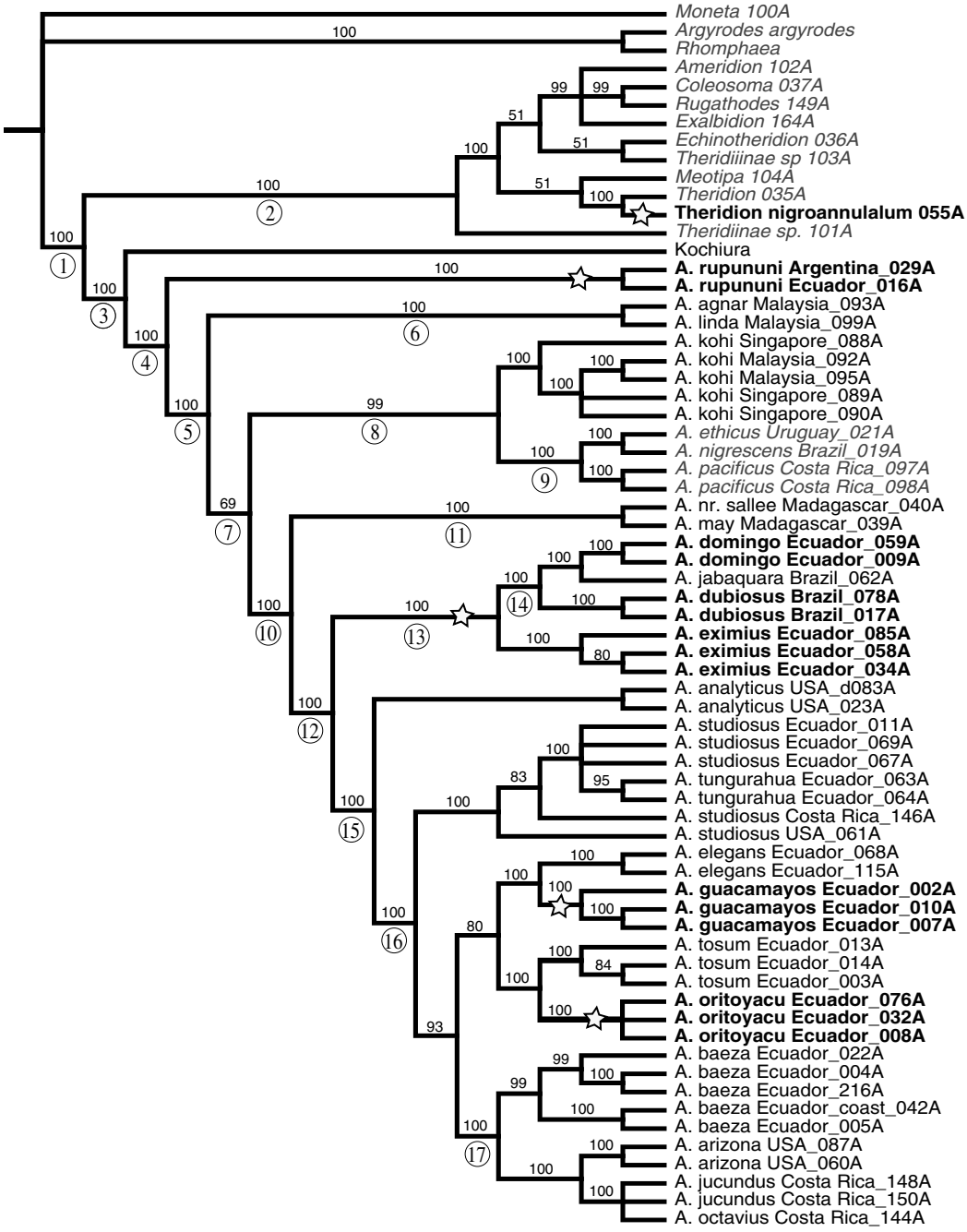

Fig. 10. Bayesian analysis of the all-data combined analyses. Numbers above branches indicate posterior probability support values, numbers in circles refer to clades as in Fig. 1, stars on nodes show independent origins of sociality. Social species are shown in **bold**, solitary in *italics*, subsocial in normal type.

4.8. Concluding remarks

We have added sequence data from six loci to existing morphological evidence to help resolve phylogenetic relationships among the social *Anelosimus* spiders. Our results continue to scatter social species phylogenetically while reconstructing only a couple of speciation events within social lineages. This implies both multiple origins of sociality and associated traits (inbreeding, sex ratio bias) and is consistent with the hypothesis that inbred social systems limit diversification in this clade. Likewise, some geographically proximal species continue to be phylogenetically separate which, in light of the likely recent divergence of the genus, suggests several successful intercontinental dispersal events. It remains puzzling why, despite multiple social origins and evidence for long distance dispersal, social *Anelosimus* species only occur in the Americas. Ecological factors, phylogenetic constraints, or simply lack of knowledge of species outside the New World might explain this. In sum, the current phylogeny offers a robust hypothesis of *Anelosimus* interrelationships that serves as a backbone to future, more

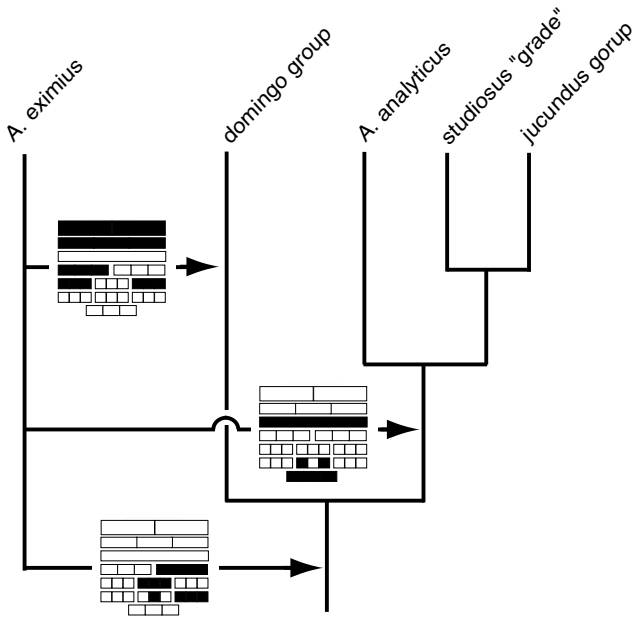

Fig. 11. The possible placements of *A. eximius* according to different data parititons.

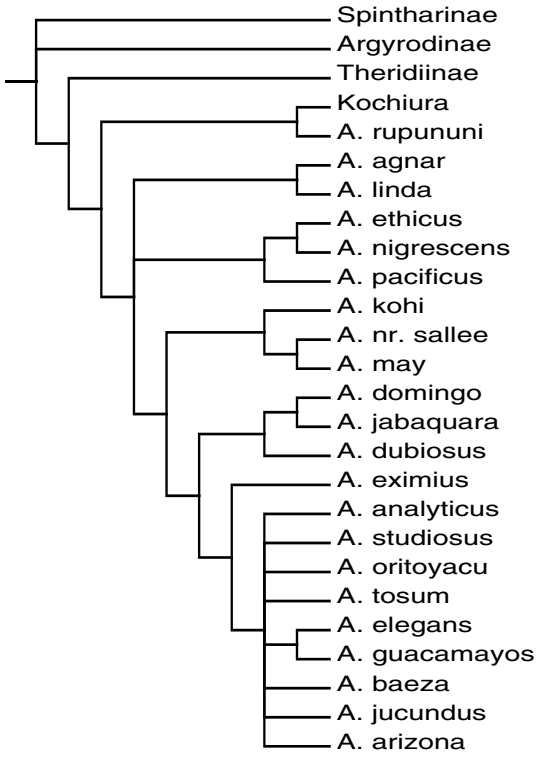

Fig. 12. Parsimony analysis of the COI-NDI dataset analyzed at the amino acid level. These results differ from the nucleotide analysis of COI mainly in the position of *A. eximius*. Bayesian analysis of the same data resulted in a less well resolved tree, however, with identical placement of *A. eximius* (posterior probability support for *A. eximius* plus clade 15 is 75).

detailed phylogenetic studies, but already facilitates the study of the many traits that make *Anelosimus* a popular model system.

Acknowledgments

We are indebted to the Museo Ecuatoriano de Ciencias Naturales for sponsoring our research in Ecuador and the Instituto Ecuatoriano de Areas Naturales y Vida Silvestre (Ecuador), and Ministerio de Energíay Ambiente (Costa Rica) for collecting permits. Some specimens for this study were borrowed from the Museum of Comparative Zoology, Harvard (H. Levi, loan to L. Avilés) and Instituto Butantan (A.D. Brescovit, loan to I. Agnarsson). The manuscript was improved by comments from Stefan Richter and two anonymous reviewers. We are grateful to Patricio Salazar, Gabriel Iturralde, Jessica Purcell, Jun-Xia Zhang, Daquin Li, William Eberhard, Gilbert Barrantes, Ju-Lin Weng, Matjaž Kuntner, and Laura May-Collado for help collecting specimens, and to Carol Ritland, Karen Needham, and Laura May-Collado for help with the molecular work. Thanks to Charles Griswold, Darrell Ubick, and Fernando Costa who donated some important specimens. This research was funded by grants from the National Sciences and Engineering Research Council of Canada to L.A. and W.P.M., and a Killam postdoctoral fellowship to I.A.

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