Zoologica Scripta

KUNGL. VETENSKAPS-AKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES

Shifting continents, not behaviours: independent colonization of solitary and subsocial *Anelosimus* spider lineages on Madagascar (Araneae, Theridiidae)

INGI AGNARSSON, MATJAŽ KUNTNER, JONATHAN A. CODDINGTON & TODD A. BLACKLEDGE

Submitted: 25 April 2009
Accepted: 12 August 2009
doi:10.1111/j.1463-6409.2009.00406.x

Agnarsson, I., Kuntner, M., Coddington, J. A. & Blackledge, T. A. (2010). Shifting continents, not behaviours: independent colonization of solitary and subsocial *Anelosimus* spider lineages on Madagascar (Araneae, Theridiidae). — *Zoologica Scripta*, *39*, 75–87.

Madagascar is a biodiversity hotspot, thought to be colonized mostly via Cenozoic dispersal from Africa, followed by endemic radiation of multiple lineages. *Anelosimus* spiders are diverse in Madagascar, and, like their congeners in the Americas, are most diverse in wet montane forests. Most *Anelosimus* species are social in that they cooperate in web building and prey capture either during a part of their life cycles (subsocial), including hitherto studied Malagasy species, or permanently (quasisocial). One Central American coastal species, *Anelosimus pacificus*, has secondarily switched to solitary living, and available evidence suggests that its closest relatives from S. America and Europe are likely also solitary. Here, we show that the only known coastal *Anelosimus* species in Madagascar and Comoros – *Anelosimus decaryi* and *Anelosimus amelie* sp. n. – are also solitary. Using a phylogenetic approach, we test two competing hypotheses: (i) that Malagasy *Anelosimus* are monophyletic and thus represent a second example of reversal to solitary living in a littoral habitat or (ii) that solitary and subsocial lineages independently colonized Madagascar. We find that solitary Malagasy *Anelosimus* are closely related to their solitary counterparts from Europe and the Americas, while subsocial Malagasy species nest sister to *Anelosimus nelsoni* from S. Africa. This finding suggests that (i) the two *Anelosimus* lineages colonized Madagascar independently and (ii) a reversal to solitary behaviour has occurred only once in *Anelosimus*. Thus, solitary littoral Malagasy species did not descend from Malagasy mountains, but arrived from much further afar. African and possibly American origin of the two lineages is implied by our findings. To restore natural classification of *Anelosimus*, *Seycellocesa* Koçak & Kemal is synonymized with it.

Corresponding author: *Ingi Agnarsson, Department of Biology, University of Puerto Rico, PO Box 23360, San Juan, PR 00931-3360, USA. E-mail: iagnarsson@gmail.com*

Ingi Agnarsson, Matjaž Kuntner, Scientific Research Centre, Institute of Biology, Slovenian Academy of Sciences and Arts, Novi Trg 2, PO Box 306, SI-1001 Ljubljana, Slovenia. E-mail: kuntner@gmail.com

Ingi Agnarsson, Matjaž Kuntner, Jonathan A. Coddington, Department of Entomology, National Museum of Natural History, Smithsonian Institution, NHB-105, PO Box 37012, Washington, DC 20013-7012, USA. E-mail: Coddington@si.edu

Ingi Agnarsson, Todd A. Blackledge, Department of Biology and Integrated Bioscience Program, University of Akron, Akron, OH 44325-3908, USA. E-mail: tab27@uakron.edu

Introduction

Madagascar is a prominent biodiversity hotspot containing highly diverse, mostly endemic biota. The origin of the Madagascar biota is much debated, especially the relative roles of Gondwanan vicariance vs. Cenozoic dispersal (Yoder & Nowak 2006). A recent review across multiple lineages (Yoder & Nowak 2006) found that the most common pattern was a sister relationship of Malagasy fauna to taxa from the African continent, and that most Malagasy lineages were too recent to be explainable by vicariance. Examples of taxa that probably colonized via dispersal from Africa include a variety of organisms such as plants (Schaefer *et al.* 2009), freshwater crabs (Cumberlidge 2008), lizards (Raselimanana *et al.* 2009) and bats (Weyeneth *et al.* 2008). While there are also several examples of Gondwanan vicariance lineages in Madagascar (Janssen *et al*. 2008), Cenozoic (up to 65 Ma) dispersal from Africa appears to have been a prominent biogeographical force shaping the Madagascar biota.

The spider genus *Anelosimus* Simon, 1891 (Theridiidae) is a model system for the study of sociality and its evolution, because the genus mostly contains species that range from temporarily (subsocial) to permanently social (Aviles 1997; Agnarsson 2006; Lubin & Bilde 2007). Only a small number of *Anelosimus* species are solitary. The Central American *Anelosimus pacificus* Levi, 1956 has secondarily switched to solitary living (Agnarsson *et al*. 2006b). Other *Anelosimus* species that are suspected to be solitary based on preliminary evidence, such as web size and content (L. Avilés, pers. comm.) are all close relatives of *A. pacificus*, namely *Anelosimus ethicus* and *Anelosimus nigrescens* from South America (Agnarsson 2005), and the European *Anelosimus vittatus* (IA pers. obs.) and likely also *Anelosimus pulchellus*, although we are aware of no data on its behaviour. *Anelosimus* is diverse in Madagascar and hitherto described species are all subsocial and monophyletic, hence forming a single Malagasy radiation (Agnarsson & Kuntner 2005).

Here, we report on the biology of two solitary *Anelosimus* species from northern Madagascar and Mayotte (an overseas department of France, forming a continuous island chain with the Comoro Islands, situated NW of Madagascar). We use a phylogenetic approach to determine the origin of these spiders. Specifically, we test two alternative hypotheses: (i) solitary behaviour evolved independently from (sub)social ancestors and thus represents a secondary reversal within Madagascar and the Comoros or (ii) a solitary lineage independently dispersed to Madagascar and the Comoros. A monophyletic Madagascar/Comoros clade that would include subsocial and solitary species treated here would support the first hypothesis, and a closer association of these species to other Old World *Anelosimus* would support the latter. We thus provide a better insight into the biogeography of *Anelosimus* spiders, and the evolution of behaviours, in Madagascar and neighbouring islands. We describe one new species, redescribe another, and also make taxonomic amendments to restore natural classification of *Anelosimus*.

Materials and methods

Solitary vs. social behaviour

Following prior work (Agnarsson *et al*. 2006a), we distinguish between solitary and social behaviour on the basis of cooperation. Many solitary theridiids show some degree of maternal care, beyond care of egg sacs (Aviles 1997; Agnarsson 2004; Lubin & Bilde 2007). However, in solitary species, juveniles are passive receivers of care and disperse from the natal nest at early instars, typically I–III, without contributing to the colony. In contrast, in all temporarily and permanently social species, juveniles stay in the natal nest until near or after adulthood. Older instars also actively cooperate and contribute to common colony tasks such as web spinning, prey capture and brood care. The webs of solitary and social species also differ in that the former are flimsier and typically less three dimensional (Fig. 1; see Results).

Observations

The two focal species, *Anelosimus decaryi* from mangrove forests of NE Madagascar and Mayotte (Fig. 2) and *Anelosimus amelie* sp. n. from lowland forest of Mayotte (Fig. 3), appeared common in their type habitats. Webs of *A. decaryi* were encountered at the tips of branches of small leaved mangrove species at the beachfront (Fig. 1). They occurred between Diego Suarez and Orangea, in the Diana region of Madagascar (S12°15.546′; E49°20.609′, sea level, 3–4.iv.2008, coll. by I. Agnarsson and M. Kuntner), and on Plage Tahiti on the western coast of the island Mayotte (S12°51.817′; E45°06.657′, sea level, 8.iv.2008, coll. by I. Agnarsson and M. Kuntner). Webs of *A. amelie* were found at the slope of Mt. Choungui, Mayotte (S12°56′43.872; E45°7′19.1994, 200-m elevation, 8.iv.2008) in open canopy growth on tips of branches of lone trees. Webs were photographed and measured in the field, the contents of webs were examined. The ages or instars of juveniles were estimated based on their size, based on comparison of the instars to other *Anelosimus* species with similarly sized adult females.

Phylogenetics

Specimens were fixed in 95% ethanol. Voucher specimens were deposited at the National Museum of Natural History, Smithsonian Institution. We obtained sequences of mitochondrial (16S, COI) and nuclear (28S) loci from *A. decaryi* and *A. amelie* and added these species to the existing species level *Anelosimus* phylogeny based both on molecular and morphological data (Agnarsson 2006; Agnarsson *et al*. 2007). GenBank accession numbers of new sequences are: Anelosimus_amelie_Mayotte 16S: GQ980270, 28S: GQ980273, COI: GQ980276Anelosimus_decaryi_Mayotte_AN03_ 16S: GQ980271, 28S: GQ980274, COI: GQ980277 Anelosimus_decaryi_Madagascar 16S: GQ980272, 28S: GQ980275, COI: GQ980278. The total dataset contained 70 individuals representing 25 *Anelosimus* species. The data matrix is available upon request from the first author. The molecular data were aligned and analysed using the same methods and settings as in the previous study (Agnarsson *et al*. 2007). In sum, for the protein coding gene COI, the alignment was trivial with no gaps implied. The other genes were aligned in CLUSTALW (Thompson *et al*. 1997), followed by minor

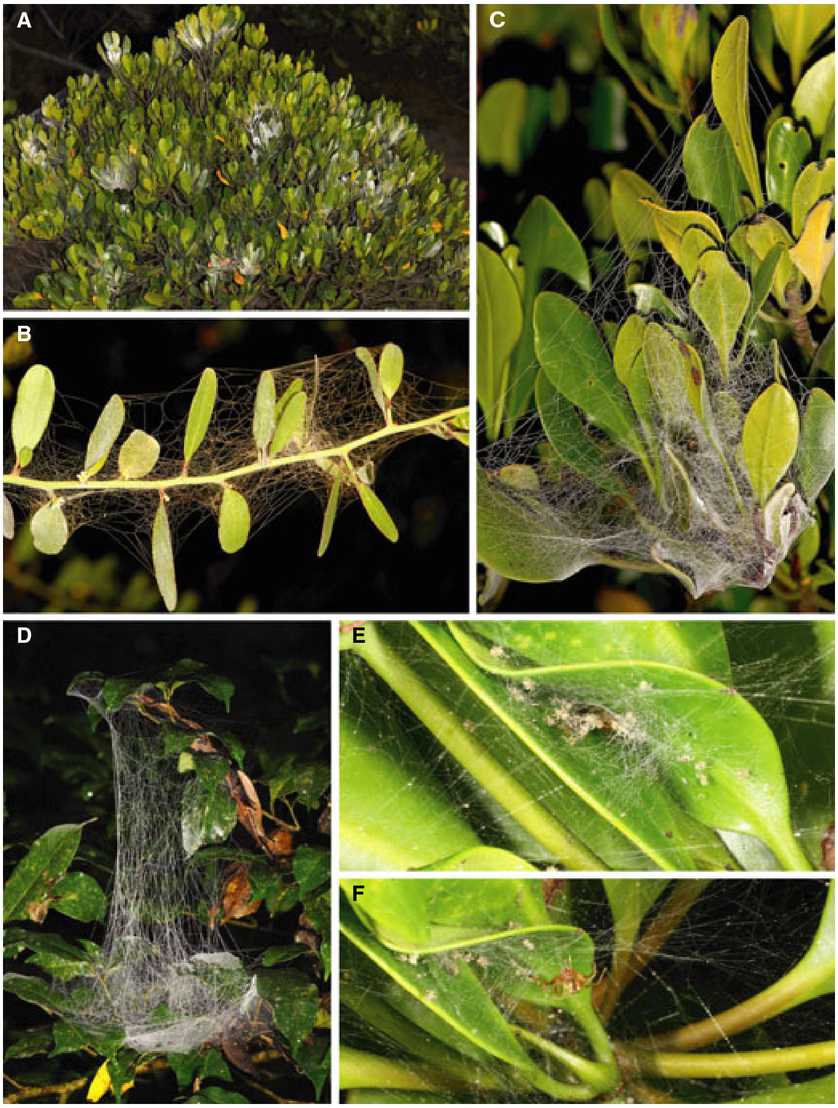

Fig. 1 A. Multiple *Anelosimus decaryi* webs on tips of leaves of a plant on the beach in Orangea, northern Madagasacar.—B,C. Webs of *Anelosimus decaryi*, a flimsy tangle of silk lines with conspicuous globules of glue throughout.—D. A typical subsocial *Anelosimus* web, this one is of an undescribed species from Montagne d'Ambre, a Madagascar montane rainforest.—E,F. retreat of *A. decaryi*, two leaves sandwiched together with silk, juveniles cluster in and around the retreat.

manual editing in Mesquite (Maddison & Maddison 2009) to correct conspicuously misaligned blocks mostly near each end of the alignments. Based on sensitivity analyses of Agnarsson *et al*. (2007), we chose gap opening and gap extension costs of 24/6, resulting in a relatively compressed alignment for our analyses. We treated gaps as missing data (see Agnarsson *et al*. 2007, for justification). Bayesian analysis was performed using MrBayes V3.1.1 (Huelsenbeck & Ronquist 2001), using the GTR+Γ+I model for molecular data partitions (as chosen by ModelTest, see Agnarsson *et al*. 2007), and partitioning the data by source (morphology, 16S, CO1, 28S) and the protein coding CO1 also by codon position. Parameters were estimated independently for each partition ['unlink statefreq = (all) revmat = (all) shape = (all) pinvar = (all)']. The model employed six substitution types (nst = 6), with rates and proportion of invariable sites (rates = invgamma), and base frequencies, estimated from the data. For each analysis, four Markov chain Monte Carlo chains (one cold and three heated) were run for 10 000 000 generations, and the sample points of the first 5 000 000 generations were discarded as 'burn-in', after which the chain reached stationarity.

Parsimony analyses were conducted with NONA (Goloboff 1993). All matrices were analysed using a heuristic search with 10 000 random additions, keeping a maximum of 10 trees per iteration.

Biogeography and sociality

We used the phylogenetic results to test the two alternative hypotheses that (i) solitary behaviour evolved secondarily within Madagascar and the Comoros or (ii) that solitary and social lineages dispersed independently

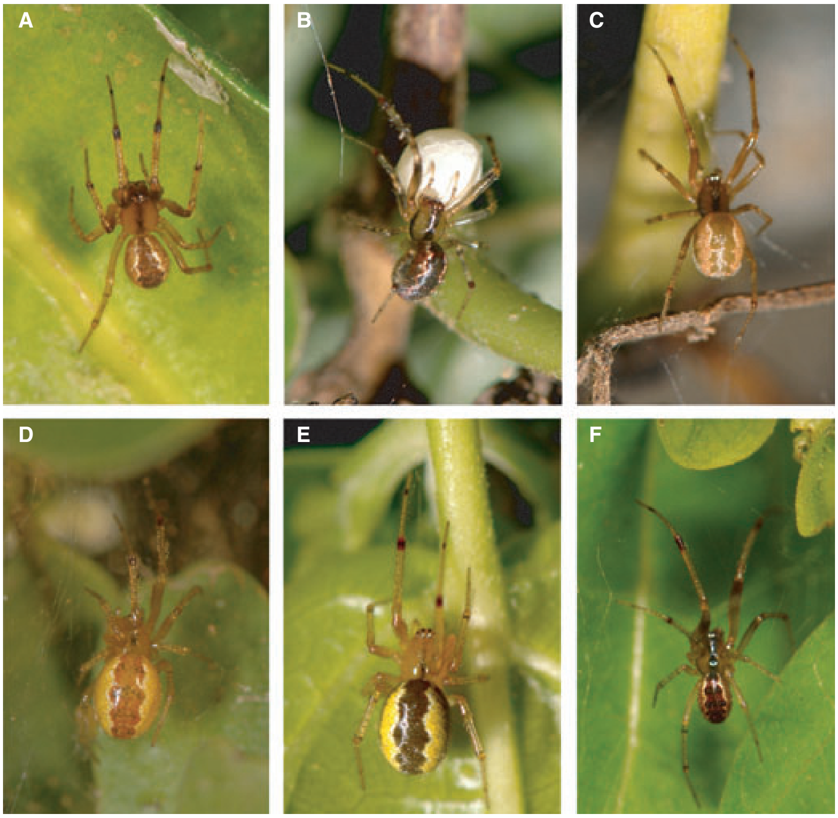

Fig. 2 Colour variation in *Anelosimus decaryi* from Madagascar and Mayotte.—A. Female from Orangea, Madagascar.—B. Female with egg sac from Tahiti Beach, Mayotte.—C. Female from Tahiti Beach, Mayotte.—D. Female from Orangea, Madagascar.—E. Female from Tahiti Beach, Mayotte.—F. Male from Orangea, Madagascar. Similar colour variation exists in the littoral species *Anelosimus kohi* from Malaysia and Singapore (Agnarsson & Zhang 2006).

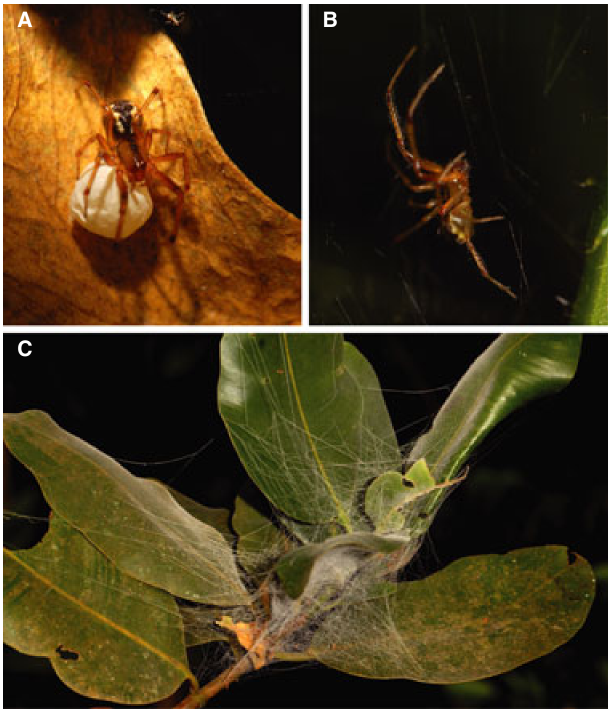

Fig. 3 *Anelosimus amelie* n. sp. from Mt. Choungui, Mayotte.—A. Female with egg sac.—B. Male.—C. Female web.

to Madagascar. For this, we used Fitch parsimony and maximum-likelihood ancestral character reconstruction (Pagel 1999), treating level of sociality and geographical distribution as discrete characters and optimizing them on the preferred phylogeny. For maximum-likelihood estimation, we used the Markov k-state 1-parameter model as implemented in Mesquite. Similar optimizations of geographical distribution as a discrete character (Fig. 5) have been used in prior biogeographical analyses (e.g. Ree & Smith 2008). Such methods seek ancestral areas for lineages that minimize subsequent dispersal or vicariance events needed to explain the current distribution of the lineage.

Sequence divergence and lineage age estimation

Intra- and interspecific sequence divergence was calculated in PHYLIP (Felsenstein, 2005) and used as a rough estimate of lineage age, assuming approximate rates of mitochondrial evolution of 2–3% per million years, following (Johannesen *et al.* 2007), for other social spiders. Currently available data only justify preliminary analyses of lineage age. More sequence data are currently being collected (Agnarsson laboratory) to allow more detailed lineage age estimation in *Anelosimus* and relatives worldwide, including fossil calibrations.

Taxonomy

Taxonomic methodology was detailed in (Agnarsson 2006). Specimens were examined under a Wild M-5A dissecting microscope. Illustrations were prepared using a Visionary Digital imaging system, the core components being a Canon 40D (Canon USA Inc. Lake Success, NY, USA) digital camera body and a K2 Infinity microscope equipped with Olympus (Olympus America Inc., Center Valley, PA, USA) metallurgical objectives. Successive images were combined with Helicon Focus 4.0, and thereafter minimally processed with Photoshop CS3 to adjust contrast, brightness, and to remove background blemishes. For photography, anatomical preparations were temporarily mounted as described in Coddington (1983). Measurements were made using an Infinity K2 long-distance microscope images processed in Photoshop CS3.

Results

Observations

The webs of most social *Anelosimus* species are similar, a dome-shaped sheet reinforced with leaves and above the sheet non-sticky aerial threads that intercept insects in flight (Agnarsson 2006; Aviles *et al*. 2001) (Fig. 1D). *Anelosimus decaryi* (Fig. 1A–C,E,F), and *A. amelie* (Fig. 3C), however, build webs that appear identical to those constructed by the solitary *A. pacificus* from littoral Central America (Agnarsson *et al*. 2006b). Typically, the web architecture is a three-dimensional silk mesh surrounding vegetation, with sticky droplets distributed throughout the mesh (Fig. 1B). Some webs included a basal, relatively planar, sheet that lacked sticky droplets and was reminiscent of subsocial species nests (Fig. 1C). Webs differed from *A. pacificus* in only sometimes containing a retreat. When a retreat was present, it consisted of two leaves sandwiched together using silk (Fig. 1E,F), as in *A. pacificus*, or alternatively, of a silk-enforced rolled leaf.

In the field, mostly solitary webs contained an adult female, an adult male or a single juvenile. We estimated the solitary juveniles to represent III or IV instars. Two webs contained a female and her young, but the young were very small, estimated to be first or second instars. These observations, while sparse, imply dispersal from the natal nest at an early ontogenetic stage, like in *A. pacificus*. As juveniles in social *Anelosimus* species typically start to contribute to the common tasks of the colony only after reaching instar III (Vollrath 1986), cooperative behaviour is likely absent in *A. decaryi* and *A. amelie*.

As in the coastal *Anelosimus kohi* from Asia, colour variation in *A. decaryi* is profuse (Fig. 2). *Anelosimus decaryi* and *A. amelie* lay globular white egg sacs (Figs 2B and 3A), which they may hide in the retreat or carry around in the chelicerae, as in the solitary *A. pacificus*. Most *Anelosimus* species in the Americas produce greyish egg sacs, while the solitary *A. pacificus* produces a white egg sac. African and Asian species also produce white egg sacs, indicating two lineages within *Anelosimus* that have switched from grey to white egg sacs.

Solitary behaviour in *Anelosimus* was first documented only recently, in *A. pacificus* (Agnarsson *et al*. 2006b). Preliminary data suggest solitary behaviour also occurs in its close relatives, the South American *A. ethicus* and *A. nigrescens* (L. Avilés pers. comm.). Our phylogeny suggests that *A. decaryi* and *A. amelie* belong to this same solitary lineage.

Phylogenetics, biogeography and sociality

Both Bayesian and parsimony analyses, irrespective of alignment, unequivocally place the new species as sister taxa (Figs 4 and 5). They nest within a clade containing all other known solitary *Anelosimus* species, including the Central American littoral species *A. pacificus* (Figs 4 and 5). Hence, a single reversal to solitary living is indicated in the common ancestor of this lineage. As suspected by Agnarsson & Kuntner (2005) based on morphological evidence these do not belong to the same lineage as the subsocial montane *Anelosimus* from Madagascar (Agnarsson & Kuntner 2005), which here are sister to *Anelosimus nelsoni* from South Africa, whose behaviour is unknown but presumed to be subsocial. This implies that, instead of a habitat and behavioural switch occurring within Madagascar, solitary and subsocial lineages arrived to Madagascar independently of one another and supports our dispersal hypothesis. Our results imply that *Anelosimus* originally diversified in the Americas, and that the lineage here described colonized Madagascar from the Americas. This result is strongly supported, the 'solitary Anelosimus clade' is supported by posterior probability of 100%. This indicates that the placement of *A. amelie* and *A. decaryi* anywhere outside this clade, or of *Anelosimus may* and *Anelosimus sallee* within it, is a significantly worse explanation of the data than the current hypothesis, given the current data and the model. Similarly, the placement of the group *A. nelsoni* plus the two other Madagascar species, *A. may* and *A. sallee* sister to the large 'eximius clade' (Fig. 5), is strongly supported (97%). In the phylogeny, specimens of *A. decaryi* from Madagascar and Mayotte are sisters and are sister to the very similar *A. amelie* from Mayotte.

The phylogeny places the two included *Seycellocesa* Koçak & Kemal species deep within *Anelosimus*, *Seycellocesa* (along with the names it replaced, the unavailable homonyms *Selimus* Saaristo and *Saaristoa* Koçak & Kemal) is therefore synonymized with *Anelosimus* (see Discussion and Appendix 1).

Fig. 4 Bayesian majority rule phylogeny of *Anelosimus*, showing the newly added species in bold. Numbers represent posterior probability support values. The genus *Seycellocesa* Koçak & Kemal, is indicated, and shown to be invalid.

Sequence divergence

Among the *Anelosimus* species here included, the maximum uncorrected mitochondrial sequence divergence was 26.9% (see Table S1, Supporting Information). Assuming rate of evolution at 2–3% per million years, the base of *Anelosimus* can be estimated to be approximately

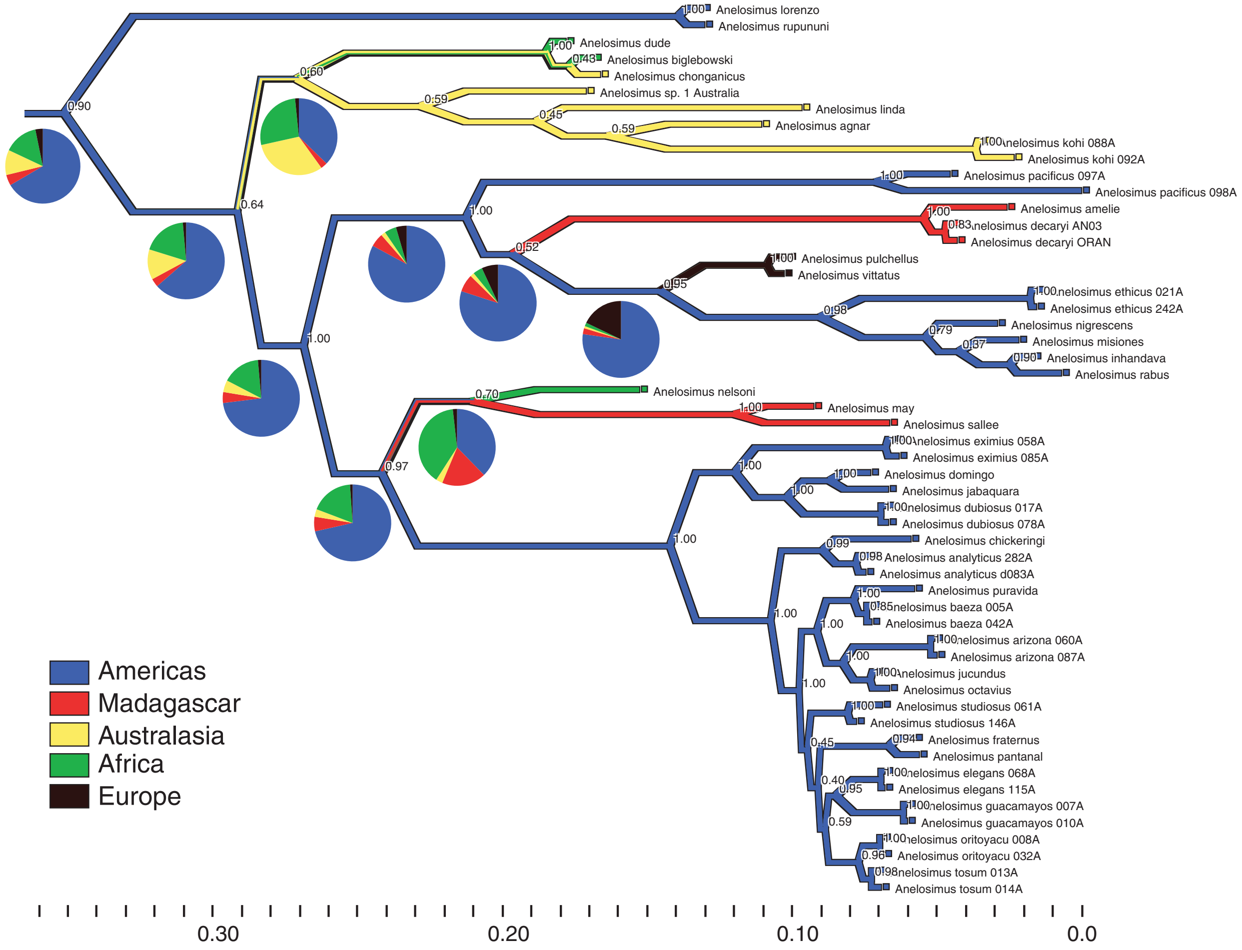

Fig. 5 A phylogenetic reconstruction of the geographical distribution of *Anelosimus* lineages. The base of the tree is roughly estimated to be dated around 11 Ma. Colours represent ancestral character optimization under parsimony of geographical distributions of lineages scored as discrete character states. Pie charts show maximum likelihood probabilities of ancestral distributions for nodes of interest. Origin and initial diversification of *Anelosimus* in the Americas is implied, with numerous subsequent dispersal events explaining colonization of other continents. There is clear scattering of solitary vs. subsocial *Anelosimus* lineages from Madagascar, as well as scattering of two mainland African lineages, implying dual dispersal to both landmasses. All *Anelosimus* species known to be solitary group together in a clade (the 'solitary *Anelosimus* clade' indicated by arrow (↙)), which also contains three species of unknown behaviour. These we predict, based on these results, also to be solitary. Branches are proportional to their lengths, indicated in scale below the tree.

9–13.4 Ma. We use the average value of 11.2 as our working estimate (Fig. 5). We note that although a crude estimation, the entire range indicates far younger age of these lineages than would be necessary to support a vicariance biogeographical scenario for *Anelosimus*. The sequence divergence between the solitary Malagasy species *A. amelie* and *A. decaryi* is about 4%, and that between the subsocial Malagasy species *A. may* and *A. sallee* about 5%. These are typical divergences between closely related *Anelosimus* species worldwide (Agnarsson, pers. obs.). In contrast, the divergence among the solitary and subsocial Malagasy species is about 16%, consistent with the hypothesis that the solitary Malagasy lineage is not derived from the subsocial Malagasy lineage. Maximum sequence divergence within the 'solitary *Anelosimus* clade' is about 18%, indicating that colonization of Madagascar by the solitary lineage took place at most 6–9 Ma.

Taxonomy

Appendix 1, Figs 6–8.

Discussion

Biogeography

Our phylogeny supports the hypothesis that two *Anelosimus* lineages independently colonized Madagascar, one solitary and the other social. Our crude estimate of the age

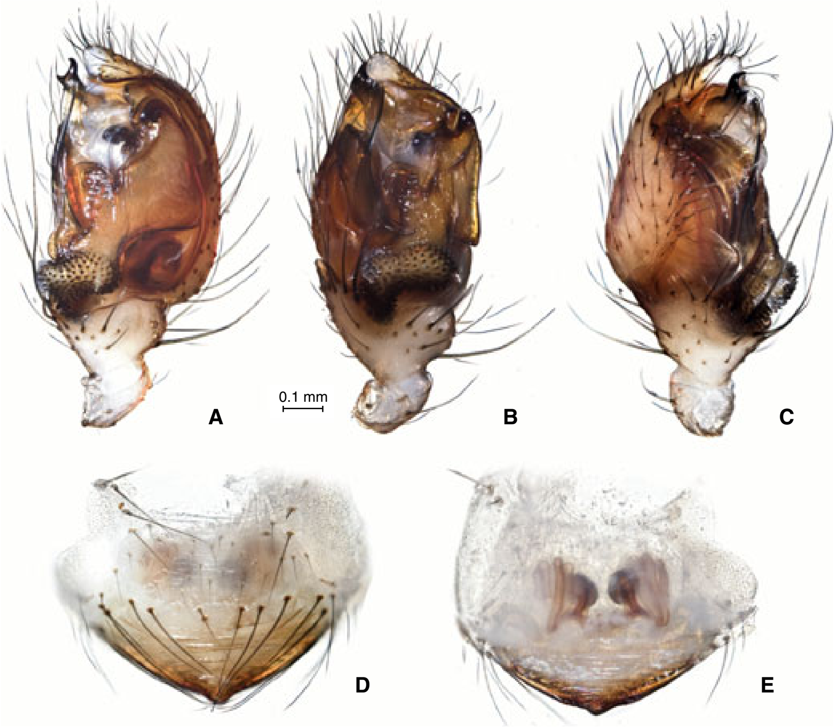

Fig. 6 Genital morphology of *Anelosimus decaryi*.—A. Left palp, prolateral.—B. Same, ventral.—C. Same, retrolateral. —D. Epigynum, ventral.—E. Same, dorsal.

of *Anelosimus* suggests a range from 9 to 13.4 Ma, which even if loosely interpreted rules out vicariance explanations for broad biogeographical patterns in *Anelosimus*. Hence, two dispersal events to Madagascar and adjacent islands (Comoros) must be postulated. The closest living relatives of the highland *Anelosimus* lineage in Madagascar, represented in the phylogeny by *A. may* and *A. sallee*, is *A. nelsoni* from South Africa (Fig. 4). Cenozoic colonization of Madagascar from mainland Africa is hypothesized to be the main force shaping the Madagascar biota (Yoder & Nowak 2006; Weyeneth *et al*. 2008; Raselimanana *et al*. 2009; Schaefer *et al*. 2009). Thus, our observations fit this expected pattern. However, for the solitary coastal lineage we describe here, the closest living relatives are found in the Americas and in Europe (Fig. 4). This indicates dispersal over very long distances, that took place at most 6–9 Ma (see Table S1), and likely more recently given that the presence of this lineage on mainland Africa has not been ruled out (see below). Given the presence of *Anelosimus* on islands such as Madagascar, Mayotte, Seychelles (Saaristo 2006), Aldabra (Roberts 1983), they are clearly capable of long-distance dispersal. However, their absence on more isolated islands such as Reunion, Mauritius and Rodriguez (own data) indicates that dispersal to remote oceanic islands is uncommon in these spiders.

Currently, the knowledge of *Anelosimus* in Africa is very fragmented. We find it likely that the solitary lineage is present on the Eastern coast of Africa. We predict that this yet to be discovered lineage will be a close relative of the Malagasy solitary species. Strangely, the *Anelosimus* lineage that current knowledge suggests is the most diverse and abundant in Eastern Africa (Agnarsson & Zhang 2006), lacks representatives in Madagascar. Because the first author has examined large collections of *Anelosimus* from Madagascar without encountering any representatives of this lineage, we argue that their absence is real rather than reflecting poor knowledge. Instead, the lack of this diverse African lineage may reflect how haphazard colonization of islands is when long-distance dispersal events are very rare (Ricklefs & Bermingham 2008). Our results indicate that *Anelosimus* originally diversified in the Americas, with at least seven subsequent dispersal events to other continents (Fig. 5). We point out that although the current results do not strongly support the exact placement of the African/Asian clade (posterior probability of 0.64), there is no evidence suggesting it might be 'basal' to the American *Anelosimus rupununi/ Anelosimus lorenzo* clade and thus affect optimization of the genus origin. The sister relationship of the *A. rupununi* clade to the remaining *Anelosimus* was supported by 27 of 30 data partitions in the analyses of Agnarsson *et al*. (2007), including total evidence analyses, molecular and morphological data analysed separately, and by five of six molecular loci independently.

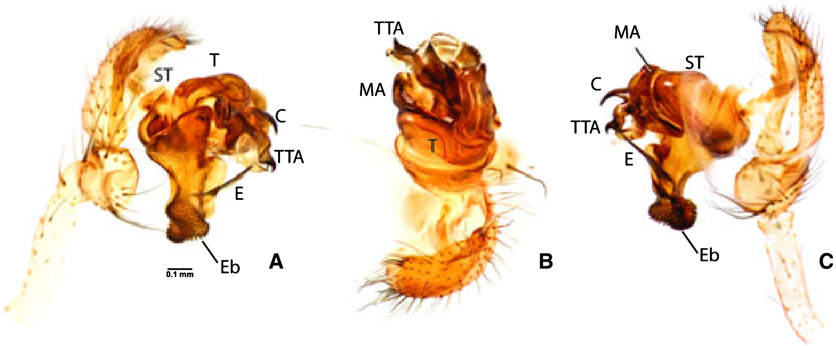

Fig. 7 Palpal anatomy of *Anelosimus decaryi*.—A. Expanded left palp, retrolateral.—B. Same, ventro-apical.—C. Same, prolateral. ST, subtegulum; T, tegulum; C, conductor; TTA, theridioid tegular apophysis; E, embolus; Eb, embolic base; MA, median apophysis.

As is the case for Madagascar, two lineages independently reached Africa, the *A. nelsoni* group that includes a number of undescribed species (Agnarsson, unpublished), and the lineage represented in Africa by *Anelosimus dude* and *Anelosimus biglebowski*, which also occurs in Asia. Our results are likewise consistent with colonization of the Comoros from Madagascar (Fig. 5). This pattern is also seen, for example, in bats (Weyeneth *et al*. 2008). However, the current phylogeny contains fewer than half of the *Anelosimus* species, and is missing genetic data for several key species, such as *A. nelsoni*. Hence, more detailed understanding of the biogeographical history of the genus and timing of events requires better sampling of *Anelosimus* across the globe, a project that is ongoing.

Colour variation

Colour variation in *A. decaryi* is profuse (Fig. 2), as also recently documented in *A. kohi* from Malaysia, as well as *A. dude* and *A. biglebowski* from Africa (Agnarsson & Zhang 2006). Such colour variation can result from any number of factors ranging from diet to genes (see Oxford & Gillespie (1998), for review). Colour polymorphism is well studied in only very few theridiid species, such as the Hawaiian 'happy face spider', *Theridion grallator* Simon, 1900 (Oxford & Gillespie 1998, 2001), *Theridion californicum* (Oxford 2009) and *Enoplognatha* (Oxford 2005; Oxford & Gunnarsson 2006). While completely unknown, *Anelosimus* represents another promising lineage for understanding the mechanics of colour variation in theridiid spiders.

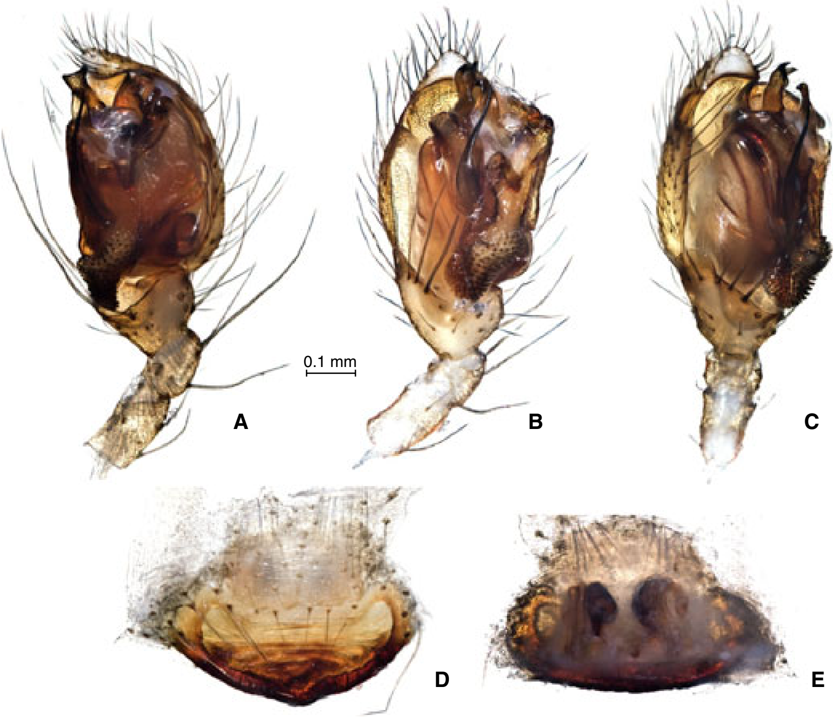

Fig. 8 Genital morphology of *Anelosimus amelie* n. sp.—A. Left palp, prolateral.—B. Same, ventral.—C. Same, retrolateral. —D. Epigynum, ventral.—E. Same, dorsal.

Sociality

Our results support our second hypothesis explaining the existence of solitary and subsocial *Anelosimus* spiders on Madagascar and adjacent islands based upon independent colonization by two lineages. Thus, solitary littoral Malagasy species did not descend from Malagasy mountains and change their behaviour, but rather arrived from much further afar. Subsocial Malagasy *Anelosimus* probably colonized via Africa, and we predict that the solitary lineage did so also, although it has yet to be documented in Africa. Prior work suggests that while sociality in spiders frequently evolves and is beneficial in the short run (Bilde *et al*. 2007), at least in certain environments (Aviles *et al*. 2007, Jones & Riechert 2008), it may represent an evolutionary dead end (Agnarsson *et al*. 2006a; but see Johannesen *et al*. 2007). Reversing to solitary behaviour might represent a 'way out' from social dead end, however, our results imply that such shifts must be very rare, and that a shift from social to solitary behaviour happened only once in *Anelosimus*. The behaviour of several *Anelosimus* species is as yet unknown, but the phylogeny allows the prediction that behaviourally unknown members of the solitary clade such as *Anelosimus misiones*, *Anelosimus inhandava* and *Anelosimus rabus*, also are solitary.

Acknowledgements

This is contribution number three resulting from the 2008 Indian Ocean expedition, funded by the Slovenian Research Agency (grant Z1-9799-0618-07 to I. Agnarsson, and program financing grant to M. Kuntner) and the National Science Foundation (grant DEB-0516038 to T. Blackledge). Additional funding came from the European Community 6th Framework Programme (a Marie Curie International Reintegration Grant MIRG-CT-2005 036536 to M. Kuntner). We are grateful to two anonymous referees whose careful comments helped improve the manuscript.

References

Agnarsson, I. (2004). Morphological phylogeny of cobweb spiders and their relatives (Araneae, Araneoidea, Theridiidae). *Zoological Journal of the Linnean Society*, *141*, 447–626.

Agnarsson, I. (2005). A revision and phylogenetic analysis of the American *ethicus* and *rupununi* groups of *Anelosimus* (Araneae, Theridiidae). *Zoologica Scripta*, *34*, 389–413.

Agnarsson, I. (2006). A revision of the New World *eximius* lineage of *Anelosimus* (Araneae, Theridiidae) and a phylogenetic analysis using worldwide exemplars. *Zoological Journal of the Linnean Society*, *146*, 453–593.

Agnarsson, I. & Kuntner, M. (2005). Madagascar: an unexpected hotspot of social *Anelosimus* spider diversity (Araneae: Theridiidae). *Systematic Entomology*, *30*, 575–592.

Agnarsson, I. & Zhang, J. X. (2006). New species of *Anelosimus* (Araneae: Theridiidae) from Africa and Southeast Asia, with notes on sociality and color polymorphism. *Zootaxa*, *1147*, 1–34.

Agnarsson, I., Aviles, L., Coddington, J. A. & Maddison, W. P. (2006a). Sociality in Theridiid spiders: repeated origins of an evolutionary dead end. *Evolution*, *60*, 2342–2351.

Agnarsson, I., Barrantes, G. & May-Collado, L. J. (2006b). Notes on the biology of *Anelosimus pacificus* Levi, 1963 (Theridiidae, Araneae) – evidence for an evolutionary reversal to a less social state. *Journal of Natural History*, *40*, 2681–2687.

Agnarsson, I., Maddison, W. P. & Aviles, L. (2007). The phylogeny of the social *Anelosimus* spiders (Araneae: Theridiidae) inferred from six molecular loci and morphology. *Molecular Phylogenetics and Evolution*, *43*, 833–851.

Aviles, L. (1997). Causes and consequences of cooperation and permanent-sociality in spiders. In J. C. Choe & B. J. Crespi (Eds) *The Evolution of Social Insects and Arachnids* (pp. 476–498). Cambridge: Cambridge University Press.

Aviles, L., Maddison, W. P., Salazar, P. A., Estevez, G., Tufino, P. & Canas, G. (2001). Social spiders of the Ecuadorian Amazonia, with notes on six previously undescribed social species. *Revista Chilena De Historia Natural*, *74*, 619–638.

Aviles, L., Agnarsson, I., Salazar, P. A., Purcell, J., Iturralde, G., Yip, E. C., Powers, K. S. & Bukowski, T. C. (2007). Natural history miscellany – Altitudinal patterns of spider sociality and the biology of a new midelevation social *Anelosimus* species in Ecuador. *The American Naturalist*, *170*, 783–792.

Barrion, A. T. & Litsinger, J. A. (1995). *Riceland Spiders of South and Southeast Asia*. CAB, Wallingford, Oxon.

Bilde, T., Coates, K. S., Birkhofer, K., Bird, T., Maklakov, A. A., Lubin, Y. & Aviles, L. (2007). Survival benefits select for group living in a social spider despite reproductive costs. *Journal of Evolutionary Biology*, *20*, 2412–2426.

Coddington, J. A. (1983). A temporary slide-mount allowing precise manipulation of small structures. In O. Kraus (Ed.) *Taxonomy, Biology and Ecology of the Araneae* (pp. 291–292). Hamburg: Naturwiss Verein Hamburg.

Cumberlidge, N. (2008). Insular species of Afrotropical freshwater crabs (Crustacea: Decapoda: Brachyura: Potamonautidae and Potamidae) with special reference to Madagascar and the Seychelles. *Contributions to Zoology*, 77, 71–81.

Felsenstein (2005). *J. Felsenstein, PHYLIP (Phylogeny Inference Package)*. Department of Genome Sciences, Seattle: University of Washington.

Goloboff, P. A. (1993). NONA. Available via http://www.cladistics.com

Huelsenbeck, J. P. & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, *17*, 754–755.

Janssen, T., Bystriakova, N., Rakotondrainibe, F., Coomes, D., Labat, J. N. & Schneider, H. (2008). Neoendemism in Madagascan scaly tree ferns results from recent, coincident diversification bursts. *Evolution*, *62*, 1876–1889.

Johannesen, J., Lubin, Y., Smith, D. R., Bilde, T. & Schneider, J. M. (2007). The age and evolution of sociality in Stegodyphus spiders: a molecular phylogenetic perspective. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *274*, 231–237.

Jones, T. C. & Riechert, S. E. (2008). Patterns of reproductive success associated with social structure and microclimate in a spider system. *Animal Behaviour*, *76*, 2011–2019.

Koçak, A. Ö. & Kemal, M. (2008a). New synonyms and replacement names in the genus group taxa of Araneida. *Centre for Entomological Studies Ankara Miscellaneous Papers*, *139–140*, 1–4.

Koçak, A. Ö. & Kemal, M. (2008b). Miscellaneous nomenclatural notes. *Centre for Entomological Studies Ankara Miscellaneous Papers*, *145*, 8–9.

Lubin, Y. & Bilde, T. (2007). The evolution of sociality in spiders. In H. Jane Brockmann, T. J. Roper, M. Naquib, K. E. Wynne-Edwards, C. Barnard & J. Mitani (Eds) *Advances in the Study of Behavior*, *Vol. 37* (pp. 83–145). San Diego: Elsevier Academic Press Inc.

Maddison, W. P. & Maddison, D. R. (2009). Mesquite: a modular system for evolutionary analysis, version 2.6. Available via http://mesquiteproject.org

Oxford, G. S. (2005). Genetic drift within a protected polymorphism: Enigmatic variation in color-morph frequencies in the candy-stripe spider, *Enoplognatha ovata*. *Evolution*, *59*, 2170–2184.

Oxford, G. S. (2009). An exuberant, undescribed colour polymorphism in *Theridion californicum* (Araneae, Theridiidae): implications for a theridiid pattern ground plan and the convergent evolution of visible morphs. *Biological Journal of the Linnean Society*, *96*, 23–34.

Oxford, G. S. & Gillespie, R. G. (1998). Evolution and ecology of spider coloration. In M. R. E. Berenbaum (Ed.) *Annual Review of Entomology* (pp. 619–643). El Camino Way, Palo Alto, CA: Annual Reviews Inc.

Oxford, G. S. & Gillespie, R. G. (2001). Portraits of evolution: studies of coloration in Hawaiian spiders. *BioScience*, *51*, 521–528.

Oxford, G. S. & Gunnarsson, B. (2006). Spatial variation in colour morph, spotting and allozyme frequencies in the candy-stripe spider, *Enoplognatha ovata* (Theridiidae) on two Swedish archipelagos. *Genetica Dordrecht*, *128*, 51–62.

Pagel, M. (1999). The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology*, *48*, 612–622.

Platnick, N. (1976). Are monotypic genera possible? *Systematic Zoology*, *25*, 198–199.

Raselimanana, A. P., Noonan, B., Karanth, K. P., Gauthier, J. & Yoder, A. D. (2009). Phylogeny and evolution of Malagasy plated lizards. *Molecular Phylogenetics and Evolution*, *50*, 336–344.

Ree, R. H. & Smith, S. A. (2008). Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, *57*, 4–14.

Ricklefs, R. & Bermingham, E. (2008). The West Indies as a laboratory of biogeography and evolution. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *363*, 2393–2413.

Roberts, M. J. (1983). Spiders of the families Theridiidae, Tetragnathidae and Araneidae (Arachnida: Araneae) from Aldabra Atoll. *Zoological Journal of the Linnean Society*, 77, 217–291.

Saaristo, M. I. (2006). Theridiid or cobweb spiders of the granitic Seychelles islands (Araneae, Theridiidae). *Phelsuma*, *14*, 49–89.

Schaefer, H., Heibl, C. & Renner, S. S. (2009). Gourds afloat: a dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous oversea dispersal events. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *276*, 843–851.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, *25*, 4876–4882.

Vollrath, F. (1986). Environment, reproduction and the sex-ratio of the social spider *Anelosimus eximius* (Araneae, Theridiidae). *Journal of Arachnology*, *14*, 267–281.

Weyeneth, N., Goodman, S. M., Stanley, W. T. & Ruedi, M. (2008). The biogeography of Miniopterus bats (Chiroptera: Miniopteridae) from the Comoro Archipelago inferred from mitochondrial DNA. *Molecular Ecology*, *17*, 5205–5219.

Wunderlich, J. (1992). Die Spinnen-Fauna der Makaronesischen Inseln: Taxonomie, Ökologie, Biogeographie und Evolution. *Beitraege Zur Araneologie*, *1*, 1–619.

Wunderlich, J. (1995). Zur Kenntnis der Endemiten, zur Evolution und zur Biogeographie der Spinnen Korsikas und Sardiniens, mit Neubeschreibungen (Arachnida: Araneae). *Beitraege Zur Araneologie*, *4*, 353–383.

Wunderlich, J. (2008). On extant and fossil (Eocene) European comb-footed spiders (Araneae: Theridiidae), with notes on their subfamilies, and with descriptions of new taxa. *Beiträge Zur Araneologie*, *5*, 140–469.

Yoder, A. D. & Nowak, M. D. (2006). Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. *Annual Review of Ecology Evolution and Systematics*, *37*, 405–431.

Yoshida, H. (2007). A new genus of the family Theridiidae (Arachnida: Araneae). *Acta Aracnologica Tokyo*, *56*, 67–69.

Yoshida, H. (2008). A revision of the genus *Achaearanea* (Araneae: Theridiidae). *Acta Arachnologica Tokyo*, *57*, 37–40.

Appendix 1

Taxonomy.

Recent taxonomic work in Theridiidae can be largely characterized as the creation of new, often monotypic, genera through reclassification without any regard, or reference, to modern phylogenetics (Wunderlich 1992, 1995, 2008; Barrion & Litsinger 1995; Saaristo 2006; Yoshida 2007, 2008). This approach is unfortunate. Instead of using knowledge of phylogenetic affinities of new species to help place them, lack of such knowledge is used as the impetus to generate novel genera to accommodate each new species. Not only are such genera all but empty hypotheses (Platnick 1976), this practice can wreak havoc to natural classification of groups by rendering existing genera paraphyletic. In a recent extreme example (Saaristo 2006), nearly all species of theridiid spiders found on Seychelles islands were each placed in a new monotypic genus. These species included *Anelosimus placens*, which Saaristo transferred to a new monotypic genus *Selimus* Saaristo, 2006 (now *Seycellocesa* Koçak & Kemal, see below). Wunderlich (2008) then subsequently transferred two similar European *Anelosimus* species to *Selimus*, *A. pulchellus* and *A. vittatus*. These changes ignored existing phylogenetic analyses clearly showing these species belong to

Anelosimus (Agnarsson 2006). Here, a combination of molecular and morphological data again demonstrate that the transfer of these *Anelosimus* species to *Selimus* is invalid and flatly rejected on phylogenetic grounds (Fig. 4). *Anelosimus pulchellus* and *A. vittatus* nest deep within the genus. Furthermore, although *A. placens* has not yet been included in a phylogenetic analysis, it is clearly an *Anelosimus* based on morphological synapomorphies of the genus (Agnarsson, pers. obs.). In addition, *Selimus* Saaristo is likely not monophyletic because *A. placens* is, based on morphological similarity, more closely related to *A. decaryi* and *A. amelie* than to *A. pulchellus* and *A. vittatus* (Agnarsson pers. obs.). Therefore, here we synonymize *Selimus* Saaristo with *Anelosimus* and transfer *A. placens*, *A. pulchellus*, and *A. vittatus* back to *Anelosimus* (Appendix 1). Adding to this contorted taxonomical history, *Selimus* Saaristo, nota bene, is preoccupied by a jumping spider *Selimus* Peckham & Peckham, 1901. It was then subsequently erroneously replaced by *Saaristoa* Koçak & Kemal, 2008a; as *Saaristoa* is also preoccupied by a spider, the linyphiid *Saaristoa* Millidge, 1978. This was amended by the creation of a second replacement name *Seycellocesa* Koçak & Kemal, 2008b;. Here, *Saaristoa* Koçak & Kemal, 2008a and *Seycellocesa* Koçak & Kemal, 2008b are synonymized with *Anelosimus*. All this taxonomical conundrum was needless as there was never any reason, or rationale, to remove these species from *Anelosimus*.

Family THERIDIIDAE Sundevall, 1833

Genus *Anelosimus* Simon, 1891

Selimus Saaristo, 2006; an unavailable junior homonym of *Selimus* Peckham & Peckham, 1901, **new synonymy** (see above, and Fig. 4, for justification).

Saaristoa Koçak & Kemal, 2008a, a replacement name for *Selimus* Saaristo, itself an unavailable junior homonym of *Saaristoa* Millidge, 1978, **new synonymy.**

Seycellocesa Koçak & Kemal, 2008b, a replacement name for *Saaristoa* Koçak & Kemal, 2008, **new synonymy**.

***Anelosimus decaryi* (Fage, 1930)**

Theridion decaryi Fage, 1930: 26, figs 1 and 2.

Anelosimus locketi Roberts, 1983: 240, figs 89–94.

Anelosimus decaryi Agnarsson & Kuntner, 2005: 589 (transfer and synonymy) (Figs 6 and 7).

Material examined. Theridion decaryi holotype male, see Agnarsson & Kuntner (2005) for detail, in Musée National d'Histoire Naturelle, Paris (AR 2367). About 30 specimens from MADAGASCAR, Antsiranana Prov., Orangea Peninsula S12°13′59.3"; E49°21′33", 3.iv.2008, scrubs, 15 m elev., Coll. I. Agnarsson and M. Kuntner. About 10 specimens from [FRANCE], Departmental collectivity of Mayotte, Comoros archipelago, Plage Tahiti, S12°51.817′; E45° 06.657′, sea level, 8.iv.2008, Coll. I. Agnarsson and M. Kuntner (National Museum of Natural History, Smithsonian Institution).

Diagnosis. Anelosimus decaryi can be distinguished from all other *Anelosimus* species, except *A. amelie*, by the shape of the embolus and embolic apophysis in the male, and by the internal female genitalia, including the trajectory details of the copulatory duct in the female (Figs 6–8). *Anelosimus decaryi* can be distinguished from *A. amelie* by smaller size, and the shape of the ridged tip of the embolic apophysis in the male, and by a less sclerotized epigynum in the female (Figs 6–8).

Male (*from Orangea*): Total length 2.97. Prosoma 1.45 long, 1.06 wide, 0.83 high, yellowish-brown, grey markings in centre and on rim. Sternum 0.72 long, 0.70 wide, extending between coxae IV, yellowish-brown, darker around rim. Abdomen 1.60 long, 1.19 wide, 1.18 high. Pattern as in Fig. 2M. Eyes subequal, diameter about 0.08. Leg I femur 2.05, patella 0.67, tibia 1.85, metatarsus 1.66, tarsus 0.77. Femur I about six times longer than wide, curved. Leg formula 1243. Leg base colour light, distal tips of joints dark red, especially on leg 1. Palp as diagnosed (Figs 6 and 7), with a prominent embolus bearing a characteristic embolic aphophysis, strongly ridged distally.

Female (*from Orangea*): Total length 3.56. Prosoma 1.76 long, 1.31 wide, 0.88 high, yellowish-brown, centre and rim darker. Sternum 0.98 long, 0.81 wide, extending midway between coxae IV, yellowish-brown, darker around rim. Abdomen 1.89 long, 1.39 wide, 1.30 high, pattern as in Fig. 2. Eyes subequal, diameter about 0.11. Leg I femur 1.82, patella 0.79, tibia 1.83, metatarsus 1.64, tarsus 0.80. Femur I about 4.5 times longer than wide, curved. Leg formula 1423. Leg colour as in male. Epigynum as diagnosed (Fig. 6), with copulatory ducts spiralling partially around the spermathecae.

Natural history. See Results.

Anelosimus amelie* Agnarsson, *n. sp.

Holotype. Male, [FRANCE], Departmental collectivity of Mayotte, Comoros archipelago, slope of Mt. Choungui (S12°56′ 43.872 E45°7′19.1994, 200 m elevation, 8.iv.2008), collected by I. Agnarsson and M. Kuntner (National Museum of Natural History, Smithsonian Institution) (Fig. 8).

Paratype. Same data as for holotype, one female.

Other material. Same data as for holotype, about 20 specimens.

Etymology. The species is a patronym, a noun in apposition, after the senior author's daughter, Amélie Melkorka.

Diagnosis. Anelosimus amelie can be distinguished from all other *Anelosimus* species except *A. decaryi*, by the shape of the embolus and embolic apophysis in the male, and by the internal female genitalia, including the trajectory of the copulatory duct in the female. It can be distinguished from *A. decaryi* by greater size, and the shape of the ridged tip of the embolic apophysis in the male, and by a more sclerotized epigynum in the female (Figs 6–8).

Male (*holotype*): Total length 2.83. Prosoma 1.49 long, 1.09 wide, 0.79 high, yellowish-brown, grey markings in centre and on rim. Sternum 0.79 long, 0.69 wide, extending between coxae IV, yellowish-brown, darker around rim. Abdomen 1.42 long, 1.15 wide, 1.14 high, truncated in front. Pattern as in Fig. 3. Eyes subequal, diameter about 0.09. Leg I femur 2.05, patella 0.67, tibia 2.04, metatarsus 1.59, tarsus 0.75. Femur I about 5.6 times longer than wide, curved. Leg base colour light brown, distal tips of joints on leg I and II red. Palp as diagnosed (Fig. 8).

Female (*paratype*): Total length 3.29. Prosoma 1.54 long, 1.16 wide, 0.85 high, yellowish-brown, centre and rim darker. Sternum 0.84 long, 0.77 wide, extending midway between coxae IV, yellowish-brown, darker around rim. Abdomen 1.79 long, 1.41 wide, 1.40 high, pattern as in Fig. 3. Eyes subequal, diameter about 1.10. Leg I femur 1.87, patella 0.69, tibia 1.88, metatarsus 1.50, tarsus 0.81. Femur I about 5.5 times longer than wide, curved. Leg formula 1423. Leg base colour light brown, distal joints red, especially leg 1. Epigynum as diagnosed (Fig. 8).

Natural history. See Results.

Anelosimus placens (Blackwall, 1877) – comb. nov.

Theridion placens Blackwall, 1877: 13, pl. 2, fig. 10.

Anelosimus placens Saaristo, 1978: 118, figs 192–202.

Selimus placens Saaristo, 2006: 74; figs 67–76; Wunderlich, 2008: 362.

Saaristoa placens Koçak & Kemal, 2008a: 4.

Seycellocesa placens Koçak & Kemal, 2008b: 8; Platnick, 2009.

See above for justification of transfer.

Anelosimus pulchellus (Walckenaer, 1802) – comb. nov.

Aranea pulchella Walckenaer, 1802: 208.

Anelosimus pulchellum Levi, 1956: 412.

Selimus pulchellus Wunderlich, 2008: 362.

Seycellocesa pulchellus Platnick, 2009.

See above for justification of transfer.

Anelosimus vittatus (C. L. Koch, 1836) – comb. nov.

Theridion vittatum C. L. Koch, 1836: 65, fig. 217.

Anelosimus vittatus Levi, 1956: 412.

Selimus vittatus Wunderlich, 2008: 362, figs 450–452, 452a.

Seycellocesa vittatus Platnick, 2009.

See above for justification of transfer.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Uncorrected mitochondrial sequence divergence among the *Anelosimus* specimens for which we have mitochondrial data. Taxa from Madagascar are in bold, solitary taxa are marked with a blue fill.