

A molecular phylogeny of bark spiders reveals new species from Africa and Madagascar (Araneae: Araneidae: *Caerostris*)

Matjaž Gregorič^{1,2}, Todd A. Blackledge², Ingi Agnarsson^{3,4} and Matjaž Kuntner^{1,4,5}: ¹Institute of Biology, Scientific Research Centre, Slovenian Academy of Sciences and Arts, Novi trg 2, P. O. Box 306, SI-1001 Ljubljana, Slovenia. E-mail: matjaz.gregoric@gmail.com; ²Integrated Bioscience Program, Department of Biology, University of Akron, Akron, Ohio, USA; ³Department of Biology, University of Vermont, Burlington, Vermont, USA; ⁴Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA; ⁵Centre for Behavioural Ecology and Evolution, College of Life Sciences, Hubei University, Wuhan, Hubei, China

Abstract. Bark spiders (genus *Caerostris* Thorell 1868) are important models in biomaterial research due to the remarkable biomechanical properties of the silk of *C. darwini* Kuntner & Agnarsson 2010 and its gigantic web. They also exhibit female gigantism and are promising candidates for coevolutionary research on sexual dimorphism. However, *Caerostris* spiders are taxonomically understudied and the lack of a phylogeny impedes evolutionary research. Using a combination of one mitochondrial and one nuclear marker, we provide the first species-level phylogeny of *Caerostris* including half of its species diversity but dense terminal sampling focusing on new lineages. Our phylogenetic and morphological results provide the evidence for six previously undescribed species: *C. almae* n. sp., *C. bojani* n. sp., *C. pero* n. sp. and *C. wallacei* n. sp., all from Madagascar, *C. linnaeus* n. sp. from Mozambique and *C. tinamaze* n. sp. from the Republic of South Africa.

Keywords: Biomaterial, spider silk, web gigantism, sexual size dimorphism, emasculation

Orb web spiders are model organisms in several fields, from functional morphology and physiology, predator-prey interactions, adaptive evolution, evolution of behavior and phylogeography, to sexual selection and biomaterial research (Coddington 1994; Bond & Opell 1998; Barth 2002; Gillespie 2004; Blackledge et al. 2011; Foelix 2011; Herberstein & Wignall 2011; Agnarsson et al. 2013). The “bark spiders” of the genus *Caerostris* Thorell 1868 are widespread throughout the Old World tropics (Grasshoff 1984) but understudied, and recent studies on *Caerostris* propose this clade as suitable for biomaterial and sexual selection research (Agnarsson et al. 2010; Kuntner & Agnarsson 2010).

The species diversity within this genus is incompletely known with only 12 described species worldwide (World Spider Catalog 2015); likewise, their phylogenetic affinities within the largest orb weaving family, Araneidae, remain controversial (Scharff & Coddington 1997; Kuntner et al. 2008, 2013; but, see Gregorič et al. 2015). Recent studies on *Caerostris* of Madagascar hint at undescribed diversity, with several sympatric species inhabiting single rainforest fragments of Madagascar (Fig. 1). *Caerostris* represents the most striking case of web gigantism with several species building orb webs considerably larger than those of most other spiders (Gregorič et al. 2011a, 2015). As an extreme example, *Caerostris darwini* Kuntner & Agnarsson 2010 utilizes a unique habitat by building its giant web in the air column above streams, rivers and lakes (Kuntner & Agnarsson 2010). *Caerostris darwini* builds orbs of up to 2 m in diameter that are suspended between riverbank vegetation by bridge lines that span up to 25 m (Gregorič et al. 2011a). Furthermore, *C. darwini* webs are made of silk that combines strength and elasticity such that it outperforms all other known spider silks, and even most synthetic fibers, in terms of toughness – the work required to fracture the silk (Agnarsson et al. 2010). *Caerostris* spiders also exhibit extreme sexual size dimorphism

(SSD), with large females and small males, and seem to have convergently evolved several enigmatic sexual behaviors connected to SSD, e.g., mate guarding, male-male aggressiveness, genital mutilation, mate plugging, and emasculation (Kuntner et al. 2008, 2015). Thus, comparative research on *Caerostris* spiders could yield important insights. Here we provide new taxonomic and phylogenetic hypotheses that will enable such research.

Molecular phylogenies place *Caerostris* on an early branching lineage of Araneidae (Sensenig et al. 2010; Kuntner et al. 2013; Gregorič et al. 2015), but no species-level phylogeny is available. We here provide the first species-level phylogeny of *Caerostris*, using a mitochondrial and a nuclear genetic marker, including six of the 12 described species plus new species. Grasshoff (1984) revised *Caerostris*, conservatively delimiting species, while high somatic and low genital variability within and among species is evident (Grasshoff 1984; Yin et al. 1997; Jäger 2007). Based on genetic distances, we here show that some *Caerostris* species diagnosed by Grasshoff likely represent species complexes, and describe six new species.

METHODS

Taxonomic sampling.—As ingroups we included six of the twelve currently recognized *Caerostris* species, *C. cowani* Butler 1882, *C. darwini*, *C. extrusa* Butler 1882, *C. mitralis* (Vinson 1863), *C. sexcuspidata* (Fabricius 1793) and *C. sumatrana* Strand 1915, and six new species proposed in this paper, *C. almae*, *C. bojani*, *C. linnaeus*, *C. pero*, *C. wallacei* and *C. tinamaze*. Our data set totals 50 *Caerostris* specimens (Appendix 1). As *Caerostris* represents an early araneid split (Gregorič et al. 2015), we used the araneids *Argiope* Audouin 1826 and *Acusilas* Simon 1895, and the zygelline *Zygiella* F.O. Pickard-Cambridge 1902 (sister to all other araneids, Kuntner et al. 2013; Gregorič et al. 2015) as outgroups, and

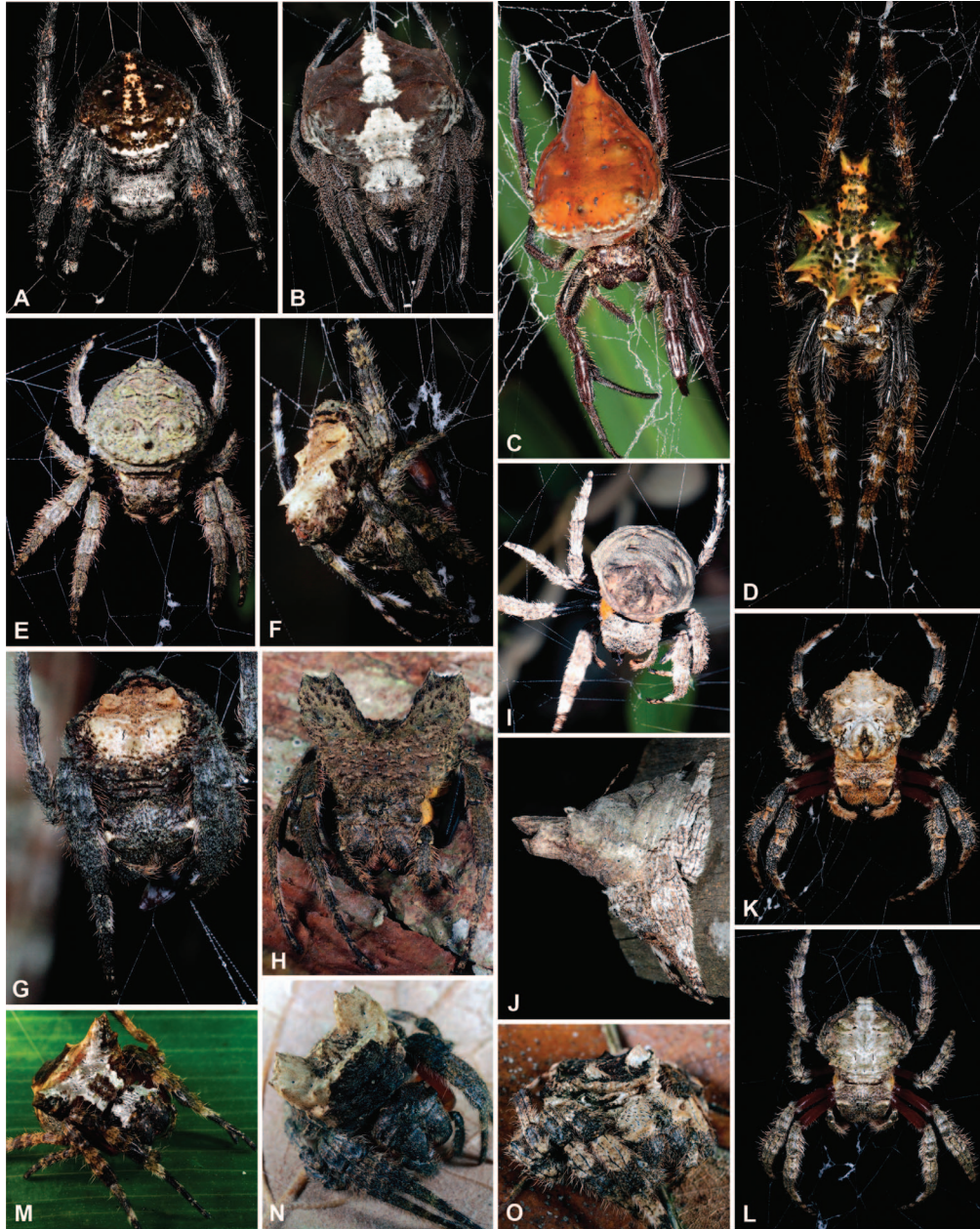


Figure 1.—*Caerostris* diversity in Africa and Madagascar. A: *C. darwini*, Madagascar; B,C: *C. extrusa*, Madagascar; D: *C. pero* new species, Madagascar; E–H: *C. bojani* new species, Madagascar; I,J: *C. linmaeus* new species, Mozambique; K,L: *C. almae* new species, Madagascar; M: *C. cowani*, Madagascar; N,O: Undetermined subadult *Caerostris* females, Madagascar.

rooted the trees with the nephilid *Nephila* Leach 1815 (Appendix 2).

We use the following museum abbreviations: CAS: California Academy of Sciences, San Francisco, California, U.S.A.; USNM: National Museum of Natural History, Smithsonian Institution, Washington DC, U.S.A.; ZMB: Museum für Naturkunde der Humboldt-Universität zu Berlin, Germany.

Morphological examination and imaging.—We performed all measurements using a Leica M165 C stereomicroscope equipped with a Leica DFC 420C camera through the Leica Application Suite 3.8 (Leica Microsystems, Wetzlar, Germany). We report all measurements in millimeters.

We captured images of external structures and epigynal anatomy using the Visionary Digital imaging system, equipped with a Canon 5D Mark II digital camera and an Infinity K2 microscope with Olympus metallurgical lenses, and we captured the images for later stacking using Adobe Lightroom 4 (Adobe Systems Incorporated, San Jose, CA, USA). We stacked the images using Zerene Stacker (Zerene Systems LLC, Richland, WA, USA) and Helicon Focus (Helicon Soft Ltd.), and further manipulated them in Adobe Photoshop CS4 (Adobe Systems Incorporated, San Jose, CA, USA).

We use the following morphological abbreviations in text and figures: ALE = anterior lateral eyes; AME = anterior

median eyes; BH = basal haematodocha; C = conductor; CB = cymbium; CD = copulatory duct; CO = copulatory opening; E = embolus; ETm = embolus-tegulum membrane; FD = fertilization duct; PME = posterior median eyes; PP = pars pendula; S = spermatheca; SD = sperm duct; ST = subtegulum; T = tegulum.

Molecular procedures.—We isolated DNA from leg muscles using the DNeasy Blood and Tissue Kit (QIAGEN, Venlo, Netherlands) following the protocol for mammals. We amplified the mitochondrial cytochrome c oxidase subunit I (CO1) gene fragment for all specimens, and the nuclear large subunit ribosomal (28S) gene fragment for all but five. All PCR reactions had a total volume of 25 μ l and consisted of 13.1 μ l dd H₂O, 5 μ l 5x PCR buffer “GoTaqFlexi” (Promega), 2.25 μ l MgCl₂ (25 mM, Promega), 0.15 μ l “5U GoTaqFlexi Polymerase” (Promega), 2.5 μ l “dNTP Mix” (2 μ M each, Biotools), 0.5 μ l of each forward and reverse 20 μ M primers, and 1.5 μ l of DNA. We included 0.15 μ l of bovine serum albumin (Promega, Fitchburg, Wisconsin; 10mg/ml) to some reactions and accordingly decreased the H₂O volume. We performed the PCR amplifications using a “2720 Thermal Cycler” (Applied Biosystems) and a “Mastercycler® ep” (Eppendorf).

We obtained ~ 1.2 kb fragments of CO1 by using several primer combinations. We used the forward “LCO1490” (GGTCAACAAATCATAAAGATATTGG) (Folmer et al. 1994) with the reverse “C1-N-2776” (aka “Maggy”; GGAT AATCAGAATATCGTCGAGG) (Hedin & Maddison 2001) primers to get the whole fragment. Alternatively, we used several combinations of the forward primers LCO1490, “degenerate LCO1490” (GGTCAACAAATCATAAAGAYAT YGG) (Folmer et al. 1994) and C1-J-2123 (aka “Tom”; GATCGAAATTTAATACTTCTTTTTTTGA) (Videngar et al. 2014), with the reverse primers Maggy, “HCO2198” (TAAACTTCAGGGTGACCAAAAATCA) (Folmer et al. 1994), “degenerate HCO2198” (TAAACTTCAGGGTGACC AAARAAYCA) (Folmer et al. 1994) and “Chelicerate-R2” (GGATGGCCAAAAAATCAAAATAAATG) (Barrett & Hebert 2005). We used a touch up program for the primer combination LCO1490 and C1-N-2776. PCR cycling conditions were 96°C for 10 min, followed by 20 cycles of 94°C for 1.5 min, 48°C–52°C for 2 min, 72°C for 2 min, followed by 15 cycles of 94°C for 1.5 min, 52°C for 1.5 min, 72°C for 2 min, and a final extension period of 72°C for 3 min. Shorter fragments using the two primer pairs were sometimes amplified using PCR conditions 94°C for 2 min, followed by 35 cycles of 94°C for 40 sec, 48°C–52°C for 1 min, 72°C for 1 min, and a final extension period of 72°C for 3 min.

We obtained the ~ 0.8 kb fragments of 28S using the forward 28Sa (GACCCGTCTTGAAACACGGA) (Whiting et al. 1997) and reverse 28S-rd5b (CCACAGCGCCAG TTCTGCTTAC) (Whiting et al. 1997) primers. We amplified the fragments using a touch down program with PCR cycling conditions 94°C for 7 min, followed by 20 cycles of 96°C for 45 sec, 62°C–52°C for 45 sec, 72°C for 1 min, followed by 15 cycles of 96°C for 45 sec, 52°C for 45 sec, 72°C for 1 min, and a final extension period of 72°C for 10 min.

Phylogenetic inference.—We aligned the protein coding CO1 sequences using ClustalW, and the ribosomal gene fragment 28S with the online version of MAFFT v.6 (Katoh & Standley

2013), using secondary structure of RNA information during the alignment process (the Q-INS-i strategy) and other values set to default. Because alignments of the 28S gene fragment contained unequal distributions of indels, we used Gblocks 0.91b to eliminate poorly aligned positions and divergent regions of the alignment in order to make our dataset more suitable for phylogenetic analyses (Talavera & Castresana 2007). We set the options to less stringent, allowing gap positions within final blocks, and less strict flanking positions. Using Mesquite 2.75 (Maddison & Maddison 2013), we concatenated gene fragments into two different matrices: first with the full 2016 bp of data, and the second containing ribosomal genes trimmed using Gblocks, summing up to 1965 bp of data.

We conducted Bayesian inference for all analyses. For both the full and Gblocks-trimmed data sets, we used unlinked models for each gene, and also used unlinked models for each gene and codon position in protein coding genes, resulting in four different analyses: the “full gene partition”, “gblocks gene partition”, “full codon partition” and “gblocks codon partition”. We used jModel Test 2.1.3 (Darriba et al. 2012) implementing the Akaike information criterion to statistically select the best-fit models of nucleotide substitutions. We conducted Bayesian analyses using MrBayes v3.1.2 run remotely at the CIPRES Science Gateway (Miller et al. 2010). For all analyses, we performed two independent runs with four simultaneous Markov Chain Monte Carlo chains, each starting with random starting trees, running for a total of 30 million generations. Using the “sump” command in MrBayes, we summarized the sampled parameters and discarded 25% generations as burnin.

Species delimitation.—We calculated genetic distances in the CO1 barcoding region among *Caerostris* individuals using Mega 6.06 (Tamura et al. 2013). We computed genetic distances using the Kimura 2 parameter (Kimura 1980) because this model represents the standard in DNA barcoding (Čandek & Kuntner 2015). We combined the results of our molecular phylogenies with morphological evidence to delimit species. We examined 401 *Caerostris* specimens, encompassing 9 of 12 described species, and only failed to obtain specimens of the Madagascan *C. ecclesigera* Butler 1882 and *C. hirsuta* (Simon 1895), and of *C. mayottensis* Grasshoff 1984 from the Comoros. Among the examined materials, we examined type specimens of *C. amanica* Strand 1907 (junior synonym of *C. vicina*), *C. insularis* Strand 1913 (junior synonym of *C. sexcuspidata*), *C. sumatrana*, and *C. rugosa* Karsch 1878 and *C. petersi* Karsch 1878 (both junior synonyms of *C. mitralis*). In addition to molecular distinction, the newly described species distinctly differ in genital morphology from all previously known species, according to diagnoses of Grasshoff (1984) and Kuntner & Agnarsson (2010). Additionally, we conservatively opted to not split certain widespread clades, despite geographical molecular structuring (e.g., *C. sumatrana* and *C. sexcuspidata*), due to limited specimen sampling outside Madagascar and South Africa (see Discussion).

RESULTS

All analyses strongly supported the monophyly of African *Caerostris* (Fig. 2, Supplemental material 1 [Online at

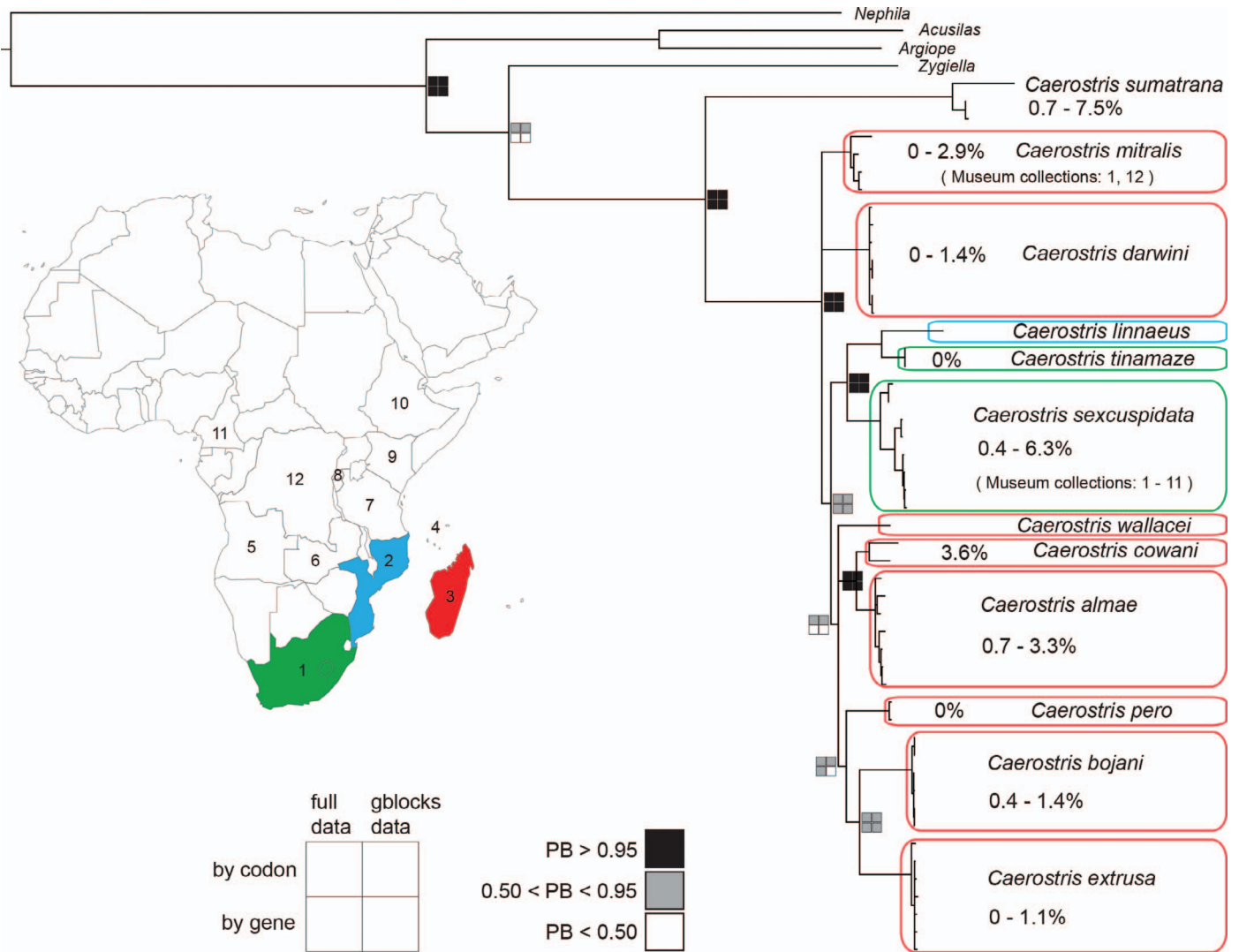


Figure 2.—Summary *Caerostris* phylogeny (full data partitioned by codon), with DNA barcode distances for species. The colored clouds enclosing species show the distribution of sequenced specimens, while the numbered countries show the distribution of species as inferred from museum collections.

dx.doi.org/10.1636/B15-05.s1]). Species from mainland Africa were recovered as monophyletic and nested within Malagasy species, but with weak support. Madagasy *Caerostris*, in turn, were never recovered as monophyletic (Fig. 2, Supplemental material 1 [Online at <http://dx.doi.org/10.1636/B15-05.s1>]).

The genetic distances among *Caerostris* species inferred from DNA barcodes ranged from 2.88% to 19.8% (ME = 7.43%, IQR = 2.36%). The median intraspecific genetic distance across all investigated *Caerostris* species was $1.25 \pm 3.2\%$. However, *C. sumatrana* and *C. sexcuspidata* likely represent species complexes and the median intraspecific genetic distance excluding these was $1.07 \pm 1.67\%$ (see Tables 1 & 2 for species details).

DISCUSSION

We present here the first species level phylogeny of *Caerostris* and describe six new species based on morphological and molecular diagnosability. DNA barcodes have proven to be a generally useful tool to aid species delimitation (Hebert et al. 2003, 2004; Barrett & Hebert 2005; Hajibabaei et al.

2006; Smit et al. 2013; but see Taylor & Harris 2012; Hamilton et al. 2014). This holds true in spiders where DNA barcodes have aided taxonomic decisions (Barrett & Hebert 2005; Arnedo & Ferrández 2007; Longhorn et al. 2007; Blagoev et al. 2009; Kuntner & Agnarsson 2011; Hendrixson et al. 2013; Agnarsson et al. 2015), and offer efficient means of species identification with 90% to 100% accuracy (Čandek & Kuntner 2015). In a sample of Araneidae, the interspecific and intraspecific genetic distances in the barcoding region were found to be $8.8 \pm 4.2\%$ and $1.1 \pm 1.8\%$, respectively (Čandek & Kuntner 2015), and the *Caerostris* species investigated here are close to araneid averages (interspecific $7.4 \pm 2.4\%$, intraspecific $1.1 \pm 1.7\%$). The newly described *Caerostris* species are genetically distinct, and are also clearly morphologically diagnosable, further justifying species hypotheses. However, while all species named here are diagnosable by morphology, molecular data imply the existence of further “cryptic species”. For example, based on DNA barcodes, *C. almae*, *C. bojani*, *C. darwini* and *C. extrusa* are well defined

Table 1.—DNA barcode distances among individuals across the investigated *Caerostris* species.

Species	Range	ME ± IQR
<i>C. almae</i> (N = 7)	0.71–3.275	1.434±1.27
<i>C. bojani</i> (N = 6)	0–1.434	1.072±1.07
<i>C. cowani</i> (N = 2)	3.622	3.622
<i>C. darwini</i> (N = 7)	0–1.43	1.069±0.36
<i>C. extrusa</i> (N = 7)	0–1.427	0.710±0.54
<i>C. mitralis</i> (N = 4)	0–2.9	1.981±2.09
<i>C. pero</i> (N = 2)	0	0
<i>C. sexcuspidata</i> (N = 8)	0.354–6.292	4.007±4.27
<i>C. sumatrana</i> (N = 3)	0.712–7.52	6.717
<i>C. tinamaze</i> (N = 2)	0	0

species with genetic distances among species far exceeding that within species (average 7.4% vs ~ 1%; Tables 1, 2). On the other hand, *C. sexcuspidata* and *C. sumatrana* show intraspecific geographical genetic structuring (average/max. of 4%/6.3% and 6.7%/7.5%, respectively; Tables 1, 2). However, these genetic clusters cannot be morphologically diagnosed with the limited specimens available at present. Furthermore, species delimitation might be influenced by an incomplete or biased sampling, and by population level processes (Hamilton et al. 2014). Thus, further molecular, ecological and biogeographical data are necessary to test whether these lineages represent genetically structured populations or “cryptic” species complexes.

Relationships among species from mainland Africa are not fully resolved, but together they form a strongly supported clade, likely nested among Madagascan species. As we obtained molecular data for 12 of the now 18 known *Caerostris* species, the recovered monophyly of African *Caerostris* is preliminary, but quite likely to persist given the morphological resemblance of African species. Only two *Caerostris* species are currently recognized in Asia, *C. sumatrana* occurring from India to Indonesia, and *C. indica* Strand 1915 known only from Myanmar (Grasshoff 1984; World Spider Catalog 2015). The Asian *Caerostris* we sampled have been identified as *C. sumatrana* based on genital morphology. However, the genetic distance among specimens

from South China and Laos reach 7.5% suggesting that broader geographical sampling across Asia will reveal even higher genetic structuring. Similarly, while museum material of *C. sexcuspidata* suggests a wide distribution across southern Africa (Fig. 2; Appendix 1), our results show “intraspecific” genetic distances of 6.3% within South Africa alone. Furthermore, museum material of “*C. sexcuspidata*” from Madagascar are in fact misidentified *C. darwini*. Both *C. sexcuspidata* and *C. sumatrana* as currently circumscribed therefore represent species complexes, and further sampling needs to test this assertion. No fewer than five *Caerostris* species inhabit a single forest fragment in Eastern Madagascar (*C. darwini*, *C. almae*, *C. bojani*, *C. pero* and *C. wallacei*) and this further indicates that *Caerostris* is much more diverse than hitherto appreciated.

Bark spiders are diverse and widespread throughout the Old World tropics. They range from fairly small to large in size, are sexually size dimorphic (Grasshoff 1984), make large to gigantic webs utilizing tough silks, and several species occupy different microhabitats even within small forest fragments (Gregorič et al. 2011a). Thus this charismatic genus offers ample opportunities for evolutionary research. For example, larger orb weaving species in general produce tougher silk, where web architecture and silk material properties coevolve with body size, improving web energy absorbing potential (Sensenig et al. 2010). Also, within individual size classes of species, orb webs undergo compensatory evolution of web performance where silk quality trades off with web architecture and the amount of silk used, a coevolutionary pattern not clearly demonstrated in many other common biomaterials such as byssal threads, tendon and keratin (Sensenig et al. 2010; Blackledge et al. 2012). The evolution of web size and material properties reaches extremes in *Caerostris*, and *C. darwini* represents an extreme in the compensatory evolution of web performance (Sensenig et al. 2010; Gregorič et al. 2015). Furthermore, *C. darwini* web biology strengthens the evidence for coevolution of silk mechanics with ecological and behavioral traits (Gregorič et al. 2011b). Because *Caerostris* species level phylogeny has been lacking, the origin and evolutionary mechanisms shaping web gigantism and silk mechanics remain ambiguous. Our species level *Caerostris*

Table 2.—Average DNA barcode distances among the investigated *Caerostris* species.

DNA barcode distance (%)	<i>C. almae</i>	<i>C. bojani</i>	<i>C. cowani</i>	<i>C. darwini</i>	<i>C. extrusa</i>	<i>C. mitralis</i>	<i>C. pero</i>	<i>C. linnaeus</i>	<i>C. wallacei</i>	<i>C. sexcuspidata</i>	<i>C. sumatrana</i>
<i>C. bojani</i>	6.4										
<i>C. cowani</i>	4.8	6.5									
<i>C. darwini</i>	6.8	7.9	4.9								
<i>C. extrusa</i>	6.3	6.4	6.4	6.6							
<i>C. mitralis</i>	4.6	8.5	5.3	6.5	6.0						
<i>C. pero</i>	6.7	7.5	7.2	8.0	7.4	6.8					
<i>C. linnaeus</i>	10.2	8.6	8.8	8.9	8.3	8.5	8.6				
<i>C. wallacei</i>	7.3	9.6	7.1	8.5	9.8	8.9	9.4	13.5			
<i>C. sexcuspidata</i>	7.5	9.3	7.4	7.3	7.0	6.2	8.7	8.2	10.6		
<i>C. sumatrana</i>	18.0	18.3	17.9	19.0	17.0	15.7	17.1	18.3	18.8	17.1	
<i>C. tinamaze</i>	9.5	9.5	8.8	10.1	8.7	7.1	9	7.5	13.6	9.8	18.7

phylogeny thus represents a first step towards developing a platform for understanding the evolution of extraordinary biomaterials.

Beyond web and silk evolution research, *Caerostris* may provide a promising additional clade to the more established model spider clades in studies of sexual dimorphism and related biologies (Cheng & Kuntner 2014, 2015; Kuntner & Elgar 2014). Sexual size dimorphism in araneoid spiders may predictably coevolve with behaviors such as emasculation, genital plugging and sexual cannibalism, judging from their convergent co-occurrence in the families Theridiidae, Nephilidae and Araneidae (Kuntner et al. 2015). The first species level phylogeny of *Caerostris* represents a new clade to complement ongoing work on the evolutionary patterns, causes and consequences of SSD in the spider family Nephilidae (Kralj-Fišer et al. 2011; Zhang et al. 2011; Danielson-Francois et al. 2012; Kuntner et al. 2012; Li et al. 2012; Kuntner & Elgar 2014), the araneid *Argiope* (Nessler et al. 2007; Foellmer 2008; Cheng & Kuntner 2014) and the theridiid *Latrodectus* Walckenaer 1805 (Andrade 1996; Kasumovic & Andrade 2009; Modanu et al. 2013).

TAXONOMY

Family Araneidae Clerck 1757

Genus *Caerostris* Thorell 1868 (bark spiders)

(Figs. 1, 3–10)

Aranea: Fabricius 1793: 427, description of *Aranea sexcuspidata* (= *Caerostris sexcuspidata*).

Epeira: Walckenaer, 1805: 67, description of *Epeira imperialis* (= *Caerostris sexcuspidata*).

Gasteracantha: C. L. Koch 1837: 36, description of *Gasteracantha sexcuspidata* (= *Caerostris sexcuspidata*).

Eurysoma: C. L. Koch 1850: 9, description of *Eurysoma sexcuspidata* (= *Caerostris sexcuspidata*).

Caerostris Thorell 1868: 4, 7, 8.

Trichocharis Simon 1895: 835, description of *Trichocharis hirsuta* (= *Caerostris hirsuta*).

Type species.—*Epeira mitralis* Vinson 1863, designated by Thorell 1868: 4.

Diagnosis.—*Caerostris* of both sexes differ from other araneids by the following combination of somatic features: prosoma and opisthosoma wider than long, head region wide and elevated from thoracic region, two pairs of median prosomal projections (none or one pair in males), the sternal tubercle adjacent to coxae IV, the median and lateral eyes grouped on separate tubercles, a frontal rostrum, cheliceral furrow smooth rather than denticulated, the abdominal sigillae, the flattened and hairy patellae, tibiae and metatarsi of legs I, II and IV, the spatulate setae on femur IV, and the ventro-lateral abdominal sclerotization in several rather than one line of small dots (Grasshoff 1984; Kuntner et al. 2008; Kuntner & Agnarsson 2010). *Caerostris* differ from other araneids by the following genital features: female epigynum with paired epigynal hooks (Figs. 3–5, 7–10), male palp with subtegulum of exaggerated size, cymbial ectal margin sclerotized as cymbium rather than transparent, no paracymbium (Kuntner et al. 2008; Kuntner & Agnarsson 2010). *Caerostris* differ from the Zygiellinae, a group sister to all other araneids

(Gregorič et al. 2015), by a hairy carapace and extensive rows of hairs on the carapace edge, the posterior eye row procurved rather than straight or recurved, straight rather than sigmoidal first femora, the abdominal humps and a truncated rather than rounded abdomen tip, abdominal dorso-lateral and dorso-central sclerotizations, the strongly sclerotized area around the book lung spiracle, the extensive rather than sparse PMS aciniform field, central rather than peripheral PLS mesal cylindrical gland spigot position, and by distal aggregate spigots embracing flagelliform spigots. *Caerostris* differ from most araneids but not zygiellines by the sustentaculum being parallel to other setae rather than divergent (Kuntner et al. 2008).

Caerostris almae Gregorič new species

(Figs. 1K–L, 3, 4)

Types.—Female holotype deposited at CAS, and labeled: *Caerostris almae* CAE301, Ranomafana NP, Madagascar; Gregorič, Agnarsson, Kuntner 2010. Male paratype deposited at CAS, and labeled: *Caerostris almae* CAE347, Analamazaotra, Madagascar; Griswold, Saucedo, Wood 2009.

Etymology.—The species epithet, a noun in genitive case, honors the first author's mother Alma Gregorič.

Diagnosis.—As in *C. extrusa*, *C. mitralis* (Grasshoff 1984: 19, 20, 29, 30), *C. tinamaze* (Fig. 9C) and *C. wallacei* (Fig. 10C), and in contrast to other *Caerostris* species, the epigynal hooks in *C. almae* (Figs. 3D; 4D, F) are short rather than long, positioned medially on the epigynal plate rather than anteriorly and pointing laterally rather than posteriorly. *C. almae* and *C. mitralis* differ from the above mentioned *Caerostris* species by the posterior epigynal margin that circles around the copulatory openings, and *C. almae* differs from *C. mitralis* by the relatively larger and bulkier epigynal hooks (Figs. 3D; 4D, F; 9C; 10C; Grasshoff 1984: 19, 20). Male *C. almae* differs from other *Caerostris* species by the relatively larger palp bulb, and the large and blunt conductor (Fig. 3I–K).

Description.—*Female* (Fig. 3A–E): Total length 10.1. *Prosoma* 4.8 long, 5.8 wide, 4.2 high. Carapace orange to brown, chelicerae dark reddish brown, both covered with white setae. Sternum 2.5 long, 3.2 wide, widest between second leg coxae, light brownish red with white setae in the center. AME diameter 0.2, PME diameter 0.22, AME separation 0.42, PME separation 0.86, PME–PLE separation 2.49, ALE–PLE separation 0.04. Clypeus height 0.43. *Appendages.* Palps brown. Coxae, trochanters and femora of legs orange, femora distally darkened, and patellae, tibiae, metatarsi and tarsi light to dark reddish brown, light brownish annulated. Leg I femur 5.2, patella 3.2, tibia 4.3, metatarsus 4.8, tarsus 1.8. *Opisthosoma* 7.8 long, 8.7 wide, 4.4 high. Base dorsum color light brown and largely covered in dark brown to dark green, with two large pointy light brown tubercles and several small tubercles. Venter brown, black in the middle, with two white transverse bands that end in bright white specks. *Epigynum* as diagnosed (Figs. 3D; 4D, F), spermathecae spheroid (Figs. 3 E; 4E, G).

Male (CAE347 from Analamazaotra, Madagascar, Fig. 3 F–K): Total length 2.8. *Prosoma* 2.1 long, 1.5 wide, 1 high. Carapace orange brown to reddish brown, chelicerae dark reddish brown, both covered with white setae. Sternum 0.7

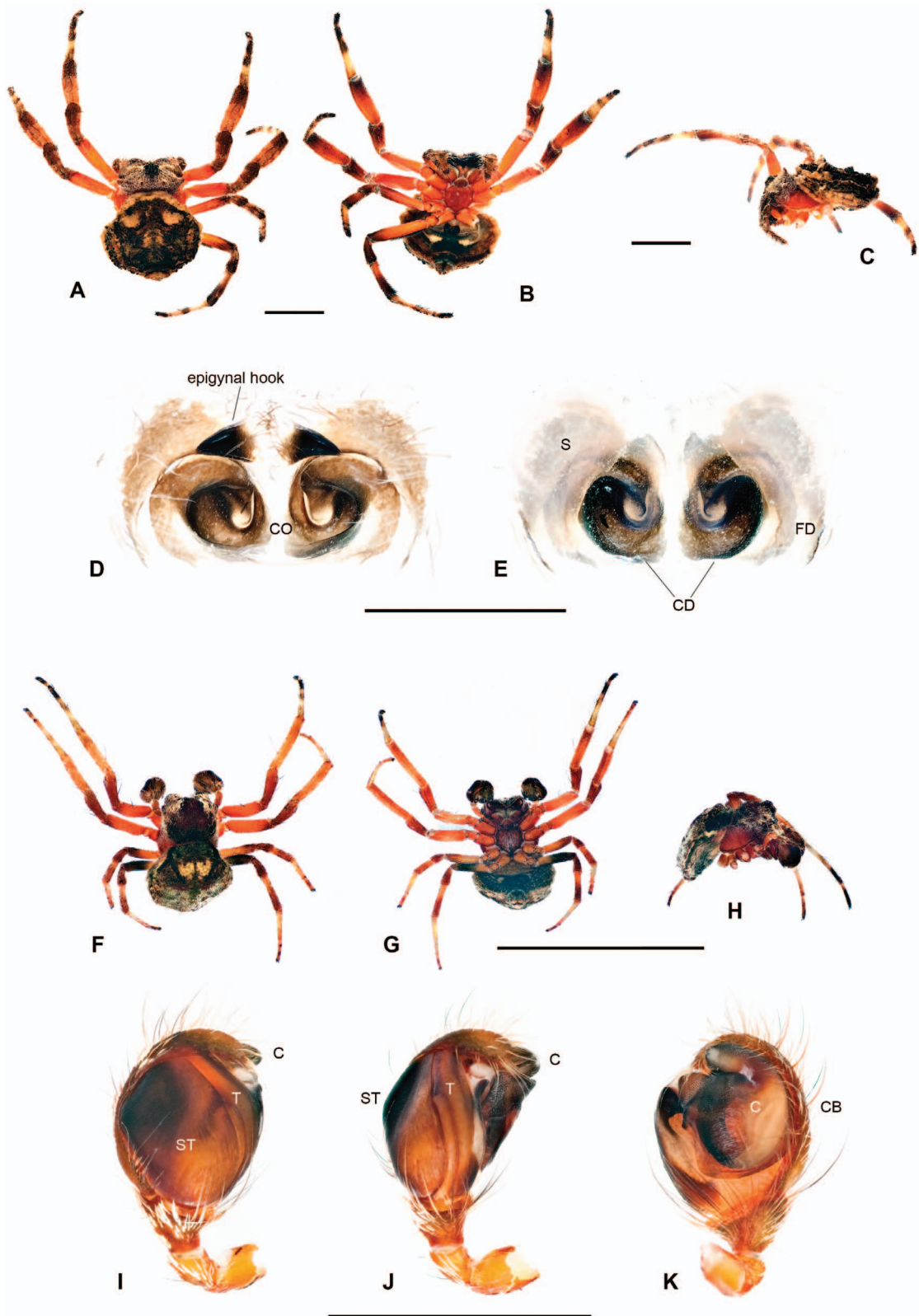


Figure 3.—*Caerostris almae*, female (A–E: CAE301) and male (F–K: CAE347) somatic and genital morphology. D: Female epigynum, ventral; E: Same, dorsal; I: Male right palp, lateral; J: Same, mesal; K: Same, ventral. Somatic scale bars = 5 mm, genital scale bars = 1 mm.

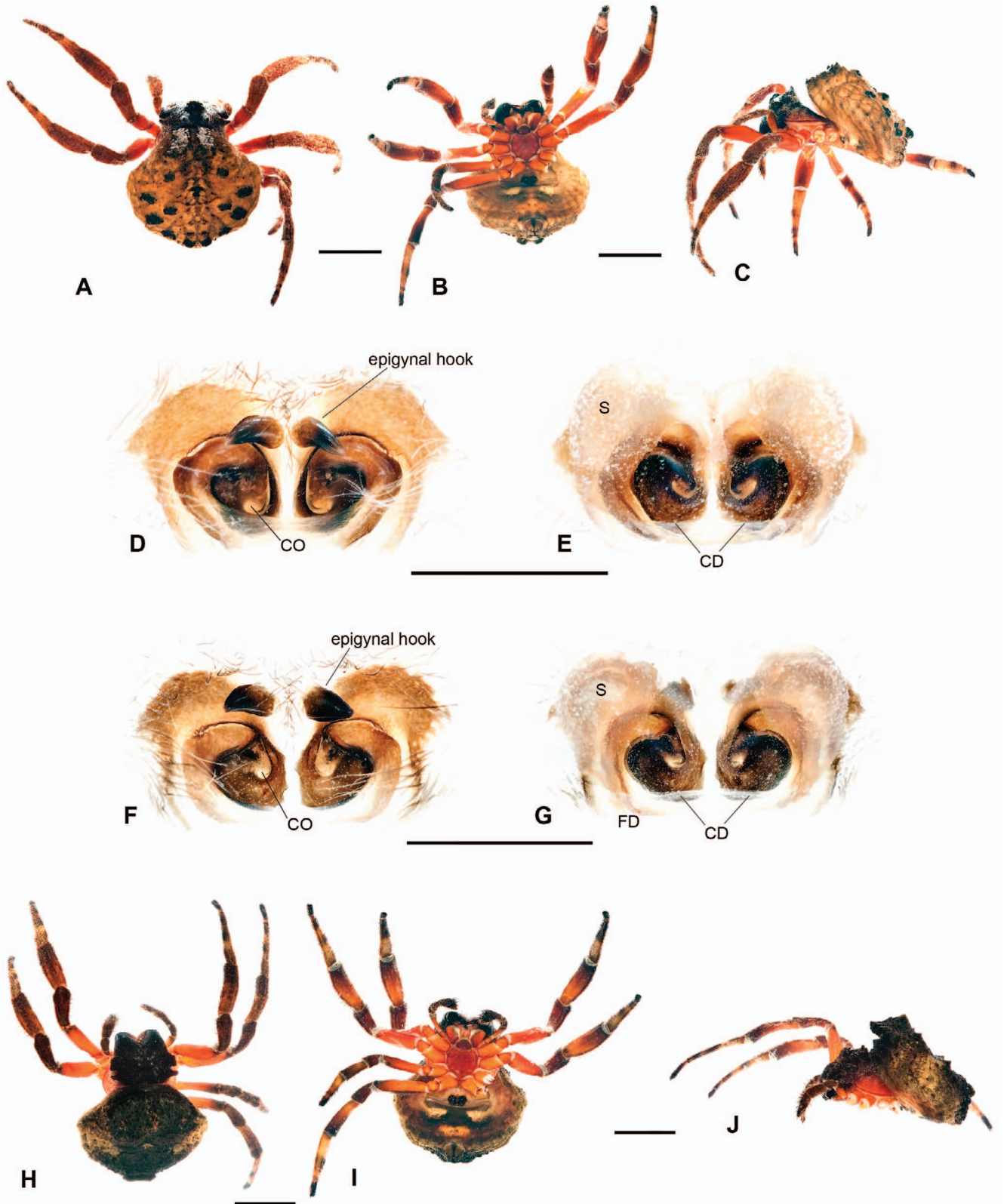


Figure 4.—*Caerostris almae*, female somatic and genital morphology, both from Andasibe-Mantadia, Madagascar. A–C: Female CAE305 somatic morphology; D: Female CAE305 epigynum, ventral; E: Same, dorsal; F: Female CAE303 epigynum, ventral; G: Same, dorsal; H–J: Female CAE303 somatic morphology. Somatic scale bars = 5 mm, genital scale bars = 1 mm.

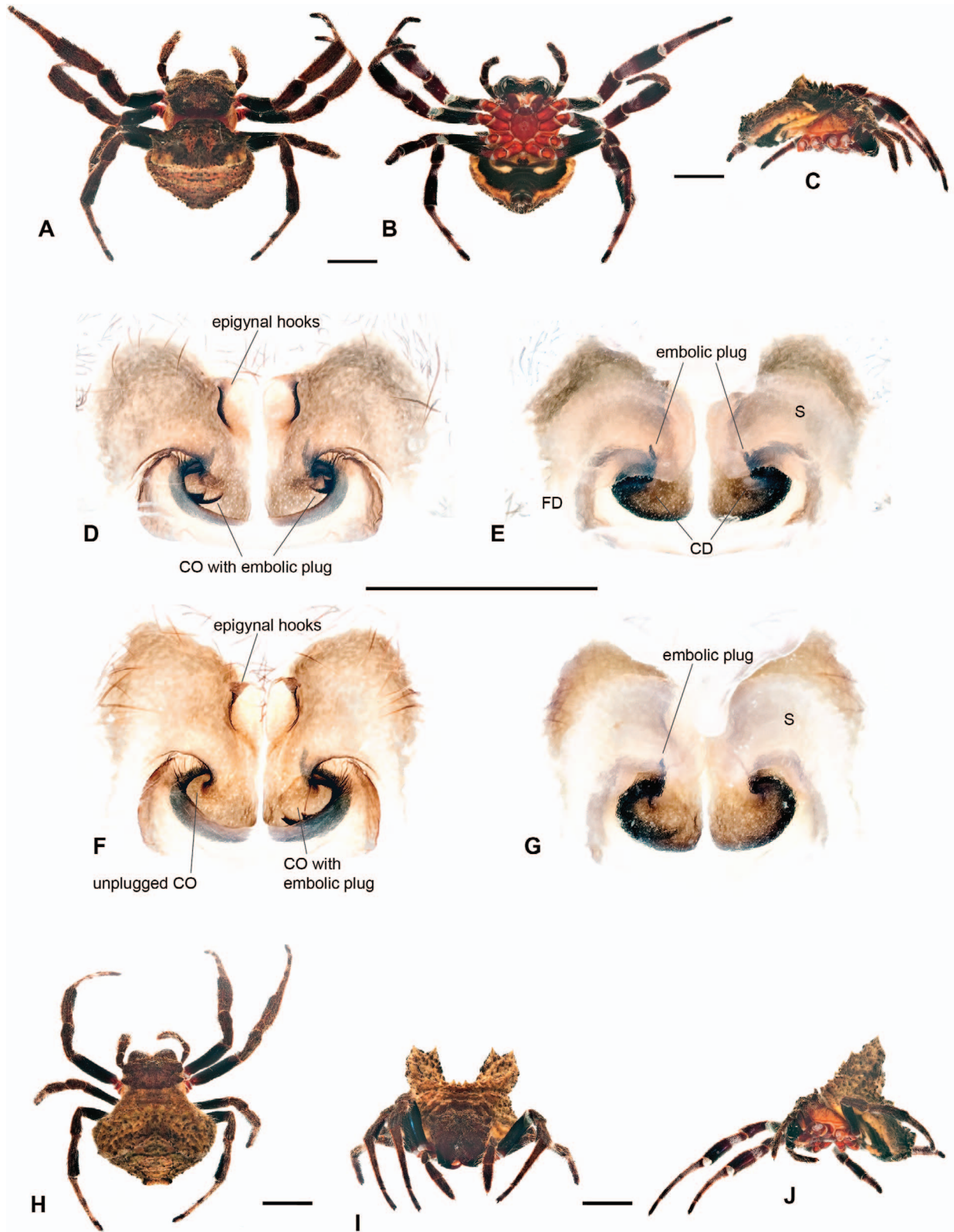


Figure 5.—*Caerostris bojani*, female somatic and genital morphology, all from Andasibe-Mantadia, Madagascar. A–C: Female CAE254 somatic morphology; D: Female CAE254 epigynum, ventral; E: Same, dorsal; F: Female CAE255 epigynum, ventral; E: Same, dorsal; H–J: Female CAE255 somatic morphology. Somatic scale bars = 5 mm, genital scale bar = 1 mm.

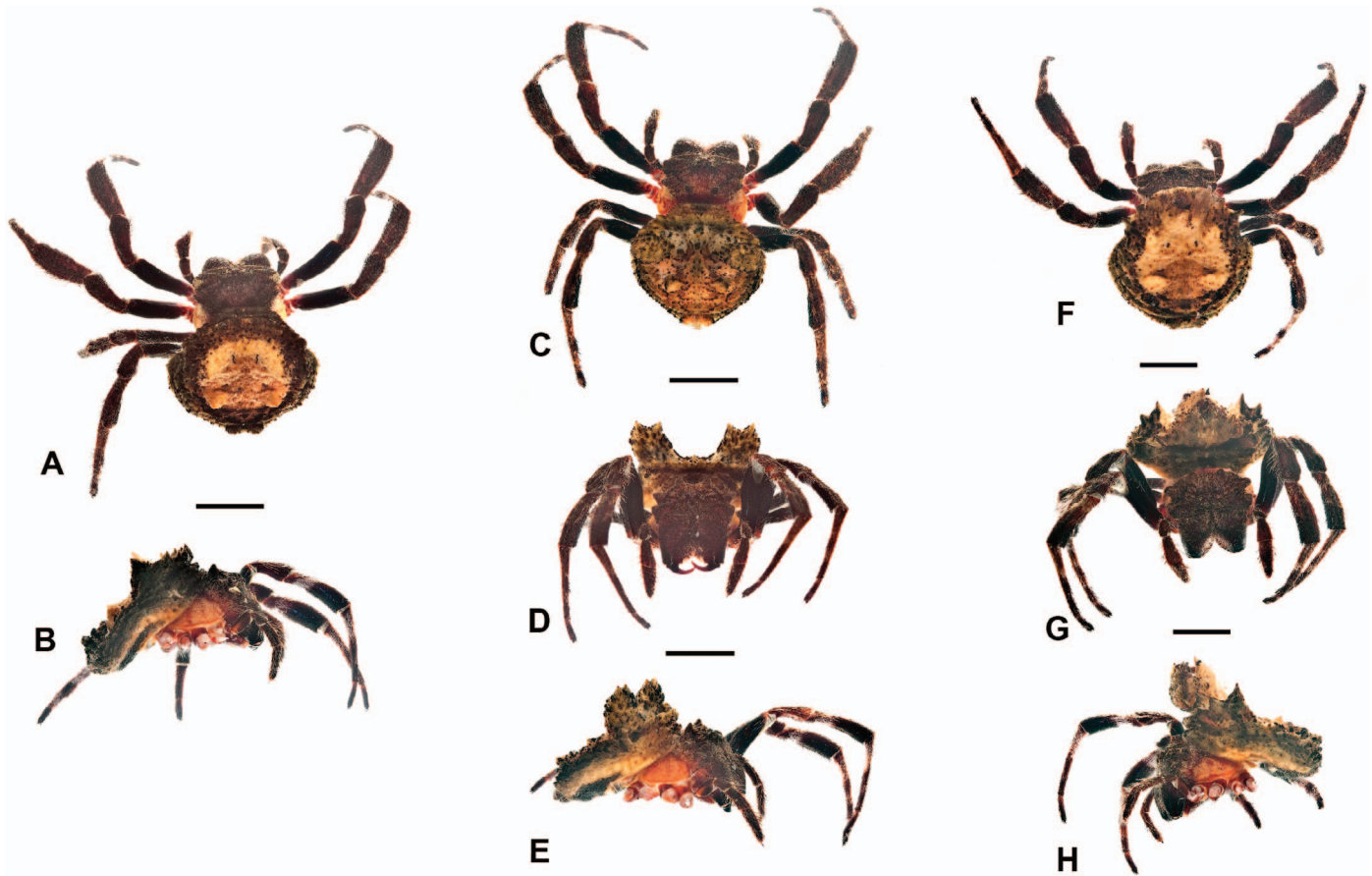


Figure 6.—*Caerostris bojani*, female somatic morphology, all from Andasibe-Mantadia, Madagascar. A, B: CAE263; C–E: CAE262; F–H: CAE252. Somatic scale bars = 5 mm.

long, 0.7 wide, widest between second leg coxae, reddish brown with white setae in the center. AME diameter 0.15, PME diameter 0.1, AME separation 0.16, PME separation 0.42, PME–PLE separation 0.91, ALE–PLE separation 0.03. Clypeus height 0.52. *Appendages*. Palps brown. Coxae, trochanters and femora of legs I and II orange brown to orange. Coxae, trochanters and femora of legs III and IV brown. Femora distally darkened, patellae, tibiae, metatarsi and tarsi light to dark reddish brown. Leg I femur 1.0, patella 1.0, tibia 1.4, metatarsus 1.5, tarsus 0.6. *Opisthosoma* 2.1 long, 2.1 wide, 1 high. Base dorsum color brown and largely covered in dark green with a pair of whitish specks anteriorly. Venter greenish brown. *Palp* as diagnosed (Fig. 3I–K).

Variation.—*Female*: Total length 8.4–13.1; prosoma length 3.9–5.2. Base color of opisthosoma dorsum light brown to brown, sometimes light grey, and covered with dark brown to dark green and black coloration, sometimes yellowish in the center, with several large and/or small tubercles. Opisthosoma venter sometimes black with three pairs of white specks, sometimes one transverse white band, sometimes white speck anteriorly to spinnerets (Figs. 3, 4).

Additional material examined.—Ten females collected at several localities in Madagascar (Appendix 1).

Distribution.—Eastern Madagascar, known from Ranomafana NP, Andasibe-Mantadia NP, Razanaka and Analama-zotra, all Toamasina Province, and from Antsirakambiaty, Fianarantsoa Province.

Natural history.—The species inhabits montane rainforests of Eastern Madagascar. All specimens were found at dawn or night, at forest edge close to water. Web typical for *Caerostris*, capture area 0.45 m² (Gregorič et al. *in prep*). Of the material investigated here, the specimen CAE398 had an embolic plug in the left copulatory opening, while others had no embolic plugs.

Caerostris bojani Gregorič new species
(Figs. 1E–H, 5, 6)

Types.—Female holotype deposited at USNM, and labeled: *Caerostris bojani* CAE254, Andasibe-Mantadia NP, Madagascar; Gregorič, Agnarsson, Kuntner 2010.

Etymology.—The species epithet, a noun in genitive case, honors the first author's father Bojan Gregorič.

Diagnosis.—As in *C. pero* (Fig. 8E, G), *C. linnaeus* (Fig. 7C) and *C. mayottensis* (Grasshoff 1984: 37), and in contrast to all other *Caerostris* species, the epigynal hooks in *C. bojani* (Fig. 5D, F) are short rather than long and positioned anteriorly on the epigynal plate rather than medially. *C. bojani* differs from *C. pero*, *C. linnaeus* and *C. mayottensis* by the short epigynal hooks with a wide rather than narrow base, and from *C. mayottensis* by the posterior epigynal margin not circling around the copulatory openings (Figs. 5D, F; 7C; 8E, G; Grasshoff 1984: 37).

Description.—*Female* (CAE254 from Andasibe-Mantadia NP, Madagascar, Fig. 5): Total length 14.8. *Prosoma* 7.6 long,

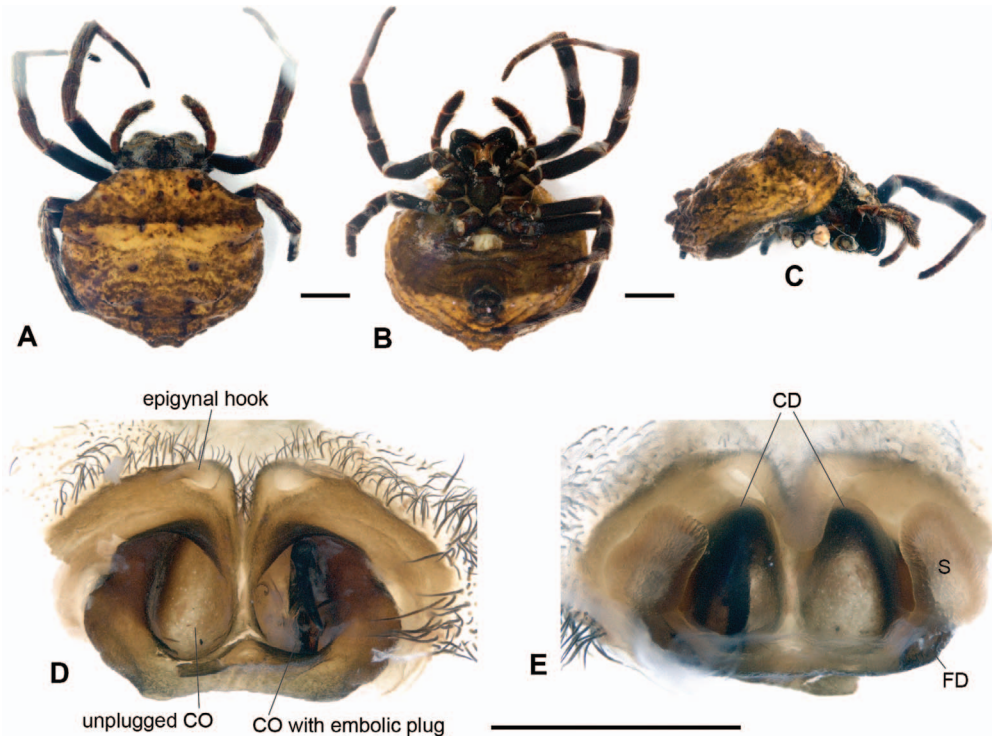


Figure 7.—*Caerostris linnaeus*, female ARA784 somatic and genital morphology, all from Maputo, Mozambique. A–C: Female somatic morphology; D: Female epigynum, ventral; E: Same, dorsal. Somatic scale bar = 5 mm, genital scale bar = 1 mm.

7.8 wide, 6 high. Carapace and chelicerae dark reddish brown, covered with light brown setae. Sternum 3.1 long, 3.1 wide, widest between second leg coxae, brownish red with white setae in the center. AME diameter 0.39, PME diameter 0.33, AME separation 0.44, PME separation 1.17, PME–PLE separation 3.05, ALE–PLE separation 0.08. Clypeus height 0.83. *Appendages*. Palps dark reddish brown. Coxae and trochanters ventrally brownish red. Femora black, patellae, tibiae, metatarsi and tarsi dark brown, ventrally annulated with white hair. Leg I femur 7.1, patella 4.1, tibia 5.6, metatarsus 7.25, tarsus 2.2. *Opisthosoma* 11.3 long, 11.3 wide, 6.3 high. Base color of dorsum grey and brown, covered with dark brown and black spots, with two larger and several smaller tubercles on anterior half. Venter black, outlined with a yellowish brown band, two white transverse bands. *Epigynum* as diagnosed (Fig. 5D), spermathecae kidney-shaped (Fig. 5E).

Variation.—*Female*: Total length 13.2–14.8; prosoma length 5.6–7.6. *Opisthosoma* grey with greenish tint to brown in color, median dorsum sometimes light brown. Dorsum with several small tubercles, or with a small to big pair of anterior tubercles (Figs. 1E–H, 5, 6).

Additional material examined.—Fifteen females collected in Andasibe-Mantadia NP, Madagascar (Appendix 1).

Distribution.—Known only from the type locality.

Natural history.—The species inhabits mountain rainforests of Eastern Madagascar. It builds its webs at dawn, under closed canopy, and hides on vegetation without web during the day. Web typical for *Caerostris*, capture area $0.16 \pm 0.1 \text{ m}^2$ (Gregorič et al. 2011a). Eleven of 15 examined females had their genitals plugged with male embolic parts, eight of these in both copulatory openings.

Caerostris linnaeus Gregorič new species
(Figs. 1I–J, 7)

Types.—Female holotype deposited at USNM, and labeled: *Caerostris linnaeus* ARA784, Maputo, Mozambique; Agnarsson, Kuntner 2013.

Etymology.—The species epithet, a noun in apposition, honors the Swedish biologist and physician Carl Linnaeus.

Diagnosis.—As in *C. bojani* (Fig. 5D, F), *C. mayottensis* (Grasshoff 1984: 37) and *C. pero* (Fig. 8E, G), and in contrast to all other *Caerostris* species, the epigynal hooks in *C. linnaeus* (Fig. 7C) are short rather than long and positioned anteriorly on the epigynal plate rather than medially. *C. linnaeus* differs from *C. mayottensis* by the posterior epigynal margin not circling around the copulatory openings, and from *C. bojani* by the short epigynal hooks with a narrow rather than wide base (Figs. 5D, F; 7C; Grasshoff 1984: 37). *C. linnaeus* differs from *C. pero* by the arch- rather than S-shaped copulatory ducts (Figs. 7D, 8F, H).

Description.—*Female* (ARA784 from Maputo, Mozambique, Fig. 7): Total length 20.7. *Prosoma* 8.9 long, 9 wide, 5.9 high. Carapace and chelicerae dark brown, covered with light brown setae. Sternum 4 long, 3.6 wide, widest between second leg coxae, uniform dark brown. AME diameter 0.34, PME diameter 0.32, AME separation 0.44, PME separation 0.99, PME–PLE separation 3.06, ALE–PLE separation 0.14. Clypeus height 1.03. *Appendages*. Palps brown. Coxae, trochanters and femora dark brown. Patellae, tibiae, metatarsi and tarsi dorsally covered with white hair, tibiae, metatarsi and tarsi ventrally annulated with white hair. Leg I femur 8, patella 4.9, tibia 6.6, metatarsus 7.6, tarsus 2.5. *Opisthosoma* 18.5 long, 20.2 wide, 9.5 high. Base color of dorsum light

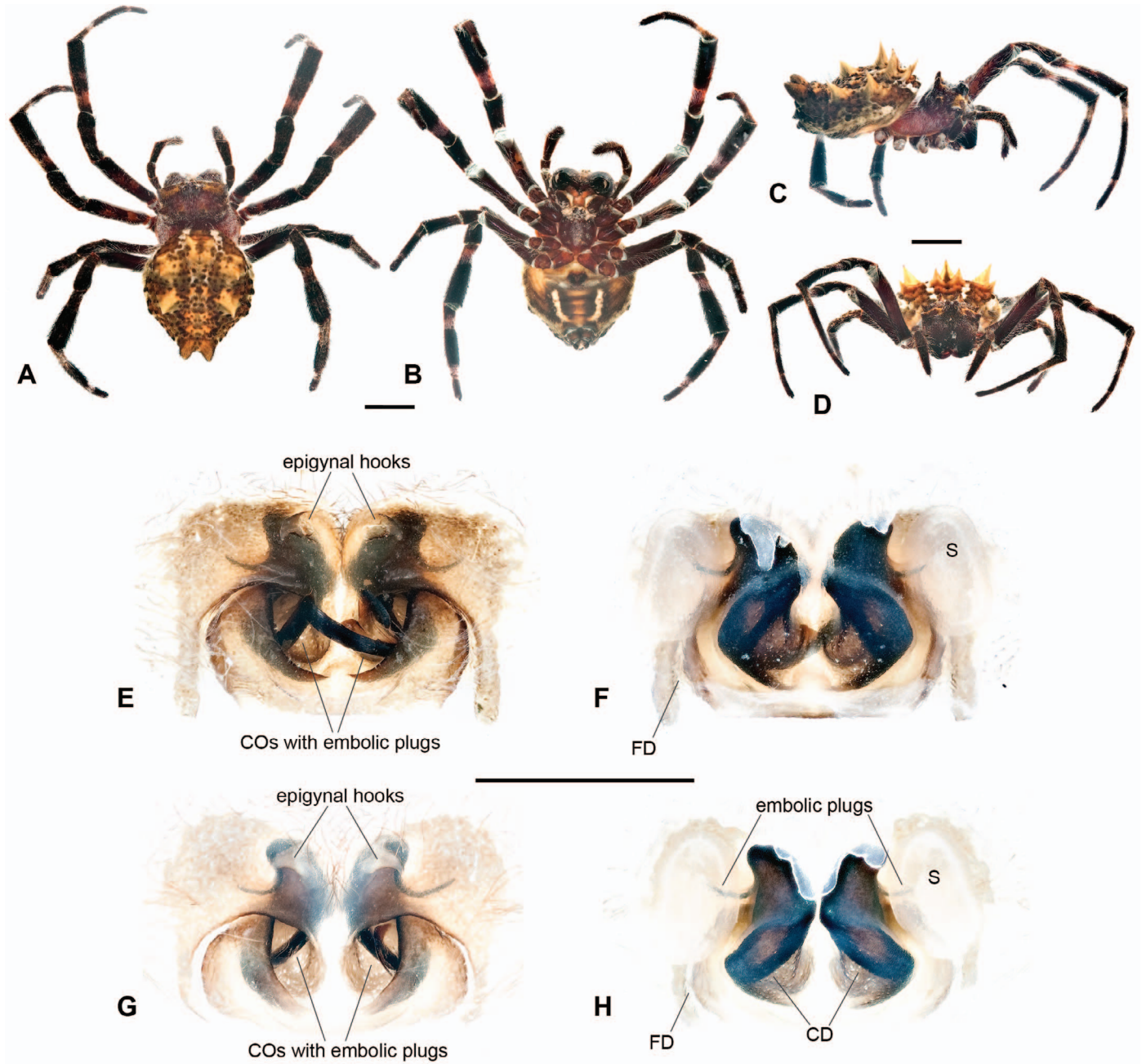


Figure 8.—*Caerostris pero*, female somatic and genital morphology, Andasibe-Mantadia NP, Madagascar. A–C: Female CAE216 somatic morphology; D: Female CAE215 somatic morphology; E: Female CAE215 epigynum, ventral; F: Same, dorsal; G: Female CAE216 epigynum, ventral; H: Same, dorsal. Somatic scale bars = 5 mm, genital scale bar = 1 mm.

brown to yellowish brown, covered with dark brown specks, with two larger and several smaller tubercles on anterior half. Venter dark brown. *Epigynum* as diagnosed (Fig. 7C), spermathecae kidney-shaped (Fig. 7D).

Variation.—Unknown.

Additional material examined.—None.

Distribution.—South Mozambique, known only from the type locality.

Natural history.—The examined specimen inhabited a forest edge around Maputo, Mozambique. The web typical for the

genus *Caerostris*, more than a meter in diameter. The examined female plugged with male embolic parts in the left copulatory opening.

Caerostris pero Gregorič new species
(Figs. 1D; 8)

Types.—Female holotype deposited at USNM, and labeled: *Caerostris pero* CAE215, Andasibe-Mantadia NP, Madagascar; Gregorič, Agnarsson, Kuntner 2010.

Etymology.—The species epithet, a noun in apposition, honors the first author's brother Peter "Pero" Gregorič.

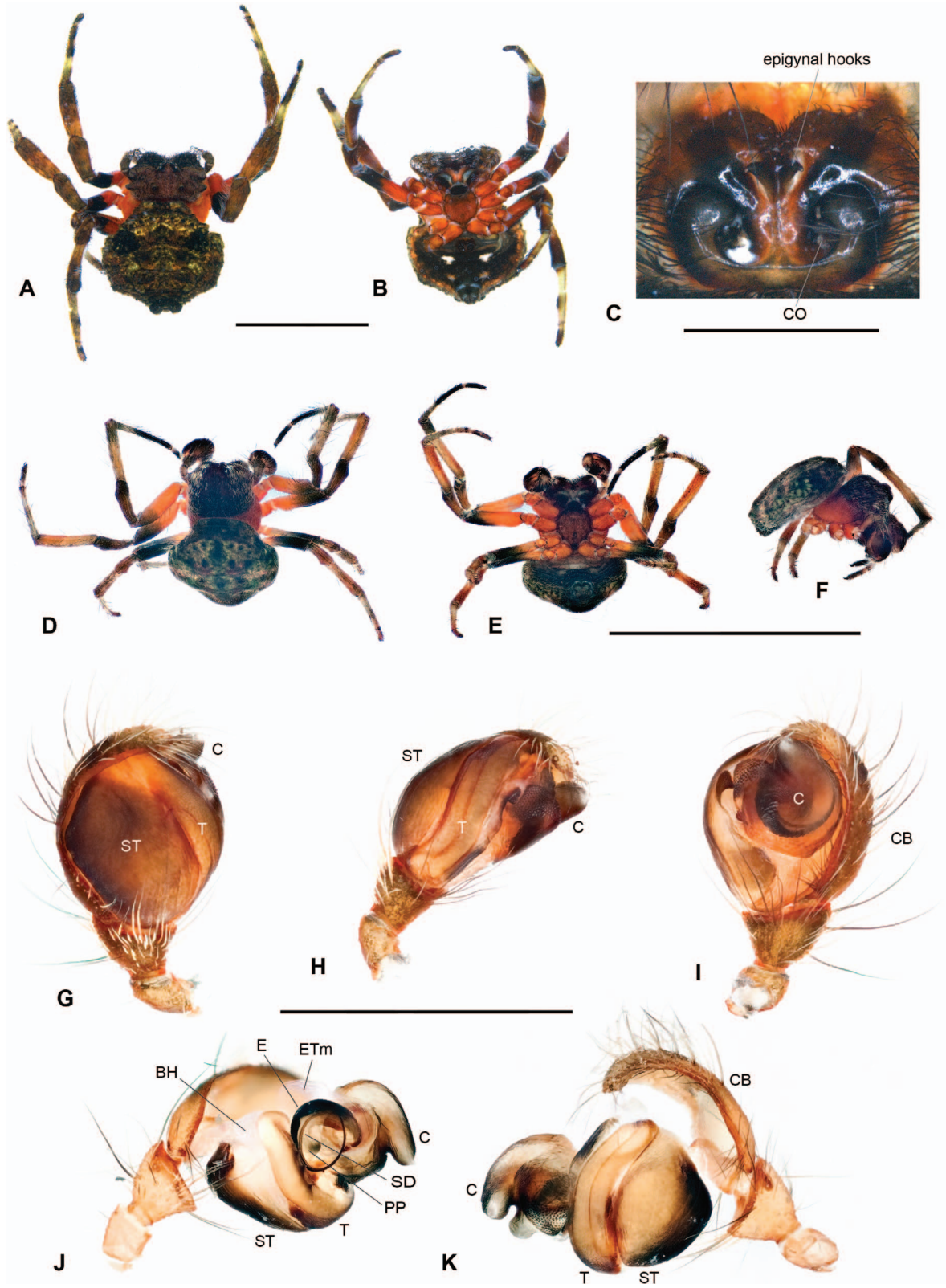


Figure 9.—*Caerostris tinamaze*, female (A–C: CAE341) and male (D–K: CAE341) somatic and genital morphology, Entabeni NR, Republic of South Africa. C: Female epigynum, ventral. G: Male right palp, lateral; H: Same, mesal; I: Same, ventral; J: Male right palp, expanded, mesal; K: Same, ventral. Somatic scale bars = 5 mm, genital scale bars = 1 mm.

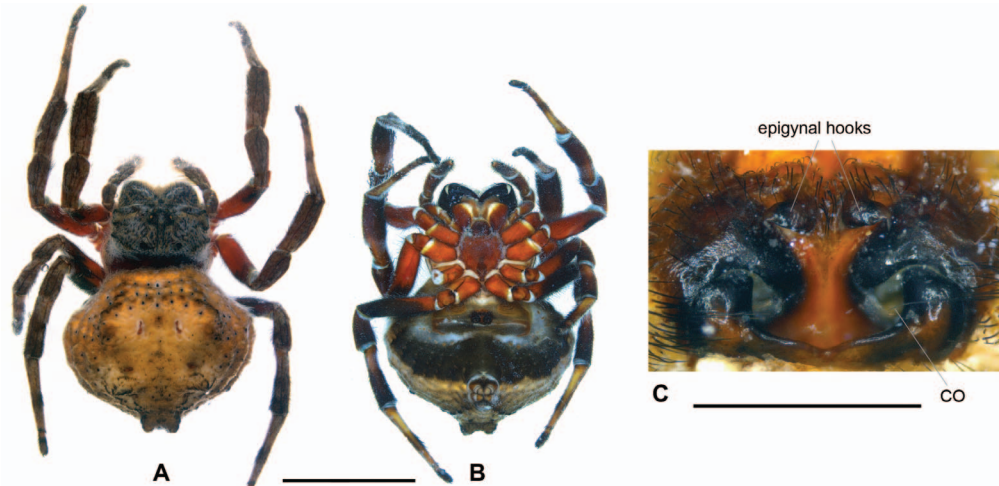


Figure 10.—*Caerostris wallacei*, female CAE334 somatic and genital morphology, Kirindy, Madagascar. C: Female epigynum, ventral. Somatic scale bars = 5 mm, genital scale bar = 1 mm.

Diagnosis.—*Caerostris pero* differs in somatic morphology from all other *Caerostris* species by the 11 pointy tubercles on the opisthosoma dorsum (Fig. 8A, C, D). As in *C. bojani* (Fig. 5D, F), *C. linnaeus* (Fig. 7C) and *C. mayottensis* (Grasshoff 1984: 37), and in contrast to all other *Caerostris* species, the epigynal hooks in *C. pero* (Fig. 8E, G) are short rather than long and positioned anteriorly on the epigynal plate rather than medially. *C. pero* differs from *C. mayottensis* by the posterior epigynal margin not circling around the copulatory openings, from *C. bojani* by the short epigynal hooks with a narrow rather than wide base (Figs. 5D, F; 8E, G; Grasshoff 1984: 37), and from *C. linnaeus* by the S- rather than arch-shaped copulatory ducts (Figs. 7D, 8F, H).

Description.—*Female* (CAE215 from Andasibe-Mantadia NP, Madagascar, Fig. 8): Total length 16.4. *Prosoma* 6.6 long, 6.9 wide, 3.1 high. Carapace and chelicerae dark reddish brown, covered with white setae. Sternum 2.5 long, 3.2 wide, widest between second leg coxae, dark reddish brown with white setae longitudinally in the center. AME diameter 0.34, PME diameter 0.27, AME separation 0.41, PME separation 0.76, PME–PLE separation 2.25, ALE–PLE separation 0.27. Clypeus height 0.82. *Appendages*. Palps dark reddish brown. Legs dorsally dark brown, light brownish annulated. Coxae, trochanters and femora of legs I and II ventrally reddish brown, patellae, tibiae, metatarsi and tarsi ventrally dark brown. Coxae and trochanters of legs III and IV ventrally brown, femora ventrally reddish brown, patellae, tibiae, metatarsi and tarsi ventrally dark brown. Leg I femur 8.5, patella 6.1, tibia 6, metatarsus 7.2, tarsus 2.3. *Opisthosoma* 13.2 long, 10.9 wide, 4 high. Dorsum brown covered with dark brown spots, with light brown longitudinal band, with 11 pointy light brown tubercles. Venter brown with two narrow, white median longitudinal bands. *Epigynum* as diagnosed (Fig. 8E), spermathecae spheroid (Fig. 8F).

Variation.—*Female*: Total length 14.3–18.6; prosoma length 5.8–6.7.

Additional material examined.—Eighteen females collected in Andasibe-Mantadia NP, Madagascar (Appendix 1).

Distribution.—Eastern Madagascar, known only from the type locality.

Natural history.—The species inhabits montane rainforests of Eastern Madagascar. It suspends its large orb web in the air column over small forest streams under closed canopy. Web typical for *Caerostris*, capture area $0.48 \pm 0.21 \text{ m}^2$ (Gregorič et al. 2011a). Ten of the 18 examined females had their genitals plugged with male embolic parts, five of these in both copulatory openings.

Caerostris tinamaze Gregorič new species
(Fig. 9)

Types.—Female holotype and male paratype deposited at CAS, and labeled: *Caerostris tinamaze* CAE341, Entabeni NR, Republic of South Africa; Miller, Wood 2006.

Etymology.—The species epithet, a noun in apposition, honors the Slovenian alpine skiing champion Tina Maze.

Diagnosis.—As in *C. extrusa*, *C. mitralis* (Grasshoff 1984: 19, 20, 29, 30), *C. almae* (Figs. 3D; 4D, F) and *C. wallacei* (Fig. 10C), and in contrast to other *Caerostris* species, the epigynal hooks in *C. tinamaze* (Fig. 9C) are short rather than long, positioned medially on the epigynal plate rather than anteriorly and pointing laterally rather than posteriorly. *C. tinamaze* differs from *C. almae* and *C. mitralis* by the posterior epigynal margin not circling around the copulatory openings (Figs. 3D; 4D, F; 9C; 10C; Grasshoff 1984: 19, 20, 29, 30). *C. tinamaze* differs from *C. sexcuspidata* by the laterally pointing epigynal hooks (Fig. 9C; Grasshoff 1984: 16, 17). Male *C. tinamaze* differs from other *Caerostris* by the blunt and anteriorly pointing conductor.

Description.—*Female* (CAE341 from Entabeni NR, Limpopo province, Republic of South Africa, Fig. 9A–C): Total length 9. *Prosoma* 4.3 long, 4.6 wide, 3.8 high. Carapace and chelicerae brown, covered with light brown setae. Sternum 2.1 long, 2.3 wide, widest between second leg coxae, orange. AME diameter 0.21, PME diameter 0.22, AME separation 0.38, PME separation 0.72, PME–PLE separation 1.77, ALE–PLE separation 0.05. Clypeus height 0.55. *Appendages*. Palps greenish brown. Coxae and trochanters orange. Femora

orange in proximal half and black in distal half. Patellae and tibiae dorsally greenish brown, and ventrally brown with annulation of yellowish brown pigment and white setae. Metatarsi proximally pale yellowish and dark brown distally, tarsi brown. Leg I femur 4.2, patella 2.6, tibia 3.6, metatarsus 4.3, tarsus 1.7. *Opisthosoma* 7 long, 7.1 wide, 3.7 high. Dorsum greenish brown with several small tubercles. Venter outlined with light brown, median black with two pairs of white specks. *Epigynum* as diagnosed (Fig. 9C), spermathecae unknown.

Male (CAE341 from Entabeni NR, Madagascar, Fig. 9D–K): Total length 2.9. Prosoma 1.6 long, 1.5 wide, 1 high. Carapace reddish brown to brown, chelicerae dark reddish brown, both covered with white setae. Sternum 0.8 long, 0.8 wide, widest between second leg coxae, brown. AME diameter 0.11, PME diameter 0.13, AME separation 0.16, PME separation 0.37, PME–PLE separation 0.47, ALE–PLE separation 0.07. Clypeus height 0.2. *Appendages*. Palps brown. Coxae, trochanters and femora of legs I and II orange brown. Coxae, trochanters and femora of legs III and IV brown. Femora distally darkened, patellae, tibiae, metatarsi and tarsi light to dark brown. Metatarsi and tarsi of leg I almost entirely black. Leg I femur 1.3, patella 0.81, tibia 1.3, metatarsus 1.2, tarsus 0.5. *Opisthosoma* 2.1 long, 2.3 wide, 1 high. Base dorsum color dark brown and largely covered in dark green. Venter dark brown to black. *Palp* as diagnosed (Fig. 9G–K).

Variation.—Unknown.

Additional material examined.—None.

Distribution.—Known only from the type locality.

Natural history.—The examined specimens inhabited an afro-montane forest fragment in a pine plantation. The examined female was plugged with male embolic parts in the right copulatory opening, the examined male intact.

Caerostris wallacei new species
(Fig. 10)

Types.—Female holotype deposited at CAS, and labeled: *Caerostris wallacei* CAE334, Kirindy, Madagascar; Wood, Miller 2006.

Etymology.—The species epithet, a noun in genitive case, honors the “other father” of evolutionary biology, Alfred R. Wallace.

Diagnosis.—As in *C. extrusa*, *C. mitralis* (Grasshoff 1984: 19, 20, 29, 30), *C. almae* (Figs. 3D; 4D, F) and *C. tinamaze* (Fig. 9C), and in contrast to other *Caerostris* species, the epigynal hooks in *C. wallacei* (Fig. 10C) are short rather than long, positioned medially on the epigynal plate rather than anteriorly and pointing laterally rather than posteriorly. *C. wallacei* differs from *C. almae* and *C. mitralis* by the posterior epigynal margin not circling around the copulatory openings, and from *C. extrusa* and *C. tinamaze* by bulky and straight epigynal hooks (Figs. 3D; 4D, F; 9C; 10C: Grasshoff 1984: 19, 20, 29, 30).

Description.—*Female* (CAE334 from Kirindy, Toliara, Madagascar, Fig. 10): Total length 15.9. Prosoma 6.5 long, 7.3 wide, 5.6 high. Carapace and chelicerae brown, covered with white and yellowish setae. Sternum 3 long, 3.1 wide, widest between second leg coxae, orange. AME diameter 0.26, PME diameter 0.26, AME separation 0.53, PME separation 1.09, PME–PLE separation 2.61, ALE–PLE separation 0.11. Clypeus height 0.76. *Appendages*. Palps brown. Coxae and

trochanters orange. Femora ventrally I–II orange, distally dark brown, greyish dorsally. Femora III–IV orange proximally, dark brown distally, greyish dorsally. Patellae brown, greyish dorsally. Tibiae brown, light and annulated with white setae proximally, greyish dorsally. Metatarsi yellowish ventrally and greyish dorsally. Tarsi brown. Leg I femur 5.7, patella 3.5, tibia 4.5, metatarsus 5.9, tarsus 1.9. *Opisthosoma* 12.1 long, 12.3 wide, 7.8 high. Dorsum yellowish brown, with several small tubercles and sclerotized dots. Venter brown. *Epigynum* as diagnosed (Fig. 10C).

Variation.—Unknown.

Additional material examined.—None.

Distribution.—Southern Madagascar, known only from the type locality.

Natural history.—The type specimen inhabited the dry deciduous Kirindy forest of Southern Madagascar. The examined female genitalia were not plugged with male embolic parts.

ACKNOWLEDGMENTS

We thank Ren-Chung Cheng, Shakira G. Qiñones Lebron, Heine C. Kiesbüy, Tjaša Lokovšek, Laura May-Collado, Yadira Ortiz and Joel Duff for their laboratory and logistic help. We thank Jonathan Coddington (USNM), Jason Dunlop (ZMB), Charles Griswold (CAS), Peter Jäger, Rudy Jocqué, Daiqin Li, Wenjin Gan, Liu Shengjie, Honore Rabarison, Sahondra Lalao Rahanitriniaina, and MICET and ICTE crews for museum loans, fresh material and help in the field. We thank Jason Bond and an anonymous reviewer for useful comments. This contribution was funded by the Slovenian Research Agency (grants P1-0236, J1-2063), the United States National Science Foundation (IOS-0745379) and the National Geographic Society (grant 8655-09).

LITERATURE CITED

- Agnarsson, I., J.A. Coddington & M. Kuntner. 2013. Systematics: Progress in the study of spider diversity and evolution. Pp. 58–111. *In* Spider Research in the 21st Century: Trends and Perspectives. (D. Penney, ed.). Siri Scientific Press, Rochdale, UK.
- Agnarsson, I., B.B. Jencik, G.M. Veve, S. Rahanitriniaina, D. Agostini & S.P. Goh, et al. (2015). Systematics of the Madagascar *Anelosimus* spiders: remarkable local richness and endemism, and dual colonization from the Americas. *ZooKeys* 509:13–52.
- Agnarsson, I., M. Kuntner & T.A. Blackledge. 2010. Bioprospecting finds the toughest biological material: Extraordinary silk from a giant riverine orb spider. *Plos One* 5:e11234.
- Andrade, M.C.B. 1996. Sexual selection for male sacrifice in the Australian redback spider. *Science* 271:70–72.
- Arnedo, M.A. & M.A. Ferrández. 2007. Mitochondrial markers reveal deep population subdivision in the European protected spider *Macrothele calpeiana* (Walckenaer, 1805) (Araneae, Hexathelidae). *Conservation Genetics* 8:1147–1162.
- Barrett, R.D.H. & P.D.N. Hebert. 2005. Identifying spiders through DNA barcodes. *Canadian Journal of Zoology* 83:481–491.
- Barth, F.G. 2002. *A Spider's World: Senses and Behavior*. Springer-Verlag, Berlin.
- Blackledge, T.A., M. Kuntner & I. Agnarsson. 2011. The form and function of spider orb webs: Evolution from silk to ecosystems. Pp. 175–262. *In* Advances in Insect Physiology, Vol 41: Spider Physiology and Behaviour—Behaviour. (J. Casas, ed.). Academic Press, Burlington.

- Blackledge, T.A., M. Kuntner, M. Marhabaie, T.C. Leeper & I. Agnarsson. 2012. Biomaterial evolution parallels behavioral innovation in the origin of orb-like spider webs. *Scientific Reports* 2:833.
- Blagoev, G., P. Hebert, S. Adamowicz & E. Robinson. 2009. Prospects for using DNA barcoding to identify spiders in species-rich genera. *ZooKeys* 16:27–46.
- Bond, J.E. & B.D. Opell. 1998. Testing adaptive radiation and key innovation hypotheses in spiders. *Evolution* 52:403–414.
- Čandek, K. & M. Kuntner. 2015. DNA barcoding gap: Reliable species identification over morphological and geographical scales. *Molecular Ecology Resources* 15:268–277.
- Cheng, R.-C. & M. Kuntner. 2014. Phylogeny suggests non-directional and isometric evolution of sexual size dimorphism in argiopine spiders. *Evolution* 68:2861–2872.
- Cheng, R.-C. & M. Kuntner. 2015. Disentangling the size and shape components of sexual dimorphism. *Evolutionary Biology* 42:223–234.
- Coddington, J.A. 1994. The roles of homology and convergence in studies of adaptation. Pp. 53–78. *In* *Phylogenetics and Ecology*. (P.V. Eggleton & R. Vane-Wright, eds.). The Linnean Society of London, London.
- Danielson-Francois, A., C. Hou, N. Cole & I.M. Tso. 2012. Scramble competition for moulting females as a driving force for extreme male dwarfism in spiders. *Animal Behaviour* 84:937–945.
- Darriba, D., G.L. Taboada, R. Doallo & D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9:772.
- Foelix, R.F. 2011. *Biology of Spiders*. 3rd ed. Oxford University Press, Oxford.
- Foellmer, M.W. 2008. Broken genitals function as mating plugs and affect sex ratios in the orb-web spider *Argiope aurantia*. *Evolutionary Ecology Research* 10:449–462.
- Folmer, O., M. Black, W. Hoeh, R. Lutz & R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3:294–299.
- Gillespie, R. 2004. Community assembly through adaptive radiation in Hawaiian spiders. *Science* 303:356–359.
- Grasshoff, M. 1984. Die Radnetzspinnen-Gattung *Caerostris* (Arachnida: Araneae). *Revue Zoologique Africaine* 98:725–765.
- Gregorič, M., I. Agnarsson, T.A. Blackledge & M. Kuntner. 2011a. Darwin's bark spider: giant prey in giant orb webs (*Caerostris darwini*, Araneae: Araneidae)? *Journal of Arachnology* 39:287–295.
- Gregorič, M., I. Agnarsson, T.A. Blackledge & M. Kuntner. 2011b. How did the spider cross the river? Behavioral adaptations for river-bridging webs in *Caerostris darwini* (Araneae: Araneidae). *PLoS One* 6:e26847.
- Gregorič, M., I. Agnarsson, T.A. Blackledge & M. Kuntner. 2015. Phylogenetic position and composition of Zyiellinae and *Caerostris*, with new insight into orb-web evolution and gigantism. *Zoological Journal of the Linnean Society* doi: 10.1111/zoj.1.12281.
- Hajibabaei, M., D.H. Janzen, J.M. Burns, W. Hallwachs & P.D. Hebert. 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences, USA* 103:968–971.
- Hamilton C.A., B.E. Hendrixson, M.S. Brewer & J.E. Bond. 2014. An evaluation of sampling effects on multiple DNA barcoding methods leads to an integrative approach for delimiting species: A case study of the North American tarantula genus *Aphonopelma* (Araneae, Mygalomorphae, Theraphosidae). *Molecular Phylogenetics and Evolution* 71:79–93.
- Hebert, P.D., A. Cywinska, S.L. Ball & J.R. deWaard. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B-Biological Sciences* 270:313–321.
- Hebert, P.D., E.H. Penton, J.M. Burns, D.H. Janzen & W. Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences, USA* 101:14812–14817.
- Hedin, M.C. & W.P. Maddison. 2001. A combined molecular approach to phylogeny of the jumping spider subfamily Dendryphantinae (Araneae: Salticidae). *Molecular Phylogenetics and Evolution* 18:386–403.
- Hendrixson, B.E., B.M. DeRussy, C.A. Hamilton & J.E. Bond. 2013. An exploration of species boundaries in turret-building tarantulas of the Mojave Desert (Araneae, Mygalomorphae, Theraphosidae, *Aphonopelma*). *Molecular Phylogenetics and Evolution* 66:327–340.
- Herberstein, M. & A. Wignall. 2011. Introduction: spider biology. Pp. 1–30. *In* *Spider Behaviour: Flexibility and Versatility*. (M. Herberstein, ed.). Cambridge University Press, Cambridge.
- Jäger, P. 2007. Spiders from Laos with descriptions of new species (Arachnida: Araneae). *Acta Arachnologica* 56:29–58.
- Kasumovic, M.M. & M.C.B. Andrade. 2009. A change in competitive context reverses sexual selection on male size. *Journal of Evolutionary Biology* 22:324–333.
- Katoh, K. & D.M. Standley. 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution* 30:772–780.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111–120.
- Kralj-Fišer, S., M. Gregorič, S. Zhang, D.Q. Li & M. Kuntner. 2011. Eunuchs are better fighters. *Animal Behaviour* 81:933–939.
- Kuntner, M. & I. Agnarsson. 2010. Darwin's bark spider: Web gigantism in a new species of bark spiders from Madagascar (Araneidae: *Caerostris*). *Journal of Arachnology* 38:346–356.
- Kuntner, M. & I. Agnarsson. 2011. Biogeography and diversification of hermit spiders on Indian Ocean islands (Nephilidae: *Nephilengys*). *Molecular Phylogenetics and Evolution* 59:477–488.
- Kuntner, M. & M.A. Elgar. 2014. Evolution and maintenance of sexual size dimorphism: aligning phylogenetic and experimental evidence. *Frontiers in Ecology and Evolution* 2:26.
- Kuntner, M., I. Agnarsson & D.Q. Li. 2015. The eunuch phenomenon: adaptive evolution of genital emasculation in sexually dimorphic spiders. *Biological Reviews* 90:279–296.
- Kuntner, M., M.A. Arnedo, P. Trontelj, T. Lokovšek & I. Agnarsson. 2013. A molecular phylogeny of nephilid spiders: Evolutionary history of a model lineage. *Molecular Phylogenetics and Evolution* 69:961–979.
- Kuntner, M., J.A. Coddington & G. Hormiga. 2008. Phylogeny of extant nephilid orb-weaving spiders (Araneae, Nephilidae): testing morphological and ethological homologies. *Cladistics* 24:147–217.
- Kuntner, M., M. Gregorič, S. Zhang, S. Kralj-Fišer & D.Q. Li. 2012. Mating plugs in polyandrous giants: Which sex produces them, when, how and why? *Plos One* 7:e40939.
- Li, D.Q., J. Oh, S. Kralj-Fišer & M. Kuntner. 2012. Remote copulation: male adaptation to female cannibalism. *Biology Letters* 8:512–515.
- Longhorn, S.J., M. Nicholas, J. Chuter & A.P. Vogler. 2007. The utility of molecular markers from non-lethal DNA samples of the CITES II protected “tarantula” *Brachypelma vagans* (Araneae, Theraphosidae). *Journal of Arachnology* 35:278–292.
- Maddison, W.P. & D.R. Maddison. 2013. Mesquite: a modular system for evolutionary analysis. Online at <http://mesquiteproject.org>.
- Miller, M.A., W. Pfeiffer & T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 Nov 2010, New Orleans, Louisiana, 1–8.
- Modanu, M., P. Michalik & M.C.B. Andrade. 2013. Mating system does not predict permanent sperm depletion in black widow spiders. *Evolution & Development* 15:205–212.

- Nessler, S.H., G. Uhl & J.M. Schneider. 2007. Genital damage in the orb-web spider *Argiope bruennichi* (Araneae: Araneidae) increases paternity success. *Behavioral Ecology* 18:174–181.
- Scharff, N. & J.A. Coddington. 1997. A phylogenetic analysis of the orb-weaving spider family Araneidae (Arachnida, Araneae). *Zoological Journal of the Linnean Society* 120:355–434.
- Sensenig, A., I. Agnarsson & T.A. Blackledge. 2010. Behavioural and biomaterial coevolution in spider orb webs. *Journal of Evolutionary Biology* 23:1839–1856.
- Smit, J., B. Reijnen & F. Stokvis. 2013. Half of the European fruit fly species barcoded (Diptera, Tephritidae); a feasibility test for molecular identification. *ZooKeys* 365:279–305.
- Talavera, G. & J. Castresana. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* 56:564–577.
- Tamura, K., G. Stecher, D. Peterson, A. Filipiński & S. Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis, Version 6.0. *Molecular Biology and Evolution* 30:2725–2729.
- Taylor, H.R. & W.E. Harris. 2012. An emergent science on the brink of irrelevance: a review of the past 8 years of DNA barcoding. *Molecular Ecology Resources* 12:377–388.
- Videgar, N., N. Toplak & M. Kuntner. 2014. Streamlining DNA barcoding protocols: Automated DNA extraction and a new *cox1* primer in arachnid systematics. *Plos One* 9:e113030.
- Whiting, M.F., J.C. Carpenter, Q.D. Wheeler & W.C. Wheeler. 1997. The Strepsiptera problem: Phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Systematic Biology* 46:1–68.
- World Spider Catalog. 2015. Natural History Museum Bern. Online at <http://wsc.nmbe.ch>
- Yin, C.M., J.F. Wang, M.S. Zhu, L.P. Xie, X.J. Peng & Y.H. Bao. 1997. *Fauna Sinica: Arachnida: Araneae: Araneidae*. Science Press, Beijing.
- Zhang, S., M. Kuntner & D.Q. Li. 2011. Mate binding: male adaptation to sexual conflict in the golden orb-web spider (Nephilidae: *Nephila pilipes*). *Animal Behaviour* 82:1299–1304.
- Manuscript received 25 January 2015, revised 6 July 2015.*

APPENDICES

Appendix I.—Taxonomic and distribution information of the *Caerostris* material examined in this study: information for specimens of each species is given as the database code, sex and number, and locality details.

Caerostris almae

- CAE301, 1 female, Madagascar, Ranomafana, elev. 1000 m, 21.256514S 47.437372E, 22.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE303, 1 female, Madagascar, Ranomafana, elev. 1000 m, 21.256514S 47.437372E, 19.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE305, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 7–8.v.2001, Agnarsson I., Kuntner M.
- CAE337, 2 female, Madagascar, Antsirakambiaty, elev. 1550 m, 20.594S 46.564E, 22–26.i.2003, Griswold C., Fisher
- CAE338, 1 female, Madagascar, Analamazaotra, elev. 960 m, 18.9297167S 48.4116E, 31.i.–3.ii.2009, Griswold C., Saucedo A., Wood H.
- CAE347, 1 male, Madagascar, Analamazaotra, elev. 960 m, 18.9297167S 48.4116E, 31.i.–3.ii.2009, Griswold C., Saucedo A., Wood H.

- CAE399, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 8.iii.–21. iv. 2012, Gregorič M., Cheng R.C., Šuen K.

Caerostris bojani

- CAE252, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 8.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE253, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 8.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE254, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 7.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE255, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 7.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE256, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 12.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE257, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 8.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE258, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 12.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE260, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 8.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE261, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 7.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE262, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 8.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE263, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 7.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE304, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 23.iv.2008, Agnarsson I., Kuntner M.
- CAE306, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 7–8.v.2001, Agnarsson I., Kuntner M.
- CAE308, 2 females, Madagascar, Andasibe-Mantadia NP, elev. 900–1000 m, 18.937172S 48.420053E, 7–8.v.2001, Agnarsson I., Kuntner M.

Caerostris cowani

- CAE300, 1 female, Madagascar, Ranomafana, elev. 1000 m, 21.256514S 47.437372E, 19.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE340, 1 female, Madagascar, Ambohitantely, elev. 1620 m, 18.171389S 47.28194E, 19–21.iii.2003, Andriamalala D., Silva D.

Caerostris darwini

- CAE233, 1 female, Madagascar, Mantadia, elev. 950 m, 18.783784S 48.427617E, 28.ii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE236, 1 female, Madagascar, Antananarivo, elev. 1280 m, 18.930325S 47.526810E, 25.ii.2010, Agnarsson I., Kuntner M., Gregorič M.

- CAE270F, 1 female, Madagascar, Madraka private reserve, elev. 1370 m, 18.912647S 47.892627E, 2.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE270M, 1 male, Madagascar, Madraka private reserve, elev. 1370 m, 18.912647S 47.892627E, 2.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE289, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900-1000 m, 18.9472S 48.418394E, 4.iv.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE294, 1 female, Madagascar, Andasibe-Mantadia NP, elev. 900-1000 m, 18.937172S 48.420053E, 30.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE298, 1 female, Madagascar, Ranomafana, elev. 1000 m, 21.256514S 47.437372E, 22.iii.2010, Agnarsson I., Kuntner M., Gregorič M.

Caerostris extrusa

- CAE218, 1 female, Madagascar, Mantadia, elev. 950 m, 18.783784S 48.427617E, 28.ii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE220, 1 female, Madagascar, Mantadia, elev. 950 m, 18.783784S 48.427617E, 28.ii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE221, 1 female, Madagascar, Mantadia, elev. 950 m, 18.783784S 48.427617E, 28.ii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE227, 1 female, Madagascar, Mantadia, elev. 950 m, 18.783784S 48.427617E, 28.ii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE279, 1 female, Madagascar, Ranomafana, elev. 1000 m, 21.256514S 47.437372E, 22.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE281, 1 female, Madagascar, Ranomafana, elev. 1000 m, 21.256514S 47.437372E, 22.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE331, 1 female, Madagascar, Analamazaotra, elev. 960 m, 18.9297167S 48.4116E, 31.i.-3.ii.2009, Griswold C., Saucedo A., Wood H.

Caerostris linnaeus

- ARA784, 1 female, Mozambique, Maputo, elev. 30 m, N -25.922183S 32.552909E, Kuntner M., Agnarsson I.

Caerostris mitralis

- CAE332, 1 female, Madagascar, Montagne d'Ambre, elev. 1000 m, 12.5234167S 49.1734E, 14.xii.2005, Wood H., Raholiarisendra H., Rabemahafaly J.
- CAE333, 1 female, Madagascar, Montagne d'Ambre, elev. 800 m, 12.4713S 49.21283E, 17.xii.2005, Wood H., Raholiarisendra H., Rabemahafaly J.
- CAE345F, 1 female, Madagascar, Analalava, elev. 700 m, 22.59167S 45.1283E, 1-5.ii.2003, Griswold C., Fisher
- CAE345M, 2 males, Madagascar, Analalava, elev. 700 m, 22.59167S 45.1283E, 1-5.ii.2003, Griswold C., Fisher

Caerostris pero

- CAE210, 1 female Madagascar, Mantadia, elev. 950 m, 18.783784S 48.427617E, 26.ii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE212, 1 female Madagascar, Mantadia, elev. 950 m, 18.783784S 48.427617E, 28.ii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE213, 1 female Madagascar, Mantadia, elev. 950 m, 18.783784S 48.427617E, 26.ii.2010, Agnarsson I., Kuntner M., Gregorič M.

- CAE214, 1 female Madagascar, Mantadia, elev. 950 m, 18.783784S 48.427617E, 26.ii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE215, 1 female Madagascar, Mantadia, elev. 950 m, 18.783784S 48.427617E, 26.ii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE216, 1 female Madagascar, Mantadia, elev. 950 m, 18.783784S 48.427617E, 26.ii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE245, 1 female Madagascar, Andasibe-Mantadia NP, elev. 900-1000m, 18.937172S 48.420053E, 12.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE246, 1 female Madagascar, Andasibe-Mantadia NP, elev. 900-1000m, 18.937172S 48.420053E, 12.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE247, 1 female Madagascar, Andasibe-Mantadia NP, elev. 900-1000m, 18.937172S 48.420053E, 11.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE248, 1 female Madagascar, Mantadia, elev. 950 m, 18.783784S 48.427617E, 26.ii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE249, 1 female Madagascar, Andasibe-Mantadia NP, elev. 900-1000m, 18.937172S 48.420053E, 12.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE250, 1 female Madagascar, Andasibe-Mantadia NP, elev. 900-1000m, 18.937172S 48.420053E, 11.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE251, 1 female Madagascar, Andasibe-Mantadia NP, elev. 900-1000m, 18.937172S 48.420053E, 12.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE266, 1 female Madagascar, Andasibe-Mantadia NP, elev. 900-1000m, 18.937172S 48.420053E, 11.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE267, 1 female Madagascar, Andasibe-Mantadia NP, elev. 900-1000m, 18.937172S 48.420053E, 11.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE268, 1 female Madagascar, Andasibe-Mantadia NP, elev. 900-1000m, 18.937172S 48.420053E, 11.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE269, 1 female Madagascar, Andasibe-Mantadia NP, elev. 900-1000m, 18.937172S 48.420053E, 11.iii.2010, Agnarsson I., Kuntner M., Gregorič M.
- CAE397, 1 female Madagascar, Andasibe-Mantadia NP, elev. 900-1000m, 18.937172S 48.420053E, 12.iii.2010, Agnarsson I., Kuntner M., Gregorič M.

Caerostris sexcuspidata

- CAE187, 1 female, Tanzania, Mpafu NR, elev. 15 m, 7.283654S 39.349953E, 29.i.2009, Pienke S.
- CAE205, 1 female, RS. Africa, Hogsback, elev. 1070 m, 32.60205S 26.944783E, 28.iii.2011, Haddad C.
- CAE206, 1 female, RS. Africa, Hogsback, elev. 1070 m, 32.60205S 26.944783E, 28.iii.2011, Haddad C.
- CAE207, 1 female, RS. Africa, Hogsback, elev. 1250 m, 32.595483S 26.931567E, 27.iii.2011, Haddad C.
- CAE208, 1 female, RS. Africa, Hogsback, elev. 1250 m, 32.595483S 26.931567E, 27.iii.2011, Haddad C.
- CAE339, 1 female, RS. Africa, Tsitsikamma National Park, elev. 15 m, 34.023483S 23.8903E, 17-18.ii.2006, Miller J., Wood H.
- CAE344F, 1 juvenile female, RS. Africa, Tsitsikamma NP, elev. 15 m, 34.023483S 23.8903E, 17-18.ii.2006, Miller J., Wood H.

CAE344M, 3 males, RS. Africa, Tsitsikamma NP, elev. 15 m, 34.023483S 23.8903E, 17-18.ii.2006, Miller J., Wood H.

Caerostris sumatrana

CAE004, 2 females, Laos, Muong Sing, elev. 640 m, N21.190367S 101.1575E, 3.xi.2004, Jäger P., Vedel V.

CAE203, 1 female, China, Baka, elev. 690 m, N21.713675S 100.783023E, 6.i.2011, Gregorič M., Kuntner M.

CAE204, 1 juvenile female, China, Baka, elev. 690 m, N21.713 675S 100.783023E, 6.i.2011, Gregorič M., Kuntner M.

Caerostris tinamaze

CAE341F, 1 female, RS. Africa, Entabeni NR, elev. 1375 m, 22.9960278S 30.264472E, iii.2006, Miller J., Wood H.

CAE341M, 1 male, RS. Africa, Entabeni NR, elev. 1375 m, 22.9960278S 30.264472E, iii.2006, Miller J., Wood H.

Caerostris wallacei

CAE334, 1 female, Madagascar, Kirindy forest, elev. 50 m, 20.0671S 44.65723E, 20-30.i.2006, Wood H., Miller J.

Appendix 2.—Taxonomic and genetic information about the terminals used in our analyses, with GenBank accession numbers (four 28S accession codes are missing because we lacked the nucleotide data).

Database code	Family	Genus	Species	COI acc. code	28S acc. code
	Nephilidae	<i>Nephila</i>	<i>fenestrata</i>	KC849084	KC849002
	Araneidae	<i>Zygiella</i>	<i>atrica</i>	KR526594	KR526501
	Araneidae	<i>Acusilas</i>	<i>coccineus</i>	KR526559	KR526466
	Araneidae	<i>Argiope</i>	<i>argentata</i>	FJ607554	FJ607519
CAE301	Araneidae	<i>Caerostris</i>	<i>almae</i>	KT267101	KT267150
CAE303	Araneidae	<i>Caerostris</i>	<i>almae</i>	KT267102	KT267151
CAE305	Araneidae	<i>Caerostris</i>	<i>almae</i>	KT267103	
CAE337	Araneidae	<i>Caerostris</i>	<i>almae</i>	KT267104	KT267152
CAE338	Araneidae	<i>Caerostris</i>	<i>almae</i>	KT267105	KT267153
CAE347	Araneidae	<i>Caerostris</i>	<i>almae</i>	KT267106	KT267154
CAE399	Araneidae	<i>Caerostris</i>	<i>almae</i>	KT267107	
CAE252	Araneidae	<i>Caerostris</i>	<i>bojani</i>	KT267093	KT267143
CAE253	Araneidae	<i>Caerostris</i>	<i>bojani</i>	KT267094	KT267144
CAE256	Araneidae	<i>Caerostris</i>	<i>bojani</i>	KT267095	KT267145
CAE257	Araneidae	<i>Caerostris</i>	<i>bojani</i>	KT267096	KT267146
CAE263	Araneidae	<i>Caerostris</i>	<i>bojani</i>	KT267097	KT267147
CAE304	Araneidae	<i>Caerostris</i>	<i>bojani</i>	KT267098	
CAE300	Araneidae	<i>Caerostris</i>	<i>cowani</i>	KT267064	KT267114
CAE340	Araneidae	<i>Caerostris</i>	<i>cowani</i>	KT267065	KT267115
CAE233	Araneidae	<i>Caerostris</i>	<i>darwini</i>	KT267066	KT267116
CAE236	Araneidae	<i>Caerostris</i>	<i>darwini</i>	KT267067	KT267117
CAE270F	Araneidae	<i>Caerostris</i>	<i>darwini</i>	KT267068	KT267118
CAE270M	Araneidae	<i>Caerostris</i>	<i>darwini</i>	KT267069	KT267119
CAE289	Araneidae	<i>Caerostris</i>	<i>darwini</i>	KT267070	KT267120
CAE294	Araneidae	<i>Caerostris</i>	<i>darwini</i>	KT267071	KT267121
CAE298	Araneidae	<i>Caerostris</i>	<i>darwini</i>	KT267072	KT267122
CAE218	Araneidae	<i>Caerostris</i>	<i>extrusa</i>	KT267073	KT267123
CAE220	Araneidae	<i>Caerostris</i>	<i>extrusa</i>	KT267074	KT267124
CAE221	Araneidae	<i>Caerostris</i>	<i>extrusa</i>	KT267075	KT267125
CAE227	Araneidae	<i>Caerostris</i>	<i>extrusa</i>	KT267076	KT267126
CAE279	Araneidae	<i>Caerostris</i>	<i>extrusa</i>	KT267077	KT267127
CAE281	Araneidae	<i>Caerostris</i>	<i>extrusa</i>	KT267078	KT267128
CAE331	Araneidae	<i>Caerostris</i>	<i>extrusa</i>	KT267079	KT267129
ARA765	Araneidae	<i>Caerostris</i>	<i>linnaeus</i>	KT267092	KT267142
CAE332	Araneidae	<i>Caerostris</i>	<i>mitralis</i>	KT267080	KT267130
CAE333	Araneidae	<i>Caerostris</i>	<i>mitralis</i>	KT267081	KT267131
CAE345F	Araneidae	<i>Caerostris</i>	<i>mitralis</i>	KT267083	KT267133
CAE345M	Araneidae	<i>Caerostris</i>	<i>mitralis</i>	KT267082	KT267132
CAE212	Araneidae	<i>Caerostris</i>	<i>pero</i>	KT267099	KT267148
CAE213	Araneidae	<i>Caerostris</i>	<i>pero</i>	KT267100	KT267149
CAE187	Araneidae	<i>Caerostris</i>	<i>sexcuspidata</i>	KT267084	KT267134
CAE205	Araneidae	<i>Caerostris</i>	<i>sexcuspidata</i>	KT267085	KT267135
CAE206	Araneidae	<i>Caerostris</i>	<i>sexcuspidata</i>	KT267086	KT267136
CAE207	Araneidae	<i>Caerostris</i>	<i>sexcuspidata</i>	KT267087	KT267137
CAE208	Araneidae	<i>Caerostris</i>	<i>sexcuspidata</i>	KT267088	KT267138
CAE339	Araneidae	<i>Caerostris</i>	<i>sexcuspidata</i>	KT267089	KT267139
CAE344F	Araneidae	<i>Caerostris</i>	<i>sexcuspidata</i>	KT267091	KT267141
CAE344M	Araneidae	<i>Caerostris</i>	<i>sexcuspidata</i>	KT267090	KT267140
CAE004	Araneidae	<i>Caerostris</i>	<i>sumatrana</i>	KT267113	
CAE203	Araneidae	<i>Caerostris</i>	<i>sumatrana</i>	KT267111	KT267158
CAE204	Araneidae	<i>Caerostris</i>	<i>sumatrana</i>	KT267112	KT267158
CAE341F	Araneidae	<i>Caerostris</i>	<i>tinamaze</i>	KT267109	KT267156
CAE341M	Araneidae	<i>Caerostris</i>	<i>tinamaze</i>	KT267110	KT267157
CAE334	Araneidae	<i>Caerostris</i>	<i>wallacei</i>	KT267108	KT267155