UNIVERZA V LJUBLJANI

BIOTEHNIŠKA FAKULTETA

Matjaž GREGORIČ

FILOGENETSKA SISTEMATIKA IN EVOLUCIJA MREŽ PRI PAJKIH MREŽARJIH SKUPIN *Zygiella* SENSU LATO IN *Caerostris*

DOKTORSKA DISERTACIJA

Ljubljana, 2013

UNIVERZA V LJUBLJANI

BIOTEHNIŠKA FAKULTETA

Matjaž GREGORIČ

FILOGENETSKA SISTEMATIKA IN EVOLUCIJA MREŽ PRI PAJKIH MREŽARJIH SKUPIN *Zygiella* SENSU LATO IN *Caerostris*

DOKTORSKA DISERTACIJA

PHYLOGENETIC SYSTEMATICS AND WEB EVOLUTION IN THE ORB WEAVING *Zygiella* SENSU LATO AND *Caerostris*

DOCTORAL DISSERTATION

Ljubljana, 2013

Doktorska disertacija je bila opravljena na Biološkem inštitutu Jovana Hadžija, ZRC SAZU, Slovenija. Dodatno laboratorijsko delo je bilo opravljeno na Department of Biology, University of Akron, Ohio, ZDA in na Department of Biology, University of Puerto Rico, San Juan, Portoriko, ZDA. Terensko delo je bilo opravljeno v Sloveniji, Indoneziji, Singapurju, Maleziji, Portoriku, na Kitajskem in na Madagaskarju.

Komisija za doktorski študij Univerze v Ljubljani je za mentorja doktorske disertacije imenovala viš. znan. sod. doc. dr. Matjaža Kuntnerja.

Komisija za oceno in zagovor:

Predsednik: prof. dr. Rok KOSTANJŠEK
Univerza v Ljubljani, Biotehniška fakulteta, Odelek za biologijo

Član: doc. dr. Cene FIŠER
Univerza v Ljubljani, Biotehniška fakulteta, Odelek za biologijo

Član in mentor: višji znan. sod. doc. dr. Matjaž KUNTNER
Biološki Inštitut Jovana Hadžija ZRC SAZU

Datum zagovora: 27. 9. 2013

Doktorsko delo je rezultat lastnega raziskovalnega dela. Podpisani se strinjam z objavo svoje naloge v polnem tekstu na spletni strani Digitalne knjižnice biotehniške fakultete. Izjavljam, da je naloga, ki sem jo oddal v elektronski obliki, identična tiskani verziji.

Matjaž Gregorič

KLJUČNA DOKUMENTACIJSKA INFORMACIJA (KDI)

ŠD	Dd
DK	UDK 565.4:575.85(043.3)
KG	Zygiellidae/*Caerostris*/evolucija mrež/filogenetska sistematika/vedenjska plastičnost
AV	GREGORIČ, Matjaž, univ. dipl. biol.
SA	KUNTNER, Matjaž (mentor)
KZ	SI-1000 Ljubljana, Večna pot 111
ZA	Univerza v Ljubljani, Biotehnična fakulteta, Oddelek za biologijo
LI	2013
IN	FILOGENETSKA SISTEMATIKA IN EVOLUCIJA MREŽ PRI PAJKIH MREŽARJIH SKUPIN *Zygiella* SENSU LATO IN *Caerostris*
TD	Doktorska disertacija
OP	XIII, 164 str., 7 pregl., 27 sl., 3 pril., 164 vir.
IJ	sl
JI	sl / en

AI Pajki mrežarji iz naddružine Araneoidea so pomembni in pogosto celo modelni organizmi v mnogih evolucijskih študijah, npr. v raziskovanju adaptivne evolucije, evolucijske ekologije in evolucije vedenja v najširšem smislu. V doktorski disertaciji smo tako raziskovali ali različne lastnosti pajčjih mrež, ki vplivajo na vrstno pestrost pajkov, med seboj koevoluirajo, in ali ontogenetske spremembe v vedenju resnično sledijo biogenetskemu pravilu in kako individualni pajki glede na svojo velikost spreminjajo energijski vložek v mreže. Večino študij smo izvajali na rodovih *Zygiella* in *Caerostris*, oba iz družine križevcev (Araneidae), ki sta filogenetsko problematična in sta zanimiva tudi po biologiji mrež. Ta rodova smo v disertaciji filogenetsko umestili in raziskali evolucijo njunih mrež. Pokazali smo, da ločene filogenetske linije obeh rodov kažejo značilne vedenjske posebnosti. Rezultati raziskav v doktorski disertaciji nakazujejo, da prvič, arhitektura mrež in mehanske lastnosti dejansko koevoluirajo z ekološkimi lastnostmi mrež in vedenjem ob njihovi gradnji. Drugič, pajčje mreže med ontogenetskim razvojem pajkov ne sledijo splošnemu biogenetskemu pravilu, ampak se spreminjajo tako, da osebku omogočajo čim učikovitejše bivanje v svojem mikrookolju, v našem primeru učinkovito plenjenje. Tretjič, energijski vložek v plenjenje je verjetno pod močnim selekcijskim pritiskom, pajki pa ga spreminjajo s prirejanjem arhitekture mrež in spreminjanjem svilenih niti in lepila, zato je za natančno oceno materialnega vložka v plenjenje potrebna kvantifikacija vsega naštetega. Četrtič, skupino Zygiellidae lahko obravnavamo kot veljaven taksonomski pojem in je verjetno sestrska skupina ostalim predstavnikom družine Araneidae. Petič, moji rezultati rod *Caerostris* filogenetsko uvrščajo med evolucijsko izvorne predstavnike družine Araneidae, rod je verjetno precej raznovrstnejši, opisali pa smo tudi pet novih vrst.

KEYWORDS DOCUMENTATION (KWD)

DN Dd
DC UDK 565.4:575.85(043.3)
CX Zygiellidae/*Caerostris*/web evolution/phylogenetic systematics/behavioral plasticity
AU GREGORIČ, Matjaž, univ. dipl. biol.
AA KUNTNER, Matjaž (mentor)
PP SI-1000 Ljubljana, Večna pot 111
PB Univerza v Ljubljani, Biotehnična fakulteta, Oddelek za biologijo
PY 2013
TI PHYLOGENETIC SYSTEMATICS AND WEB EVOLUTION IN THE ORB WEAVING *Zygiella* SENSU LATO AND *Caerostris*
DT Doctoral dissertation
NO XIII, 164 p., 7 tabl., 27 fig., 3 ann., 164 ref.
LA sl
AL sl / en

AB The orb weaving araneoid spiders are considered important or even model organisms in several topics of evolutionary research, like adaptive evolution, evolutionary ecology and evolution of behavior in the broadest sense. In my doctoral thesis, I investigated whether spider webs coevolve with other traits that influence spider diversification, whether ontogenetic changes in spider webs really follow the biogenetic law, and how individual spiders alter their foraging investment in relation to body size. I conducted most studies on the genera *Zygiella* s.l. and *Caerostris* from the family Araneidae. Both genera are taxonomically controversial and exhibit interesting web biology. Thus, I phylogenetically placed both genera and investigated the evolution of their webs. The results in the doctoral dissertation show that first, the results continue to build evidence for the coevolution of behavioral (web building), ecological (web microhabitat) and biomaterial (silk biomechanics) traits. Second, biogenetic law cannot explain spider orb web allometry, but our results instead support optimization of foraging area in response to spider size. Third, in relation to body size, spiders alter their webs through i) web architecture, ii) investment in silk, and iii) investment in glue, and that quantifying all three parameters is necessary for meaningful estimates of total foraging investment. Fourth, Zygiellidae can be regarded a valid taxonomic concept and is probably sister to other araneids. Fifth, my results recover *Caerostris* as basal araneid, the genus probably contains several more species, and we also describe five new species.

KAZALO VSEBINE

KLJUČNA DOKUMENTACIJSKA INFORMACIJA III

KEY WORDS DOCUMENTATION IV

KAZALO VSEBINE V

KAZALO ZNANSTVENIH DEL VIII

KAZALO PREGLEDNIC IX

KAZALO SLIK X

KAZALO PRILOG XII

OKRAJŠAVE IN SIMBOLI XIII

1 PREDSTAVITEV PROBLEMATIKE IN HIPOTEZE 1

1.1 PAJKI KOT POMEMBNI RAZISKOVALNI OBJEKTI 2

1.2 MREŽE PAJKOV KOT POMEMBNI RAZISKOVALNI OBJEKTI 2

1.2.1 Vloga v raziskavah biomaterialov 3

1.3 ZGRADBA KOLESASTE MREŽE 4

1.4 EVOLUCIJA KOLESASTE MREŽE 7

1.5 DELOVANJE KOLESASTE MREŽE 10

1.5.1 Individualna variabilnost v mrežah 11

1.6 CILJI DOKTORSKE NALOGE 12

1.6.1 Koevolucija vedenja, ekologije in biomateriala 12

1.6.2 Ontogenetske spremembe v arhitekturi mrež 13

1.6.3 Energijski vložek v plenjenje in vedenjska plastičnost.............................. 13

1.6.4 Filogenetska sistematika rodov *Zygiella* s.l. in *Caerostris*........................ 14

2 ZNANSTVENA DELA...16

2.1 OBJAVLJENA ZNANSTVENA DELA.. 16

2.1.1 Kako je pajek prečkal reko? Vedenjske adaptacije čez reke razpetih mrež pri vrsti *Caerostris darwini* (Araneae: Araneidae)... 16

2.1.2 Darwinov drevesni pajek: Ogromen plen v ogromnih mrežah (*Caerostris darwini*, Araneae: Araneidae)?... 33

2.1.3 Optimalno prehranjevanje, ne biogenetsko pravilo, napove alometrijo pajčjih kolesastih mrež.. 56

2.2 OSTALO POVEZOVALNO ZNANSTVENO DELO 68

2.2.1 Ali večje živali prilagodijo vložek v prehranjevanje? Vzorci materialnega vložka v pajčje kolesaste mreže.. 68

2.2.2 Filogenetski položaj in evolucija mrež pri pajkih iz rodu *Zygiella* s.l. in drevesnih pajkih iz rodu *Caerostris* (Araneae: Araneidae) 95

3 RAZPRAVA IN SKLEPI...136

3.1 FILOGENETSKA SISTEMATIKA IN EVOLUCIJA MREŽ RODOV *Zygiella* S.L. IN *Caerostris*.. 136

3.1.1 Filogenetski položaj in obseg skupine Zygiellidae in rodu *Caerostris*..... 136

3.1.2 Evolucija mrež in lastnosti mrež kot taksonomski znaki......................... 139

3.2 KOEVOLUCIJA VEDENJA, EKOLOGIJE IN BIOMATERIALA 140

3.3 ONTOGENETSKE SPREMEMBE V ARHITEKTURI MREŽ........................ 142

3.4 ENERGIJSKI VLOŽEK V PLENJENJE IN VEDENJSKA PLASTIČNOST ... 143

4 POVZETEK 145

4.1 POVZETEK 145

4.2 SUMMARY 148

5 VIRI 151

KAZALO ZNANSTVENIH DEL

Gregorič M., Agnarsson I., Blackledge T.A., Kuntner M. 2011. How did the spider cross the river? Behavioral adaptations for river-bridging webs in *Caerostris darwini* (Araneae: Araneidae). PLoS ONE, 6(10): e26847 16

Gregorič M., Agnarsson I., Blackledge T.A., Kuntner, M. 2011. Darwin's bark spider: Giant prey in giant orb webs (*Caerostris darwini*, Araneae: Araneidae)? Journal of Arachnology, 39: 287-295 33

Gregorič M., Kiesbüy H.C., Quiñones Lebrón S.G., Rozman A., Agnarsson I., Kuntner M. 2013. Optimal foraging, not biogenetic law, predicts spider orb web allometry. Naturwissenschaften, 100: 263-268........56

KAZALO PREGLEDNIC

Tabela 1: Table 1: Comparison of web size among orb web spiders. 49

Tabela 2: Table 2: Web data for *Caerostris darwini* females in Madagascar.. 50

Tabela 3: Table 1: Paerson's correlations of spider length/mass and two asymmetry indices in three inclination groups of *L. venusta* webs. 65

Tabela 4: Table 1: The volumes of invested silk and glue in *Zygiella* s.l. species. 87

Tabela 5: Table 2: Paerson's correlations of web variables and spider carapace width for the investigated *Zygiella* s.l. species.. 88

Tabela 6: Table 3: Pearson's correlations of web variables and spider body condition width for the investigated *Zygiella* s.l. species.. 89

Tabela 7: Table 4: Average invested volumes of MA silk, Flag silk and glue for species investigated by Sensenig et al. (2010a). 90

KAZALO SLIK

Slika 1: Deleži vrstne pestrosti redov pajkovcev (Arachnida) in lepo vidna dominanca pajkov (Araneae) in obeh glavnih skupin pršic – Parasitiformes in Acariformes povzeto po Harvey (2002). 1

Slika 2: Zgradba kolesaste mreže povzeto po Blackledge s sod. (2011). 5

Slika 3: Primerjava lepljive in kribelatne lovilne mreže prirejeno po Blackledge s sod. (2009)........6

Slika 4: Nedavna filogenija na podlagi molekularnih in morfoloških znakov in optimizacija arhitekture mreže na izbrano topologijo.. 9

Slika 5: Figure 1. *Caerostris darwini* webs. 30

Slika 6: Figure 2. The typical (A) and the alternative (B) bridging behaviors in *C. darwini*........31

Slika 7: Figure 3. Web building in *C. darwini* (A-C) versus the “classical” araneid web (D), shown without spirals. 31

Slika 8: Figure 1: *Caerostris darwini* in Andasibe-Mantadia NP........52

Slika 9: Figure 2: *Caerostris darwini* habitat and prey........53

Slika 10: Figure 3: Web of female *Caerostris darwini* illustrating investigated parameters. 54

Slika 11: Figure 4: Web size in *Caerostris darwini* and three congeneric species. 54

Slika 12: Fig. 1: Web of *L. venusta* illustrating investigated parameters........66

Slika 13: Fig. 2: Correlations between spider length and hub displacement, and spider mass and hub displacement, in three inclination groups of *L. venusta* webs........67

Slika 14: Figure 1: The architecture of a typical *Zygiella* s.l. web with measured parameters. 91

Slika 15: Figure 2: Orb webs and sticky spiral threads (inset, right) representing investigated species........92

Slika 16: Figure 3: Absolute web parameters among *Zygiella* s.l. species 93

Slika 17: Figure 4: Correlations of major ampullate silk volume, flagelliform volume and glue with spider carapace width. 94

Slika 18: Figure 1: Web pictures of Zygiellidae, several other sector webs, and of *Caerostris*...120

Slika 19: Figure 2: *Caerostris* diversity from Africa and Madagascar. 121

Slika 20: Figure 3: The preferred Orbicularian phylogeny. 122

Slika 21: Figure 4: The *Caerostris* phylogeny from Bayesian inference of COI, 28S, H3 and ITS2, and under the “full codon partition” scheme, with COI gene fragment differences for species clusters. 123

Slika 22: Figure 5: The evolution of sector webs and web size plotted on the preferred topology (Bayesian inference under the “full codon partition” scheme)........................124

Slika 23: Figure 6: Web size evolution among the investigated *Caerostris* species, plotted on the trimmed preferred topology (Bayesian inference under the “full codon partition” scheme). 125

Slika 24: Fig. 7: *Caerostris almae*, female somatic and genital morphology, all from Andasibe-Mantadia, Madagascar..128

Slika 25: Fig. 8: *Caerostris bojani*, female somatic and genital morphology, all from Andasibe-Mantadia, Madagascar..130

Slika 26: Fig. 9: *Caerostris pero*, female somatic and genital morphology, Andasibe-Mantadia NP, Madagascar. 132

Slika 27: Fig. 10: A: *C. pulpfictioni* somatic and genital morphology; B: *C. tinamaze* somatic and genital morphology. 135

KAZALO PRILOG

Priloga 1: Dovoljenje založnika za objavo članka How did the spider cross the river? Behavioral adaptations for river-bridging webs in *Caerostris darwini* (Araneae: Araneidae) v tiskani in elektronski verziji doktorske disertacije.

Priloga 2: Dovoljenje založnika za objavo članka Darwin's bark spider: Giant prey in giant orb webs (*Caerostris darwini*, Araneae: Araneidae)? v tiskani in elektronski verziji doktorske disertacije.

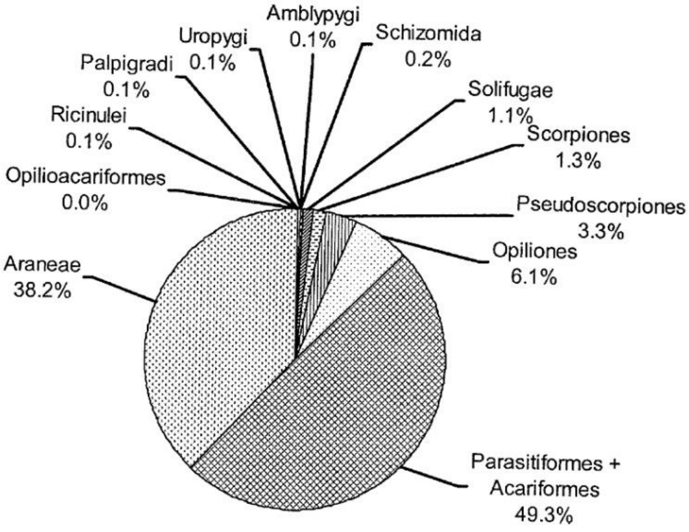
Priloga 3: Dovoljenje založnika za objavo članka Optimal foraging, not biogenetic law, predicts spider orb web allometry v tiskani in elektronski verziji doktorske disertacije.

OKRAJŠAVE IN SIMBOLI

s.l.	sensu lato, v širšem smislu
ALE	anterior lateral eyes, sprednje stranske oči
AME	anterior median eyes, sprednje sredinske oči
CD	copulatory duct, kopulacijski vod
FD	fertilization duct, oploditveni vod
PME	posterior median eyes, zadnje sredinske oči
S	spermatheca, spermateka

1 PREDSTAVITEV PROBLEMATIKE IN HIPOTEZE

Red pajkov (Araneae, Arachnida) je ena pestrejših skupin živali. Po globalni vrstni pestrosti se uvrščajo na sedmo mesto, med pajkovci je raznovrstnejši rod pršic (Acari; Slika 1), ostalih pet raznovrstnejših redov pa predstavljajo žuželke hrošči (Coleoptera), kožekrilci (Hymenoptera), metulji (Lepidoptera), dvokrilci (Diptera) in polkrilci (Hemiptera) (Coddington in Colwell, 2000; Harvey, 2002). Pajki so razširjeni po celem svetu in so zavzeli vsa terestrična življenjska okolja (Foelix, 2011). So izredno številčni. Določene raziskave poročajo o gostoti, ki v skrajnosti dosežejo 5 milijonov osebkov na hektar nekega angleškega travnika in 29.000 osebkih v kubičnem metru nekega angleškega gozda (Coddington in Colwell, 2000). To so sicer ekstremi, vendar lahko trdimo, da se na kvadratnem metru ne-puščavskega habitata nahaja vsaj eden pa vse do 800 osebkov. Vrstna pestrost pajkov na enem hektarju variira od 100 vrst v zmernem pasu pa do 600 in več v tropih (Coddington in Colwell, 2000).



Slika 1: Deleži vrstne pestrosti redov pajkovcev (Arachnida) in lepo vidna dominanca pajkov (Araneae) in obeh glavnih skupin pršic – Parasitiformes in Acariformes povzeto po Harvey (2002).

Glede na njihovo izjemno vrstna pestrost, so pajki edinstveni po obligatnem plenilstvu kot prehranjevalni strategiji. Za razliko od pajkov je vrstna pestrost žuželk in pršic verjetno povezana z raznolikostjo v prehranjevalnih strategijah, predvsem s fitofagijo in parazitizmom (Mitter s sod., 1988; Coddington in Levi, 1991 Blackledge s sod., 2009). Osrednjo vlogo pri povečevanju raznovrstnoti pajkov je v evoluciji predstavljala sposobnost proizvodnje svilene niti, ki se po evolucijski vlogi lahko primerja celo z letenjem pri žuželkah ali toplokrvnostjo pri sesalcih (Foelix, 2011). Pajki niso edini organizmi, ki proizvajajo lepljivo nit, vendar so edini, ki jo uporabljajo v vseh stopnjah svojega življenja in v različne namene (Foelix, 2011). Pajki svileno nit uporabljajo za gradnjo zatočišč, kokonov in lovilnih mrež, svilena nit pa omogoča tudi komunikacijo med osebki in daje pajkom izjemne možnosti razširjanja (Blackledge s sod., 2011; Herberstein in Wignall, 2011).

1.1 PAJKI KOT POMEMBNI RAZISKOVALNI OBJEKTI

Pajki so zaradi svoje vrstne pestrosti pomembni in celo modelni organizmi ne samo v araneologiji, ampak širše. Nekatere skupine pajkov so modelni organizmi za študije funkcionalne morfologije in fiziologije ter strupov (npr. Higgins, 2000; Dechant s sod., 2006; Fratzl in Barth, 2009; Kuhn-Nentwig s sod., 2012). Zaradi obligatnega plenilstva so pomembni objekti v študijah odnosov med plenom in plenilcem (npr. Craig s sod., 1996; Elgar s sod., 1996; Pekar s sod., 2012). Pri pajkih se pojavljajo skupine z različnimi stopnjami socialnosti, ki tako v primerjavi z eusocialnimi žuželkami predstavljajo dober alternativen objekt za raziskovanje evolucije socialnosti (npr. Agnarsson s sod., 2006; Lubin in Bilde, 2007; van Veelen s sod., 2010). Podobno se pri pajkih skozi evolucijo večkrat pojavlja ekstremni spolni velikostni dimorfizem, kar pajke postavlja med modelne organizme v študijah vzrokov in posledic evolucije tega pojava (npr. Coddington s sod., 1997; Blanckenhorn s sod., 2007; Kuntner in Coddington, 2009). Zaradi svoje raznolikosti in zanimive spolne biologije, so skupaj z določenimi skupinami žuželk tudi pomembni objekti raziskav spolne selekcije (npr. Andrade, 1996; Schneider in Lubin, 1996; Arnqvist in Rowe, 2002; Lubin in Bilde, 2007). Nekatere skupine pajkov imajo izjemne sposobnosti razširjanja, spet druge so v tem segmentu precej omejene, zaradi česar so pajki pogosto raziskovani tudi v mnogih biogeografskih študijah (npr. Roderick in Gillespie, 1998; Gillespie, 2002; Garb in Gillespie, 2009; Bidegaray-Batista in Arnedo, 2011; Agnarsson in Kuntner, 2012).

1.2 MREŽE PAJKOV KOT POMEMBNI RAZISKOVALNI OBJEKTI

Večina pajčjih skupin, ki se pojavljajo kot objekti zgoraj omenjenih študij pripada naddružini Orbiculariae, ki je z več kot 12.000 opisanimi vrstami ena največjih skupin pajkov (Platnick, 2013). Orbiculariae, t.i. pajke mrežarje, opredeljuje gradnja kolesastih mrež (Griswold s sod., 1998; Blackledge s sod., 2009). Prav slednja je vzrok za to, da so pajki mrežarji tako primeren raziskovalni objekt, saj mreže predstavljajo fizičen odraz vedenja, ki ga je mogoče neposredno kvantificirati (Eberhard, 1990b; Benjamin in Zschokke, 2004). Zato so pajki mrežarji še posebej primerni objekti v študijah adaptivne evolucije, evolucijske ekologije, evolucijskih inovacij, fenotipske plastičnosti itd. (Coddington, 1994; Bond in Opell, 1998; Blackledge s sod., 2011; Herberstein in Wignall, 2011). Pajčje mreže so merljiv energijski vložek pajkov v plenjenje. Ker jih lahko neposredno izmerimo predstavljajo edinstven objekt za raziskave odnosov med plenom in plenilcem (npr. Eberhard, 1986; Craig s sod., 1996; Opell in Schwend, 2007), hkrati pa so pajče mreže lahko tudi habitat drugim organizmom in so tako primerne za študij odnosov med producenti in porabniki (npr. Higgins in Buskirk, 1998; Agnarsson, 2003, 2011). Ker so mreže fizičen odraz pajčjega vedenja, so primerne tudi za študij vedenjske plastičnosti (npr. Watanabe, 2000; Blamires, 2010), rabe okolja (npr. Blackledge in Gillespie, 2004; Gillespie, 2004), evolucije vedenja (npr. Vollrath in Selden, 2007; Kuntner in Agnarsson, 2009; Blackledge s sod., 2012a), in mnogih drugih vprašanj iz področja vedenja živali (npr. Eberhard, 1982, 2007; Blackledge in Zevenbergen, 2007; Gregorič s sod., 2010; Kuntner s sod., 2010a).

1.2.1 Vloga v raziskavah biomaterialov

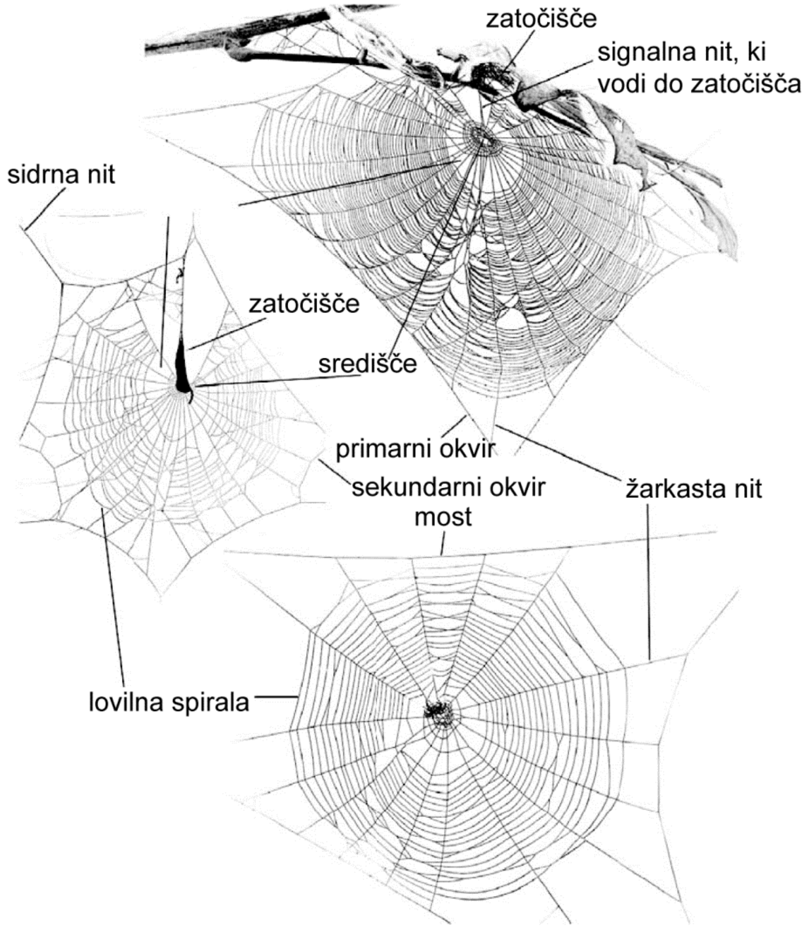
Večina pajkov proizvaja različne tipe svilene niti, ki jih uporabljajo v različne namene (Blackledge s sod., 2011; Foelix, 2011). T.i. vlečna nit (angl. »dragline«) se je razvila zgodaj v evoluciji pajkov in je bila prva diskretna svilena nit, proizvedena namesto ploskev svilenih vlaken. Izvor vlečne niti se smatra kot ključno pridobitev za evolucijski uspeh pajkov (Bond in Opell, 1998). Večina od več kot 43.000 opisanih vrst pajkov uporablja vlečno nit za različne namene, kot so npr. preproste varovalne niti (angl. »lifeline«) ali okvirji lovilnih mrež, poleg katerih pa pajki mrežarji gradijo tudi druge tipe niti (Blackledge s sod., 2011; Foelix, 2011). Ob očitni evolucijski pomembnosti pajčjega prediva, ima le-ta tudi splošno znane izjemne mehanske lastnosti (Foelix, 2011). Zahvaljujoč nenavadni kombinaciji trdnosti in elastičnosti imajo svilene niti, predvsem vlečna nit, izjemno visoko natezno trdnost (angl. »toughness«) (Blackledge s sod., 2011). Natezna trdnost je največja natezna obremenitev, ki jo material še prenese (Kraut, 2001). Trdnost in elastičnost sta v sintetičnih polimerih značilno v negativni korelaciji, tako da vlečne niti pajkov v natezni trdnosti prekašajo tudi sintetične materiale z visoko absorpcijo energije. Dobro poznan sintetični material Kevlar™ tako po natezni trdnosti prekašajo za okoli 300% (Agnarsson s sod., 2010). Pajčja svilena nit po natezni trdnosti prekaša vse biološke materiale, tudi kite, kosti in celo celulozo, po razmerju med maso in trdnostjo pa za okoli petkrat prekaša jeklo (Agnarsson s sod., 2009a).

Pajki mrežarji po natezni trdnosti svojega prediva prekašajo ostale skupine pajkov, zaradi česar ni presenetljivo, da so prav ti pajki postali modelni organizmi v raziskavah funkcije in evolucije pajčjega prediva (Sensenig s sod., 2010; Harmer s sod., 2011). Ti raziskovalci poskušajo bolje razumeti povezavo med molekulsko zgradbo in mehanskimi lastnostmi svilene niti in odgovarjajo na številna vprašanja, povezana tako z vrstno pestrostjo pajkov kot tudi uporabnostjo prediv za človeka (npr. Hayashi in Lewis 2000; Agnarsson s sod., 2009b, 2010; Swanson s sod., 2009; Vollrath in Porter, 2009; Sensenig s sod., 2010; Sahni s sod., 2011; Blackledge s sod., 2012a, b).

1.3 ZGRADBA KOLESASTE MREŽE

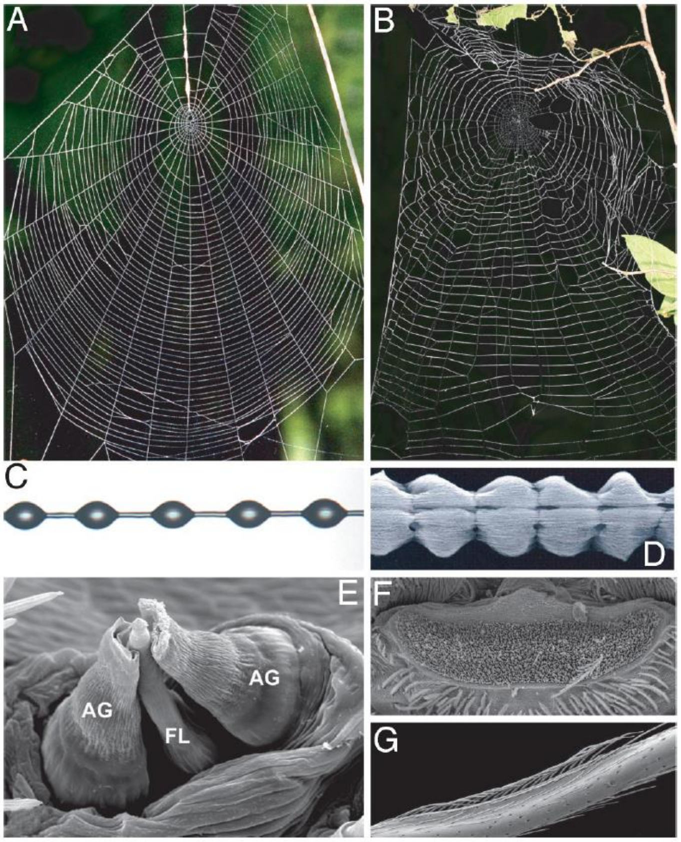
Gradnja kolesaste mreže se pojavlja znotraj velike in raznovrstne skupine pajkov mrežarjev, med katerimi klasično kolesasto mrežo (slika 2, 3) gradijo predstavniki sedmih pajčjih skupin (Griswold s sod., 1998; Blackledge s sod., 2011). Kolesaste mreže so tipično razpete v zračnem stolpcu in imajo dvodimenzionalno lovilno površino, pajki pa jih zgradijo s značilnimi vedenjskimi zaporedji (Foelix, 2011). Kolesaste mreže so zelo raznolike, v premeru segajo od le nekaj centimetrov pa do več kot metra in pol, in se po modifikacijah v zgradbi razlikujejo med skupinami pajkov (Harmer s sod., 2011; Herberstein in Tso, 2011).

Glavni sestavni deli kolesaste mreže so naslednji (Slika 2, 3). Primarni okvir (angl. »primary frame«) s sidrnimi nitmi (angl. »anchor thread«) mrežo pritrja na podlago. Vodoravnemu delu okvirja rečemo tudi most (angl. »bridge (thread/line)«). Lovilni kolesasti del mreže sestavljajo žarkaste niti (angl. »radial thread«), ki potekajo od središča mreže (angl. »hub«) do primarnega ali sekundarnega okvirja, in pravokotno na njih postavljena lovilna spirala (angl. »capture spiral«) (Zschokke, 2002; Blackledge s sod., 2011). Med gradnjo mreže pajki zgradijo tudi pomožno oz. začasno spiralo (angl. »auxiliary/temporary spiral«), ki jo večinoma podrejo med gradjo lovilne spirale (Eberhard, 1982). Nekatere skupine gradijo dodatne strukture, kot so trodimenzionalni prepleti prediva ob lovilni ploskvi, ali »dekoracije« v obliki stabilimentov ali odpadlega organskega materiala, praviloma rastlinskega izvora (Herberstein in Tso, 2011), mreže pa so lahko modificirane še na mnoge druge načine.



Slika 2: Zgradba kolesaste mreže povzeto po Blackledge s sod. (2011).

Kolesaste mreže po svoji značilni zgradbi delimo v dve osnovni skupini – poznamo t.i. kribelatne in lepljive kolesaste mreže – ki sta značilni vsaka za svojo naddružino pajkov mrežarjev (Slika 3). Kribelatna naddružina Deinopoidea obsega 2 družini (Deinopidae, Uloboridae) in nekaj čez 20 rodov, ekribelatna naddružina Araneoidea pa obsega okoli 15-20 družin in več kot 1000 rodov (Agnarsson s sod., 2013; Platnick, 2013). Predstavniki obeh naddružin gradijo mreže podobne v osnovni zgradbi, glavne razlike pa so v zgradbi lovilnih spiralnih niti. Predstavniki Deinopoidea izdelujejo spiralne niti iz kribelatnega prediva tako, da najprej spletejo jedrno vlakno (angl. »core axial fibre«) in šele nato na ta jedrna vlakna fizično »razčešejo« stotine tankih kribelatnih vlaken. Takšni skupki vlaken nato tvorijo nit, katere adhezivnost temelji na van der Waalsovih silah (Opell, 1997a). Pravkar opisana gradnja kribelatne spirale je energetsko in časovno potraten proces (Zschokke in Vollrath, 1995a; Opell, 1997a). Za razliko od kribelatnih pajkov mrežarjev, predstavniki naddružine Araneoidea hkrati s produkcijo jedrne niti le-to prevlečejo s plastjo lepila, ki se samodejno oblikuje v drobne kapljice vzdolž jedrne niti (Opell s sod., 2008).



Slika 3: Primerjava lepljive in kribelatne lovilne mreže prirejeno po Blackledge s sod. (2009). A: Kolesasta mreža predstavnika Araneoidea. B: Kolesasta mreža predstavnika Deinopoidea. C: Nit lepljive spirale araneoidne kolesaste mreže. D: Nit lovilne spirale deinopoidne kolesaste mreže iz kribelatnega prediva. E: Predstavniki Araneoidea s pomočjo t.i. triade izvodil predilnih žlez spletejo jedrno nit in istočasno nanjo nanesejo lepilo. Omenjeno triado sestavljajo izvodilo flageliformne žleze (FL), ki proizvede jedrno nit lepljive spirale, in izvodila agregatnih predilnih žlez (AG), ki na jedrno nit naneseta lepilo. F in G: Kribelatni pajki stotine tankih svilenih vlaken iz t.i. kribeluma (F) razčešejo z glavniku podobnim kalamistrum-om (G).

Predvsem zaradi različnega tipa lovilne spirale je bil monofiletski izvor skupine Orbiculariae dolgo sporen in znatno testiran, sinapomorfije te skupine pa so v glavnem vključevale zgolj vedenjske znake, predvsem podrobnosti v gradnji mrež (Blackledge s sod., 2011; Foelix, 2011). Z napredkom v poznavanju filogenetskih metod in uporabnosti vedenjskih znakov v filogenetiki (Eberhard, 1982; Coddington, 1986b; Freudenstein, 2005; Kuntner s sod., 2008), je monofiletski status skupine postajal vse bolje podprt

(Coddington, 1986b; Scharff in Coddington, 1997; Griswold s sod., 1998), danes pa ga podpirajo še molekularne analize (Blackledge s sod., 2009; Agnarsson s sod., 2012; Dimitrov s sod., 2012). Naddružina Araneoidea je mnogo pestrejša od naddružine Deinopoidea in je ekološko dominantna (Blackledge s sod., 2011).

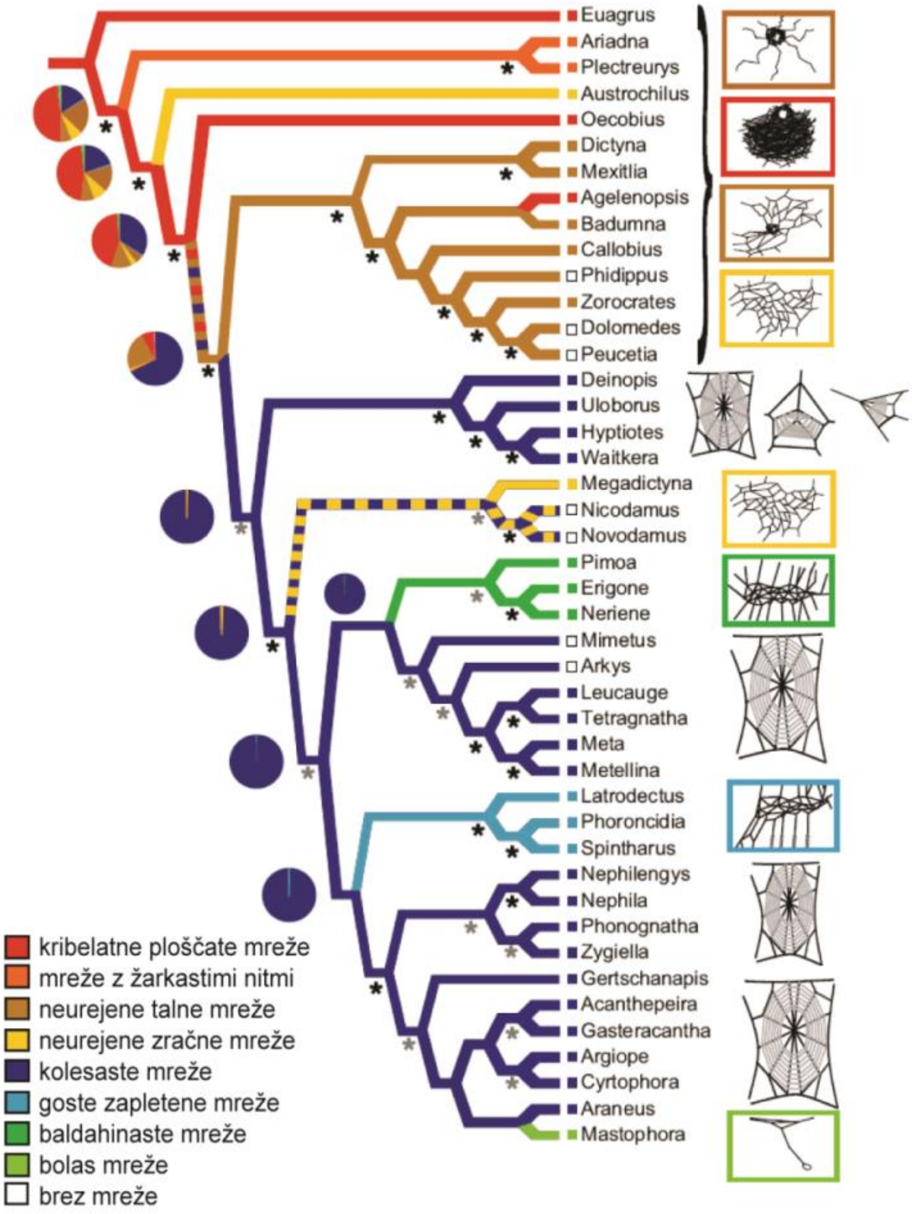
1.4 EVOLUCIJA KOLESASTE MREŽE

Evolucijska diferzifikacija pajkov je povezana z evolucijskimi inovacijami prediva in mrež (Harmer s sod., 2011). Predniki pajkov mrežarjev so verjetno gradili ploščate mreže pritrjene na podlago, kolesasta mreža, postavljena v zračni stolpec, pa je verjetno pomenila ključno evolucijsko pridobitev skupine Orbicularie. Ločenost od podlage je tako bil pogoj za evolucijo novih oblik mrež, hkrati pa so takšne mreže prestrezale plen, ki ga prejšnje niso, kar je pomenilo izkoriščanje novih ekoloških niš in s tem večanje vrstne pestrosti skupine (Blackledge s sod., 2009). Prehod na mreže, razpete v zraku, verjetno predstavlja tudi začetek oboroževalne tekme med letečimi žuželkami in pajki, ki kažejo vzorce skupne radiacije (Vollrath in Selden, 2007; Harmer s sod., 2011).

Zaradi osvajanja novih habitatov so se ob gradnji kolesastih mrež pajki srečevali tudi z vrsto novih selekcijskih pritiskov, ki so pogojevali nadaljno večanje vrstne pestrosti. Tako so kolesaste mreže podvržene okoljskim dejavnikom kot npr. vetru, odsotnosti čvrste podlage v primeru ko so te razpete na prožni podlagi, npr. travah, ter obremenitvam mrež z veliko kinetično energijo ob lovu letečega plena (Harmer s sod., 2011). Čeprav so že mehanske lastnosti prediva pajkov, ki ne gradijo kolesastih mrež, izjemne, je kot odgovor na zgoraj omenjene selekcijske pritiske pri pajkih mrežarjih prišlo do evolucije še znatno kvalitetnejšega prediva (Swanson s sod., 2006), prišlo pa je tudi do evolucije lepiljive spirale, ki je menjala kribelatno. Prav slednje predstavlja drugo ključno evolucijsko pridobitev pajkov mrežarjev in je verjetno predstavljajo podlago za naglo večanje vrstne pestrosti skupine Araneoidea (Bond in Opell, 1998; Blackledge s sod., 2009). Predstavniki Araneoidea tako izdelujejo lovilne niti, ki so bolj »lepljive« od kribelatnih lovilnih niti, izdelujejo pa jih znatno hitreje in bolj ekonomično v smislu porabe materiala (Opell, 1997b; 1999). Skupaj s še nekaj dodatnimi prednostmi so takšne mreže danes sposobne ujeti tudi največje leteče žuželke in celo manjše ptice in netopirje (Harmer s sod., 2011; Nyffeler in Knornschild, 2013)

Izvorna gradnja kolesaste mreže pa ne pomeni, da vsi predstavniki pajkov mrežarjev takšne mreže tudi resnično gradijo. V evoluciji pajkov mrežarjev je dvakrat prišlo do prehoda na trodimenzionalno zračno mrežo: tako poznamo goste zapletene mreže družine Theridiidae in baldahinaste mreže družine Linyphiidae (Slika 4; Blackledge s sod. (2009)). Vendar tudi med sedmimi družinami pajkov, ki danes gradijo klasične kolesaste mreže, najdemo mnoge modifikacije. Tako npr. poznamo t.i. »lestvičaste mreže«, ki so vertikalno podaljšane in predstavljajo adaptacije na omejen prostor, npr. debla dreves

(Harmer in Herberstein, 2009; Kuntner s sod., 2010b), lahko pa gre tudi za adaptacije na določen plen, npr. metulje (Eberhard, 1975). Lestvičaste mreže se pojavljajo v družinah Araneidae, Tetragnathidae in Nephilidae, še posebej značilne pa so za slednje (Kuntner s sod., 2010b). Predstavniki družine Synotaxidae gradijo ploščate, nekoncentrične, t.i. kvadrataste mreže, sestavljene iz navideznih majnih kvadratov (Eberhard, 1995). Rod *Wixia* iz družine Araneidae izdeluje mreže, ki jim manjka lovilna spirala in so sestavljene zgolj iz radialnih niti (Stowe, 1986; Levi, 1993). Reducirane so tudi t.i. mreže s sektorjem, ki jim v blažjih oblikah redukcije lahko manjka zgolj en sektor lovilne ploskve (npr. rod *Zygiella*, Araneidae) ali pa so reducirane na zgolj en trikoten sektor lovilnega dela (npr. rod *Hyptiotes*, Uloboridae) (Lubin, 1986; Gregorič s sod., 2010). Predstavniki družine Theridiosomatidae gradijo različno modificirane mreže, najbolj znane pa so tiste, ki vsebujejo vse elemente kolesaste mreže, vendar so iz planarne oblike modificirane v dežnikasto (Coddington, 1986a).



Slika 4: Nedavna filogenija na podlagi molekularnih in morfoloških znakov in optimizacija arhitekture mreže na izbrano topologijo. Črne zvezdice nakazujejo močno podporo razvejišča tako po metodi varčnosti (jackknife > 75%), kot po Bayesovi metodi (posterior probabilities > 90%). Sive zvedice nakazujejo močno podporo po zgolj eni od obeh metod, ali z jackknife vrednostjo 50-74%. Barve vej prikazujejo rekonstrukcijo mrež po metodi varčnosti, tortni diagrami prikazujejo relativne verjetnosti po metodi največjega verjetja povzeto po Blackledge s sod. (2009).

1.5 DELOVANJE KOLESASTE MREŽE

Pajki so pogosto opisani kot plenilci generalisti, ki pojedo vse, kar pade v njihove mreže (Foelix, 2011). Čeprav to do neke mere drži, so mnoge študije pokazale, da impresiven nabor različnih pajčjih mrež predstavlja adaptacije na zelo različen tip plena in mikrookolja (Nentwig, 1983, 1985; Eberhard, 1986, 1990b; Blackledge s sod., 2003). Kritično za delovanje pajčje mreže je tako koliko in kakšen plen bodo prestregle, kako ga bodo ustavile ter kako zadržale.

Kot za večino drugih živali je tudi za pajke kritična izbira prehranjevalnega okolja. Različne pajčje vrste in celo različni stadiji znotraj vrst tako v nekem habitatu za gradnjo mrež izbirajo različna mikrookolja (Wise, 1993). Simpatrične vrste kažejo jasne preference za podlago, na katero so mreže pritrjene, koliko so oddaljene od tal, kako odprt je habitat itd. (Brown, 1981; Blackledge s sod., 2003; Richardson in Hanks, 2009). Sama velikost mreže seveda neposredno vpliva na število žuželk, ki bo priletelo v lovilno ploskev, vendar ta odnos ni tako preprost, saj je vsaka mreža narejena iz omejene količine prediva (Eberhard, 1988). Gostota mreže namreč pomembno vpliva na velikost potencialnega plena, pomembno pa je tudi kolikšno kinetično energijo plena mreža še lahko zadrži (Blackledge in Eliason, 2007; Sensenig s sod., 2010). Potencialen plen kolesastih mrež so v glavnem leteče in skakajoče žuželke, za uspešen ulov pa morajo mreže absorbirati njihovo kinetično energijo, kar naj bi bil tudi eden glavnih selekcijskih faktorjev v evoluciji izrednih materialnih lastnosti pajčjega prediva (Denny, 1976). Nedavne raziskave so pokazale, da žarkaste niti močno dominirajo v absorpciji kinetične energije plena, in jo običajno absorbirajo skoraj v celoti. Glavna vloga lovilne spirale, ki je pribl. 1000-krat bolj elastična od žarkastih niti, je zadržanje ustavljenega plena (Sensenig s sod., 2012). Takšni rezultati nakazujejo, da na različne tipe niti delujejo različni selekcijski pritiski (Sensenig s sod., 2012).

Ključno vlogo v makroevoluciji arhitekture pajčjih mrež je verjetno imela prav povezava med prestrezanjem, ustavljanjem in zadržanjem plena (Blackledge in Eliason, 2007). Ker so mreže predvsem lovilne naprave, njihova arhitektura verjetno odseva adaptacije na plen, ki prispeva največ ulovljene biomase (Blackledge, 2011). Nedavne študije so celo pokazale, da je večina mrež adaptiranih na t.i. »redek velik plen«, torej na plen z veliko maso, ki pa se le redko ujame v mreže, vendar k ujeti biomasi prispeva bistveno več kot mnoge majhne ulovljene žuželke (Venner in Casas 2005; Blackledge 2011; vendar Eberhard (2013) hipotezo redkega velikega plena zavrača kot pretirano posploševanje). V evoluciji kolesaste mreže torej glavno vlogo igrajo tako spremembe v kvaliteti različnih svilenih niti, ki sestavljajo mrežo (t.j. v mehanskih lastnostih materiala), kakor tudi spremembe v vedenju, povezanim s tem kako pajki te niti uporabijo v mreži, torej z gradnjo in arhitekturo mrež (Craig, 1987; Lin s sod., 1995). Nedavne študije so tako celo pokazale, da materialne lastnosti pajčjega prediva koevoluirajo z arhitekturo kolesaste mreže. Natančneje, pri večjih vrstah pajkov mrežarjev je večinoma prišlo do evolucije kvalitetnejšega prediva, hkrati pa je koevoluirala tudi arhitektura mrež, in sicer na način, ki pajkom omogoča ulov velike biomase plena tudi z uporabo relativno manjše količine prediva (Sensenig s sod., 2010).

1.5.1 Individualna variabilnost v mrežah

Zgoraj našteti selekcijski pritiski so privedli do tega, da je osnovno vedenje osebka pri gradnji mrež v glavnem prirojeno, mreže pa se značilno razlikujejo med družinami, rodovi ali celo vrstami pajkov (Eberhard, 1982; Kuntner s sod., 2008; Gregorič s sod., 2010). Vseeno pa osebki skozi svoje življenje spreminjajo določene podrobnosti svojih mrež (Blackledge s sod., 2011). Takšna vedenjska plastičnost je dobro dokumentirana, do nje pa lahko pride zaradi različnih razlogov (Harmer s sod., 2011). Pajki prilagajajo lastnosti svojih mrež glede na količino in tip plena, ki je na voljo (Higgins, 1992; Sherman, 1994; Herberstein s sod., 2000), glede na lastno velikost (Heiling in Herberstein, 1998; Herberstein in Heiling, 1999), nahranjenost (Vollrath in Samu, 1997; Mayntz s sod., 2009; Blamires, 2010), starost (Hesselberg, 2010; Kuntner s sod., 2010a, b) in izkušnje (Heiling in Herberstein, 1999; Nakata, 2007), glede na prisotnost določenih plenilcev in parazitov (Higgins, 1992; Eberhard, 2000; Nakata, 2008), glede na vremenske dejavnike (Eberhard, 1990b; Liao s sod., 2009) idr.

V zadnjih letih so kolesaste mreže pogosto tudi objekt raziskovanja individualne variabilnosti na ontogenetskem nivoju, predvsem v študijah ontogenetskih sprememb v živalskem vedenju (ontogenija vedenja je v vedenjski biologiji definirana kot čas od izvalitve/skotitve do odraslosti). Na tem področju so kolesaste mreže še posebej primeren objekt, saj predstavljajo merljivo vedenje skozi celotno ontogenijo vedenja (Benjamin in Zschokke, 2004; Vollrath in Selden, 2007). Zgradba kolesaste mreže se skozi ontogenijo spreminja pri predstavnikih večine raziskovanih družin pajkov mrežarjev (Eberhard s sod., 2008), vendar so razlogi za te spremembe nasprotujoči. Nekatere študije razlagajo ontogenetske spremembe v arhitekturi mrež s t.i. biogenetskim pravilom (Eberhard s sod., 2008; Hesselberg, 2010). Biogenetsko pravilo, ali »ontogenija povzema filogenijo« kot je fenomen leta 1872 definiral Haeckel (Olsson s sod., 2010), predvideva, da ontogenija organizma sledi vzorcem evolucijskih sprememb v sorodstveni liniji tega organizma (Nelson, 1978; Olsson s sod., 2010). Druge študije nakazujejo, da ontogenetske spremembe v arhitekturi mrež odsevajo druge dejavnike, ki neposredno vplivajo na fitnes (prispevek gen(fen)otipa h genetskemu skladu naslednje generacije) organizma, npr. optimizacijo plenjenja, izogibanje plenilcem ipd. (Kuntner s sod., 2010a; Nakata, 2010; Nakata in Zschokke, 2010; Zschokke in Nakata, 2010).

1.6 CILJI DOKTORSKE NALOGE

Kot smo omenili v prejšnjih poglavjih, so pajki, predvsem pa pajki mrežarji iz naddružine Araneoidea pomembni in pogosto celo modelni organizmi v mnogih evolucijskih študijah. Med temi študijami so pajki mrežarji še posebej primerni objekti v raziskovanju adaptivne evolucije, evolucijske ekologije in evolucije vedenja v najširšem smislu (Coddington, 1994; Herberstein in Wignall, 2011). V doktorski disertaciji smo tako raziskovali ali različne lastnosti pajčjih mrež, ki vplivajo na vrstno pestrost pajkov, med seboj koevoluirajo, ali ontogenetske spremembe v vedenju resnično sledijo biogenetskemu pravilu in kako individualni pajki glede na svojo velikost spreminjajo energijski vložek v mreže. Večino študij smo izvajali na rodovih *Zygiella* in *Caerostris*, oba iz družine križevcev (Araneidae), ki sta filogenetsko problematična in sta zanimiva tudi po biologiji mrež. Ta rodova smo v doktorski disertaciji filogenetsko umestili in raziskali evolucijo njunih mrež.

1.6.1 Koevolucija vedenja, ekologije in biomateriala

Koevolucija je sprememba neke lastnosti, ki jo sproži sprememba druge lastnosti (Yip s sod., 2008). Koevolucija se lahko pojavlja na več hierarhičnih nivojih, od aminokislin do medvrstnih oboroževalnih bitk (Ridley, 1993; Futuyma, 1997; Pellegrini s sod., 1999). Slednje so sicer dobro dokumentirane, slabo pojasnjena pa ostaja koevolucija med lastnostmi, ki neki vrsti omogočajo zasedanje specifične ekološke niše. To je še posebej očitno za potencialno koevolucijo lastnosti, ki nimajo očitne genetske povezave, npr. ekoloških lastnosti »razširjenih fenotipov« (npr. pajčje mreže in njihovo mikrookolje), vedenjskih lastnosti (npr. gradnja mreže) in biomehanskih lastnosti (npr. materialne lastnosti prediva) (Sensenig s sod., 2010). Najpomembnejšo vlogo v evoluciji pajčjih mrež predstavljajo spremembe v materialnih lastnostih prediva in vedenje povezano s tem kako je to predivo uporabljeno v mrežah (gradnja in arhitektura mrež) (Lin s sod., 1995; Craig s sod., 1996). Nedavno je postalo znano, da mehanske lastnosti prediva resnično koevoluirajo z arhitekturo mrež (Sensenig s sod., 2010), dejansko vedenje ob gradnji mrež pa je v tem kontekstu skoraj nepoznano. Ker pajki gradijo mreže z vedenjskimi zaporedji, ki so ustaljeni, evolucijsko konzervativni in filogenetsko informativni (Eberhard, 1982; Kuntner s sod., 2008), smo pričakovali, da bomo ob evoluciji novih arhitektur opazil tudi nova vedenja ob sami gradnji mrež. Za objekt študije smo zato izbrali nedavno odkrito vrsto *Caerostris darwini* (Darwinov drevesni pajek), ki je znana po gradnji največjih mrež, ki so narejene iz najmočnejšega poznanega prediva in postavljene v edinstveno mikrookolje – razpete so čez potoke ter manjše reke in jezera (Agnarsson s sod., 2010; Kuntner in Agnarsson, 2010). Ker so mreže predvsem adaptacije na plen v določenem mikrookolju, me je zanimalo tudi, ali so ogromne mreže *C. darwini* adaptirane na lov specifičnega plena, npr. ptičev ali netopirjev, ki uporabljajo reke kot letalne poti, in ali te mreže morda izkoristijo množično pojavljanje vodnih žuželk, npr. enodnevnic (Kuntner in Agnarsson, 2010).

1.6.2 Ontogenetske spremembe v arhitekturi mrež

Biogenetsko pravilo je bilo predmet številnih razprav skozi celotno preteklo stoletje (Nelson, 1978; Olsson s sod., 2010). Čeprav raziskave morfoloških lastnostih zavračajo biogenetsko pravilo kot splošen biološki koncept, so mnoge študije pokazale nekatere vzporednice med ontogentskim in filogenetskim razvojem (Gould, 1992; Richardson in Keuck, 2002). Biogenetsko pravilo je nedavno ponovno postalo predmet raziskav, med drugim tudi v vedenjskih študijah, ki so v tem pogledu slabo raziskane (Richardson in Keuck, 2002). Pajčje mreže so idealen objekt za raziskave ontogenije vedenja, vendar si avtorji, ki so nedavno raziskovali ta pojav, niso povsem enotni ali ontogenija pajčjih mrež ustreza biogenetskemu pravilu ali je pogojena z drugimi dejavniki (Eberhard s sod., 2008; Nakata, 2010). Rezultati nekaterih predhodnih študij tako kažejo, da masa pajkov najbolj vpliva na spremembe v mrežah med njihovim ontogenetskim razvojem (Kuntner s sod., 2010a) in nakazujejo, da mreže med ontogenetskim razvojem pri vsaj nekaterih rodovih družine Nephilidae ne sledijo evolucijskim spremembam v sorodstveni liniji (Kuntner s sod., 2010b). Kot objekt v nalogi predstavljene študije ontogenetskih sprememb v pajčjih mrežah smo izbrali vrsto *Leucauge venusta*, ki po biologiji mreže predstavlja idealen objekt za testiranje nasprotujočih si ontogenetskih predpostavk, ki jih postavljata biogenetsko pravilo in optimizacija plenjena.

1.6.3 Energijski vložek v plenjenje in vedenjska plastičnost

Vedenje med fenotipskimi lastnostmi predstavlja najvišjo raven organizacije, naravna in spolna selekcija pa je na tem nivoju najmočnejša (West-Eberhard, 1989; Garland in Kelly, 2006). Živali tako večajo svoj fitnes z ustreznim razporejanjem časa in energije med vedenjskimi lastnostimi kot so prehranjevanje, razmnoževanje in izogibanje plenilcem (Lima in Dill, 1990; Ferrari s sod., 2009). Da bi bolje razumel odnose (»trade-off«) med takšnimi lastnostmi, smo pri petih ozkosorodnih vrstah rodu *Zygiella* s.l. raziskovali vložek materiala v plenjenje (mreže) v odvisnosti od telesne velikosti. Čeprav so spremembe v mrežah v odvisnosti od velikosti pajka dobro dokumentirane, se rezultati preteklih študij ne ujemajo in si včasih celo nasprotujejo (Witt s sod., 1968; Sherman, 1994; Venner s sod., 2000; Nakata, 2007; Mayntz s sod., 2009; Blamires, 2010). V naši raziskavi smo zato energijski vložek v plenjenje merili na nov način. Namesto preprostega merjenja velikosti in gostote mreže ali skupne dolžine različnih niti, smo ocenili skupen volumen treh tipov prediva, ki ga pajki v glavnem uporabijo v gradnji mrež. Dodatno smo raziskali še lastnosti mrež, ki so jih raziskovale pretekle študije.

1.6.4 Filogenetska sistematika rodov *Zygiella* s.l. in *Caerostris*

Vrstna pestrost pajkov mrežarjev je tesno povezana z evolucijo delovanja mrež (Blackledge s sod., 2011). Za razumevanje evolucije lastnosti, ki vplivajo na delovanje mrež, bi v idealnem primeru poznali filogenetske odnose med sorodstvenimi linijami pajkov mrežarjev. Kljub napredku filogenetskih metod so filogenetski odnosi med družinami in celo znotraj njih slabo poznani (Agnarsson s sod., 2013).

Večina pajkov, ki gradi kolesaste mreže, pripada družini Araneidae. Z več kot 3000 opisanimi vrstami ta družina po vrstni pestrosti med pajki zaseda tretje mesto (Platnick, 2013) in zajema morfološko, vedenjsko in ekološko izjemno raznolike pajke, ki sicer izvorno gradijo kolesaste mreže, vendar razen tega nimajo nujno veliko skupnih lastnosti. Na poti k učinkovitemu orodju za evolucijske raziskave je torej nujno boljše poznavanje odnosov med skupinami pajkov mrežarjev, morda pa bi bilo celo potrebno na novo definirati tradicionalno družino Araneidae. V doktorski nalogi smo se zato osredotočili na dva filogenetsko problematična rodova križevcev, ki sta zanimiva modela mnogih evolucijskih raziskav: to sta rod *Zygiella* Pickard-Cambridge 1902 v širšem smislu (*Zygiella* s.l.) in rod *Caerostris* Thorell 1868.

Pajki rodu *Zygiella* s.l. imajo značilne mreže s praznim sektorjem (Levi, 1974; Gregorič s sod., 2010) in so bili med prvimi objekti raziskav arhitekture, funkcije in gradnje kolesaste mreže (Wiehle, 1927, 1929; Peters, 1937; Witt s sod., 1968), vse do danes pa vztrajajo kot raziskovalni objekti v študijah odnosov med plenom in plenilcem, spolne selekcije, vedenjske plastičnosti ter vedenja in fiziologije gradnje mrež (Zschokke in Vollrath, 1995b; Venner s sod., 2000, 2003; Thevenard s sod., 2004; Venner in Casas, 2005; Bel-Venner in Venner, 2006; Bel-Venner s sod., 2008; Mayntz s sod., 2009). Holarktično razširjen rod *Zygiella* s.l. sicer že dolgo velja za filogenetsko spornega. V preteklosti je bil večkrat izmenično uvrščen v družini Araneidae in Tetragnathidae (Levi, 1980; Levy, 1987; Heimer in Nentwig, 1991; Roberts, 1995; Scharff in Coddington, 1997), pred kratkim pa celo razdeljen v štiri rodove, ki bi naj pripadali svoji družini Zygiellidae (Wunderlich, 2004). Novi rodovi so sprejeti in katalogizirani, medtem ko status družine Zygiellidae ostaja sporen (Platnick, 2013). Nedavne molekularne filogenetske analize sicer vključujejo zgolj eno ali dve vrsti rodu *Zygiella*, vendar ju neodvisno združujejo z določenimi drugimi rodovi križevcev, potencialno družino »Zygiellidae« pa negotovo postavljajo med družini Araneidae in Nephilidae, kar nakazuje, da je koncept samostojne družine morda smiselen (Alvarez-Padilla s sod., 2009; Blackledge s sod., 2009; Sensenig s sod., 2010; Agnarsson s sod., 2012; Dimitrov s sod., 2012; Kuntner s sod., 2013).

Drevesni pajki iz rodu *Caerostris* so do nedavnega bili precej neraziskani tako ekološko, vedenjsko, kot tudi taksonomsko, čeprav ta skrivnostni rod vsebuje velike, raznolike pajke mrežarje, ki so široko razširjeni po tropih starega sveta (Grasshoff, 1984), biologija njihovih mrež pa je izredno zanimiva (Kuntner in Agnarsson, 2010). Predstavniki rodu *Caerostris* gradijo največje znane kolesaste mreže, zgrajene iz najmočnejšega znanega prediva, nekatere vrste pa te mreže zgradijo v unikatnem mikrookolju in ob tem uporabijo unikatno kombinacijo vedenjskih sekvenc (Agnarsson s sod., 2010; Kuntner in Agnarsson, 2010; Gregorič s sod., 2011a, b). Zaradi njihovih izredno velikih mrež in izredno močnega prediva pajki rodu *Caerostris* postajajo modelni organizmi v raziskovanju biomaterialov. Sistematsko je rod drevesnih pajkov slabo poznan in je bil v preteklosti vključen v le nekaj filogenetskih analiz. Študije, ki so temeljile na morfoloških znakih rod uvrščajo v Araneidae blizu rodov *Argiope* in *Gasteracantha* (Kuntner s sod., 2008; Scharff in Coddington, 1997), molekularne analize pa jih uvrščajo sestrsko vsem ostalim predstavnikom Araneidae (Sensenig s sod., 2010; Kuntner s sod., 2013), kar nakazuje, da imata rodova *Caerostris* in *Zygiella* s.l. morda podobne filogenetski položaj.

V doktorski disertaciji smo tako na podlagi šestih molekulskih markerjev preverjali monofiletski izvor in obseg potencialne družine Zygiellidae in rodu *Caerostris* ter ugotavljal njun filogenetski položaj. Ker so ustrezno podprti filogenetski odnosi ključnega pomena v primerjalni biologiji, ki lahko napove selekcijske pritiske in rezultate (Felsenstein, 1985; Kiontke s sod., 2004), in ker arhitektura in gradnja kolesaste mreže očitno dobro opredeljujeta glavne skupine pajkov mrežarjev (Eberhard, 1982; Kuntner s sod., 2008), smo testirali ali podrobnosti v arhitekturi in gradnji mrež resnično odsevajo filogenetske odnose med skupinami in se posebej osredotočil na evolucijo gradnje in gigantizma mrež.

2 ZNANSTVENA DELA

2.1 OBJAVLJENA ZNANSTVENA DELA

2.1.1 Kako je pajek prečkal reko? Vedenjske adaptacije čez reke razpetih mrež pri vrsti *Caerostris darwini* (Araneae: Araneidae)

How did the spider cross the river? Behavioral adaptations for river-bridging webs in *Caerostris darwini* (Araneae: Araneidae)

Matjaž Gregorič, Ingi Agnarsson, Todd A. Blackledge, Matjaž Kuntner

2011, PLoS ONE 6(10): e26847

Izvleček

Ozadje: Koevolucija na nivoju medvrstnih interakcij je dobro dokumentirana, slabo pojasnjeno pa ostaja kako koevoluirajo lastnosti znotraj vrste, predvsem vedenjske in biomehanske lastnosti živalskih »razširjenih fenotipov«. Pri pajkih mrežarjih je pričakovana koevolucija vedenja z ekološkimi in fizičnimi lastnostmi njihovih mrež. Darwinov drevesni pajek (*Caerostris darwini*) je znan po gradnji največjih mrež, ki so narejene iz najmočnejšega poznanega prediva in postavljene v edinstveno mikrookolje – razpete so čez potoke ter manjše reke in jezera. V članku testiramo domneve, da edinstvena ekologija in arhitektura mrež te vrste koevoluira z vedenjem ob gradnjih mrež.

Metodologija: *C. darwini* smo opazovali v njihovem naravnem habitatu in snemali gradnjo njihovih mrež. Ovrednotili smo 90 dogodkov ob gradnji mrež in ta vedenja primerjali z drugimi vrstami pajkov mrežarjev.

Sklepi: *C. darwini* za gradnjo mrež uporablja edinstveno kombinacijo vedenj, med katerimi so tudi nekatera vedenja, ki pri drugih pajkih niso poznana. Prvič, pajki z vetrom spustijo nenavadno veliko količino prediva, ki ga veter nese čez vodno telo, s čimer se oblikuje svilen most. Drugič, pajki skoraj ne raziskujejo okolja, kjer bodo gradili mrežo. Tretjič, celotno lovilno ploskev zgradijo pod prvotnim mostom. Za razliko od ostalih pajkov mrežarjev, središče mreže ni del prvotnega mosta, ampak je zgrajen *de novo*. Četrtič, lovilni del mreže vsebuje dva tipa žarkastih niti, kjer so tiste v zgornji polovici podvojene. To edinstveno vedenje rezultira v izredno velikih, a precej preprosto zgrajenih mrežah. Naši rezultati podpirajo koevolucijo vedenjskih (arhitektura mrež), ekoloških (mikrookolje mreže) in biomaterialnih (mehanske lastnosti prediva) lastnosti, ki skupaj omogočajo vrsti *C. darwini*, da je zasedla to med pajki edinstveno ekološko nišo.

Abstract

Background: Interspecific coevolution is well described, but we know significantly less about how multiple traits coevolve within a species, particularly between behavioral traits and biomechanical properties of animals' "extended phenotypes". In orb weaving spiders, coevolution of spider behavior with ecological and physical traits of their webs is expected. Darwin's bark spider (*Caerostris darwini*) bridges large water bodies, building the largest known orb webs utilizing the toughest known silk. Here, we examine *C. darwini* web building behaviors to establish how bridge lines are formed over water. We also test the prediction that this spider's unique web ecology and architecture coevolved with new web building behaviors.

Methodology: We observed *C. darwini* in its natural habitat and filmed web building. We observed 90 web building events, and compared web building behaviors to other species of orb web spiders.

Conclusions: *Caerostris darwini* uses a unique set of behaviors, some unknown in other spiders, to construct its enormous webs. First, the spiders release unusually large amounts of bridging silk into the air, which is then carried downwind, across the water body, establishing bridge lines. Second, the spiders perform almost no web site exploration. Third, they construct the orb capture area below the initial bridge line. In contrast to all known orb-weavers, the web hub is therefore not part of the initial bridge line but is instead built *de novo*. Fourth, the orb contains two types of radial threads, with those in the upper half of the web doubled. These unique behaviors result in a giant, yet rather simplified web. Our results continue to build evidence for the coevolution of behavioral (web building), ecological (web microhabitat) and biomaterial (silk biomechanics) traits that combined allow *C. darwini* to occupy a unique niche among spiders.

Introduction

Coevolution, change of one trait triggered by shifts in a related trait [1], can occur at many hierarchical levels from amino acids to interspecific arms races [2-4]. While species coevolution is well documented, we lack a broad understanding of how multiple traits coevolve to enable resource use within a species. This particularly holds true for the potential coevolution of traits that lack obvious genetic linkage, such as ecological "extended phenotypic" (e.g. spider webs and their microhabitat), behavioral (e.g. web building behaviors), and biomechanical (e.g. intrinsic properties of silk) traits [5].

Spider webs are physical manifestations of web building behaviors and are built using some of the world's "highest performance" biomaterials – spider silks. Spider webs are thus ideal for studying coevolution between behaviors, ecology, and performance of biomaterials [6-8]. The orb web's evolutionary origin defines a single clade, Orbiculariae, a large and diverse group with more than 12.000 species [9-12]. Architectural evolution of orb webs through time has resulted in novel web types [9,13,14], such as the linyphiid sheetwebs and theridiid cobwebs [10,15,16], the deinopid casting web [17], as well as many modifications of the classical orb web [7,9,18-20]. Because spiders build orb webs using highly stereotypical behaviors that are evolutionarily conserved and phylogenetically informative [13,20], the evolution of new web architectures are expected to coincide with novel behaviors.

The impressive range of web designs within the Orbiculariae represents adaptations to a large range of prey types in diverse habitats [7,8]. Two major components in spider web evolution are the changes in quality (intrinsic material properties) of the different types of spider silk composing webs and the changes in behaviors associated with how those silks are assembled to produce the finished web (web building and architecture) [21,22]. In particular, material properties of spider silk coevolve with web design among orb spiders, a coevolutionary pattern not clearly demonstrated in many other common biomaterials such as byssal threads, tendon and keratin [5]. However, the actual behaviors that orb web spiders use are largely unstudied in this context.

Due to its amazing web architecture and silk toughness [23], the recently discovered Darwin's bark spider (*Caerostris darwini* Kuntner and Agnarsson 2010) is a promising system for studying the coevolution of behavioral traits with biomaterials during adaptation to new habitats. This species is endemic to Madagascar and is unique in building giant webs across streams, rivers and lakes. Some other spider species build smaller webs at the edges of waterways. However, no other spider builds webs that utilize the air column above large water bodies as habitat (Fig. 1) [7,23]. The webs of *C. darwini* are made of silk combining strength and great elasticity such that it outperforms all other known spider silks, and even most synthetic fibers, in terms of toughness (work required to fracture the silk) [24]. Furthermore, capture areas of *C. darwini* webs regularly exceed 1 m in diameter and are suspended on bridge lines that often exceed 10 meters, while the largest capture areas reach almost 2 meters in diameter and are suspended on bridge lines up to 25 meters in length. These webs surpass even the gigantic *Nephila* webs, making *C. darwini* orb webs the largest known [23,25,26]. However, nothing is known about potential behavioral adaptations used to construct these giant webs in such unique microhabitats.

We thus pose several questions. First, bridging open space is a challenge for "typical" orb web spiders [18]. How then, do the enigmatic Darwin's bark spiders bridge such enormous distances across water? Second, did *C. darwini* evolve adaptations in web building behavior that accompany novel silk properties in achieving their unique webs? If so, did these behaviors evolve as modifications of existing orb web-weaving behaviors or are these behaviors evolutionary novelties? To address these questions, we designed a field study in eastern Madagascar and collected data on *C. darwini* web building and the site exploration behavior that precede web building [27].

Results

We observed numerous *C. darwini* establishing bridges over open water bodies (Fig. 1) by descending on a dragline from their resting places in vegetation and releasing large amounts of silk into the wind (hereafter "bridging silk"; Fig. 2A). Bridging silk always constituted tens of silk threads that broadly exit the spinnerets and then formed into a single line after 24 seconds (median (ME), interquartile range (IQR) = 18.75; N = 14; Video S1, S2). After the bridging silk eventually became entangled in vegetation or other substrates, typically on the other side of the water body, spiders (N = 19) started reeling in the silk, thus increasing its tension. If the attachment broke, the spiders reeled the loose silk up and consumed it, then continued attempting to establish bridge lines. If the attachment held, the spider crossed over the bridge line. When the spiders first crossed open spaces, they all (N = 19) cut and reeled the original bridging line as they laid a new one behind, as seen in other orb spiders (e.g. [27-29]). The spiders then reinforced the bridge line and both attachment points several times. To connect the bridge line with a third attachment point, all spiders (N = 32) gradually descended towards ground on a dragline while simultaneously releasing a new bridging silk thread into the air. The spider continued descending its dragline, until either successfully attaching the bridging silk to some distant substrate or reaching solid ground. We never observed connections to the water surface, but silk was instead always connected to vegetation sticking out of water or to shore vegetation. Spiders that contacted water crawled up the dragline thread to the original bridge line where they established a new dragline connection and then repeated the above mentioned behaviors until the spiders found solid surface for attachment. This apparent constraint on the placement of anchor lines implies that *C. darwini* webs typically could only be constructed close to the shore. However, this was not the case as many water bodies in these habitats were populated by semiaquatic plants that were used as substrate for web attachments. Also, webs were often constructed in the middle of water bodies, attached not only using long bridge lines but also long third anchor lines.

In a few cases, *C. darwini* departed from the usual bridging behavior by first attaching a bridging line on the dragline from which they were hanging, and then continuing bridging attempts with a second bridging line (Fig. 2B). However, these spiders never successfully completed such bridging threads.

Up to this point, all spiders (N = 32) constructed a structure consisting of a more or less horizontal bridge line and a more or less vertical thread (Fig. 3A). This structure never resembled the textbook "Y" built by other orb spiders (e.g. [30,31]), but rather a "T" (hereafter “T structure”). The junction of the two threads (hereafter “T junction”) never matched the proto-hub (hub of the future web), and the capture area was always built entirely below the bridge line (Fig. 3). The horizontal thread of the T structure was always converted into the bridge line and two horizontal anchor threads in the finished web, and the vertical thread was converted into two vertical radii and the lower anchor line. In contrast, other orb weavers build a Y shaped initial structure, where the three arms meet at the proto-hub and are converted into (replaced by) radii and anchor lines in the finished web so that the capture area is built around them (Fig. 3) [27,31-34].

Caerostris darwini never built more than three anchor points (N = 32). Although some spiders showed certain levels of web site exploration by establishing up to three bridging silk attachments, we never observed exploration after establishing the T structure. Thus, the proto-orbs (primary frame, proto-hub and proto-radii, constructed together just prior to construction of the rest of the radii and spirals) were highly stereotypical, i.e. always consisted of the same arrangement of threads (Fig. 3B). To build a frame, all spiders walked down a part of the vertical thread of the T structure, laying a new silk thread behind, thus doubling this part of the vertical thread. In finished webs, the point where spiders attached the second vertical thread became the hub (Fig. 3A-C), and the doubled part of the vertical thread became a vertical radius. The spiders then built the orb web’s side frames together with the first radii (Fig. 3B). We never observed secondary frame construction, which occurs in other orb weaving spiders when building radii (the secondary frame is thus connected to the threads of the primary frame; Fig. 3SF) [34,35].

All observed spiders (N = 9) constructed single radii in the lower orb half (Fig. 3C). The spiders first laid a silk thread when moving from hub to frame, then cutting and reeling it using their third legs when returning to the hub, and simultaneously laying a new thread behind. Radii in the upper orb half were constructed as double radii, lacking the cut and reel of the previous thread. The silk remains of the cut and reeled threads in the lower orb were deposited at/near the hub, which sometimes appeared like rudimentary stabilimenta [36] in finished orbs. During radius construction, spiders reinforced the hub several times with additional loops of silk.

All spiders (N = 43) built the non-sticky spiral (NSS) from the hub to orb periphery, and the sticky spiral (SS) on the way back towards the hub. They attached the SS to every radius crossed and removed the NSS in the process. They determined the SS attachment point by tapping with the outer leg I. After finishing SS construction, spiders tested the web tension by shaking at the hub. We never observed hub destruction behavior and never observed building of ‘web decorations’ (stabilimenta), although the latter were occasionally found in webs in the field. Additionally, we observed one individual of an undescribed *Caerostris* species building radii, spirals and the hub the same way as *C.*

darwini, and webs of all other encountered *Caerostris* species also lacked secondary frames.

The time *C. darwini* used to cross a water body (time from start of bridging behavior to reinforcement of future bridge) was 5-163 min (ME = 52.5, IQR = 118.25; N = 7). The spiders then spent 6-43 min (ME = 15, IQR = 13.13; N = 8) building the vertical anchor line, reinforcing the attachments and building the web frame. After that, the spiders used 3-9 min (ME = 6.75, IQR = 2.15; N = 10) and 22-64 min (ME = 42.5, IQR = 18.38; N = 6) to construct the radii and spirals, respectively. During web renewal, *C. darwini* (N = 29) completely removed and rebuilt the radii and SS, as well as frame threads outlining the capture area. They only reinforced the anchor lines, and thus both bridge lines as well as third anchor lines established across the water bodies are maintained long term.

Discussion

Cearostris darwini uses a set of previously unknown behaviors to build orb webs in the air column above large water bodies. The spider produces unusually large amounts of bridging silk, almost completely lacks web site exploration behavior, has highly stereotypical proto-orb construction, builds the whole capture area below the initial bridge line, and constructs two types of radii in the same web. *Caerostris darwini* also anchors the web at only three points, and lacks both secondary web frames and hub modification. Other web building behaviors, such as spiral construction, are typical of other araneids [13,20,37]. We hypothesize that both the extreme mechanical properties of silk and the combination of web building behaviors in *C. darwini* represent adaptations to their novel environment.

Recent literature reports that orb spiders typically initiate web building by bridging using a single silk thread composed of minor ampullate silk, which is tightly interconnected with strands of aciniform silk [28,38-40]. This behavior is also used by larger spiders to move to new web sites. In contrast, most small spiders disperse aerially (balloon) using a similar silk thread but with sail like terminus composed of numerous spread out silk strands, providing larger surface area [41]. However, our observations agree with older literature on bridging silk that suggest it also initially consists of numerous spread out silk strands [18,35,42,43]. During ballooning, spiders typically climb to, and release silk from higher ground, while bridging behavior in spiders starts with a descent on a dragline. Our observations indicate that *C. darwini* does not differ from other orb weavers in the general structure of the bridge thread but rather in the quantity of threads attached to the main line (Video S1, S2). Such large amounts of silk are probably necessary to carry the bridge line over a sufficient distance to span large rivers and lakes, similar to dispersal via ballooning. Furthermore, the similarity between the bridging and ballooning behaviors of orb spiders suggest that the bridging behavior probably evolved from the ballooning behavior, the latter being known in almost all araneomorph spiders [6,38].

Caerostris darwini bridging behavior is somewhat flexible. Several *C. darwini* individuals exhibited an alternative bridging behavior, attaching bridging lines while descending on their draglines and additionally releasing new bridging silk. Similar behavior is either facultative or predominant in other orb weavers [18,42]. However, this alternative behavior was rare and never successful in our observations so that it likely plays only a minor role in bridging relatively short distances. As in other spiders [29], prior to crawling on the new bridge line, *C. darwini* reels the newly attached bridging silk, thus increasing the tension and testing the attachment strength. However, this is the first observation of spiders using bridging behavior to establish the third anchor lines (Fig. 3AL). Although this behavior might be present but simply not reported for other spider species, it would be more advantageous in spiders building over water where there are no or few attachment opportunities below the web, such as in *C. darwini*, compared to the majority of orb weavers who build over land.

According to the “refined gravity hypothesis” bridges sag under the weight of spiders and bridging to move between web sites could thus be less efficient in larger spiders that produce long bridges with more elastic silk [40,44]. Ultimately, movement by larger orb spiders could be limited to short distances if their bridge lines sag too much. Our findings may contradict the refined gravity hypothesis as *C. darwini* are among the largest orb weavers and their silk is extremely elastic [5,24,26], yet they bridge larger distances than any other known orb spider. However, orb webs are suspended on bridge lines made of the unusually elastic major ampullate silk, while the initial bridging line when crossing open space is thought to consist of minor ampullate silk, whose mechanical properties are not yet known for *C. darwini*.

Caerostris darwini webs are relatively simple and this may relate to the webs’ habitat. For example the web site exploratory behavior, as performed by most orb weavers and preceding web building *per se*, probably serves to avoid obstacles for the web’s capture area [27,32,34]. These exploratory behaviors are not stereotypical as the environment is usually highly variable [27]. The resulting proto-orbs thus vary even within the same individual, and some components of the proto-orbs are not part of the finished webs [32,34]. However, the air column above open water is typically obstacle free, and hence *C. darwini* need not perform additional exploration beyond the T-structure. We hypothesize that the open habitat above water led to the evolutionary loss of complex exploratory behaviors thereby resulting in the highly stereotypical and simplified proto-orb construction in *C. darwini*.

Uniform proto-orbs in *C. darwini* are always followed by suspension of the web on three anchor lines, the minimum necessary for a planar orb web. Searching for additional anchor points in the same plane would be uneconomical considering the scarcity of anchor points over water (e.g. vegetation) and the relative distance between shores. Webs of *C. darwini* also lack secondary frames, which most other orb weavers incorporate into their webs to lower the tension along radii [45-47]. Radii in a web as large as this may be under lower tension and therefore secondary frames may not be needed, but this remains to be tested. Other simplified features of *C. darwini* orb webs include few radii (15-30 [23,25]), broad spiral mesh (5.9-30.5 [23,25]) and the lack of hub destruction behavior [48]. After finishing spiral construction, *C. darwini* leave the hub intact, which while typical of species from other orb-weaving families, is unusual in araneids, most of which bite out and replace the hub silk [10,13,20,49].

Perhaps the most striking differences between *C. darwini* webs and those of other known orb weavers are two features: i) the unique building of the whole capture area under the initial bridge line (Fig. 3) [30,32-34], and ii) the combination of two types of radii in the same web – single radii in the lower and double radii in the upper orb web half. First, our results indicate that in *C. darwini*, bridging instead of web site exploration is the more energetically costly part of web building [34], here probably even intensified by large amounts of bridging silk used. We argue that retaining the long bridge in its entirety, as *C. darwini* does, is advantageous because both the silk and time investment, and thus energy investment in the functional bridge, is higher in an over-river habitat compared with terrestrial air columns with relatively shorter bridges. Other orb weavers typically modify and subsequently destroy and rebuild the initial bridge.

Second, *C. darwini* combines single and double radii in the same web, whereas most orb weavers only build single radii, except uloborids and nephilids which construct double radii throughout their webs [13,20,37]. However, a handful of other araneids also double their radii near the periphery of the web [50,51], where the tension within a radius is higher [45,46,48]. Double radii in *C. darwini* could have several functions. First, in other orb weavers, radii in the upper half of orb webs are under higher tension [52]. As *C. darwini* build large webs with few radii, but also do not build secondary frames that reduce tension of long radii [45,47], doubled radii may thus simply have the advantage to distribute force across more silk. Second, hub modification after orb construction is associated with adjusting tension in radii [48]. As *C. darwini* do not modify hubs after orb construction, adding another thread to radii in the upper half of the orb, perhaps pulled more/less tightly, might serve as a mechanism of adjusting tension. As at least some other *Caerostris* species also build simple webs containing two types of radii, their web structure might represent a preadaptation for building oversized webs across rivers and lakes, but may also not play a role in conquering this unique habitat at all.

Our results provide a strong evidence for the coevolution of behavioral web building traits with ecological traits such as web microhabitat, which in turn is linked to the exceptional material characteristics of silk in *C. darwini*. However, in the absence of a species level phylogeny, we cannot precisely pinpoint the exact origins of each of these traits. Nevertheless, the fact that some of *C. darwini* building behaviors, e.g. the simplified web and the building of two types of radii, are shared with at least some congeners, implies that these behaviors may have arisen at a deeper hierarchical level. Future research should focus on the precise order of evolutionary events in *Caerostris*. Therefore, we plan to integrate phylogenetic, taxonomic, behavioral and mechanical research of additional species of *Caerostris* into a coherent picture elucidating the fascinating web biology of these spiders.

Materials and Methods

We documented web building behavior of *Caerostris darwini* females (adult or subadult) at several localities in Andasibe-Mantadia National Park (between S18.94760 E48.41972 and S18.79841 E48.42631 at roughly 960 m elevation), Toamasina Province, eastern Madagascar, between 24 February and 4 April 2010. In total, we observed 90 whole or partial web building events. We filmed and photographed selected behavioral sequences using camcorders (Sony DCR-SR87 HDD) and Canon SLR cameras (EOS 5D Mark II and EOS 7D).

Research, collecting, and export permits were obtained from The National Association for the Management of Protected Areas in Madagascar and Ministère de L'environnement, des Forêts et du Tourisme (permits Nu 087/08, Nu 088/08, and Nu 091N-EA04/MG08), through the Institute for the Conservation of Tropical Environments offices in Stony Brook and Antananarivo. Permits are on file with IA.

In 32 of 90 web building events, we started our observations at the beginning of web building. To do so, we at least partially destroyed *C. darwini* webs and then monitored them. To force the spiders to build a new bridge line, we sometimes (N = 19) destroyed the entire web including the bridge. In others (N = 13), we destroyed the capture area and all frame threads below the bridge, leaving the latter intact. We observed whole web building events in 18 of these 32 web building events. In the other 14, we had to terminate our observations prior to spiral construction, but did observe web building until the construction of the whole web frame and at least some radii. In 58 of all 90 web building events, we started our observations during spiral construction (N = 43) or radius construction (N = 15). Additionally, we sampled all radii of four webs on microscope glass slides to subsequently examine them under 1000x magnification, in order to confirm that all radii in the upper and lower orb web half are double and single stranded, respectively.

Acknowledgments

We thank Jonathan Coddington, William Eberhard and Samuel Zschokke for helpful discussions about our results, and an anonymous reviewer for the valuable comments. This is the second contribution resulting from the 2010 Madagascar expedition. We thank the MICET crew in Antananarivo, and Sahondra Lalao Rahanitriniaina, Honore Rabarison and Dina Andrianoely for their help in the field.

References

1. Yip KY, Patel P, Kim PM, Engelman DM, McDermott D, et al. (2008) An integrated system for studying residue coevolution in proteins. Bioinformatics 24: 290-292.

2. Futuyma DJ (1997) Evolutionary Biology. Sunderland: Sinauer Publications. 763 p.

3. Pellegrini M, Marcotte EM, Thompson MJ, Eisenberg D, Yeates TO (1999) Assigning protein functions by comparative genome analysis: Protein phylogenetic profiles. Proceedings of the National Academy of Sciences of the United States of America 96: 4285-4288.

4. Ridley M (1993) Evolution. Oxford: Blackwell Scientific Publications. 670 p.

5. Sensenig A, Agnarsson I, Blackledge TA (2010) Behavioural and biomaterial coevolution in spider orb webs. Journal of Evolutionary Biology 23: 1839-1856.

6. Blackledge TA, Agnarsson I, Kuntner M (2011) The form and function of spider orb webs: evolution from silk to ecosystems. Advances in Insect Physiology. In press.

7. Eberhard WG (1990) Function and phylogeny of spider webs. Annual Review of Ecology and Systematics 21: 341-372.

8. Shear WA, editor (1986) Spiders, webs, behavior, and evolution. Stanford: Stanford University Press. 492 p.

9. Coddington JA (1986) The monophyletic origin of the orb web. In: Shear WA, editor. Spiders: Webs, Behavior,and Evolution. Stanford, CA: Stanford Univ. Press. pp. 319-363.

10. Griswold CE, Coddington JA, Hormiga G, Scharff N (1998) Phylogeny of the orb-web building spiders (Araneae, Orbiculariae : Deinopoidea, Araneoidea). Zoological Journal of the Linnean Society 123: 1-99.

11. Garb JE, DiMauro T, Vo V, Hayashi CY (2006) Silk genes support the single origin of orb webs. Science 312: 1762-1762.

12. Blackledge TA, Scharff N, Coddington JA, Szuts T, Wenzel JW, et al. (2009) Reconstructing web evolution and spider diversification in the molecular era. Proceedings of the National Academy of Sciences of the United States of America 106: 5229-5234.

13. Eberhard WG (1982) Behavioral characters for the higher classification of orb-weaving spiders. Evolution 36: 1067-1095.

14. Coddington JA, Levi HW (1991) Systematics and evolution of spiders (Araneae). Annual Review of Ecology and Systematics 22: 565-592.

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

16. Eberhard WG, Agnarsson I, Levi HW (2008) Web forms and the phylogeny and theridiid spiders (Araneae: Theridiidae): chaos from order. Systematics and Biodiversity 6: 415-475.

17. Coddington JA (1986) Orb webs in "non-orb weaving" ogre-faced spiders (Araneae: Dinopidae): A question of genealogy. Cladistics 2: 53-67.

18. Wiehle H (1927) Beiträge zur Kenntnis des Radnetzbaues der Epeiriden, Tetragnathiden und Uloboriden. Zeitschrift für Morphologie und Ökologie der Tiere 8(3-4): 468-537.

19. Kuntner M, Kralj-Fišer S, Gregorič M (2010) Ladder webs in orb-web spiders: ontogenetic and evolutionary patterns in Nephilidae. Biological Journal of the Linnean Society 99: 849-866.

20. Kuntner M, Coddington JA, Hormiga G (2008) Phylogeny of extant nephilid orb-weaving spiders (Araneae, Nephilidae): testing morphological and ethological homologies. Cladistics 24: 147-217.

21. Craig CL (1987) The significance of spider size to the diversification of spider-web architectures and spider reproductive modes. American Naturalist 129: 47-68.

22. Lin LH, Edmonds DT, Vollrath F (1995) Structural-engineering of an orb-spiders web. Nature 373: 146-148.

23. Kuntner M, Agnarsson I (2010) Darwin's bark spider: Web gigantism in a new species of bark spiders from Madagascar (Araneidae: *Caerostris*). Journal of Arachnology 38: 346-356.

24. Agnarsson I, Kuntner M, Blackledge TA (2010) Bioprospecting finds the toughest biological material: Extraordinary silk from a giant riverine orb spider. Plos One 5(9): e11234.

25. Gregorič M, Agnarsson I, Blackledge TA, Kuntner M (2011) Darwin's bark spider: giant prey in giant orb webs (*Caerostris darwini*, Araneae: Araneidae)? Journal of Arachnology. In press.

26. Kuntner M, Coddington JA (2009) Discovery of the largest orbweaving spider species: The evolution of gigantism in *Nephila*. Plos One 4(10): e7516.

27. Eberhard WG (1972) Web of *Uloborus diversus* (Araneae-Uloboridae). Journal of Zoology 66: 417-465.

28. Foelix RF (1996) Biology of Spiders. Oxford: Oxford University Press. 336 p.

29. Moya-Larano J, Vinkovic D, De Mas E, Corcobado G, Moreno E (2008) Morphological evolution of spiders predicted by pendulum mechanics. Plos One 3(3): e1841.

30. Foelix RF (1996) Biology of spiders. New York: Oxford University Press. 330 p.

31. Peters HM (1939) Probleme des Kreuzspinnen-Netzes. Zeitschrift für Morphologie und Ökologie der Tiere 36: 180-226.

32. Eberhard WG (1990) Early stages of orb construction by *Philoponella vicina*, *Leucauge mariana*, and *Nephila clavipes* (Araneae, Uloboridae and Tetragnathidae), and their phylogenetic implications. Journal of Arachnology 18: 205-234.

33. Zschokke S (1999) Nomenclature of the orb-web. Journal of Arachnology 27: 542-546.

34. Zschokke S (1996) Early stages of orb web construction in *Araneus diadematus* Clerck. Revue Suisse de Zoologie hors série 2: 709-720.

35. Witt PN, Reed CF, Peakall DB (1968) A spider's web: Problems in regulatory biology. New York: Springer Verlag. 107 p.

36. Eberhard WG (1973) Stabilimenta on the webs of *Uloborus diversus* (Araneae: Uloboridae) and other spiders. Journal of Zoology 171: 367-384.

37. Scharff N, Coddington JA (1997) A phylogenetic analysis of the orb-weaving spider family Araneidae (Arachnida, Araneae). Zoological Journal of the Linnean Society 120: 355-434.

38. Foelix RF (2010) Biology of Spiders. Oxford: Oxford University Press. 432 p.

39. Peters HM (1990) On the structural and glandular origin of the bridging lines used by spiders for moving to distant places. Acta Zoologica Fennica 190: 309-314.

40. Rodriguez-Girones MA, Corcobado G, Moya-Larano J (2010) Silk elasticity as a potential constraint on spider body size. Journal of Theoretical Biology 266: 430-435.

41. Weyman GS, Sunderland KD, Jepson PC (2002) A review of the evolution and mechanisms of ballooning by spiders inhabiting arable farmland. Ethology Ecology & Evolution 14: 307-326.

42. Eberhard WG (1987) How spiders initiate airborne lines. Journal of Arachnology 15: 1-9.

43. Savory TH (1952) The Spider's Web. New York: Warner, New York. 153 p.

44. Corcobado G, Rodriguez-Girones MA, De Mas E, Moya-Larano J (2010) Introducing the refined gravity hypothesis of extreme sexual size dimorphism. Bmc Evolutionary Biology 10.

45. Zschokke S (2002) Form and function of the orb web. In Toft S, Scharff N, editors. European Arachnology 2000. Aarhus University Press, Aarhus, Denmark. pp. 99-106.

46. Zschokke S (2002) Ultraviolet reflectance of spiders and their webs. Journal of Arachnology 30: 246-254.

47. Eberhard WG (1986) Effects of orb-web geometry on prey interception and retention. In: Shear WA, editor. Spiders, webs, behavior and evolution. Stanford: Stanford University Press. pp. 70-100.

48. Eberhard WG (1981) Construction behaviour and the distribution of tensions in orb webs. Bulletin of the British Arachnological Society 5: 189-204.

49. Hormiga G, Eberhard WG, Coddington JA (1995) Web-construction behavior in australian *Phonognatha* and the phylogeny of nephiline and tetragnathid spiders (Araneae, Tetragnathidae). Australian Journal of Zoology 43: 313-364.

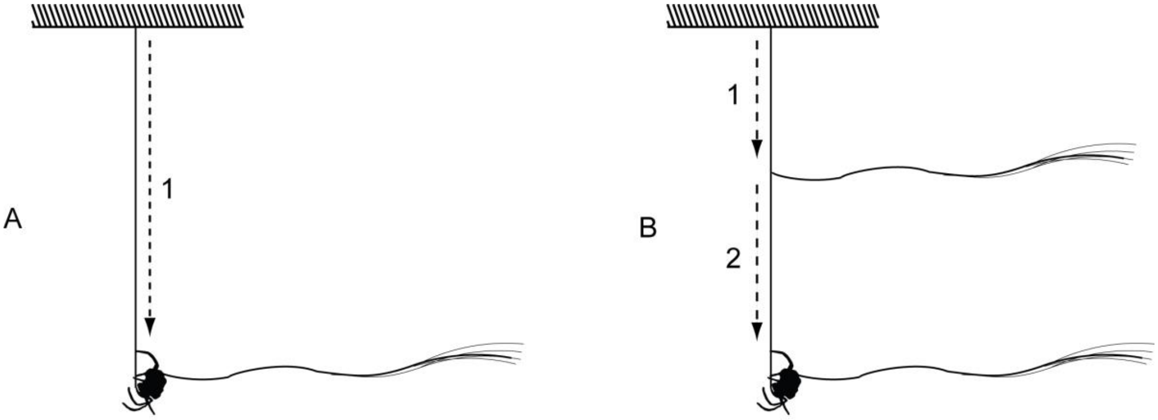
50. Zschokke S (2000) Radius construction and structure in the orb-web of *Zilla diodia* (Araneidae). Journal of Comparative Physiology a-Sensory Neural and Behavioral Physiology 186: 999-1005.

51. Zschokke S, Vollrath F (2000) Planarity and size of ore-webs built by *Araneus diadematus* (Araneae : Araneidae) under natural and experimental conditions. Ekologia-Bratislava 19: 307-318.

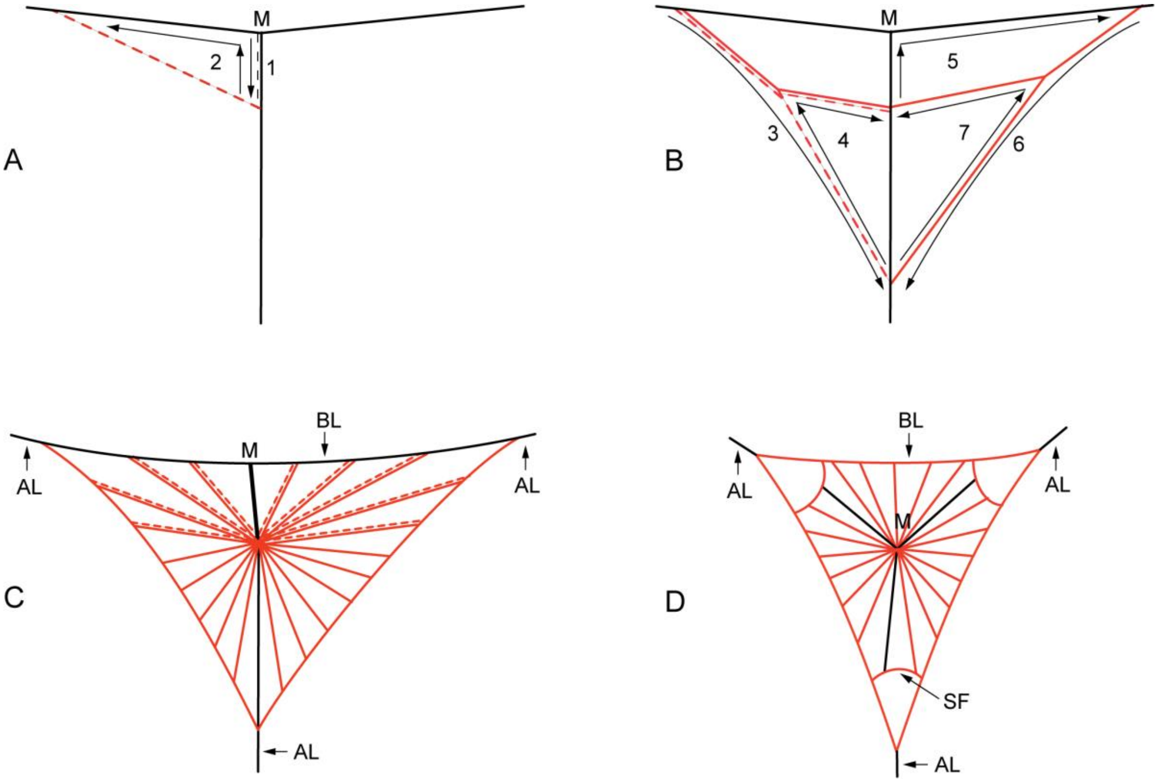
52. Wirth E, Barth FG (1992) Forces in the spider orb web. Journal of Comparative Physiology A 171: 359-371.

Figure Legends

Slika 5: Figure 1. *Caerostris darwini* webs (white arrows indicate bridge lines) suspended above water in Andasibe Mantadia NP with whole orbs (A) and only bridge lines (B) visible.



Slika 6: Figure 2. The typical (A) and the alternative (B) bridging behaviors in *C. darwini*.



Slika 7: Figure 3. Web building in *C. darwini* (A-C) versus the "classical" araneid web (D), shown without spirals. AL. Anchor line. BL. Bridge line. M. Connection point of the initial bridge line and vertical anchor line. SF. Secondary frame. Numbered arrows show building sequence of web frame. A dashed and a solid line in "C" indicate doubled radii.

Supporting Information Legends

Video S1, S2. *C. darwini* using bridging silk. Note the sail-like terminus of the bridging silk.

2.1.2 Darwinov drevesni pajek: Ogromen plen v ogromnih mrežah (*Caerostris darwini,* Araneae: Araneidae)?

Darwin's bark spider: Giant prey in giant orb webs (*Caerostris darwini*, Araneae: Araneidae)?

Matjaž Gregorič, Ingi Agnarsson, Todd A. Blackledge, Matjaž Kuntner

2011, Journal of Arachnology 39: 287-295.

Izvleček. Kljub izredni raznolikosti pajčjih kolesastih mrež je pri le nekaj skupinah prišlo do evolucije kolesastih mrež, ki so v premeru večje od 1 m. Do pred kratkim so bili primeri takšnega gigantizma mrež znani le pri nekaj predstavnikih družin Nephilidae in Araneidae, vendar nove študije na drevesnih pajkih (*Caerostris*) iz Madagaskarja poročajo o edinstvenem primeru gigantizma mrež: Darwinov drevesni pajek (*C. darwini*) razpne svoje mreže čez znatna vodna telesa, te mreže pa so narejene iz svile, ki po mehanskih lastnostih prekaša vse ostale pajčje svile. V članku raziskujemo arhitekturo mrež vrste *C. darwini* in začenjamo odgovarjati na dvoje zanimivih vprašanj: 1. Ali so mreže vrste *C. darwini* specializirane za lov specifičnega plena, morda celo vretenčarskega? 2. Ali te ogromne mreže morda izkoristijo množično pojavljanje vodnih insektov? Med terenskim delom na Madagaskarju smo raziskovali arhitekturo in ekologijo mrež vrste *C. darwini* ter zabeležili tudi interakcije s plenom. Tukaj karakteriziramo mrežo vrste *C. darwini* kot relativno preprosto, z majhnim številom žarkastih niti in redko lepljivo spiralo. Primerjamo tudi lastnosti mrež pri simpatričnih vrstah rodu *Caerostris*, med katerimi je pri *C. darwini* poznan najbolj ekstremen primer gigantizma mreže, kjer lovilni del mreže meri tudi do 2,76m^2 in je razpet čez reke do razdalje 25,5 m. Trenutni podatki kažejo, da so mreže *C. darwini* učinkovite pasti za vodne žuželke, npr. enodnevnice in kačje pastirje, medtem ko vretenčarskega plena v mrežah omenjene vrste nismo opazili. Predvidevamo, da množično pojavljanje vodnih žuželk za to vrsto lahko pomeni ulov, analogen velikemu, redkemu plenu, ki ga nedavne študije omenjajo kot ključnega za razmnoževanje pajkov mrežarjev.

Abstract. Although the diversity of spider orb web architectures is impressive, few lineages have evolved orb webs larger than 1m diameter. Until recently, such web gigantism was reported only in a few nephilids and araneids. However, new studies on bark spiders (*Caerostris*) of Madagascar report a unique case of web gigantism: Darwin's bark spider (*C. darwini*) casts its webs over substantial water bodies, and these webs are made from silk whose toughness outperforms all other known spider silks. Here, we investigate *C. darwini* web architecture and provide data to begin to answer two intriguing questions to explain these extraordinary web characteristics: 1. Are *C. darwini* webs specialized to subdue unusually large, perhaps even vertebrate prey? 2. Do these large, riverine webs allow the spiders to capitalize on catching numerous small semi-aquatic insects? During field work in Madagascar, we studied *C. darwini* web architecture and ecology, as well as interactions with prey. We characterize *C. darwini* webs as having relatively simple capture areas with very open sticky spirals and few radial lines. We also compare web features in several sympatric *Caerostris* species, among which *C. darwini* represents the most extreme case of web gigantism with the largest orbs up to 2.76 m^2 and longest bridge lines reaching 25.5 m. While preliminary, current data suggest that *C. darwini* webs are effective snares for semi-aquatic insects such as mayflies and dragonflies, while vertebrate prey were never observed. We suggest that mass emergence of aquatic insects may function analogously to the capture of rare, large prey that recent studies suggest are critical for reproduction in orb weaving spiders.

Keywords: Web gigantism–web architecture–web size–bridge line–kleptoparasite–silk–biomaterial–prey capture

Spider orb webs are highly efficient and specialized traps that have diversified greatly through time (Eberhard 1982; Coddington 1986a; Coddington & Levi 1991; Blackledge et al. 2009, 2011; Foelix 2010; Herberstein & Tso 2011). In addition to the classical “wagon-wheel” shaped orb itself, derived web forms include linyphiid sheetwebs and theridiid cobwebs (Griswold et al. 1998; Agnarsson 2004; Eberhard et al. 2008), uloborid and araneid sector webs (Wiehle 1927; Gregorič et al. 2010), nephilid and araneid ladder webs (Robinson & Robinson 1972; Eberhard 1975; Harmer & Framenau 2008; Kuntner et al. 2008a, b, 2010b; Harmer 2009), the deinopid casting web (Coddington 1986b) and others. Even for “standard” orb webs, the details of architecture and overall web size also vary substantially among taxa and include several instances of “web gigantism”. For example, webs within the family Nephilidae encompass extremes ranging from small arboricolous ladders in *Clitaetra* Simon 1889 to the dramatically elongated ladder webs of *Herennia* Thorell 1877 and *Nephilengys* Koch 1872, which often exceed a meter in height (Kuntner 2007; Kuntner et al. 2008a, b, 2010; Kuntner & Agnarsson 2009), and the giant aerial orbs of *Nephila* that reach 1.5 m diameter (Kuntner et al. 2008a). The largest known orb webs are built by the recently described Darwin’s bark spider (*Caerostris darwini*, Kuntner and Agnarsson 2010) from Madagascar. These spiders produce webs close to 2 m in diameter that are suspended upon the longest bridge lines ever recorded, allowing the webs to span rivers and small lakes (Agnarsson et al. 2010; Kuntner & Agnarsson 2010).

Bark spiders (genus *Caerostris* Thorell, 1868) are a diverse group, widespread in the old world tropics that are poorly studied taxonomically, ecologically and behaviorally (Kuntner & Agnarsson 2010). Grasshoff (1984) revised this genus, but its phylogenetic placement remains controversial (Scharff & Coddington 1997; Kuntner et al. 2008a;

Sensenig et al. 2010). Only 12 *Caerostris* species are currently considered valid (Platnick 2010; Kuntner & Agnarsson 2010).

The most recent studies on *Caerostris* of Madagascar hint at further, as yet undescribed diversity (Kuntner & Agnarsson 2010). Up to seven sympatric species inhabit a single reserve, the Andasibe-Mantadia National Park. Some of these species are nocturnal and others diurnal, but almost all of them construct sizeable webs at forest edges or clearings (Agnarsson et al. 2010; Kuntner & Agnarsson 2010). However, one species, the recently described Darwin's bark spider, *C. darwini* (Fig. 1) exhibits exceptional web biology and behavior in utilizing a unique habitat by building webs above streams, rivers and lakes (Fig. 2A-C; Kuntner & Agnarsson 2010). These webs can reach extreme sizes – suspended between vegetation on the river banks by bridge lines that often span more than 10 meters with the orbs frequently exceeding 1m in diameter (Kuntner & Agnarsson 2010). A second, apparently undescribed, *Caerostris* species in Andasibe-Mantadia NP also builds its webs over water, but only spanning relatively small streams inside closed canopy forest. Although other spiders build webs on edges of water bodies, or even attach webs to water (Eberhard 1990), individual spiders in no other species routinely utilize the air column above large streams, rivers and lakes as a habitat (Kuntner & Agnarsson 2010). How the spiders cross these large water bodies is only now being researched (Gregorič et al. in prep.), but *C. darwini* webs are constructed of silk that outperforms all other spider silks in combining high strength and elasticity into the toughest known biological material, outperforming even most synthetic fibers (Agnarsson et al. 2010).

Thus, it is certainly desirable to expand our understanding of *Caerostris* biology, in particular of key species such as *C. darwini*. In this paper, we broaden knowledge of *C. darwini* natural history by characterizing their webs, including a comparison with three congeners. We also begin to test whether or not their giant, riverine webs are specialized for capturing exceptional prey. The combination of web gigantism and the high material toughness of *C. darwini* silk, as well as the webs' location across rivers that could act as flyways, suggests that these webs could be specialized in part for capture of small flying vertebrates – birds or bats (Agnarsson et al. 2010; Kuntner & Agnarsson 2010). Both birds and bats are occasionally captured in the webs of several species of orb spiders (Levi 1970; Graham 1997; Peloso & de Sousa 2007; Sakai 2007; Timm & Losilla 2007). Sensenig et al. (2010) used web architecture and silk biomechanics to estimate that *C. darwini* webs could resist up to 62 $\mu J/cm^2$ of prey energy without breaking during impact, which approaches the flight energy of some birds and bats, the smallest of which are less than 2 g body mass and fly at speeds less than 5 m/s. Alternatively, or additionally, *C. darwini* webs could present large surface areas for the simultaneous capture of numerous aquatic insects, especially during mass emergences.

Methods

Field site.–We studied *C. darwini* and three other syntopic *Caerostris* species (labeled as "sp.1", "sp.2" and "sp.3") in Andasibe-Mantadia National Park (between S18.94760 E48.41972 and S18.79841 E48.42631 at roughly 960 m elevation), Toamasina Province, eastern Madagascar, between 24.ii.2010 and 4.iv.2010. Voucher specimens are deposited in the collections of the National Museum of Natural History, Smithsonian Institution.

Behavioral recording.–We video recorded and photographed prey capture behavior and web architecture using camcorders (Sony DCR-SR87 HDD) and SLR cameras (Canon EOS 5D Mark II and EOS 7D).

Web characteristics.–To measure how long *C. darwini* webs persisted, we monitored 20 webs of mature females for three days and documented web building time. We also quantified web parameters for an additional 26 mature females' webs illustrated in Fig. 3a-c as well as length of bridge line (Fig. B, C), number of radii, number of sticky spirals (SS) along the vertical axis, number of kleptoparasitic spiders associated with the web, stabilimentum (defined as absent (0), rudimentary (1) or robust (2) (Fig. 2D, E)), habitat (defined as above water (0) or not above water (1)), canopy (defined as open (0) or closed (1) if estimated that more than 50% of the canopy was covered by tree crowns). Most of the above parameters have been used previously in the literature (e.g. Risch 1977; Opell 1999; Herberstein & Tso 2000; Blackledge & Gillespie 2002; Kuntner et al. 2010; Kuntner & Agnarsson 2009; Gregorič et al. 2010; Nakata & Zschokke 2010).

To quantify web shapes, we then calculated indices following Peters (1937), Blackledge & Gillespie (2002) and Kuntner et al. (2008b): Web capture area (CA), was defined by the formula:

$$CA=(a/2)*(b/2)*\pi$$

where a and b were the horizontal and vertical diameters, respectively, of the capture area. Mesh width was defined as the number of rows of SS per centimeter of web height.

We calculated a ladder index (LI) or vertical eccentricity of capture area as:

$$LI = b/a.$$

We also determined vertical web asymmetry through hub displacement index (HD) as:

$$HD = (b-c)/b$$

where *c* was the distance from the center of the hub to the bottom of the capture area.

For an additional comparison of web size, we measured web width, height, top sticky spiral to hub distance, and bridge length in *Caerostris* “sp.1” (N=16), “sp.2” (N=22) and “sp.3” (N=5). We then compared our data with published data for web sizes of other orb weaving spiders, limiting ourselves to webs at least 0.1 m^2 (Table 1).

Prey capture.–To document prey capture, we recorded every wrapped or fed upon prey item in *C. darwini* webs. Additionally, we video recorded four spiders during daytime for four days each, for a total of 113 hours. Because the video resolution precluded exact determination of prey taxa, we grouped prey items into three size categories: small (<1 cm), medium (1-2.5 cm) and large (>3 cm).

Based upon an analysis of web architecture and silk biomechanics, Sensenig et al. (2010) estimated that *C. darwini* webs could stop higher energy flying prey than a phylogenetically diverse sampling of 16 other genera of orb spiders, including other large orb-weavers such as *Nephila* and *Argiope*. However, their estimate is a theoretical measure of maximum performance, which may not be attained by actual webs, and they also did not measure how effectively the webs could retain prey. To better determine the maximum prey size that *C. darwini* webs can stop and retain, and to document the spiders’ attack behavior, we introduced 34 medium and large prey items, each into a different web. Prey included five grasshoppers (2 cm), one large grasshopper (5 cm), one mantis (10 cm), two small frogs (2 cm), two moths (10 cm), two beetles (7 cm), 13 small dragonflies of different species (4-7 cm), and eight large (10 cm) dragonflies of one species. Prey were tossed into the capture areas of webs from a distance of ~ 0.5m, more or less with the same speed and more or less perpendicular to the web plane. Although the initial impact of prey with the web using this technique did not perfectly mimic natural interceptions and we could not control for how many threads each prey contacted, our method should still provide a reasonable index of the ability of *C. darwini* webs to stop and retain several different types of potential prey.

Statistical analysis.–We checked all data for normality using Kolmogorov-Smirnov tests. Because the distribution of data was not normal for bridge length and web area, we report medians ($\mu_{1/2}$) ± interquartile ranges for these parameters, while we report mean values (A) ± standard deviations for other data. We tested interspecific differences in web measures using the Kruskall-Wallis test and Mann-Whitney U-tests. We set the significance level to 0.008 or lower (Bonferroni correction). We performed all analyses in PASW 18 for Windows (Field 2005).

Results

Web characteristics.–*Caerostris darwini* orbs ranged in size from 0.21 m^2 to 2.76 m^2 ($\mu_{1/2}$=0.61±0.52 m^2). Capture area did not significantly differ from those of *Caerostris* sp.1 and *Caerostris* sp.2 ($\mu_{1/2}$=0.48±0.21 m^2 and $\mu_{1/2}$=0.5±0.39 m^2, respectively), whereas *Caerostris* sp.3 had a significantly smaller capture area ($\mu_{1/2}$=0.16±0.1 m^2; Fig. 4). Bridge lines of *C. darwini* orbs were 0.95-25.5 m ($\mu_{1/2}$=3.5±2.6 m), significantly longer than those of other *Caerostris* species ($\mu_{1/2}$=1.7±1.1 m in sp.1; 1.8±1.2 m in sp.2 and 1.4±0.67 m in sp.3; Fig. 4). The webs of *C. darwini* contained 15-30 radii (A=23.5±4), no split radii, 40-155 vertical SS (A=97±25) and mesh widths of 0.51-1.7 SS/cm (A=1.04±0.33). All webs lacked secondary radii (Kuntner et al. 2008a) and only two (8%) webs had stabilimenta (both "rudimentary"). The webs were almost symmetric with the ladder index of 0.78-1.49 (A=1.19±0.18) and hub displacement of 0.47-0.69 (A=0.59±0.05). Most (63%) webs did not contain kleptoparasitic spiders. Four (21%) webs contained one or two kleptoparasites (all Argyrodinae), whereas only three (16%) webs contained more.

All webs were suspended above or at the edges of water bodies and always under open canopy (Fig. 2A-C). The webs never had retreats and the spiders sat at the hub during all weather conditions (not removing SS during rain), day and night. Our monitoring of 20 webs over three days revealed that the webs were not long lasting. In two of these 20 webs, the host spider was absent for the whole time, and two more spiders disappeared during observation. The other 15 spiders renewed their webs two to four times in three days. Web renewal usually (74%) took place between 1600 and 1800 hr. However, throughout our field work we observed numerous webs being constructed at the same location, suspended on bridge lines that were clearly retained for several days. We thus estimated that bridge lines can be maintained for at least five weeks. We never observed web destruction by flying vertebrates or large insects, but regularly observed dragonflies avoiding the webs and even perching on bridge lines.

Prey capture.–We haphazardly encountered 25 prey items during web surveys: two (8%) honey bees, three small beetles (12%), one wasp (4%), one grasshopper (4%), two damselflies (8%), one fly (4%), one (4%) queen ant, one (4%) butterfly, one (4%) big unidentified prey item and four (16%) dragonflies. The 113 hours of video material revealed the capture of 50 (79.4%) small, 12 (19%) medium and one (1.6%) large prey item (a papilionid butterfly). Altogether, large prey items constituted 6.8% of all caught prey: 20% in haphazardly encountered prey and 1.6% in video material, but this disparity is expected because active feeding on small, quickly consumed insects is less likely to being haphazardly observed. Video material also revealed one case of kleptoparasitic flies in *C. darwini* (*Caerostris* supplementary video 3 (http://www.nephilidae.com/videos/videos.htm)).

During our prey presentations (*Caerostris* supplementary video 1, 2 (http://www.nephilidae.com/videos/videos.htm)), frogs and large insects (i.e. large beetles and moths) were not retained in webs. The webs retained all five 2 cm, but not the 5 cm grasshopper. Out of the 21 dragonflies introduced, the webs retained all 13 “regular sized” specimens, but only four of the eight “large” ones.

All spiders performed bite-wrap attack behavior (*Caerostris* supplementary video 1 (http://www.nephilidae.com/videos/videos.htm); Eberhard 1982). The spiders carried all prey, except the largest dragonflies, back to the hub in their chelicerae (*Caerostris* supplementary video 1 (http://www.nephilidae.com/videos/videos.htm)), while the large dragonflies were lifted to the hub on a silk thread while they were still attached to other parts of web (*Caerostris* supplementary video 2 (http://www.nephilidae.com/videos/videos.htm)).

Discussion

The present study shows that *Caerostris darwini* build the longest bridge lines and largest orb webs known, with exceptional webs bridging water bodies more than 25 m across and capture areas reaching 2.76 m^2 (Table 1, 2; Fig. 4). *C. darwini* webs contain relatively sparsely spaced capture spirals and are almost symmetrical (Sensenig et al. 2010). Webs are always suspended above or next to water and their capture areas are renewed daily. In contrast, bridge lines are maintained and reinforced regularly, for up to five weeks. We found no evidence that these giant webs are an adaptation for capturing flying vertebrates, such as birds or bats. First, no such large prey items were caught in the web, although the sample size in this study is small enough that such rare events could have been missed (Blackledge 2011). Second, numerous visually acute flying insects, such as dragonflies, were seen avoiding the webs throughout the field study. While dragonflies are exceptionally maneuverable fliers (Alexander 1984, 1986; Azuma et al. 1988; Thomas et al. 2004), this observation suggests that flying vertebrates might also perceive and avoid the webs. On the other hand, orb spiders can still capture substantial numbers of insects in taxa that see and avoid spider webs (Craig 1994; Rao et al. 2008). Third, the relatively sparse packing of silk in the webs, which have significantly larger mesh widths than similarly sized orb weavers (Sensenig et al. 2010), necessarily limit their stopping and retention power. Instead, along with our previous study, we found that these webs subdue small to large flying insects, such as mayflies (Kuntner & Agnarsson 2010) and dragonflies (this study).

Web characteristics.–Although the maximal values of web size are statistical outliers (Fig. 4) in our study, we believe it likely that webs are often even larger in nature. Many *C. darwini* webs are suspended far from the shores of rivers and lakes or high in the air column such that we simply could not measure them. These open, aerial microhabitats are less spatially limited, compared to the more easily accessible space directly above the water surface that we studied. Thus, *C. darwini* may be capable of building larger webs than we measured.

With the exception of their extreme size and unusual microhabitat, *C. darwini* webs resemble typical araneid orbs in many respects (Zschokke 2002; Kuntner et al. 2008a; Kuntner & Agnarsson 2010). They are more or less vertical, almost symmetric, have closed hubs, the non-sticky spirals are removed from finished webs, they have gradual hub-loop to sticky spiral transitions, have few radii and SS compared to other orb weavers (Sensenig et al. 2010), have no split radii, lack retreats, and rarely contain stabilimenta (Eberhard 1982). Kuntner & Agnarsson (2010) reported the hub as open or closed, however, this is incorrect. We only observed the typically araneid closed hub and the complete lack of the hub bite-out behavior (Gregorič et al. in prep.). Kuntner & Agnarsson (2010) speculated that the webs last longer than typical araneid orbs, but did not have long-term observations of individual webs over several days. In fact, only the bridge lines are long lasting, while the capture areas are renewed daily as in most other orb weavers (Foelix 1996; Carico 1986). The web building behaviors of *C. darwini* depart from typical araneids (Eberhard 1982; Kuntner 2008a). *C. darwini* exhibit minimal web site exploration and build webs that lack secondary frame threads. The relatively simple capture areas contain very open sticky spirals, supported by few radii that are both single and doubled in the same web. Detailed comparison of web spinning behaviors will be fully summarized elsewhere (Gregorič et al. in prep.)

Attack behavior.–*Caerostris darwini* attacks all prey by first biting and then wrapping them. Typical araneid, tetragnathid and uloborid attack behavior is wrap-biting (Eberhard 1982), which probably evolved six to seven times within orbicularian spiders (Kuntner et al. 2008a). In contrast, bite-wrapping is probably plesiomorphic for a larger clade of orb spiders (Kuntner et al. 2008a), and is utilized by nephilids, some araneids such as *Deliochus*, *Phonognatha*, *Caerostris* (Kuntner et al. 2008a) and *Zygiella* s.l. (Gregorič et al. 2010). *Caerostris darwini* uses bite-wrapping regardless of prey size, whereas many wrap-biting spiders occasionally bite-wrap in response to different taxa of prey (Robinson & Robinson 1974; Foelix 1996). *Caerostris darwini* also uses a relatively unusual behavior for transporting subdued prey back to the hub. Instead of freeing large prey from the web and hanging them on a short thread (Foelix 1996), *C. darwini* carries even large prey back to hub in their chelicerae (*Caerostris* supplementary video 1 (http://www.nephilidae.com/videos/videos.htm)). Only the largest dragonflies were not carried using this behavior, but instead were lifted towards the hub using a longer silk thread, while the prey was still attached to other parts of web. On the other hand, this observation and the fact that many orb spider species directly carry small prey, suggest that it is the large prey size threshold at which *C. darwini* switches from the carrying behavior that is unusual, rather than the behavior itself.

Prey capture.–Our prey tossing experiments found that dragonflies were the largest prey retained by *C. darwini* webs, with larger insects and frogs always breaking through the webs. By far the most commonly observed prey entangled in webs were small insects, with larger (>3cm) prey found only at relatively low frequencies (~7%). However, the disparity between large prey encountered haphazardly (20%) and by video material (1.6%) is expected because active feeding on small, quickly consumed insects is less likely to being haphazardly observed. Although we never observed exceptionally large prey in *C. darwini* webs, such rare large prey may be fundamentally important for female fecundity in most orb spiders, even though the rarity of their capture makes them difficult to observe in field studies (Venner & Casas 2005; see Blackledge 2011, this volume for review). Therefore, more sampling effort is clearly needed to thoroughly exclude the hypothesis that the unusual size and placement of *C. darwini* webs facilitate the capture of exceptionally large prey.

The rare, large prey hypothesis is particularly tempting given the exceptional toughness of the silk in *C. darwini* webs (Agnarsson et al. 2010). However, orb spiders face functional tradeoffs between making relatively sparse webs with large capture areas to maximize interception of prey, versus building smaller and denser webs capable of stopping and retaining bigger prey (Chacon & Eberhard 1980; Eberhard 1986; Blackledge & Zevenbergen 2006; Blackledge & Eliason 2007; Sensenig et al. 2010). *Caerostris darwini* web architecture is unusually open, with fewer radii and larger mesh width than other large orb webs (Sensenig et al. 2010). Thus, the webs may instead function to maximize capture surface for large numbers of small aquatic insects, such as the mass capture of mayflies observed in *C. darwini* webs by Kuntner & Agnarsson (2010). Such mass captures could even function analogously to rare, large prey in the rarity of their occurrence and their importance for foraging success. However, no mass capture of insects was observed in this study and might be as difficult to observe during field studies as the capture of individual large prey (Blackledge 2011). Alternatively, the large, sparse capture areas of *C. darwini* webs combined with their unusually tough silk, may represent a compromise towards subduing large numbers of small aquatic insects while still maintaining the ability to capture rare larger prey. Such a “multifunctional” web would contrast with the behavior of at least one other orb weaver, *Parawixia*, that instead dramatically enlarges capture areas of webs only during mass emergences of large, easily captured reproductive termite prey (Sandoval 1994). The evolution of the extreme silk toughness in *C. darwini* may facilitate this potentially dual function of the large sparse web, making it critical to better determine patterns of evolution for both silk properties and web architecture within the genus *Caerostris*.

Conclusions.–*Caerostris darwini* exhibits several aspects of unusual web biology that allow the spiders to spin giant orbs suspended in the air column above bodies of water, thereby exploiting a unique ecological niche. Prey capture data suggest that *C. darwini* does not prey on flying vertebrates, but instead mostly consumes medium to large flying insects. We speculate that the mass emergence of aquatic insects may function analogously for spider fitness to the capture of single rare, large prey in other orb spiders. However, with the currently available data we cannot rule out alternative hypotheses for the origin of the exceptional properties of *C. darwini* silk, such as adaptation to carry the spiders' weight on long, sagging bridge lines (Rodriguez-Girones et al. 2010) or resisting abiotic factors such as wind and rain (Eberhard 1990).

Acknowledgments

This is contribution one resulting from the 2010 Madagascar expedition, funded by the National Geographic Society (grant 8655-09 to I. Agnarsson, M. Kuntner and T. Blackledge). Additional funding came from the Slovenian Research Agency (grants P1-0236, J1-2063) and NSF (IOS-0745379). We thank the MICET crew in Antananarivo, and Sahondra Lalao Rahanitriniaina and Honore Rabarison for their help in the field We also thank Dinesh Rao, William Eberhard, Sean Blamires and Wolfgang Nentwig for their help in getting web size data for other spider species.

Literature Cited

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., M. Kuntner & T.A. Blackledge. 2010. Bioprospecting finds the toughest biological material: extraordinary silk from a giant riverine orb spider. PLoS ONE 5(9):e11234.

Alexander, D.E. 1984. Unusual phase relationships between the forewings and hindwings in flying dragonflies. The Journal of Experimental Biology 109:379-383.

Alexander, D.E. 1986. Wind tunnel studies of turns by flying dragonflies. The Journal of Experimental Biology 122:81-98.

Azuma, A. & T. Watanabe. 1988. Flight performance of a dragonfly. The Journal of Experimental Biology 137:221-252.

Blackledge, T.A. 2011. Prey capture in orb weaving spiders: Are we using the best metric? Journal of Arachnology 39:000-000.

Blackledge, T.A., M. Kuntner & I. Agnarsson. 2011. The form and function of spider orb webs: evolution from silk to ecosystems. Advances in Insect Physiology 00:000-000.

Blackledge, T.A., J. Coddington, N. Scharff, T. Szuts, J. Wenzel, C. Hayashi, & I. Agnarsson. 2009. Reconstructing web evolution and spider diversification in the molecular era. Proceedings of the National Academy of Sciences of the United States of America 106:5229-5234.

Blackledge, T.A. & C.M. Eliason. 2007. Functionally independent components of prey capture are architecturally constrained in spider orb webs. Biology Letters 3:456-458.

Blackledge, T.A. & R.G. Gillespie. 2002. Estimation of capture areas of spider orb webs in relation to asymmetry. Journal of Arachnology 30:70-77.

Blackledge, T.A. & J.M. Zevenbergen. 2006. Mesh width influences prey retention in spider orb webs. Ethology 112:1194-1201.

Blamires, S.J., M.B. Thompson & D.F. Hochuli. 2007. Habitat selection and web plasticity by the orb spider *Argiope keyserlingi* (Argiopidae): Do they compromise foraging success for predator avoidance? Austral Ecology 32:551-563.

Carico, J.E. 1986. Web removal patterns in orb-weaving spiders. Pp. 306-318. *In*: Spiders: webs, behavior, and evolution (W.A. Shear, ed.).Stanford University Press, Stanford, California.

Chacón P., W.G. Eberhard. 1980. Factors affecting numbers and kinds of prey caught in artificial spider webs, with considerations of how orb webs trap prey. Bulletin of the British Arachnological Society 5:29-38.

Coddington, J.A. 1986a. The monophyletic origin of the orb web. Pp. 319-363. *In*: Spiders: webs, behavior, and evolution (W.A. Shear, ed.). Stanford University Press, Stanford, California.

Coddington, J.A. 1986b. Orb webs in ‘non-orb weaving’ ogrefaced spiders (Araneae: Deinopidae): a question of genealogy. Cladistics 2:53-67.

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

Craig, C.L. 1994. Predator foraging behavior in response to perception and learning by its prey: interactions between orb-spinning spiders and stingless bees. Behavioral Ecology and Sociobiology 35:45-52.

Eberhard, W.G. 1975. Inverted ladder orb web of *Scoloderus* sp. and intermediate orb of *Eustala* sp. Araneae – Araneidae. Journal of Natural History 9:93-106.

Eberhard, W.G. 1982. Behavioural characters for the higher classification of orb-weaving spiders. Evolution 36:1067-1095.

Eberhard, W.G. 1986. Effects of orb-web geometry on prey interception and retention. Pp. 70-100. *In*: Spiders: webs, behavior, and evolution (W.A. Shear, ed.). Stanford University Press, Stanford, California.

Eberhard, W.G. 1989. Effects of orb web orientation and spider size on prey retention. Bulletin of the British Arachnological Society 8:45-48.

Eberhard, W.G. 1990. Function and phylogeny of spider webs. Annual Review of Ecology and Systematics 21:341-372.

Eberhard, W.G., I. Agnarsson & H.W. Levi. 2008. Web forms and phylogeny of theridiid spiders (Araneae: Theridiidae): chaos from order? Systematics and Biodiversity 6:415-475.

Field, A. 2005. Discovering Statistics Using SPSS, 2nd edition. Sage Publications, London, UK. 816 pp.

Foelix, R.F. 1996. Biology of Spiders, 2nd edition. Oxford University Press, Oxford, UK. 336 pp.

Foelix, R.F. 2010. Biology of Spiders, 3rd edition. Oxford University Press, Oxford, UK. 432 pp.

Gillespie, R.G. & T. Caraco. 1987. Risk-sensitive foraging strategies of two spider populations. Ecology 68:887-899.

Graham, D.L. 1997. Spider webs and windows as potentially important sources of hummingbird mortality. Journal of Field Ornithology 68:98-101.

Grasshoff, M. 1984. Die Radnetzspinnen-Gattung *Caerostris* (Arachnida: Araneae). Revue Zoologique Africaine 98:725-765.

Gregorič, M., R. Kostanjšek & M. Kuntner. 2010. Orb web features as taxonomic characters in *Zygiella* s.l. (Araneae, Araneidae). Journal of Arachnology 38:319-327.

Griswold, C.E., J.A. Coddington, G. Hormiga & N. Scharff. 1998. Phylogeny of the orb-web building spiders (Araneae, Orbiculariae: Deinopoidea, Araneoidea). Zoological Journal of the Linnean Society 123:1-99.

Harmer, A.M.T. 2009. Elongated orb-webs of Australian ladder-web spiders (Araneidae: *Telaprocera*) and the significance of orb-web elongation. Journal of Ethology 27:453-460.

Harmer, A.M.T. & V.W. Framenau. 2008. *Telaprocera* (Araneae: Araneidae), a new genus of Australian orb-web spiders with highly elongated webs. Zootaxa 59-80.

Herberstein, M.E. & I.M. Tso. 2000. Evaluation of formulae to estimate the capture area and mesh height of orb webs (Araneoidea, Araneae). Journal of Arachnology 28:180-184.

Herberstein, M.E. & I.M. Tso. 2011. Spider webs: evolution, diversity and plasticity. Pp. 57-98. *In*: Spider Behaviour: Flexibility and Versatility (M.E. Herberstein, ed.). Cambridge University Press.

Jäger, P. 2007. Spiders from Laos with descriptions of new species (Arachnida: Araneae). Acta Arachnologica 56:29-58.

Knoflach, B. & A. Van Harten. 2000. Palpal loss, single palp copulation and obligatory mate consumption in *Tidarren cuneolatum* (Tullgren, 1910) (Araneae, Theridiidae). Journal of Natural History 34:1639-1659.

Kuntner, M. 2005. A revision of *Herennia* (Araneae : Nephilidae : Nephilinae), the Australasian 'coin spiders'. Invertebrate Systematics 19:391-436.

Kuntner, M. 2007. A monograph of *Nephilengys*, the pantropical ‘hermit spiders’ (Araneae, Nephilidae, Nephilinae). Systematic Entomology 32:95-135.

Kuntner, M & I. Agnarsson. 2009. Phylogeny accurately predicts behaviour in Indian Ocean *Clitaetra* spiders (Araneae : Nephilidae). Invertebrate Systematics 23:193-204.

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., J.A. Coddington & G. Hormiga. 2008a. Phylogeny of the extant nephilid orb-weaving spiders (Araneae, Nephilidae) - testing morphological and ethological homologies. Cladistics 24:147-217.

Kuntner, M., C.R. Haddad, G. Aljančič & A. Blejec. 2008b. Ecology and web allometry of *Clitaetra irenae*, an arboricolous African orbweaving spider (Araneae, Araneoidea, Nephilidae). Journal of Arachnology 36:147-217.

Kuntner, M., I. Agnarsson & M. Gregorič. 2009a. Nephilid spider eunuch phenomenon induced by female or rival male aggressiveness. Journal of Arachnology 37:266-271.

Kuntner, M., J.A. Coddington & J.M. Schneider. 2009b. Intersexual arms race? Genital coevolution in nephilid spiders (Araneae, Nephilidae). Evolution 63:1451-1463.

Kuntner, M., S. Kralj-Fišer, J.M. Schneider & D. Li. 2009c. Mate plugging via genital mutilation in nephilid spiders: an evolutionary hypothesis. Journal of Zoology 277:257-266.

Kuntner, M., M. Gregorič & D. Li. 2010a. Mass predicts web asymmetry in *Nephila* spiders. Naturwissenschaften 97:1097-1105.

Kuntner, M., S. Kralj-Fišer & M. Gregorič. 2010b. Ladder webs in orb-web spiders: ontogenetic and evolutionary patterns in Nephilidae. Biological Journal of the Linnean Society 99:849-866.

Levi, H.W. 1970. The *ravilla* group of the orbweaver genus *Eriophora* in North America (Araneae: Araneidae). Psyche 77:280-302.

Nakata, K. & S. Zschokke. 2010. Upside-down spiders build upside-down orb webs: web asymmetry, spider orientation and running speed in Cyclosa. Proceedings of the Royal Society B 277:3019-3025.

Nentwig, W. 1985. Prey analysis of four species of tropical orb-weaving spiders (Araneae: Araneidae) and a comparison with araneids of the temperate zone. Oecologia 66:580-594.

Opell, B.D. 1999. Redesigning spider webs: Stickiness, capture area and the evolution of modern orb-webs. Evolutionary Ecology Research 1:503–516.

Peloso, P.L. & V.P. de Sousa. 2007. Predation on *Todirostrum cinereum* (Tyrannidae) by the orb-web spider *Nephilengys cruentata* (Araneae, Nephilidae). Revista Brasileira de Ornitologia 15:461-463.

Peters, H. 1937. Studien am netz der kreuzspinne (*Aranea diadema*.). 1. Die Grundstruktur des Netzes und Beziehungen zum Bauplan des Spinnenkorpers. Morphologie und Ökologie der Tiere 33:128-150.

Platnick, N.I. 2010. The World Spider Catalog, Version 11.0. The American Museum of Natural History. Online at http://research.amnh.org/entomology/spiders/catalog/

Rao, D., K. Cheng & M.E. Herberstein. 2008. Stingless bee response to spider webs is dependent on the context of encounter. Behavioral Ecology and Sociobiology 63:209–216.

Rao, D., M. Webster, A.M. Heiling, M.J. Bruce & M.E. Herberstein. 2009. The aggregating behaviour of *Argiope radon*, with special reference to web decorations. Journal of Ethology 27:35-42.

Risch, P. 1977. Quantitative analysis of orb web patterns in four species of spiders. Behavior Genetics 7:199-238.

Robinson, M.H. & B. Robinson. 1972. The structure, possible function and origin of the remarkable ladder-web built by a New-Guinea orbweb spider (Araneae: Araneidae). Journal of Natural History 6:687–694.

Robinson, M.H. & B. Robinson. 1974. The biology of some *Argiope* species from New Guinea: predatory behavior and stabilimentum construction (Araneae: Araneidae). Zoological Journal of the Linnean Society 54:145-159.

Robinson, M.H. & B. Robinson. 1980. Comparative studies of the courtship and mating behavior of tropical araneid spiders. Pacific Insects 36:35-218.

Rodriguez-Girones, M.A., C. Guadalupe & J. Moya-Larano. 2010. Silk elasticity as a potential constraint on spider body size. Journal of Theoretical Biology 266:430-435.

Sakai, W.H. 2007. Long-billed hermit (*Phaethornis superciliosus*) caught in golden orb-spider (*Nephilia clavipes*) web. Ornitologia Neotropical 18:117–119.

Sandoval, C.P. 1994. Plasticity in web design in the spider *Parawixia bistriata*: A response to variable prey type. Functional Ecology 8:701-707.

Sensenig, A, I. Agnarsson & T.A. Blackledge. 2010. Behavioural and biomaterial coevolution in spider orb webs. Journal of Evolutionary Biology 23:1839-1856.

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.

Sherman, P.M. 1994. The orb web—an energetic and behavioral estimator of a spiders dynamic foraging and reproductive strategies. Animal Behaviour 48:19-34.

Thomas, A.L.R., K.G. Taylor, R.B. Srygley, R.L. Nudds & R.J. Bomphrey. 2004. Dragonfly flight: free-flight and tethered flow visualizations reveal a diverse array of unsteady lift-generating mechanisms, controlled primarily via angle of attack. The Journal of Experimental Biology 207:4299-4323.

Timm, R.M. & M. Losilla. 2007. Orb-weaving spider, *Argiope savignyi* (Araneidae), predation on the proboscis bat *Rhynchonycteris naso* (Emballonuridae). Caribbean Journal of Science 43:282-284.

Uhl, G. & F. Vollrath. 1998. Little evidence for size-selective sexual cannibalism in two species of *Nephila* (Araneae). Zoology 101:101-106.

Venner, S. & J. Casas. 2005. Spider webs designed for rare but life-saving catches. Proceedings of the Royal Society B 272:1587-1592.

Wiehle, H. 1927. Beiträge zur Kenntnis des Radnetzbaues der Epeiriden, Tetragnathiden und Uloboriden. Zeitschrift für Morphologie und Ökologie der Tiere 8:468-537.

Zschokke, S. 2002. Form and function of the orb-web. Pp. 99-106. *In*: European Arachnology 2000 (Toft, S. & N. Scharff, eds). Aarhus University Press, Aarhus.

Tables

Tabela 1: Table 1: Comparison of web size (if larger than 0.1 m^2) among orb web spiders, measured for adult and penultimate female webs.

Species	**Web area (m^2)**	**Max. web area (m^2)**	**Author**
Meta merianae		0.28	Wiehle 1927
Argiope katherina (N=24)	0.13	0.22	Rao pers. comm.
Herennia etruscila (N = 2)	0.13	0.14	Kuntner et al. 2010b
Talthybia depressa (N = 1)	0.21	0.21	own data
Eriowixia laglaizei (N = 1)	0.32	0.32	own data
Nephilengys dodo (N = 2)	0.44	0.56	own data
Caerostris sumatrana (N = 1)	0.62	0.62	own data
Herennia multipuncta (N = 6)	0.11 ± 0.2	0.29	Kuntner et al. 2010b
Nephila inaurata (N = 23)	0.12 ± 0.28	1.15	own data
Argiope radon (N = 103)	0.122 ± 0.055	0.49	Rao et al. 2009, pers. comm.
Argiope argentata (N = 762)	0.13 ± 0.045		Nentwig 1985
Araneus angulatus	0.13-.031	0.31	Wiehle 1929
Araneus circe	0.13-0.28	0.28	Wiehle 1928, 1931
Nephila clavipes (N = 32)	0.15 ± 0.07	0.35	own data
Caerostris sp. 3 (N = 5)	0.16 ± 0.1	0.35	this study
Nephilengys borbonica (N = 4)	0.19 ± 0.09	0.23	own data
Nephila ardentipes (N = 24)	0.19 ± 0.18	0.65	own data
Nephilengys malabarensis (N = 7)	0.23 ± 0.16	0.41	Kuntner et al. 2010b
Eriophora sp. (N = 20)	0.24 ± 0.18	0.69	own data
Nephila pilipes (N = 30)	0.28 ± 0.15	0.63	Kuntner et al. 2010a, own data
Caerostris darwini (N = 16)	0.28 ± 0.47	1.07	Kuntner & Agnarsson 2010
Argiope keyserlingi (N = 273)	0.3 ± 0.14		Blamires et al. 2007
Nephilengys livida (N = 29)	0.33 ± 0.23	0.77	own data
Eriophora fuliginea (N = 349)	0.36 ± 0.11		Nentwig 1985

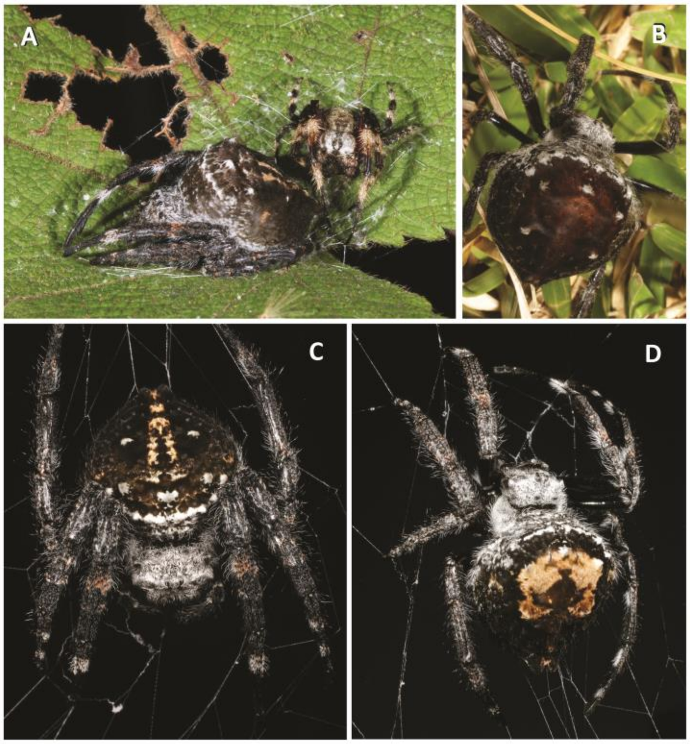
Nephila clavipes (N = 1072)	0.36 ± 0.11		Nentwig 1985
Caerostris sp. 1 (N = 16)	0.48 ± 0.21	0.86	this study
Caerostris sp. 2 (N = 22)	0.5 ± 0.39	1.1	this study
Caerostris darwini (N = 26)	0.61 ± 0.52	2.76	this study

Tabela 2: Table 2: Web data for *Caerostris darwini* females in Madagascar. SS=sticky spirals.

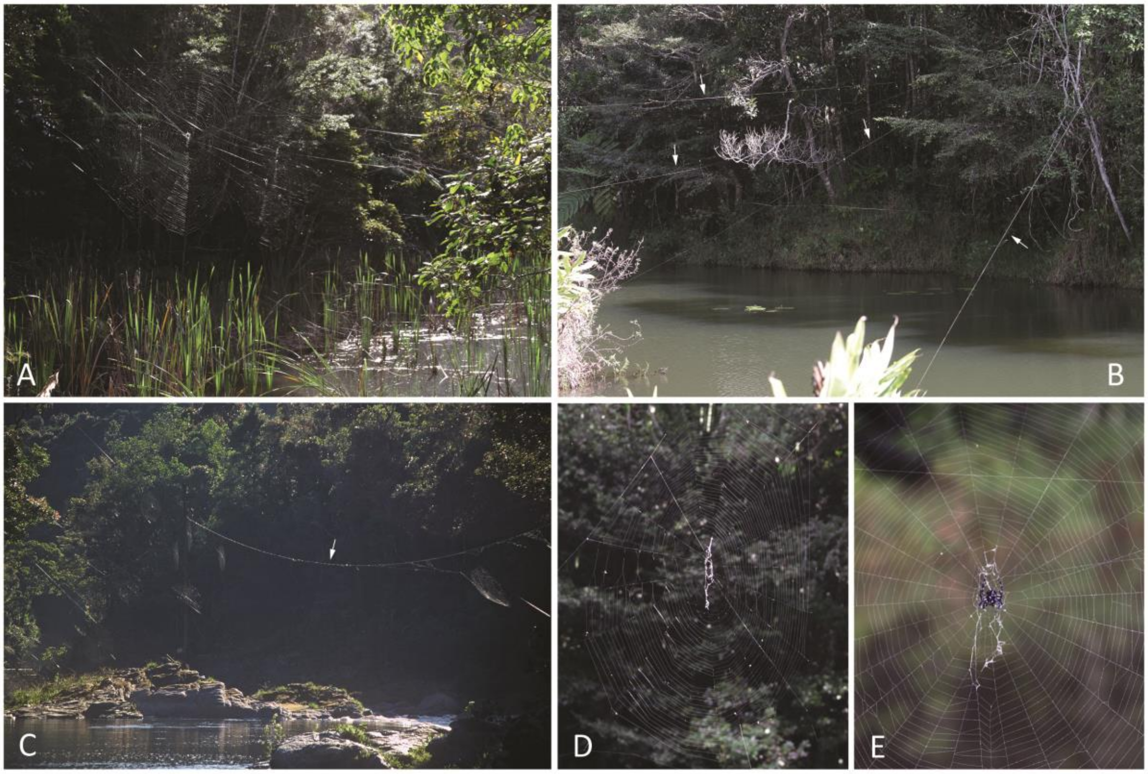
Web are (m^2)	**Bridge length (cm)**	**Radii no.**	**SS no.**	**Kleptoparasite no.**	**Mesh width (SS/cm)**	**Ladder Index**	**Hub displacement**
0.21	130	18	40	0	0.70	1.21	0.65
0.23	265	21	77	0	1.24	1.32	0.55
0.25	275	20	88	0	1.47	1.15	0.63
0.26	210	24	104	0	1.70	1.11	0.57
0.28	330	29	89	0	1.35	1.22	0.53
0.31	95		0			1.19	0.68
0.41	320	23	121	0	1.57	1.13	0.60
0.41	180		53	4	0.71	1.07	0.69
0.41	400		0			1.07	0.57
0.46	380	28	110	0	1.33	1.17	0.58
0.49	164	30	82	7	1.17	0.78	0.64
0.53	215		0			1.13	0.57
0.61	350		83	0	0.91	1.06	0.47
0.62	275	20	110	0	1.12	1.21	0.59
0.64	300	29	126	6	1.22	1.30	0.58
0.70	500	25	112	1	1.15	1.05	0.61
0.76	450	24	103	2	1.03	1.03	0.59

	0.82	670		108	0	0.90	1.38	0.50
	0.86	350	27	91	0	0.73	1.41	0.60
	0.90	430	25	94	0	0.76	1.35	0.60
	1.15	540	23	92	1	0.70	1.19	0.66
	1.19	750		117	1	0.78	1.49	0.60
	1.21	640		83		0.51	1.71	0.58
	1.36	400		0			1.02	0.50
	2.76	550	25	155		0.79	1.08	0.58
		2550						
Mean	0.61±0.52	350±260	23.5±4	97±25		1.04±0.33	1.19±0.18	0.59±0.05
Sp.1 mean	0.48±0.21	170±109						
Sp.2 mean	0.5±0.39	177±122						
Sp.3 mean	0.16±0.1	142±66.5						

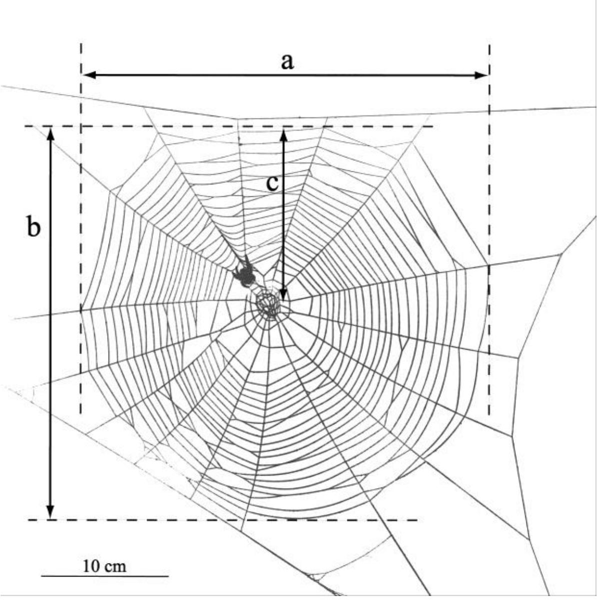
Figure Legends



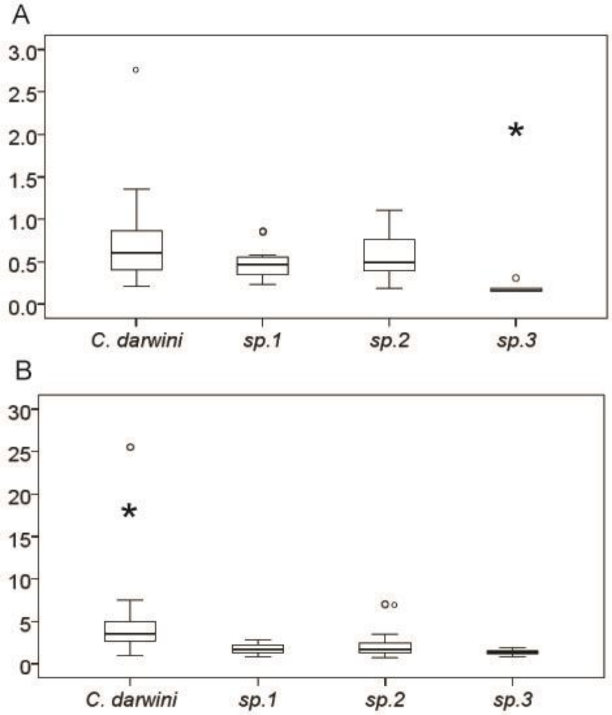
Slika 8: Figure 1: *Caerostris darwini* in Andasibe-Mantadia NP: A, male with female in vegetation; B-D, females with typical color (C) and two less common color forms (B, D).



Slika 9: Figure 2: *Caerostris darwini* habitat and prey: webs suspended above water in Ranomafana NP (C) and Andasibe-Mantadia NP with whole orbs (A) and only bridge threads (B) visible; *C. darwini* female with a robust (D) and a rudimentary (E) stabilimentum in web.



Slika 10: Figure 3: Web of female *Caerostris darwini* illustrating investigated parameters: web width (a), web height (b) and top sticky spiral to hub distance (c).



Slika 11: Figure 4: Web size in *Caerostris darwini* and three congeneric species. A. Web capture area (m^2). B. Bridge thread length (m). Asterisk marks the species that significantly differ from the others.

Electronic Supplementary Material

Caerostris supplementary video 1 (http://www.nephilidae.com/videos/videos.htm): Dragonfly introduced into subadult female *C. darwini* web. Note bite-wrap attack behavior and spider carrying the prey to hub in its chelicerae.

Caerostris supplementary video 2 (http://www.nephilidae.com/videos/videos.htm): Dragonfly introduced into female *C. darwini* web. Note spider lifting the prey towards hub while the prey is still attached to other web parts.

Caerostris supplementary video 3 (http://www.nephilidae.com/videos/videos.htm): Kleptoparasitic flies approaching female *C. darwini* and her prey.

2.1.3 Optimalno prehranjevanje, ne biogenetsko pravilo, napove alometrijo pajčjih kolesastih mrež

Optimal foraging, not biogenetic law, predicts spider orb web allometry

Matjaž Gregorič, Heine C. Kiesbüy, Shakira G. Quiñones Lebrón, Alenka Rozman, Ingi Agnarsson, Matjaž Kuntner

2013, Naturwissenschaften 100: 263-268

Izvleček

Biognetsko pravilo predvideva, da ontogenija organizma sledi vzorcem evolucijskih sprememb v sorodstveni liniji tega organizma. Nekateri morfološki dokazi to hipotezo podpirajo, vendar jo precej dokazov tudi zavrača. Vseeno ostaja biogenetsko pravilo na nivoju vedenja slabo raziskano. Pajčje mreže so idealen objekt raziskav ontogenije vedenja, saj predstavljajo fizični odraz vedenja. Pri pajkih mrežarjih se tekom ontogenetskega razvoja simetrija njihovih mrež pogosto spreminja, nekateri avtorji pa menijo, da takšne spremembe odsevajo biogenetsko pravilo. V članku smo raziskali alometrijo mrež pri vrsti *Leucauge venusta* in tako preverjali, ali opaženi vzorci ustrezajo napovedim na podlagi biogenetskega pravila ali napovedim alternativne hipoteze optimizacije plenjenja. Vrsta *L. venusta* gradi mreže, ki se v naklonu raztezajo od vodoravnih, preko poševnih, do navpičnih; biogenetsko pravilo predvideva, da alometrija ustreza ontogenetskemu stadiju, medtem ko hipoteza optimizacije plenjenja predvideva, da alometrija sledi učinkom gravitacije. Natančneje, hipoteza optimizacije plenjenja predvideva, da bomo tekom entogenetskega razvoja osebkov opazili postopno povečano asimetričnost zgolj pri navpičnih mrežah. Pokazali smo, da navpične mreže tekom ontogenetskega razvoja postajajo vse bolj asimetrične, medtem ko to ne drži za poševne in vodoravne mreže. Takšni rezultati ne ustrezajo predikcijam biogenetskega pravila, ampak podpirajo hipotezo optimizacije plena.

Abstract

The biogenetic law posits that the ontogeny of an organism recapitulates the pattern of evolutionary changes. Morphological evidence has offered some support for, but also considerable evidence against the hypothesis. However, biogenetic law in behavior remains underexplored. As physical manifestation of behavior, spider webs offer an interesting model for the study of ontogenetic behavioral changes. In orb-weaving spiders, web symmetry often gets distorted through ontogeny, and these changes have been interpreted to reflect the biogenetic law. Here, we test the biogenetic law hypothesis against the alternative, the optimal foraging hypothesis, by studying the allometry in

Leucauge venusta orb webs. These webs range in inclination from vertical through tilted to horizontal; biogenetic law predicts that allometry relates to ontogenetic stage, whereas optimal foraging predicts that allometry relates to gravity. Specifically, pronounced asymmetry should only be seen in vertical webs under optimal foraging theory. We show that, through ontogeny, vertical webs in *L. venusta* become more asymmetrical in contrast to tilted and horizontal webs. Biogenetic law thus cannot explain *L. venusta* web allometry, but our results instead support optimization of foraging area in response to spider size.

Keywords: recapitulation theory, ontogeny, ontogenetic change, asymmetry, optimal foraging, *Leucauge venusta*

Introduction

The biogenetic law, i.e. “ontogeny recapitulates phylogeny” as formulated by Haeckel in 1872 (Olsson et al. 2010), predicts that the ontogeny of an organism follows the pattern of preceding evolutionary changes in its lineage, and was extensively debated throughout the past century (Nelson 1978; Olsson et al. 2010). Although morphological evidence refutes the biogenetic law as a truly general biological concept, several studies have observed some degree of parallelism between ontogeny and phylogeny (Gould 1992; Richardson and Keuck 2002). However, the ontogenetic changes in animal behavior are underexplored in this context, but have recently been suggested to potentially recapitulate phylogeny (Richardson and Keuck 2002; Eberhard et al. 2008; Nakata 2010).

Spider webs are a particularly convenient system to study ontogenetic changes in behavior. They represent a physical record of spiders' behaviors through all ontogenetic stages, enabling a measurement of the behavioral development through ontogeny (Benjamin and Zschokke 2004; Venner and Casas 2005; Vollrath and Selden 2007), and are furthermore easily quantified (Blackledge 2011). The architecture of spider webs changes through ontogeny in most of the families studied so far (Eberhard et al. 2008). The classical vertical wagon-wheel shaped webs, or ‘orb webs’, usually retain their general architecture through ontogeny. However, larger spiders build larger webs that tend to be more asymmetrical (Herberstein and Heiling 1999). Early instar orb weaving spiders typically build symmetrical, circular orb webs with the web hub located close to the geometrical center, while adults mostly build vertically elongated webs with vertically displaced hubs (Eberhard 1990; Zschokke and Vollrath 1995). As the ancestral orb web was supposedly symmetric (Eberhard 1985; Eberhard et al. 2008; Hesselberg 2010), some studies consider such changes in web ontogeny to reflect the biogenetic law, summarizing data from 11 genera and five orb weaving families (Eberhard et al. 2008; Hesselberg 2010). However, other studies have questioned such a general rule suggesting that other factors might affect web asymmetry (e.g. Heiling and Herberstein 1999;

Kuntner et al. 2010a; Nakata 2010; Nakata and Zschokke 2010; Zschokke and Nakata 2010).

Nakata (2010) was the first to contrast biogenetic law with the alternative hypothesis, which predicts that orb weaving spiders as central place foragers maximize their prey catching by altering the symmetry of their webs as they grow. This hypothesis predicts that spiders displace the hubs of their webs somewhat above the webs' geometric center because of the difference in their upward and downward running speeds (Masters and Moffat 1983; ap Rhisiart and Vollrath 1994). Due to gravity effects, running speed in different directions is logically affected by spider mass, and several studies show that spider size and mass correlate positively with hub displacement and/or web asymmetry (e.g. Herberstein and Heiling 1999; Bleher 2000; Kuntner et al. 2008; Kuntner et al. 2010a; Kuntner et al. 2010b). Additionally, spiders typically face downwards while sitting at the hub, thus combining the effect of gravity and orientation to optimize prey catching (ap Rhisiart and Vollrath 1994; Zschokke and Nakata 2010).

We test these two alternative hypotheses by studying ontogenetic shifts in orb web allometry in the tetragnathid spider *Leucauge venusta*. This species builds orb webs that range from vertical to horizontal in inclination throughout all ontogenetic stages, and is thus ideal to investigate the effect of the optimal foraging area versus the biogenetic law on spider orb web allometry. The optimal foraging hypothesis predicts that gravity does not have an impact on the symmetry of horizontal orb webs (ap Rhisiart and Vollrath 1994; Herberstein and Heiling 1999), but should affect the symmetry of vertical orb webs, shifting it towards asymmetry. On the other hand, the biogenetic law predicts that all webs should display a more or less equal web allometry, related to ontogeny (Eberhard et al. 2008). A similar hypothesis predicts that experienced web building spiders learn to build more efficient webs (Heiling and Herberstein 1999).

Methods

Contrary to most previous studies (e.g. Masters and Moffat 1983; ap Rhisiart and Vollrath 1994; Herberstein and Heiling 1999; Bleher 2000; Kuntner et al. 2008; Kuntner and Agnarsson 2009), we investigate instars through the entire ontogenetic range, and also explore the effect of spider mass because it additionally affects vertical web asymmetry (Kuntner et al. 2010a). We used spider linear size and live mass to quantify ontogeny because they highly correlate with estimated ontogenetic stages, but explain ontogeny bettter (Kuntner et al. 2010a). As webs in captivity architecturally differ from webs in nature (Sensenig et al. 2010), we measured all webs in their natural setting.

We measured 98 webs of *L. venusta* on 28.x.2011 at El Verde, El Yunque State Forest, Luquillo (N18.32301 W65.81985) and on 20.xi.2011 at Cambelache State Forest, Barceloneta (N18.45226 W66.59711), Puerto Rico. Webs were chosen at random.

In the field, we dusted the webs with cornstarch to increase visual contrast and measured the following parameters (Fig. 1): a = web width (cm), b = web height (cm), c = top to hub distance (cm), and inclination in arc degrees (0^o to 90^o with the extremes representing a perfectly horizontal and vertical web, respectively). In the laboratory, we weighed all spiders to the nearest 0.01 mg, and measured the width of the carapace as a measure of spider linear size.

We calculated the two web asymmetry measures. First, hub displacement used as in (Kuntner et al. 2008) and similar to the hub asymmetry index (Blackledge et al. 2011), defined with the formula HD=(b-c)/b. Second, the ladder index used as in (Peters 1937; Kuntner et al. 2008) and similar to the web asymmetry index (Blackledge et al. 2011) as a measure of web shape, delimited with the outermost sticky spiral, and defined with the formula LI=b/a. We preferred hub displacement and ladder index to hub asymmetry and web asymmetry, respectively, because hub displacement and ladder index are linear and more readily visualized.

We grouped our web data into three inclination groups, horizontal ($0\text{-}30^o$, N = 39), tilted ($31\text{-}60^o$, N = 33) and vertical webs ($61\text{-}90^o$, N = 26). We tested all data for normality using the Kolmogorov-Smirnov test. Since only the data for carapace width were normally distributed, we log-transformed all other data. We then used Paerson's correlation to test if spider length and mass correlate with hub displacement and ladder index, and the analysis of variance (ANOVA) to test if ladder index differs between inclination groups. We performed all statistical tests in PASW 18 (Field 2005).

Results and Discussion

Web inclination had a clear effect on web allometry (Fig. 2). In horizontal and tilted webs, hub displacement did not correlate with spider length and mass (Table 1, Fig. 2A,B). In vertical webs however, hub displacement significantly correlated with spider length and mass (Table 1, Fig. 2C). The ladder index as a measure of web shape did not correlate with spider size (Table 1), nor did it differ between the three inclination groups ($F_{99,2}$ = 1.343, p = 0.266).

The biogenetic law has been rejected in numerous studies (Gould 1992; Theissen and Saedler 1995; Richardson et al. 1997; Richardson and Keuck 2002), but there is some evidence for it in others, e.g. in bivalve muscles (Miyazaki and Mickevich 1982), paper wasps (Wenzel 1993), and among spiders the webs of the family Theridiidae (Eberhard et al. 2008; Barrantes and Eberhard 2010). The present study investigates the ontogenetic changes in web architecture in the orb weaving spider *Leucauge venusta*. Our results show an increase in hub displacement through ontogeny in vertical webs, but not in horizontal or tilted webs, while web shape remains circular throughout the ontogeny of all spiders. Such ontogenetic change in orb web architecture is predicted by the optimal foraging hypothesis, but not by the biogenetic law.

That hubs become more displaced in vertical webs of larger spiders, but not in horizontal webs, indicates that web symmetry changes due to optimization of the spiders' foraging area, most likely because of the spiders' difference in upward and downward running speed (Herberstein and Heiling 1999; Kuntner et al. 2010a). That webs remain circular throughout ontogeny further indicates that increased differences in running speed affect web symmetry, as that difference can only explain the increased hub displacement. Changes in web shape are usually a result of limited web space (Kuntner et al. 2010b; Harmer and Herberstein 2009) or prey adaptation (Eberhard 1975). As *L. venusta* build webs in unlimited aerial space, and because circular webs are better prey catching devices than asymmetrically shaped webs (Harmer et al. 2012), web shape was not expected to change through ontogeny. While spiders also change web architecture in relation to prey and habitat (Herberstein and Tso 2011), our results are likely not affected by such factors as the webs we measured were located within a small area.

Orb webs are spiders' extended phenotypes employed in all ontogenetic stages and thus directly influence their fitness (Eberhard 1990; Herberstein and Tso 2011). Web architecture that maximizes foraging success throughout the spiders' lives is likely to be selected for. This is supported by other studies, e.g. different species of *Cyclosa* enlarge different parts of their webs in relation to which side the spiders are facing (Nakata and Zschokke 2010), *Telaprocera* spiders build highly vertically elongated webs when in horizontally limited space and the more efficient circular webs when in unlimited space (Harmer and Herberstein 2009), and heavier individuals of several species build more asymmetrical webs (Herberstein and Heiling 1999; Kuntner et al. 2010a). Additionaly, experienced spiders also learn which parts of the web are most successful in catching prey (Heiling and Herberstein 1999). Furthermore, Coslovsky & Zschokke (2009) investigated the building costs in different parts of an orb web. Contrary to predictions, they found that building of the lower half of the web is costlier than the building of the upper half. Enlarging the lower half of the web by displacing the hub upwards thus indicates that an optimal foraging area is under even stronger selection pressure with building cost as an opposing force.

Although web allometry in *L. venusta* cannot be explained by invoking the biogenetic law, the ultimate test of whether ontogeny recapitulates the evolutionary steps of *Leucauge* spiders would have to include phylogenetic data. Currently, however, a

Leucauge phylogeny is not available and thus the evolution of *Leucauge* webs is unknown. Additionally, some authors argue that the ancestral orb web was probably symmetric and circular (Eberhard et al. 2008; Hesselberg 2010), but until solid evidence exists for such assumption, the ancestral orb web remains of limited use in tests of the biogenetic law. Furthermore, since the spider group Orbiculariae comprises approximately 12,000 species (Coddington 1986; Griswold et al. 1998; Garb et al. 2006; Blackledge, Scharff et al. 2009), a reconstructed ancestral web architecture of such a diverse group would provide only a limited power in the context of testing the biogenetic law. Evolutionary tests thus will require ancestral reconstruction of the orb web at the nodes under study, rather than the common ancestor of Orbiculariae.

In conclusion, the ontogeny of *L. venusta* webs does not reflect the biogenetic law but is consistent with the foraging optimization. The extent to which our findings in *L. venusta* can be generalized to other orb web spiders is difficult to estimate. However, because the web is primarily a foraging structure, we predict that future studies of ontogenetic changes in orb webs will support foraging optimization, especially when considering phylogenetic patterns of investigated spider groups. Ontogenetic changes in non-foraging behavior and what factors influence them requires further study, and is also likely influenced by many factors under selection.

Acknowledgements

We thank Laura May-Collado, Arian Avalos and Mayte Avalos for logistic help, and Simona Kralj-Fišer for help in statistical analysis. This work was supported by the Slovenian Research Agency (grants P1-0236, J1-2063 to MK), the Slovene Human Resources Development and Scholarship Fund (grant 11010-50/2010 to MG), National Science Foundation (DEB-1050187-1050253 to IA).

References

ap Rhisiart A, Vollrath F (1994) Design-features of the orb web of the spider, *Araneus diadematus*. Behav Ecol 5:280-287

Barrantes G, Eberhard WG (2010) Ontogeny repeats phylogeny in Steatoda and Latrodectus spiders. J Arachnol 38:485-494. doi:10.1636/b10-05.1

Benjamin SP, Zschokke S (2004) Homology, behaviour and spider webs: web construction behaviour of *Linyphia hortensis* and *L. triangularis* (Araneae : Linyphiidae) and its evolutionary significance. J Evol Biol 17:120-130. doi:10.1046/j.1420-9101.2004.00667.x

Blackledge TA (2011) Prey capture in orb weaving spiders: are we using the best metric? J Arachnol 39:205-210

Blackledge TA, Kuntner M, Agnarsson I (2011) The form and function of spider orb webs: Evolution from silk to ecosystems. In: Casas J (ed) Adv Ins Physiol 41:175-262. doi:10.1016/b978-0-12-415919-8.00004-5

Blackledge TA, Scharff N, Coddington JA, Szuts T, Wenzel JW, Hayashi CY, Agnarsson I (2009) Reconstructing web evolution and spider diversification in the molecular era. P Natl Acad Sci Usa 106:5229-5234. doi:10.1073/pnas.0901377106

Bleher B (2000) Development of web-building and spinning apparatus in the early ontogeny of *Nephila madagascariensis* (Vinson, 1863) (Araneae: Tetragnathidae). B Brit Arachnol Soc 11:275-283

Coddington JA (1986) The monophyletic origin of the orb web. In: Shear WA (ed) Spiders: Webs, Behavior,and Evolution. Stanford Univ. Press, Stanford, CA, pp 319-363

Coslovsky M, Zschokke S (2009) Asymmetry in orb-webs: an adaptation to web building costs? J Insect Behav 22:29-38. doi:10.1007/s10905-008-9151-2

Eberhard WG. 1975. Inverted ladder orb web of *Scoloderus* sp. and intermediate orb of *Eustala* sp. Araneae – Araneidae. J Nat Hist 9:93–106. doi: 10.1080/00222937500770071

Eberhard WG (1985) The "sawtoothed" orb of *Eustala* sp., with a discussion of the ontogenetic patterns of change in web design in spiders. Psyche 92:105-118

Eberhard WG (1990) Function and phylogeny of spider webs. Ann Rev Ecol Syst 21:341-372

Eberhard WG, Barrantes G, Madrigal-Brenes R (2008) Vestiges of an orb-weaving ancestor? The "biogenetic law" and ontogenetic changes in the webs and building behavior of the black widow spider *Latrodectus geometricus* (Araneae Theridiidae). Ethol Ecol & Evol 20:211-244

Field A (ed) (2005) Discovering statistics using SPSS. 2 edn. Sage Publications, London

Garb JE, DiMauro T, Vo V, Hayashi CY (2006) Silk genes support the single origin of orb webs. Science 312:1762-1762. doi:10.1126/science.1127946

Gould SJ (1992) Ontogeny and phylogeny - revisited and reunited. Bioessays 14:275-279. doi:10.1002/bies.950140413

Griswold CE, Coddington JA, Hormiga G, Scharff N (1998) Phylogeny of the orb-web building spiders (Araneae, Orbiculariae : Deinopoidea, Araneoidea). Zool J Linn Soc-Lond 123:1-99

Harmer AMT, Herberstein ME (2009) Taking it to extremes: what drives extreme web elongation in Australian ladder web spiders (Araneidae: Telaprocera maudae)? Anim Behav 78:499-504. doi:10.1016/j.anbehav.2009.05.023

Harmer AMT, Kokko H, Herberstein ME, Madin JS (2012) Optimal web investment in sub-optimal foraging conditions. Naturwissenschaften 99:65–70. doi:10.1007/s00114-011-0862-4

Heiling AM, Herberstein ME (1999) The role of experience in web-building spiders (Araneidae). Anim Cogn 2:171-177

Herberstein ME, Heiling AM (1999) Asymmetry in spider orb webs: a result of physical constraints? Anim Behav 58:1241-1246. doi:10.1006/anbe.1999.1255

Herberstein ME, Tso IM (2011) Spider webs: evolution, diversity and plasticity. In: Herberstein ME (ed) Spider Behaviour: Flexibility and Versatility. Cambridge University Press, Cambridge, pp 57-98

Hesselberg T (2010) Ontogenetic Changes in Web Design in Two Orb-Web Spiders. Ethology 116:535-545. doi:10.1111/j.1439-0310.2010.01760.x

Kuntner M, Agnarsson I (2009) Phylogeny accurately predicts behaviour in Indian Ocean Clitaetra spiders (Araneae : Nephilidae). Invertebr Syst 23:193-204. doi:10.1071/is09002

Kuntner M, Gregorič M, Li DQ (2010a) Mass predicts web asymmetry in *Nephila* spiders. Naturwissenschaften 97:1097-1105. doi:10.1007/s00114-010-0736-1

Kuntner M, Haddad CR, Aljancic G, Blejec A (2008) Ecology and web allometry of *Clitaetra irenae*, an arboricolous African orb-weaving spider (Araneae, Araneoidea, Nephilidae). J Arachnol 36:583-594

Kuntner M, Kralj-Fišer S, Gregorič M (2010b) Ladder webs in orb-web spiders: ontogenetic and evolutionary patterns in Nephilidae. Biol J Linn Soc 99:849-866

Masters WM, Moffat AJM (1983) A functional explanation of top-bottom asymmetry in vertical orbwebs. Anim Behav 31:1043-1046. doi:10.1016/s0003-3472(83)80010-4

Miyazaki JM, Mickevich MF (1982) Evolution of Chesapecten (Mollusca, Bivalvia, Miocene-Pliocene) and the biogenetic law. Evol Biol 15:369-409

Nakata K (2010) Does ontogenetic change in orb web asymmetry reflect biogenetic law? Naturwissenschaften 97:1029-1032. doi:10.1007/s00114-010-0719-2

Nakata K, Zschokke S (2010) Upside-down spiders build upside-down orb webs: web asymmetry, spider orientation and running speed in *Cyclosa*. P Roy Soc B-Biol Sci 277:3019-3025. doi:10.1098/rspb.2010.0729

Nelson G (1978) Ontogeny, phylogeny, paleontology, and the biogenetic law. Syst Zool 27:324-345. doi:10.2307/2412883

Olsson L, Levit GS, Hossfeld U (2010) Evolutionary developmental biology: its concepts and history with a focus on Russian and German contributions. Naturwissenschaften 97:951-969. doi:10.1007/s00114-010-0720-9

Peters HM (1937) Studien am netz der Kreuzspinne (*Aranea diadema*.). 1. Die Grundstruktur des Netzes und Beziehungen zum Bauplan des Spinnenkorpers. Z Morphol Ökol Tiere 33:128-150

Richardson MK, Hanken J, Gooneratne ML, Pieau C, Raynaud A, Selwood L, Wright GM (1997) There is no highly conserved embryonic stage in the vertebrates: Implications for current theories of evolution and development. Anat Embryol 196:91-106. doi:10.1007/s004290050082

Richardson MK, Keuck G (2002) Haeckel's ABC of evolution and development. Biol Rev 77:495-528. doi:10.1017/s1464793102005948

Sensenig A, Agnarsson I, Gondek TM, Blackledge TA (2010) Webs in vitro and in vivo: spiders alter their orb-web spinning behavior in the laboratory. J Arachnol 38:183-191

Theissen G, Saedler H (1995) MADS-box genes in plant ontogeny and phylogeny - Haeckel's 'biogenetic law' revisited. Curr Opin Genet Dev 5:628-639. doi:10.1016/0959-437x(95)80032-8

Venner S, Casas J (2005) Spider webs designed for rare but life-saving catches. P Roy Soc B-Biol Sci 272:1587-1592. doi:10.1098/rspb.2005.3114

Vollrath F, Selden P (2007) The role of behavior in the evolution of spiders, silks, and webs. Ann Rev Ecol Evol Syst 38:819-846. doi:10.1146/annurev.ecolsys.37.091305.110221

Wenzel JW (1993) Application of the biogenetic law to behavioral ontogeny - a test using nest architecture in paper wasps. J Evol Biol 6:229-247. doi:10.1046/j.1420-9101.1993.6020229.x

Zschokke S, Nakata K (2010) Spider orientation and hub position in orb webs. Naturwissenschaften 97:43-52. doi:10.1007/s00114-009-0609-7

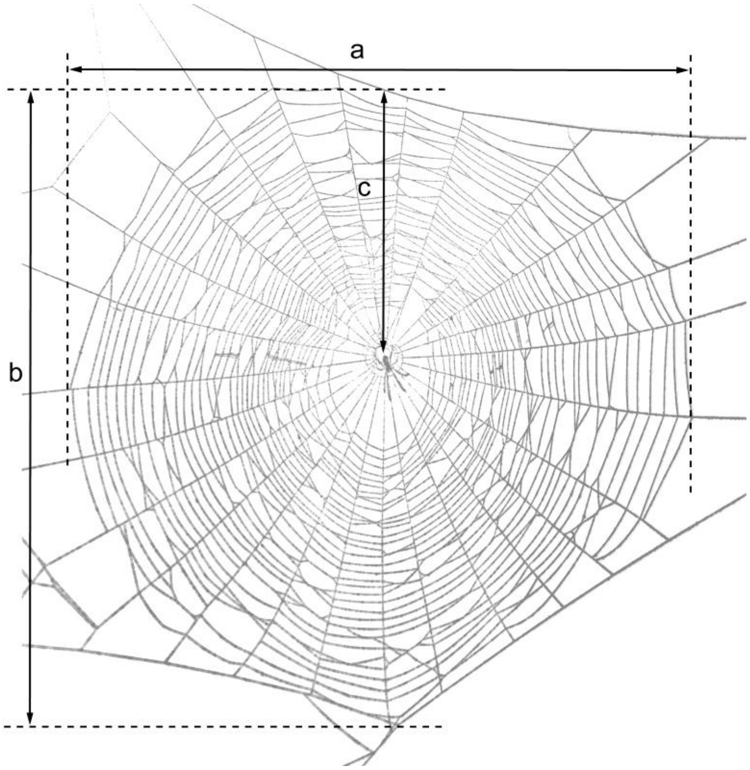
Zschokke S, Vollrath F (1995) Web construction patterns in a range of orb-weaving spiders (Araneae). Eur J Entomol 92:523-541

Tables

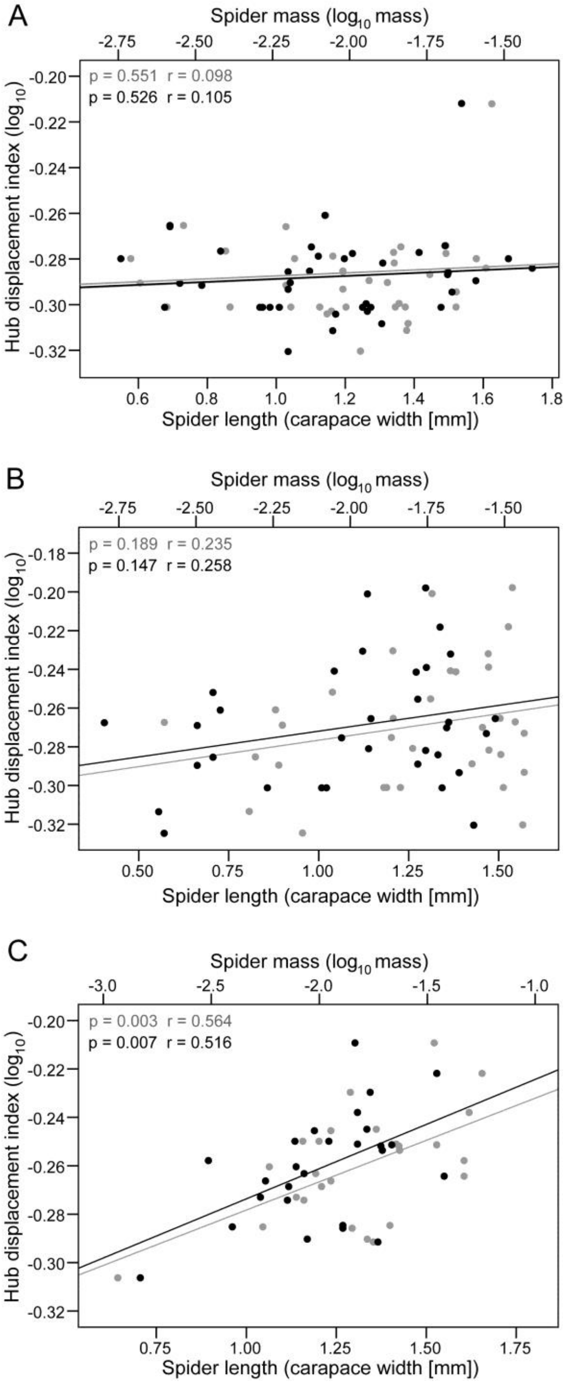
Tabela 3: **Table 1:** Paerson's correlations of spider length/mass and two asymmetry indices (hub displacement, ladder index) in three inclination groups of *L. venusta* webs.

Correlation	***P***	***r***	***N***
Spider length and hub displacement (horizontal webs)	0.551	0.098	39
Spider mass and hub displacement (horizontal webs)	0.526	0.105	39
Spider length and ladder index (horizontal webs)	0.674	-0.07	39
Spider mass and ladder index (horizontal webs)	0.557	-0.097	39
Spider length and hub displacement (tilted webs)	0.189	0.235	33
Spider mass and hub displacement (tilted webs)	0.147	0.258	33
Spider length and ladder index (tilted webs)	0.942	-0.013	33
Spider mass and ladder index (tilted webs)	0.953	-0.011	33
Spider length and hub displacement (vertical webs)	**0.003**	**0.564**	**26**
Spider mass and hub displacement (vertical webs)	**0.007**	**0.516**	**26**
Spider length and ladder index (vertical webs)	0.933	0.017	26
Spider mass and ladder index (vertical webs)	0.788	-0.055	26

Figures Captions



Slika 12: **Fig. 1:** Web of *L. venusta* illustrating investigated parameters: (a) web width; (b) web height; (c) hub to top distance.



Slika 13: **Fig. 2:** Correlations between spider length and hub displacement (grey circles), and spider mass and hub displacement (black circles), in three inclination groups of *L. venusta* webs: the horizontal (A), tilted (B) and vertical (C) webs. The black and grey p-values reflect the black and grey circles, respectively.

2.2 OSTALO POVEZOVALNO ZNANSTVENO DELO

2.2.1 Ali večje živali prilagodijo vložek v prehranjevanje? Vzorci materialnega vložka v pajčje kolesaste mreže

Do larger animals adapt their foraging effort? Patterns of material investment in spider orb webs

Matjaž Gregorič, Todd A. Blackledge, Matjaž Kuntner

Izvleček

Plenilci večajo svoj fitnes z optimalnim razporejanjem časa in energije v vedenja, povezana s plenjenjem. Pajki in njihove mreže so dober model za študije vlaganja v plenjenje, saj pajčje mreže najverjetneje predstavljajo kompromis med visokimi energetskimi stroški gradnje mreže in njeno učinkovitostjo. Predhodne študije si nasprotujejo v sklepih kako pajki spreminjajo mreže kot odgovor na nahranjenost ali tip plena. Ob tem v teh študijah poročajo predvsem o tem, kako pajki spreminjajo arhitekturo mrež, kako se spreminja uporaba prediva in lepila pa ostaja neraziskano, čeprav bi bil ravno pristop, ki bi ocenil vse te informacije, ključen za razumevanje energijskega vlaganja v plenjenje. Da bi raziskali odnos med energetskimi stroški gradnje in učinkovitostjo mrež, smo pri petih ozkosorodnih vrstah pajkov mrežarjev *Zygiella* s.l. (Araneidae) raziskali vložek materiala v odvisnosti od telesne velikosti in nahranjenosti, in z objavljenimi podatki 22 drugih vrst pajkov mrežarjev opravili dodatno meta-analizo odvisnosti materialnega vlaganja v plenjenje v odvisnosti od telesne velikosti. Za odrasle samice različnih velikosti in nahranjenosti smo izmerili skupno prostornino treh izločkov predilnih žlez, ki jih pajki mrežarji večinoma uporabijo pri gradnji mrež in raziskali razlike večih arhitekturnih lastnosti mrež in uporabe prediva. Ugotovili smo, da je material, ki ga pajki uporabijo v gradnji mrež, v pozitivno alometričnem odnosu s telesno velikostjo. Vrste *Zygiella* s.l. so se razlikovale v tem ali in kako je vložen material koreliral s telesno velikostjo in nahranjenostjo, vložek v plenjenje pa je odvisen od arhitekture mreže, vlaganja v svilene niti in vlaganja v lepilo. Pokazali smo, da je za uporabno oceno vlaganja v plenjenje potrebno izmeriti vse tri parametre. Za zaključek predpostavljamo, da pri pajkih mrežarjih odločilen vpliv na vložek v plenjenje igra del življenjskega cikla, v katerem se nahaja osebek, in da je vedenjska plastičnost, ki veča osebkov fitnes, pod močnim selekcijskim pritiskom, kar med vrstami verjetno privede do različnih vzorcev vlaganja v plenjenje.

Abstract

Predators maximize their fitness by investing optimal amounts of time and energy into behavioral efforts such as foraging. Spiders and their webs are good models for studying foraging investment because an orb web likely reflects a trade-off between the high material costs of producing silk and glue versus prey-catching efficiency. Previous studies showed conflicting results of how spiders alter their webs in response to body condition and prey. However, these studies have mostly documented only changes in web architecture, not changes in the use of silk and glue, although a combined approach assessing all such information is critical for understanding foraging investment. To investigate this trade-off, we examined the material investment in relation to body size and condition in five closely related species of orb web spiders *Zygiella* s.l. (Araneidae), and performed an additional meta-analysis of the relationship between body size and foraging investment using data of 22 other orb weaving species. We measured the total volumes of three spinning gland secretions that spiders use for building orb webs, and explored differences in several web architecture measures and silk use between females of different size and body condition. We found that orb web materials scale positively allometric with body size across orb weaving species. The *Zygiella* s.l. species varied significantly in whether and how the invested materials correlated with body size and body condition, and foraging investment was altered through web architecture, investment in silk, and investment in glue. We show that quantifying all three parameters is necessary for meaningful estimates of total foraging investment. We conclude by suggesting that foraging investment might be influenced by the stage of spider life cycles, and that plasticity to maximize foraging is under strong selection, and thus differs between species.

Keywords: allometry, static allometry, foraging investment, behavioral plasticity, phenotypic plasticity, trade-off, orb web, silk

Introduction

Predators use diverse behavioral strategies to capture prey. The amount of time predators invest into such behavioral efforts, i.e. foraging investment, plays a critical role in maximizing their fitness (Lima and Dill 1990; Ferrari et al. 2009). Web-building spiders are particularly appropriate objects for studying foraging investment, because spider webs represent physical records of the spiders' foraging behaviors that can be easily quantified (Blackledge et al. 2011b; Herberstein and Tso 2011).

The architecture of webs varies greatly among spider groups and even within species (Eberhard et al. 2008a; Blackledge et al. 2009). Spiders change web architecture throughout their ontogeny (Eberhard et al. 2008b; Kuntner et al. 2010b) and accordingly increase the amount of invested silk (Sensenig et al. 2011). Changes in web architecture and silk mechanics critically influence web performance and thus the spiders' phenotypic performance (Blackledge et al. 2011b). For instance, among orb web spiders, larger species in general evolved tougher silk concurrently with changes in silk structure and web architectures that maximize the stopping potentials of the orb webs (Sensenig et al. 2010a). Furthermore, individuals of several spider species alter their web architectures according to their size and feeding history (Blackledge et al. 2011b). They can increase or decrease the size of webs (Sherman 1994; Venner et al. 2000; Blackledge and Zevenbergen 2007), change the webs' symmetry (Herberstein and Heiling 1999; Harmer 2009; Kuntner et al. 2010a) and molecular composition of their silks (Tso et al. 2005; Townley et al. 2006), 'decorate' their webs with additional silk structures (Blackledge 1998; Li and Lee 2004), and build protective barrier webs (Higgins 1992; Uhl 2008).

Most studies investigating foraging investment in spiders used the wagon-wheel shaped (orb) webs because of their straightforward architecture. Orb webs function mainly as foraging devices, and the size and availability of prey are considered proximal cues inducing plasticity in orb webs (Vollrath and Selden 2007; Blackledge 2011). Most of these studies have documented the changes in web architecture, and have shown that hungry individuals of some species increase their foraging investment by building larger webs and/or using more threads, while sated individuals allocate their resources away from continuous foraging (e.g. Sherman 1994; Venner et al. 2000; Mayntz et al. 2009). However, several studies found no or even the opposite, effect (Witt et al. 1968; Vollrath and Samu 1997; Nakata 2007; Blamires 2010). Furthermore, few studies have examined how behavioral plasticity in orb web production is associated with the use of silk, although such information is critical for understanding the spiders' foraging investment (e.g. (Watanabe 2000; Tso et al. 2007; Liao et al. 2009), but see (Blackledge and Zevenbergen 2007; Boutry and Blackledge 2008; Boutry and Blackledge 2009) for studies on cob web spiders).

Moreover, no study investigating foraging investment in orb weaving spiders (e.g. Herberstein et al. 2000; Venner et al. 2000; Mayntz et al. 2009; Anotaux et al. 2012), explores both web architecture as well as the use of silk. For example, if individual spiders change web characteristics such as web size, the spacing between threads, or the total thread lengths, these changes do not necessarily correlate to foraging investment, as spiders can also control the diameter of silk threads and the amount of glue used (Blackledge et al. 2005; Boutry and Blackledge 2008; Sensenig et al. 2010b). Thus, a spider could potentially produce a web of twice the surface area, but the same amount of silk, simply by longer and thinner threads. These two webs would consequently function very differently at intercepting and stopping flying insects. Because orb webs likely reflect a trade-off between high material costs of producing silk and catching efficiency (Zschokke et al. 2006; Blackledge et al. 2011b), information about the interaction between web architecture and the use of silk is critical for understanding the functional consequences of variation in webs.

To investigate this trade-off, we examined the material investment in orb webs in relation to body size and condition in five closely related species of *Zygiella* sensu lato, measuring the total volume of all three spinning gland secretions that spiders mainly use for building orb webs: the major ampullate silk (MA silk), the flagelliform silk (Flag silk) and glue produced by aggregate glands. The radii of an orb web mainly consist of MA silk and function to stop the prey's impact, while the spiraling Flag silk coated with glue forms the sticky spiral functioning to retain prey (Fig. 1,2 Sensenig et al. 2012). Thus, we first investigated how spider size influences foraging investment both among and within species, and second, we investigated how body condition influences foraging investment within species.

It is logical that larger spider species and individuals produce more silk, and we thus expected that larger spider species show higher foraging investment, in terms of absolute amount of silk and glue utilized to construct a web. However, almost nothing is known about how silk production scales with body size, and in particular whether different species produce proportionally similar amounts of silk in relation to body size. In allometry studies, it is important to infer whether changes in size do or do not lead to changes in proportion, i.e. whether scaling is isometric or allometric, respectively (Damuth 2001). We thus focused on how spider size scaled with the volumes of silks and glue as a measure of foraging investment. If the scaling was isometric, spider size would alone predict foraging investment. If foraging investment scaled positively or negatively allometric, i.e. silk production allometrically increases at a faster or slower rate, foraging investment cannot be explained by body size. The latter would indicate that foraging investment is constrained by other factors, e.g. it might be energetically costlier for larger spiders to build webs.

In addition to investigating the total silk investment in foraging, we explored changes in several web architecture measures, as well as silk thread diameters and glue droplet number and size. Because orb webs are adapted to specific microhabitats (Foelix 2011), we hypothesized that such adaptations may lead to different patterns of material investment. For example, spider species in different habitats might have evolved properties of their webs according to the kinetic energies of different prey particular to each species' preferred microhabitat. To test whether such differences can be detected among closely related species, we selected the five closely related *Zygiella* s.l. species occupying different habitats in the same general geographic location in Slovenia.

Methods

Field work and sample preparation

We sampled 18 webs of *Leviellus thorelli*, 10 webs of *Parazygiella montana*, 22 webs of *Stroemiellus stroemi*, 20 webs of *Zygiella keyserlingi* and 18 webs of *Zygiella x-notata* at six localities in Slovenia, from 7. ix. 2009 to 25. x. 2009. In the field, we haphazardly selected adult female webs, measured their horizontal and vertical diameters (Fig. 1 a, b), and photographed them from a perpendicular angle to subsequently measure other web parameters (see *Web measurements* for details). We then sampled two radial threads and four outermost spiral threads for both the lower and upper part of each web (Fig. 1), using microscope glass slides with stripes of raised support to preserve glue droplets.

We photographed all sticky threads using a Leica MZ16 stereomicroscope equipped with a Leica DFC 420C camera, under various magnifications that at least 10 glue droplets were visible for each sticky thread, in order to measure the number and size of glue droplets. We processed all samples at the same time in the same laboratory with a humidity of ~ 50 %, which resembled natural conditions and is comparable with other studies (Sensenig et al. 2010a). In order to visualize the axial fibers, we then glued all samples to the microscope glass slides and photographed all radial and sticky threads on two different locations, under 1000x magnification. Using these photographs, we later measured the diameters of all threads (Blackledge et al. 2005).

We measured carapace width as a measure of spider size. We used abdomen volume as a measure of body mass (Jakob et al. 1996). We measured the width (AW), length (AL) and height (AH) of each spider abdomen in order to calculate abdomen volume using the formula:

$$V_{abdomen} = \frac{4}{3} \cdot \pi \cdot \frac{AW \cdot AL \cdot AH}{2}$$

To estimate body condition, i.e. spider mass corrected for spider size, we regressed $\log_{10}$-transformed spider abdomen volume against $\log_{10}$-transformed spider carapace width, and used the standardized residuals as a body condition index (Jakob et al. 1996).

Web architecture quantification

From photographs of webs, we measured the distance from the hub to the outermost sticky spiral along all four axis of the webs' (Fig. 1 c-f), the horizontal and vertical diameters of the web hubs (Fig. 1 g-i), counted radial threads (R_N; Fig. 1 R), and counted spiral threads on the four web axis (Fig. 1 SS_N, SS_E, SS_S, SS_W). Because webs of *Zygiella* s.l. mostly contain a spiral free sector (Gregorič et al. 2010), we also measured its angle when present (Fig. 1 j).

To quantify web architecture, we then calculated the following indices: Capture area (CA), i.e. the area covered by sticky threads, was calculated using the Ellipse-Hub formula (Blackledge and Gillespie 2002) that we adjusted for free-sector webs:

$$CA = \frac{a}{2}\cdot\frac{b}{2}\cdot\pi - \frac{g}{2}\cdot\frac{(h+i)}{2}\cdot\pi - \pi\cdot c^2\cdot\frac{j}{360}$$

The average mesh width (MW), i.e. the average distance between adjacent sticky threads, was calculated following (Herberstein and Tso 2000):

$$MW = \frac{\frac{c-h}{SS_N} + \frac{d-i}{SS_S} + \frac{f-\frac{g}{2}}{SS_W} + \frac{e-\frac{g}{2}}{SS_E}}{4}$$

The total length of capture threads in the web was calculated using the Capture thread length formula (CTL) (Sherman 1994; Sensenig et al. 2010a) that we adjusted for free-sector webs:

$$CTL = (\pi - \frac{j_{(rad)}}{2})\cdot\frac{SS_N + SS_E + SS_S + SS_W}{4}\cdot(\frac{a+b}{4} - \frac{g+h+i}{4})$$

Silk and glue quantification

We estimated the cross-sectional area of silks and size of glue droplets from photographs. Radial threads in *Zygiella* s.l. consist of four strands of major ampullate (MA) silk and four strands of minor ampullate silk. Because minor ampullate silk strands are much thinner than MA silk strands and thus add little to the total silk volume (Blackledge et al. 2011a), we measured only MA silk strands to calculate the total volume of invested radial silk. For each radial thread, we thus measured the diameter of all four MA silk strands on two parts of each radial thread (each radius consists of a pair of double-stranded threads). Sticky spiral threads consist of two strands of flagelliform (Flag) silk coated with glue. To calculate the total volume of invested Flag silk, we measured the diameter of both Flag silk strands on two parts of each sticky thread. We then averaged all measurements of MA and Flag silk strands, respectively, in order to use these average strand diameters in further calculations.

We calculated the average hypothetical MA (d_{MA}) and Flag (d_{Flag}) silk thread diameters using the formulae

$$d_{MA} = 4 \cdot r_{MAst}$$

$$d_{Flag} = 2 \cdot \sqrt{2} \cdot r_{Fst}$$

where r_{MAst} was the average measured radius of a single MA strand, and r_{Fst} was the average measured radius of a single Flag silk strand.

Following (Sensenig et al. 2010a), we calculated the total volume of invested MA (V_{MA}) and Flag (V_{Flag}) silk using the formulae

$$V_{MA} = \frac{a+b}{4} \cdot R_N \cdot \pi \cdot (\frac{d_{MA}}{2})^2$$

$$V_{Flag} = CTL \cdot \pi \cdot (\frac{d_{Flag}}{2})^2$$

When orb web spiders build the sticky spiral, they coat the two Flag strands with glue excreted from aggregate glands. The glue then forms into glue droplets according to the diameter of the Flag thread and the glue coating volume, typically forming alternating larger and smaller droplets (Opell et al. 2008; Blackledge et al. 2011a). To calculate the total volume of invested glue, we measured only larger glue droplets because the smaller ones represent a negligible amount of glue and their presence is variable (Opell et al. 2008). We measured the average distance among at least ten glue droplets per sticky thread (DD), as well as the length and width of two haphazardly chosen glue droplets for every sticky thread. Following (Opell et al. 2008), we calculated the volume of a single glue droplet using the formula

$$SDV = \frac{2 \cdot \pi \cdot droplet\ width^2 \cdot droplet\ length}{15}$$

Following (Sensenig et al. 2011), we calculated the total volume of invested glue using the formula

$$V_G = SDV \cdot \frac{CTL}{DD}$$

Data analysis

We checked all data for normality using Kolmogorov-Smirnov tests. Because the distribution of data was not always normal, we used the Kruskal-Wallis test and Mann-Whitney U-tests, including Bonferoni correction, to test interspecific differences in absolute silk and glue investment among the *Zygiella* s.l. species. We did not normalize data for these tests in order to enable direct comparison of our results with other studies. Due to non-normal distribution of data, we used medians of all species in analyses of foraging investment among species.

For all other tests, we $\log_{10}$-transformed all data to normalize them, and to get a linear relationship of compared quantities. Log-transforming both axes later enabled easier analysis of the slope of the linear functions fitting our data.

To test whether volumes of invested materials correlate with carapace width, we performed linear regression and then investigated the slopes of the regression lines. In a linear function where y is the log of silk volume and x is the log of carapace width

$$\log y = m \cdot \log x + \log b,$$

'm' represents how silk investment changes with body size. If such a linear function has a slope of $m = 1$, the investment of a material increases at an isometric rate with body size. If $m < 1$ or $m > 1$, the investment of a material allometricaly increases at a slower (negatively allometric) or faster (positively allometric) rate, respectively. Instead of the logarithmic form, the relationship between two measured quantities could also be expressed in the form of a power law $y = b \cdot x^m$, and in both cases m represents the scaling exponent. To calculate if the slope of the linear function fitting our data significantly deviates from m = 1, we used the formula

$$t = |\frac{(m-1)}{std.\ err.}|$$

to calculate *t*, and then calculated the significance.

To investigate how the volumes of materials scale among orb weaving spider species, we included data of 22 species (Table 4), published by Sensenig et al. (2010a). We also investigated how web architecture and silk use influence the observed changes in volumes of the three materials within the investigated species. For example, an increase in the total silk volume can be the result of building a larger web, using thicker silk threads or a combination of both. To investigate how spider size correlates with web architecture and silk use, we used Pearson's correlation. All statistics was done in PASW 18 (Field 2005).

Results

The absolute volumes of materials that the investigated *Zygiella* s.l. species use for building webs varied between species (Table 1; Fig. 2, 3). *L. thorelli* used more silk than all other species, followed by *Z. keyserlingi* that used more silk than *P. montana*, *S. stroemi* and *Z. x-notata*. *L. thorelli* and *Z. keyserlingi* used more glue than other species. *P. montana* and *S. stroemi* used the least silk and glue. While web architecture, e.g. web size and symmetry, radial and spiral counts etc., characteristically vary between the five *Zygiella* s.l. species (Gregorič et al. 2010), our results show that these species also used silk threads and glue droplets of different size (Fig. 3).

The results from the analysis combining the here investigated *Zygiella* s.l. species with the 22 species investigated by Sensenig et al. (2010a) showed that the investment of materials scaled positively allometric with carapace width across the 27 orb weaving species, and that the volume of all materials increased with the power ~ 2 in relation to spider size (Fig. 4A). There was no correlation between carapace width and volume of materials only across the five investigated *Zygiella* s.l. species (MA silk volume: $p = 0.135$, $r = 0.761$, $N = 5$; Flag silk volume: $p = 0.238$, $r = 0.647$, $N = 5$; Glue volume: $p = 0.139$, $r = 0.757$, $N = 5$). However, the linear regressions that best fit our data were $y = 2.2x - 2.1$ for log MA silk volume, $y = 3.2x - 2.7$ for log Flag silk volume and $y = 3.01x - 2$ for log glue volume.

The patterns of how carapace width scaled with volumes of materials varied between the species (Fig. 4). The volumes of all materials increased with carapace width in *L. thorelli* and *S. stroemi* (Fig. 4B,C), but did not correlate with carapace width in *Z. keyserlingi*, *Z. x-notata* and *P. montana* (Fig. 4D-F). In both *L. thorelli* and *S. stroemi*, the volumes of all materials scaled positively allometric to carapace width: the amount of materials increased with the power of ~ 3 in relation to spider size in *L. thorelli* and with the power of ~ 5-7 in *S. stroemi* (Fig. 4B,C).

Within the investigated *Zygiella* s.l. species, larger individuals altered their web architecture and silk use differently (Table 2). For example, larger individuals of both *L. thorelli* and *S. stroemi* used more of all three materials (Fig. 4), spread over larger capture areas (Table 2). However, larger individuals of *L. thorelli* produced thicker threads as well as less but substantially larger glue droplets, while larger individuals of *S. stroemi* produced threads of the same thickness and glue droplets of same size and number. Larger individuals of *Z. x-notata* produced larger glue droplets but less of them (Table 3), and thus the overall volume of invested glue was not correlated with spider size (Fig. 4). Carapace width in *Z. keyserlingi* and *P. montana* did not correlate with any of the investigated web parameters (Table 4).

Better fed individuals altered their web architecture and silk use differently among the investigated species (Table 3). Body condition did not correlate with any web parameter in *L. thorelli* and *Z. x-notata* (Table 3). Better-fed individuals of *S. stroemi* invested more of all three materials by building larger capture areas covered with an equal mesh of threads, and hence used more radial and spiral threads. However, both MA and Flag silk diameters as well as glue droplet size and number did not correlate with body condition in *S. stroemi* (Table 3). Better-fed individuals of *Z. keyserlingi* built thicker radial threads and thus increased total MA silk volume, and better-fed individuals of *P. montana* slightly increased the number of glue droplets, but substantially decreased their size, thus decreasing total glue volume (Table 3).

Discussion

Web performance plays a key role in the foraging effort of orb weaving spiders, and thus plays a critical role in microhabitat adapting of species and maximizing the fitness of individuals (Blackledge et al. 2011b; Harmer et al. 2011). By investigating the interplay of silk and glue investment as well as web architecture in relation to spider size and body condition, we showed that the silk invested in orb webs increases positively allometric across 27 orb weaving species (Fig 4A). However, *Zygiella* s.l. species varied significantly in whether and how the invested materials correlated with body size and body condition.

Few studies investigate how foraging investment in spiders scales with body size and no studies investigated how it scales across species. For example, in another orb weaver, *Neoscona arabesca*, body size through ontogeny scaled isometrically with MA and Flag silk investment and positively allometrically with glue investment. The material properties of MA silk remained constant while the webs became sparser and relatively smaller. These relationships then resulted in the web's stopping potential scaling isometrically with spider size while stickiness per area declined allometrically (Sensenig et al. 2011). At the interspecific level, Sensenig et al. (Sensenig et al. 2010a) showed that larger orb weaving species use more silk with improved material properties, resulting in webs with higher stopping potentials; however, how these parameters scale with spider size is not clear.

We show that across 27 orb weaving species, the volumes of materials that spiders use for building orb webs, increase positively allometric – with a power of ~ 2 – with spider size (Fig. 4A). At a finer phylogenetic scale, within the single recent lineage of *Zygiella* s.l. that we sampled in this study, spider size does not correlate with median invested volumes of materials. Although the five species sampled probably present to few data points for a reliable analysis, the slopes of linear regressions that best fit our data have positive allometric slopes, which indicates similar trends as the analysis among all 27 orb weaving species. Most traits in animals scale negatively allometric with body size and any perfect isometry is regarded a special case (Bonduriansky and Day 2003; Shingleton et al. 2007). The few traits that exhibit positive allometry are mostly "exaggerated or bizarre" and appear to be shaped by sexual selection (Bonduriansky and Day 2003; Bonduriansky 2007).

As orb webs directly influence prey catching and thus the fitness of spiders, web performance is likely under strong natural selection (Sensenig et al. 2010a; Blackledge et al. 2011b). We show that silks and glue appear to exhibit a similar case of positive allometry, which indicates that natural selection might shape material investment into spider webs similarly to how sexual selection shapes allometry of certain sexually-selective traits. As we do not yet know how web performance scales with spider size, we can only speculate that larger orb weaving species need to invest relatively more material in order for relative web performance to stay more or less constant. In other words, we hypothesize that larger spider species need relatively more silk and glue to build webs with relatively comparable interception (size and density of capture area), stopping (the amount of kinetic energy that radial threads can dissipate) and retaining (stickiness per area) potentials. A positive allometry of material investment in relation to body size additionally hints at a possibility that the amount of invested silks and glue into orb webs is a limiting factor in body size evolution of orb weaving spiders.

To infer whether increased or decreased foraging investment within a species is achieved through altering web architecture, use of silk, or both, we investigated several other web parameters that either contribute to the calculated total foraging investment or are important for the web function. Investigating these web parameters also enabled a comparison of our results with previous studies. We found that, within the five investigated *Zygiella* s.l. species, volumes of the three materials do not scale uniformly with body size of individual spiders (Table 2), nor do they scale uniformly with body condition of individual spiders (Table 3), indicating that not only body size and body condition, but other factors affect their foraging investment.

Positive allometry of web material with spider size across orb weaving spiders is only partially confirmed by patterns within the investigated *Zygiella* s.l. species. In *L. thorelli* and *S. stroemi*, material investment scaled positively allometric with spider size: the amount of materials increased with the power of ~ 3 in relation to spider size in *L. thorelli*, and with the power of ~ 5-7 in relation to spider size in *S. stroemi* (Fig. 4). On the other hand, material investment showed no correlation to spider size in *Z. keyserlingi*, *Z. x-notata* and *P. montana* (Fig. 4). Additionally, *L. thorelli* and *S. stroemi* differ in details of how web architecture and silk use correlate to body size. Namely, these two species seem to have opposite patterns of material investment. Capture area correlates positively with body size in both species, but in *L. thorelli*, the increase of total material volumes across the slightly larger web is due to using thicker threads and larger glue droplets, while in *S. stroemi*, the increase in material volumes is due to covering the substantially larger web using more radial and sticky threads of same diameters and covered with glue droplets of same size and number (Fig. 4B,C). In other words, with increasing body size, webs of *L. thorelli* are capable of dissipating more kinetic energy, while webs of *S. stroemi* are capable of intercepting more prey.

Several factors could explain such patterns. First, growing bodies might change allometrically in a different way between species of different sizes, which might favor different web traits. Indeed, *L. thorelli* is significantly larger than *S. stroemi* (Gregorič et al. 2010), and this might partially explain the differences in patterns of silk and glue use. However, as both *Z. keyserlingi*, *Z. x-notata* and *P. montana* are of intermediate size (Gregorič et al. 2010), this hardly explains why there is no correlation of body size and foraging investment in these species. Second, our preliminary molecular analyses show that *Zygiella* s.l. is a monophyletic group consisting of two clades, *Leviellus*+*Stroemiellus* and *Zygiella*+*Parazygiella*. Thus, phylogenetic constrains are another possible explanation for differences between these two species groups. Third, ecological factors might affect patterns of material investment. For example, orb webs are generally adapted to prey taxa that contribute the most caught biomass (Venner and Casas 2005; Blackledge 2011). Although most spiders are generalist predators (Birkhofer and Wolters 2012; Pekar et al. 2012), different spider species predominantly prey on certain invertebrate groups and/or certain sizes of prey (Nentwig 1983; Nentwig 1985; Pekar et al. 2012). Thus, web properties that maximize foraging have likely been selected for, and thus might reflect adaptations to different prey taxa and their kinetic characteristics. Fourth, yet another possible explanation is that spiders might respond differently to prey consumption according to the stage of their life cycles, and web building skills seem to decrease with age of adult spiders (Anotaux et al. 2012). As this is closely connected to the relation of body condition and foraging investment, we explain it further in paragraphs below.

If quantifying only web architecture parameters like capture area (CA), number of radial threads (R_N), capture thread length (CTL) and mesh width (MW), some of our results confirm previous studies that document an increased foraging investment of hungry spiders, while other results confirm a decreased foraging investment of food satiated spiders or no correlation with food intake (Table 3). For example, *Larinioides cornutus* increase CA when food deprived, and decrease CA and total thread length when food satiated (Sherman 1994), and fed individuals of *Z. x-notata* decrease CA and CTL (Venner et al. 2000) and increase CA and MW when prey is limited (Mayntz et al. 2009). Such patterns are confirmed by some of our results: first, better-fed *S. stroemi* individuals increase CA, CTL and R_N, which results in an increase of total volumes of all three materials; second, better-fed *Z. keyserlingi* individuals use thicker radial threads, which results in increasing total volume of MA silk (Table 3).

On the other hand, these seemingly logical patterns are far from universal as several studies show opposite trends. For example, Witt (Witt et al. 1968) showed no web architecture changes in well-fed *Z. x-notata*, while continuous food limitation resulted in decreased CA, thread lengths and MW, but Mayntz et al. (Mayntz et al. 2009) demonstrated an increased R_N in the same species when individuals were fed on high quality prey. *Cyclosa octotuberculata* decreases CA and total thread length when food is limited and increases those measures when well-fed, while MW remains constant (Nakata 2007). Well-fed *Argiope keyserlingi* increase CA and MW, but not R_N (Blamires 2010). These conflicting results too, are confirmed by some of our results: first, better-fed individuals of *L. thorelli* and *Z. x-notata* showed no changes in web architecture properties or total invested materials; second, better-fed individuals of *P. montana* increased the number of glue droplets, but substantially decreased their size, thus decreasing total glue volume (Table 3).

Possible explanations, for the described disparities between patterns of how body size as well as body condition of individuals affect foraging investment between different species, include phylogenetic, ecological and physiological factors. However, because different studies oppose each other even for same species, we hypothesize that spiders respond differently to prey consumption according to the stage of their life cycles. For example, individuals of the sampled *S. stroemi* and *Z. keyserlingi* population might have recently matured, were thus in the stage of acquiring nutrients as fast as possible, and accordingly responded with a rapid increase in the invested web materials as they gained mass. On the other hand, the sampled *L. thorelli*, *Z. x-notata* and *P. montana* individuals might have recently laid eggs or were getting to do so, and accordingly did not invest into web building but rather allocated resources from continuous foraging into eggs, a response previously shown for orb weaving spiders (Higgins 1990; Sherman 1994). Additionally, ageing seems to additionally decrease web building precision, (Anotaux et al. 2012), a phenomenon that seems plausible especially after egg-laying when web building should be freed from selection pressures.

There are at least three important short-comings that make comparing and synthesizing the disparate results of these studies difficult. First, many of them deal only with a binary comparison of starvation – more vs. less fed. Animals likely respond behaviorally and physiologically to foraging status in non-linear fashion, for instance maximizing foraging investment at intermediate hunger levels while physiological constrains begin to reduce that investment closer to starvation, so that two “more vs. less” comparisons could arrive at different answers simply by being at different points on that curve. Second, these studies largely consider only one aspect of foraging investment in spiders – the shapes of webs and lengths of threads, which ignores the substantial control that spiders exert over thread diameters. For example, our results show that different species might alter different parameters of webs, e.g. changing thread diameters versus changing thread lengths, which both result in changed foraging investment. However, changed diameters would remain undetected if quantifying only web architecture, and the functional consequences of orb webs cannot be inferred correctly without such data. Third, to test the hypothesis of different foraging investment in relation to the stage of the spiders' adult life cycles, one would have to measure foraging investment throughout that stage within same individuals to control for the variability between individuals.

In conclusion, we here show that the amount of material that orb weaving spiders use for building webs likely plays one of the key roles in the evolution of web size and possibly even body size of orb weavers. We also show that, according to their size and body condition, individual spiders change their webs both in web architecture and silk use, but the type of their response might be affected by several factors such as ecological, phylogenetic and life-history constrains. We additionally suggest that foraging investment might be largely influenced by the stage of spider life cycles.

Acknowledgments

This contribution was funded by the Slovenian Research Agency (grants P1-0236, J1-2063) and National Science Foundation USA. We thank A. Sensenig for providing raw data of already published work. We thank T. Knapič, L. Rozman and M. Turjak for their help in field. We thank T. Gondek, A. Sensenig and Joel Duff for their laboratory and logistic help, and S. Kralj-Fišer and C.P. Liao for help in statistics.

Literature

Anotaux M, Marchal J, Châline N, Desquilbet L, Leborgne R, Gilbert C, Pasquet A (2012) Ageing alters spider orb-web construction. Animal Behaviour 84:1113-1121

Birkhofer K, Wolters V (2012) The global relationship between climate, net primary production and the diet of spiders. Global Ecology and Biogeography 21:100-108

Blackledge TA (1998) Stabilimentum variation and foraging success in *Argiope aurantia* and *Argiope trifasciata* (Araneae : Araneidae). Journal of Zoology 246:21-27

Blackledge TA (2011) Prey capture in orb weaving spiders: are we using the best metric? Journal of Arachnology 39:205-210

Blackledge TA, Agnarsson I, Kuntner M (2011a) The form and function of spider orb webs: evolution from silk to ecosystems. Advances in Insect Physiology 41:175-262

Blackledge TA, Cardullo RA, Hayashi CY (2005) Polarized light microscopy, variability in spider silk diameters, and the mechanical characterization of spider silk. Invertebrate Biology 124:165-173

Blackledge TA, Gillespie RG (2002) Estimation of capture areas of spider orb webs in relation to asymmetry. Journal of Arachnology 30:70-77

Blackledge TA, Kuntner M, Agnarsson I (2011b) The form and function of spider orb webs: Evolution from silk to ecosystems. In: Casas J (ed) Advances in insect physiology, Vol 41: Spider physiology and behaviour - Behaviour, pp 175-262

Blackledge TA, Scharff N, Coddington JA, Szuts T, Wenzel JW, Hayashi CY, Agnarsson I (2009) Reconstructing web evolution and spider diversification in the molecular era. Proceedings of the National Academy of Sciences of the United States of America 106:5229-5234

Blackledge TA, Zevenbergen JM (2007) Condition-dependent spider web architecture in the western black widow, *Latrodectus hesperus*. Animal Behaviour 73:855-864

Blamires SJ (2010) Plasticity in extended phenotypes: orb web architectural responses to variations in prey parameters. Journal of Experimental Biology 213:3207-3212

Bonduriansky R (2007) Sexual selection and allometry: A critical reappraisal of the evidence and ideas. Evolution 61:838-849

Bonduriansky R, Day T (2003) The evolution of static allometry in sexually selected traits. Evolution 57:2450-2458

Boutry C, Blackledge TA (2008) The common house spider alters the material and mechanical properties of cobweb silk in response to different prey. Journal of Experimental Zoology Part a-Ecological Genetics and Physiology 309A:542-552

Boutry C, Blackledge TA (2009) Biomechanical variation of silk links spinning plasticity to spider web function. Zoology 112:451-460

Damuth J (2001) Scaling of growth: Plants and animals are not so different. Proceedings of the National Academy of Sciences of the United States of America 98:2113-2114

Eberhard WG, Agnarsson I, Levi HW (2008a) Web forms and the phylogeny of theridiid spiders (Araneae: Theridiidae): chaos from order. Systematics and Biodiversity 6:415-475

Eberhard WG, Barrantes G, Madrigal-Brenes R (2008b) Vestiges of an orb-weaving ancestor? The "biogenetic law" and ontogenetic changes in the webs and building behavior of the black widow spider *Latrodectus geometricus* (Araneae Theridiidae). Ethology Ecology & Evolution 20:211-244

Ferrari MCO, Sih A, Chivers DP (2009) The paradox of risk allocation: a review and prospectus. Animal Behaviour 78:579-585

Field A (2005) Discovering statistics using SPSS. In, 2 edn. Sage Publications, London, pp 816

Foelix RF (2011) Biology of Spiders, 3 edn. Oxford University Press, Oxford

Gregorič M, Kostanjšek R, Kuntner M (2010) Orb web features as taxonomic characters in *Zygiella* s.l. (Araneae, Araneidae). Journal of Arachnology 38:319-327

Harmer AMT (2009) Elongated orb-webs of Australian ladder-web spiders (Araneidae: *Telaprocera*) and the significance of orb-web elongation. Journal of Ethology 27:453-460

Harmer AMT, Blackledge TA, Madin JS, Herberstein ME (2011) High-performance spider webs: integrating biomechanics, ecology and behaviour. Journal of the Royal Society Interface 8:457-471

Herberstein ME, Craig CL, Elgar MA (2000) Foraging strategies and feeding regimes: Web and decoration investment in Argiope keyserlingi Karsch (Araneae : Araneidae). Evolutionary Ecology Research 2:69-80

Herberstein ME, Heiling AM (1999) Asymmetry in spider orb webs: a result of physical constraints? Animal Behaviour 58:1241-1246

Herberstein ME, Tso IM (2000) Evaluation of formulae to estimate the capture area and mesh height of orb webs (Araneoidea, Araneae). Journal of Arachnology 28:180-184

Herberstein ME, Tso IM (2011) Spider webs: evolution, diversity and plasticity. In: Herberstein ME (ed) Spider Behaviour: Flexibility and Versatility. Cambridge University Press, Cambridge, pp 57-98

Higgins L (1992) Developmental changes in barrier web structure under different levels of predation risk in *Nephila clavipes* (Araneae, Tetragnathidae). Journal of Insect Behavior 5:635-655

Higgins LE (1990) Variation in foraging investment during the intermolt interval and before egg-laying in the spider Nephila clavipes (Araneae, Araneidae). Journal of Insect Behavior 3:773-783

Jakob EM, Marshall SD, Uetz GW (1996) Estimating fitness: A comparison of body condition indices. Oikos 77:61-67

Kuntner M, Gregorič M, Li DQ (2010a) Mass predicts web asymmetry in *Nephila* spiders. Naturwissenschaften 97:1097-1105

Kuntner M, Kralj-Fišer S, Gregorič M (2010b) Ladder webs in orb-web spiders: ontogenetic and evolutionary patterns in Nephilidae. Biological Journal of the Linnean Society 99:849-866

Li DQ, Lee WS (2004) Predator-induced plasticity in web-building behaviour. Animal Behaviour 67:309-318

Liao CP, Chi KJ, Tso IM (2009) The effects of wind on trap structural and material properties of a sit-and-wait predator. Behavioral Ecology 20:1194-1203

Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation - a review and prospectus. Canadian Journal of Zoology-Revue Canadienne De Zoologie 68:619-640

Mayntz D, Toft S, Vollrath F (2009) Nutrient balance affects foraging behaviour of a trap-building predator. Biology Letters 5:735-738

Nakata K (2007) Prey detection without successful capture affects spider's orb-web building behaviour. Naturwissenschaften 94:853-857

Nentwig W (1983) The non-filter function of orb webs in spiders. Oecologia 58:418-420

Nentwig W (1985) Prey analysis of 4 species of tropical orb-weaving spiders (Araneae, Araneidae) and a comparison with araneids of the temperate zone. Oecologia 66:580-594

Opell BD, Markley BJ, Hannum CD, Hendricks ML (2008) The contribution of axial fiber extensibility to the adhesion of viscous capture threads spun by orb-weaving spiders. Journal of Experimental Biology 211:2243-2251

Pekar S, Coddington JA, Blackledge TA (2012) Evolution of stenophagy in spiders (Araneae): Evidence based on the comparative analysis of spider diets. Evolution 66:776-806

Sensenig A, Agnarsson I, Blackledge TA (2010a) Behavioural and biomaterial coevolution in spider orb webs. Journal of Evolutionary Biology 23:1839-1856

Sensenig A, Agnarsson I, Gondek TM, Blackledge TA (2010b) Webs in vitro and in vivo: spiders alter their orb-web spinning behavior in the laboratory. Journal of Arachnology 38:183-191

Sensenig AT, Agnarsson I, Blackledge TA (2011) Adult spiders use tougher silk: ontogenetic changes in web architecture and silk biomechanics in the orb-weaver spider. Journal of Zoology 285:28-38

Sensenig AT, Lorentz KA, Kelly SP, Blackledge TA (2012) Spider orb webs rely on radial threads to absorb prey kinetic energy. Journal of the Royal Society Interface 9:1880-1891

Sherman PM (1994) The orb-web - an energetic and behavioral estimator of a spiders dynamic foraging and reproductive strategies. Animal Behaviour 48:19-34

Shingleton AW, Frankino WA, Flatt T, Nijhout HF, Emlen DJ (2007) Size and shape: the developmental regulation of static allometry in insects. Bioessays 29:536-548

Townley MA, Tillinghast EK, Neefus CD (2006) Changes in composition of spider orb web sticky droplets with starvation and web removal, and synthesis of sticky droplet compounds. Journal of Experimental Biology 209:1463-1486

Tso IM, Chiang SY, Blackledge TA (2007) Does the giant wood spider Nephila pilipes respond to prey variation by altering web or silk properties? Ethology 113:324-333

Tso IM, Wu HC, Hwang IR (2005) Giant wood spider *Nephila pilipes* alters silk protein in response to prey variation. Journal of Experimental Biology 208:1053-1061

Uhl G (2008) Size dependent occurrence of different types of web decorations and a barrier web in the tropical spider *Argiope argentata* (Fabricius 1775) (Araneae Araneidae). Tropical Zoology 21:97-108

Venner S, Casas J (2005) Spider webs designed for rare but life-saving catches. Proceedings of the Royal Society B-Biological Sciences 272:1587-1592

Venner S, Pasquet A, Leborgne R (2000) Web-building behaviour in the orb-weaving spider *Zygiella x-notata*: influence of experience. Animal Behaviour 59:603-611

Vollrath F, Samu F (1997) The effect of starvation on web geometry in an orb-weaving spider. Bulletin of the British Arachnological Society 10:295-298

Vollrath F, Selden P (2007) The role of behavior in the evolution of spiders, silks, and webs. Annual Review of Ecology Evolution and Systematics 38:819-846

Watanabe T (2000) Web tuning of an orb-web spider, *Octonoba sybotides*, regulates prey-catching behaviour. Proceedings of the Royal Society of London Series B-Biological Sciences 267:565-569

Witt PN, Reed CF, Peakall DB (1968) A spider's web: Problems in regulatory biology. Springer Verlag, New York

Zschokke S, Henaut Y, Benjamin SP, Garcia-Ballinas JA (2006) Prey-capture strategies in sympatric web-building spiders. Canadian Journal of Zoology-Revue Canadienne De Zoologie 84:964-973

Tables

Tabela 4: Table 1: The volumes of invested silk and glue in *Zygiella* s.l. species: ME = median; IQR = interquartile range.

	L. thorelli N = 18	***P. montana*** N = 10	***S. stroemi*** N = 22	***Z. keyserlingi*** N = 20	***Z. x-notata*** N = 18
MA silk (mm^3)	0.033-0.855 ME = 0.0967 IQR = 0.3	0.0087-0.0422 ME = 0.0229 IQR = 0.03	0.0046-0.0308 ME = 0.0134 IQR = 0.01	0.0277-0.0913 ME = 0.0511 IQR = 0.03	0.0077-0.0592 ME = 0.0283 IQR = 0.02
Flag. silk (mm^3)	0.023-0.688 ME = 0.119 IQR = 0.335	0.002-0.013 ME = 0.006 IQR = 0.008	0.002-0.02 ME = 0.006 IQR = 0.005	0.01-0.066 ME = 0.039 IQR = 0.028	0.003-0.028 ME = 0.012 IQR = 0.088
Glue (mm^3)	0.056-1.718 ME = 0.283 IQR = 0.68	0.0095-0.119 ME = 0.048 IQR = 0.052	0.0039-0.148 ME = 0.0198 IQR = 0.031	0.0076-0.402 ME = 0.1926 IQR = 0.172	0.0094-0.261 ME = 0.0973 IQR = 0.086
Capture area (cm2)	44.1-246.6 ME = 145.05 IQR = 87.8	42.6-196.8 ME = 91.05 IQR = 69.5	8.3-81.5 ME = 32.6 IQR = 21	93.9-320.1 ME = 217.9 IQR = 111	48.1-186.3 ME = 131.4 IQR = 74.7
No. of radial threads	18-36 ME = 25 IQR = 7	14-31 ME = 19 IQR = 8	16-39 ME = 30 IQR = 7	25-54 ME = 40 IQR = 4	12-29 ME = 20.5 IQR = 7
Sticky silk length (m)	0.17-5.54 ME = 2.94 IQR = 1.87	0.53-2.36 ME = 1.24 IQR = 1.33	0.47-3.67 ME = 1.62 IQR = 1.29	1.71-9.46 ME = 6.46 IQR = 3.18	0.48-3.73 ME = 2.31 IQR = 1.28
Radial silk diameter (mm)	0.017-0.068 ME = 0.027 IQR = 0.03	0.0126-0.0193 ME = 0.015 IQR = 0.003	0.01-0.016 ME = 0.013 IQR = 0.0015	0.012-0.016 ME = 0.014 IQR = 0.002	0.013-0.02 ME = 0.016 IQR = 0.003
Sticky silk diameter (mm)	0.01-0.041 ME = 0.023 IQR = 0.022	0.007-0.009 ME = 0.008 IQR = 0.001	0.006-0.008 ME = 0.007 IQR = 0.0007	0.007-0.011 ME = 0.009 IQR = 0.002	0.008-0.011 ME = 0.009 IQR = 0.001
Glue droplet (µm3)	875-74815 ME = 17423 IQR = 54553	303-18846 ME = 1372 IQR = 12306	123.5-12731 ME = 688 IQR = 386	257-2709 ME = 1522 IQR = 1399.9	584-8176 ME = 3975 IQR = 2425
Glue droplets per 1 mm	3.2-43.6 ME = 6.1 IQR = 7.4	4.9-42.1 ME = 16.7 IQR = 23	4.9-35 ME = 19.1 IQR = 6.6	3.8-25.7 ME = 19.7 IQR = 9.9	7-21.3 ME = 13.4 IQR = 4.9

Tabela 5: Table 2: Paerson's correlations of web variables and spider carapace width for the investigated *Zygiella* s.l. species. Significant results are bolded.

	L. thorelli N = 18 carapace width (log)	***S. stroemi*** N = 22 carapace width (log)	***Z. keyserlingi*** N = 20 carapace width (log)	***Z. x-notata*** N = 18 carapace width (log)	***P. montana*** N = 10 carapace width (log)
d_R (log)	**p = 0.001** **r = 0.701**	p = 0.695 r = 0.088	p = 0.331 r = 0.229	p = 0.745 r = 0.082	p = 0.442 r = - 0.275
d_{SS} (log)	**p = 0.001** **r = 0.713**	p = 0.237 r = 0.263	p = 0.193 r = 0.304	p = 0.356 r = - 0.231	p = 0.580 r = - 0.200
R_N (log)	p = 0.986 r = - 0.005	**p < 0.001** **r = 0.683**	p = 0.876 r = 0.037	p = 0.359 r = 0.230	p = 0.525 r = - 0.228
CTL (log)	p = 0.096 r = 0.404	**p < 0.001** **r = 0.542**	p = 0.325 r = 0.232	p = 0.265 r = 0.278	p = 0.804 r = 0.073
SDV (log)	**p < 0.001** **r = 0.786**	p = 0.426 r = 0.179	p = 0.548 r = 0.143	**p = 0.037** **r = 0.495**	p = 0.990 r = - 0.005
DD (log)	**p < 0.001** **r = - 0.816**	p = 0.966 r = - 0.01	p = 0.678 r = - 0.099	**p = 0.009** **r = - 0.598**	p = 0.776 r = - 0.104
CA (log)	**p = 0.023** **r = 0.533**	**p = 0.003** **r = 0.604**	p = 0.396 r = 0.201	p = 0.283 r = 0.268	p = 0.279 r = 0.380
MW (log)	p = 0.125 r = 0.375	**p = 0.028** **r = 0.468**	p = 0.854 r = - 0.044	p = 0.309 r = 0.256	p = 0.100 r = 0.549

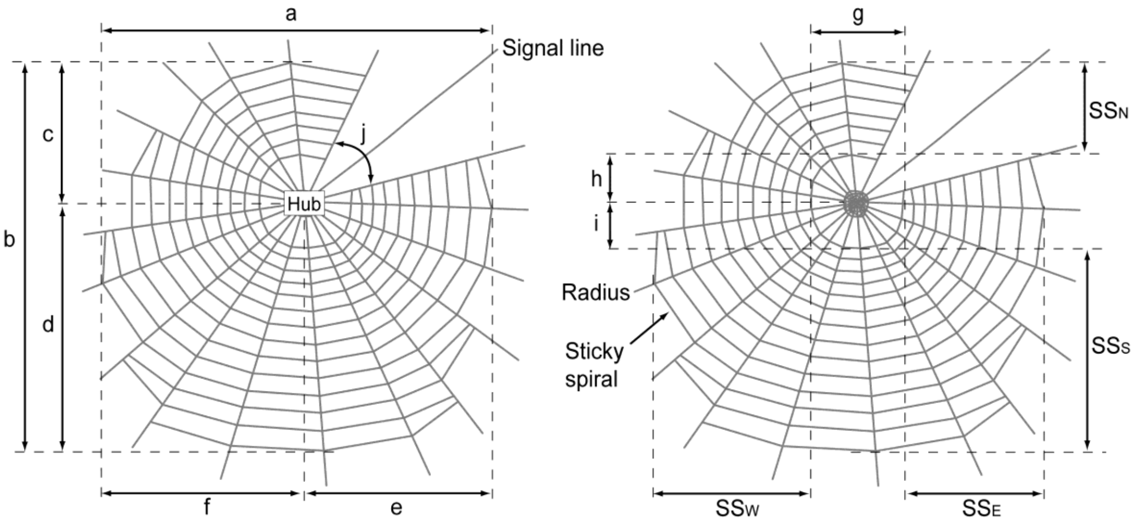
Tabela 6: Table 3: Pearson's correlations of web variables and spider body condition width for the investigated *Zygiella* s.l. species. Significant results are bolded.

	L. thorelli N = 18 body condition (log)	***S. stroemi*** N = 22 body condition (log)	***Z. keyserlingi*** N = 20 body condition (log)	***Z. x-notata*** N = 18 body condition (log)	***P. montana*** N = 10 body condition (log)
MA silk volume (log)	p = 0.665 r = 0.109	**p = 0.021** **r = 0.487**	**p = 0.024** **r = 0.501**	p = 0.158 r = 0.347	p = 0.864 r = 0.062
Flag silk volume (log)	p = 0.616 r = 0.126	**p = 0.001** **r = 0.648**	p = 0.055 r = 0.434	p = 0.377 r = 0.221	p = 0.78 r = 0.101
Glue volume (log)	p = 0.444 r = 0.192	**p = 0.001** **r = 0.638**	p = 0.721 r = - 0.085	p = 0.065 r = 0.442	**p = 0.02** **r = - 0.714**
No. of radial threads (log)	p = 0.694 r = 0.099	**p = 0.035** **r = 0.451**	p = 0.911 r = 0.026	p = 0.851 r = 0.047	p = 0.298 r = 0.366
Sticky silk length (log)	p = 0.607 r = 0.129	**p<0.001** **r = 0.739**	p = 0.199 r = 0.299	p = 0.554 r = 0.149	p = 0.867 r = 0.061
Radial silk diam. (log)	p = 0.726 r = 0.088	p = 0.182 r = - 0.295	**p = 0.011** **r = 0.556**	p = 0.067 r = 0.44	p = 0.925 r = - 0.034
Sticky silk diam. (log)	p = 0.668 r = 0.108	p = 0.291 r = - 0.235	p = 0.132 r = 0.348	p = 0.318 r = 0.249	p = 0.647 r = - 0.166
Glue droplet volume (log)	p = 0.809 r = 0.061	p = 0.109 r = 0.351	p = 0.933 r = - 0.02	p = 0.065 r = 0.443	**p = 0.001** **r = - 0.866**
Glue droplets per mm (log)	p = 0.683 r = 0.103	p = 0.198 r = - 0.285	p = 0.152 r = - 0.332	p = 0.948 r = - 0.016	**p = 0.022** **r = 0.707**
Capture area (log)	p = 0.462 r = 0.184	**p = 0.002** **r = 0.62**	p = 0.1 r = 0.377	p = 0.337 r = 0.239	p = 0.66 r = - 0.159
Mesh width (log)	p = 0.724 r = 0.089	p = 0.341 r = 0.213	p = 0.455 r = 0.176	p = 0.399 r = 0.211	p = 0.921 r = - 0.036

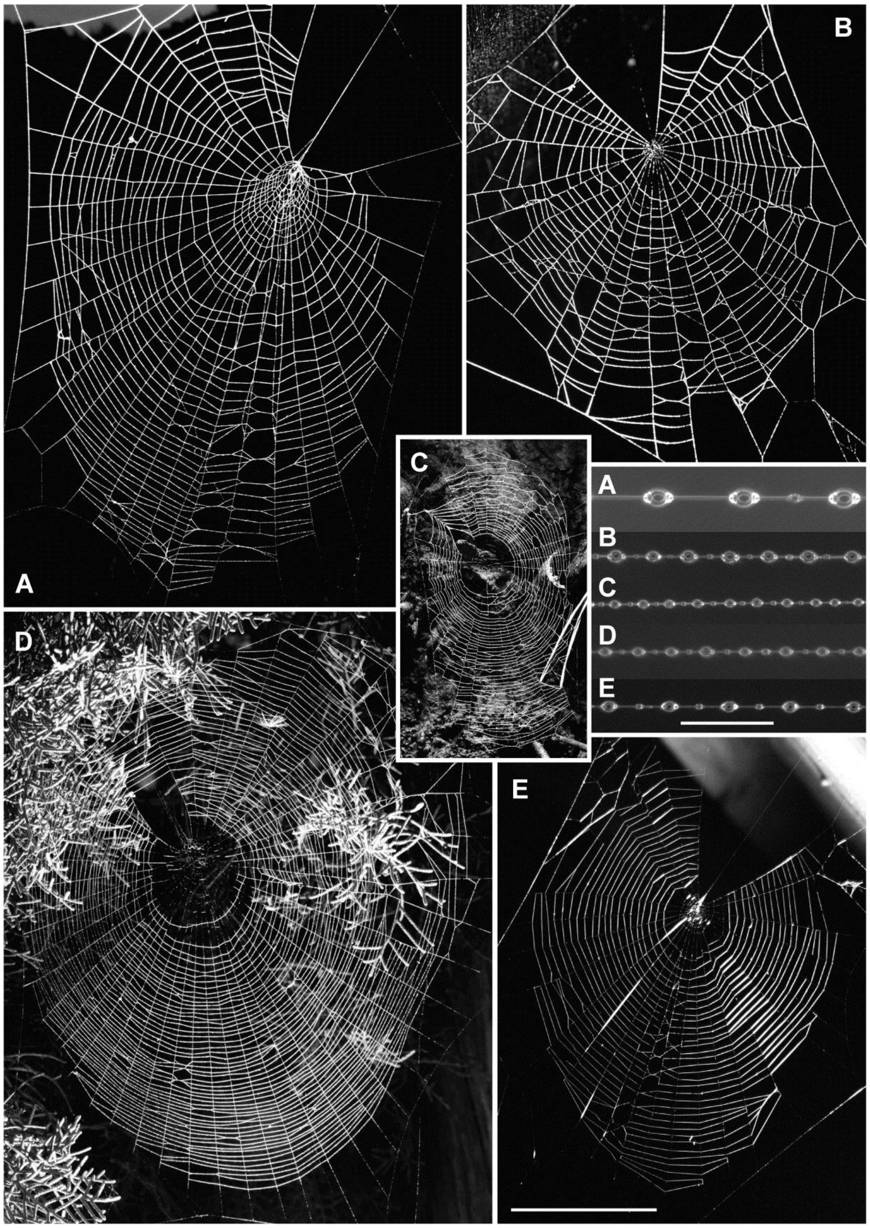
Tabela 7: Table 4: Average invested volumes of MA silk, Flag silk and glue for species investigated by Sensenig et al. (2010a).

Species	**MA silk volume** (mm^3)	**Flag silk volume** (mm^3)	**Glue volume** (mm^3)
Araneus diadematus	0.0293	0.0390	1.389
Araneus marmoreus	0.0254	0.0280	1.430
Araneus trifolium	0.0402	0.0472	1.979
Argiope aurantia	0.0585	0.0745	0.872
Argiope trifasciata	0.0373	0.0374	0.547
Caerostris darwini	0.0529	0.0298	3.340
Cyclosa conica	0.0042	0.0022	0.477
Eustala sp.	0.0051	0.0067	0.134
Gasteracantha cancriformis	0.0172	0.0072	0.491
Larinioides cornutus	0.0228	0.0347	0.580
Leucauge venusta	0.0066	0.0075	0.185
Mangora gibberosa	0.0018	0.0035	0.388
Metepeira labyrinthea	0.0050	0.0080	0.194
Micrathena gracilis	0.0165	0.0094	0.414
Neoscona arabesca	0.0064	0.0107	0.232
Neoscona crucifera	0.0596	0.1668	2.227
Neoscona domiciliorum	0.0464	0.0650	1.810
Nephila clavipes	0.0839	0.0720	0.510
Nuctenea umbratica	0.0278	0.0216	0.665
Tetragnatha versicolor	0.0044	0.0030	0.066
Verrucosa arenata	0.0097	0.0190	0.544
Zygiella x-notata	0.0050	0.0039	0.168

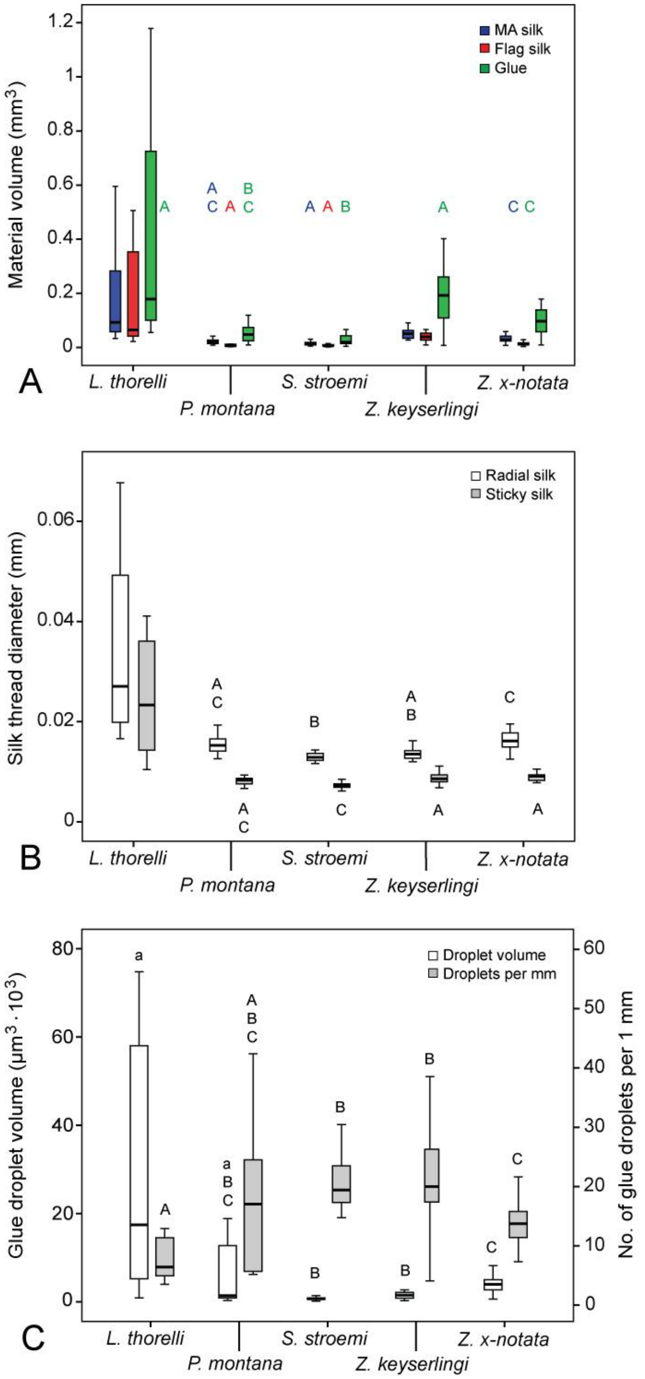
Figure Captions



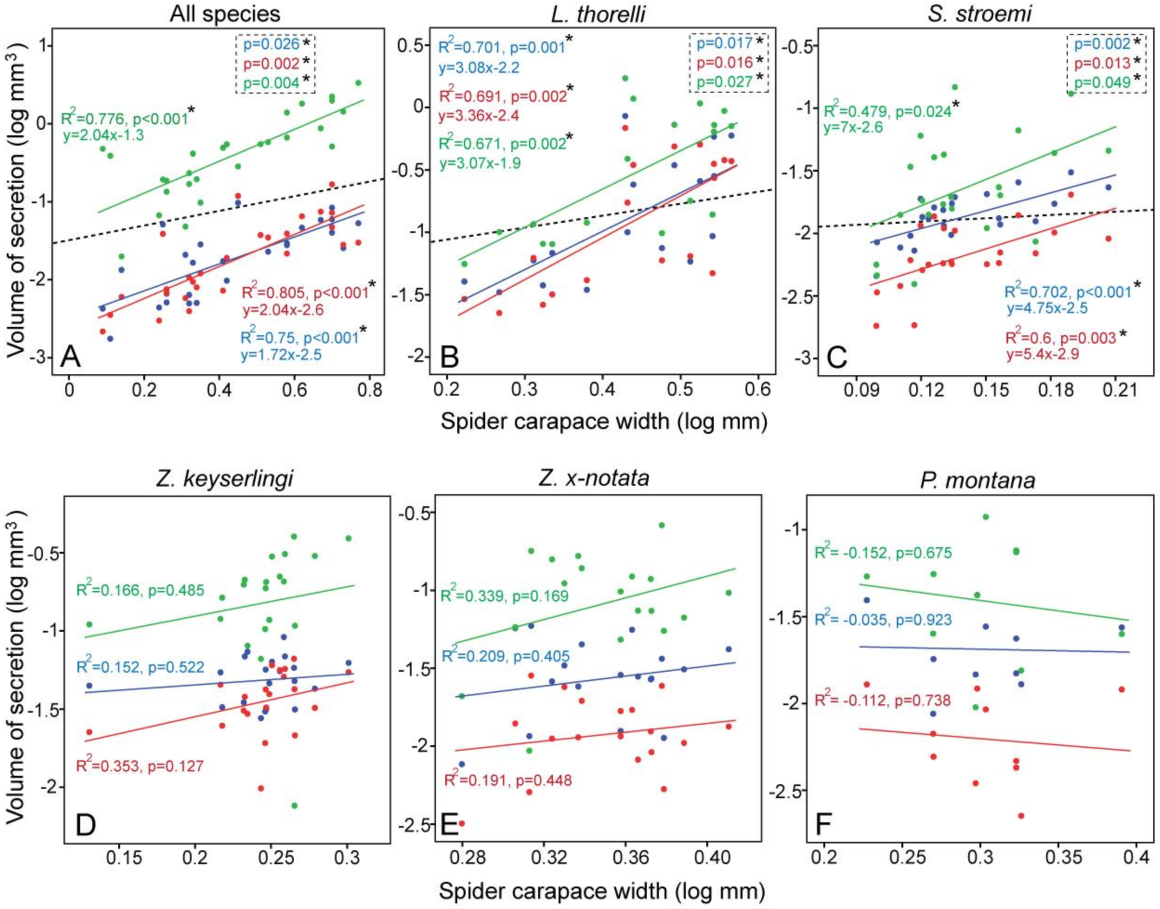
Slika 14: Figure 1: The architecture of a typical *Zygiella* s.l. web with measured parameters: a = web width; b = web height; c = hub to top; d = hub to bottom; e = hub to east side; f = hub to west side; g = free zone width; h = hub to spiral above; i = hub to spiral below; j = free sector angle; SS_N, SS_S, SS_E, SS_W = number of sticky spirals north, south, east and west of hub, respectively.



Slika 15: Figure 2: Orb webs and sticky spiral threads (inset, right) representing investigated species. A-E: *L. thorelli*, *P. montana*, *S. stroemi*, *Z. keyserlingi* and *Z. x-notata*, respectively. Scale bars, 5 cm for webs and 150 μm for spiral threads.



Slika 16: Figure 3: Absolute web parameters among *Zygiella* s.l. species: A, total material volumes; B, silk diameters; C, volume of single glue droplets. Sharing at least one capital letter denotes non-significance ($p > 0.1$), sharing at least one small letter denotes a trend ($0.05 < p < 0.1$), no shared letter denotes significance ($p < 0.05$).



Slika 17: Figure 4: Correlations of major ampullate (radial) silk volume (blue circles), flagelliform (sticky silk) volume (red circles) and glue (green circles) with spider carapace width. In the linear function $y = m \cdot x + b$, 'm' represents its slope and 'x' represents the log of spider size. No linear function is given for uncorrelated parameters. Dashed lines represent $m = 1$ slopes, and p-values inside the dashed square represent the significance of whether the slope of the linear function fitting our data differs from the $m = 1$ slope. Significant correlations are marked using asterisk.

2.2.2 Filogenetski položaj in evolucija mrež pri pajkih iz rodu *Zygiella* s.l. in drevesnih pajkih iz rodu *Caerostris* (Araneae: Araneidae)

The phylogenetic placement and web evolution of the sector web spiders *Zygiella* s.l. and bark spiders *Caerostris* (Araneae: Araneidae)

Matjaž Gregorič, Ingi Agnarsson, Todd A. Blackledge, Matjaž Kuntner

Izvleček

Pajki mrežarji iz naddružine Araneoidea so pomembni in pogosto celo modelni organizmi v mnogih evolucijskih študijah, vendar so sorodstveni odnosi med in v sorodstvenih linijah slabo raziskani. Tukaj predstavljamo prvi obsežnejši test filogenetskega položaja filogenetsko spornih skupin »Zygiellidae« in *Caerostris*. Rod *Zygiella* je pomemben raziskovalni objekti v študijah odnosov med plenom in plenilcem, spolne selekcije, vedenjske plastičnosti ter vedenja in fiziologije gradnje mrež, vendar je filogenetsko sporen. Čeprav trenutno uvrščen v Araneidae, je ta rod v preteklosti bil večkrat premeščen v različne družine, nedavno pa celo razdeljen v štiri rodove, ki bi naj pripadali svoji družini Zygiellidae. Drevesni pajki iz rodu *Caerostris* so precej neraziskani tako ekološko, vedenjsko, kot tudi taksonomsko, vendar postajajo pomembni objekti v raziskavah evolucije mrež in biomaterialov: predstavniki rodu namreč gradijo največje znane kolesaste mreže, zgrajene iz najmočnejšega znanega biomateriala. Z obsežnim izborom notranjih in zunanjih taksonov smo sestavili podatkovni set, ki temelji na mitohondrijskih (COI, 16S) in jedrnih (H3, 18S, 28S, ITS2) delih genov ter obsega 114 terminalov pajkov mrežarjev, s poudarkom na skupinah, ki gradijo kolesaste mreže. Dodatno smo testirali ali podrobnosti v arhitekturi in gradnji mrež odsevajo filogenetske odnose med skupinami in se posebej osredotočili na evolucijo gradnje mrež in gigantizma mrež. Pokazali smo, da Zygiellidae vsebujejo holarktični rod *Zygiella* s.l. ter rodova *Deliochus* in *Phonognatha*, ki sta razširjena v Avstraliji in Aziji. Skupina Zygiellidae je verjetno sestrska ostalim predstavnikom Araneidae in je verjetno veljavna taksonomska skupina. Rod *Caerostris* se uvršča blizu bazalnih razvejišč skupine Araneidae, vendar je natančen filogenetski položaj rodu nejasen. Predstavljamo tudi prvo vrstno filogenijo rodu *Caerostris* vključno z opisi petih novih vrst. Naši rezultati podpirajo vedenjske posebnosti filogenetskih linij in kažejo, da se tako Zygiellidae kot *Caerostris* od ostalih pajkov mrežarjev ločijo po kombinaciji določenih vedenjskih lastnosti.

Abstract

The orb weaving araneoid spiders are considered important or even model organisms in several topics of evolutionary research, but phylogenetic relationships among and within major orb weaving lineages are poorly understood. To build towards a good tool for evolutionary research, we here provide the first robust tests of the phylogenetic placement of the taxonomically controversial “Zygiellidae” and *Caerostris*. *Zygiella* is an important object in studies of orb web biology, predator-prey interactions, sexual selection, behavioral plasticity and web-building behavior and physiology, but is taxonomically controversial. Although currently in Araneidae, *Zygiella* was transferred between families in the past, and was recently split into four genera and proposed to belong to its own family Zygiellidae. The enigmatic araneid bark spiders of the genus *Caerostris* are understudied taxonomically, ecologically and behaviorally, but are becoming important objects in research of web evolution and biomaterials: they build the largest orb webs known that are made of nature’s toughest material. Using extensive ingroup and outgroup sampling, we here gathered a molecular data set using mitochondrial (COI, 16S) and nuclear (H3, 18S, 28S, ITS2) gene fragments for 114 orbicularian taxa, focusing on orb weaving representatives. We additionally test whether phylogenetic relationships among orb weaving lineages are reflected in behavior connected to orb web biology, and we specifically discuss the evolution of web building behavior and web gigantism. We show that Zygiellidae contains the Holarctic *Zygiella* s.l., and the Australasian *Phonognatha* and *Deliochus*. Zygiellidae is probably sister to other araneids, and might be considered a valid taxonomic concept. We recover *Caerostris* as basal araneids, but its exact phylogenetic affinities are ambiguous. We conclude that phylogenetic exclusivity reflects behavioral differences, as both Zygiellidae and *Caerostris* behavior differs from other orb weavers.

Keywords:web gigantism, web building, orb web, biomaterial, spider silk, molecular phylogenetics

Introduction

Orbiculariae, defined with the orb web origin, is one of the largest spider groups with over 12,000 described species (Griswold *et al.*, 1998; Blackledge *et al.*, 2009; Platnick, 2013). Many orb weavers are considered important or even model organisms not only for the araneological community but wider, e.g. in studies of development, functional morphology and physiology, adaptive evolution, sexual selection, sexual size dimorphism, phylogeography, evolutionary ecology etc. (Coddington, 1994; Bond & Opell, 1998; Gillespie, 2004; Blackledge *et al.*, 2011; Herberstein & Wignall, 2011). Furthermore, orb webs are directly measurable manifestations of behavior and are thus ideal objects for addressing a variety of topics like predator-prey interactions (e.g. Eberhard, 1986; Craig *et al.*, 1996; Opell & Schwend, 2007), producer-consumer interactions (e.g. Higgins & Buskirk, 1998; Agnarsson, 2003; 2011), behavioral plasticity (e.g. Watanabe, 2000; Blamires, 2010), resource use (Blackledge & Gillespie, 2004; Gillespie, 2004), evolution of behavior (e.g. Vollrath & Selden, 2007; Kuntner & Agnarsson, 2009; Blackledge *et al.*, 2012a) and various other behavioral questions (e.g. Eberhard, 1982; Eberhard, 2007; Kuntner *et al.*, 2010; Gregorič *et al.*, 2013). Moreover, because orb webs are made of nature’s toughest material, orb weavers are routinely used in studies of biomaterials (e.g. Hayashi & Lewis, 2000; Agnarsson *et al.*, 2009; Swanson *et al.*, 2009; Vollrath & Porter, 2009; Agnarsson *et al.*, 2010; Sensenig *et al.*, 2010; Sahni *et al.*, 2011; Blackledge *et al.*, 2012b).

The diversification of orb weavers is closely linked with the evolution of traits associated to orb web biology (Blackledge *et al.*, 2011). To understand the evolution of these traits, ideally, one would rely on well resolved phylogenetic relationships among orb weaving lineages. However, the phylogenetic relationships among orb weaving families and even within them are poorly understood (Agnarsson *et al.*, 2013). Within Orbiculariae, several lineages evolved web architectures very different from the ancestral orb web (Griswold *et al.*, 1998; Blackledge *et al.*, 2009). Nonetheless, the majority of studies focusing on the above mentioned topics, choose the classical orb webs as study objects, due to their simple architecture, superior material properties and large diversity in size, shape and resource use (Blackledge *et al.*, 2011).

Most spiders building classical orb webs are grouped into the family Araneidae, which is the third largest spider family with more than 3000 species (Platnick, 2013). Besides primitively building orb webs, these spiders greatly diversified morphologically, behaviorally and genetically. For building towards a robust tool for evolutionary research, better resolved relationships among orb weaving groups, and even a redefinition of the classical Araneidae might be needed. We here focus on two araneid groups that are both taxonomically controversial, as well as interesting models for several evolutionary questions, including web biology: the genera *Zygiella* Pickard-Cambridge 1902 in the broad sense (from here on "*Zygiella* s.l.") and *Caerostris* Thorell 1868.

Zygiella s.l. spiders were among the first objects of studies focusing on the architecture, function and building of orb webs (e.g. Wiehle, 1927; 1929; Peters, 1937; Witt *et al.*, 1968), and are still widely used as objects of studies focusing on predator-prey interactions, sexual selection, behavioral plasticity and web-building behavior and physiology (e.g. Zschokke & Vollrath, 1995; Venner *et al.*, 2000; Venner *et al.*, 2003; Thevenard *et al.*, 2004; Venner & Casas, 2005; Bel-Venner & Venner, 2006; Bel-Venner *et al.*, 2008; Mayntz *et al.*, 2009). The genus *Zygiella* s.l. contains mostly widespread Palearctic species that possess a characteristic orb web feature – a spiral-free sector in the upper part of the orb with a signal line leading to the tubular silk retreat (Levi, 1974; Gregorič *et al.*, 2010; Fig. 1). *Zygiella* s.l. has traditionally been placed in Araneidae, and is catalogued there again at the time (Platnick, 2013), but its taxonomic placement remains controversial. In the past, *Zygiella* s.l. was transferred between the families Tetragnathidae (Levi, 1980; Heimer & Nentwig, 1991; Wunderlich, 2004) and Araneidae (Levy, 1987; Roberts, 1995; Scharff & Coddington, 1997), and several species were transferred to other genera. Recently, in the absence of phylogenetic evidence, Wunderlich (2004) split *Zygiella* into four smaller genera and proposed them to belong to their own family, Zygiellidae. According to Wunderlich (2004), Zygiellidae consists of *Leviellus*, *Parazygiella*, the monotypic *Stroemiellus*, *Zygiella* in the strict sense (from here on "*Zygiella*"), but also *Chrysometa*, traditionally a tetragnathid that also builds a sector web – a proposed zygiellid synapomorphy. While the new genera have been catalogued, the family Zygiellidae has not been generally accepted (Platnick, 2013).

Several recent molecular phylogenies of araneoid spiders cast new doubt on the araneid affinity of *Zygiella* s.l. (Alvarez-Padilla *et al.*, 2009; Blackledge *et al.*, 2009; Sensenig *et al.*, 2010; Agnarsson *et al.*, 2012; Blackledge *et al.*, 2012a; Dimitrov *et al.*, 2012). These analyses include only two *Zygiella* species (*Z. x-notata* and/or *Z. atrica*), and they establish a clade that unites *Zygiella* with the Australasian leaf-curling araneids *Deliochus* and *Phonognatha*. They ambiguously place the "zygiellid" clade somewhere between all other araneids and the family Nephilidae, which suggests that perhaps Zygiellidae may be a valid taxonomic concept.

Until recently, the spiders of the genus *Caerostris* were grossly understudied ecologically, behaviorally and taxonomically. However, this enigmatic genus contains diverse, large orb weavers that are widespread through the Old World tropics (Fig. 2; Grasshoff, 1984) and exhibit peculiar web biology (Kuntner & Agnarsson, 2010). *Caerostris* spiders build the largest orb webs utilizing the toughest silk (Agnarsson *et al.*, 2010; Gregorič *et al.*, 2011b), and some species even utilize a unique habitat by suspending their webs above large water bodies, using a unique set of web building behaviors (Kuntner & Agnarsson, 2010; Gregorič *et al.*, 2011a). Because of their gigantic webs made of extremely tough silk, *Caerostris* spiders have the potential to become new models for biomaterial research. Additionally, the genus *Caerostris* represents a promising model for the research of extreme sexual size dimorphism, as it exhibits several peculiar behavioral syndromes associated with it, e.g. mate guarding, male-male aggressiveness, genital mutilation, mate plugging and self-castration (Kuntner *et al.*, 2008; Kuntner & Agnarsson, 2010).

Systematically, *Caerostris* spiders are largely unknown. Beyond the outdated revision (Grasshoff, 1984), *Caerostris* has been included in only three phylogenetic analyses, but with at most two species per study). Morphological data place *Caerostris* deep within araneids in the analysis of (Scharff & Coddington, 1997), but as basal araneids sister to *Argiope* in the analysis of (Kuntner *et al.*, 2008). Molecular data place *Caerostris* sister to all other araneids (Sensenig *et al.*, 2010). The tentative placement among basal araneids indicates that *Caerostris* might have similar phylogenetic affinities as zygiellids. Besides the uncertain phylogenetic position of *Caerostris*, only 12 species are currently catalogued, but future research might discover a much larger *Caerostris* diversity as the remaining pockets of Madagascar alone seem to harbor several undescribed species (Fig. 2; Kuntner & Agnarsson, 2010; Gregorič *et al.*, 2011b).

To build towards a robust tool for evolutionary research, we here test the monophyly and phylogenetic placement of *Zygiella* s.l. and *Caerostris*. We provide a phylogenetic analysis of most orbicularian families with emphasis on orb web building spider families. Using two mitochondrial (COI, 16S) and four nuclear (H3, 18S, 28S, ITS2) gene fragments, our molecular analysis includes all *Zygiella* s.l. genera, several former and potential zygiellid genera, as well as several described and undescribed *Caerostris* species with an extensive outgroup selection. Because phylogenies are essential in comparative biology as they can predict evolutionary pressures and outcomes (Felsenstein, 1985; Kiontke *et al.*, 2004), and because details in orb web architecture and building seem to characterize major orb weaving lineages (Eberhard, 1982; Kuntner *et al.*, 2008), we additionally discuss whether phylogenetic relationships among orb weaving lineages are reflected in behavior connected to orb web biology, specifically, whether the free sector in fact represents a zygiellid synapomorphy. We also construct the first partial species level phylogeny of *Caerostris*, describe five new *Caerostris* species, and discuss the evolution of web gigantism.

Methods

Taxonomic sampling for phylogenetic inference

As ingroup taxa, we first aimed to include as many species that were at any point associated with the genus *Zygiella*. We thus included all four *Zygiella* s.l. genera with at least two species per genus (except for the monotypic *Stroemiellus*) summing up to nine of the 17 described *Zygiella* s.l. and two undescribed species. We also included *Phonognatha* and *Deliochus*, the two genera consistently recovered sister to *Zygiella* in recent analyses (e.g. Sensenig *et al.*, 2010; Dimitrov *et al.*, 2012), as well as the tetragnathid *Chrysometa aureola*, a member of Zygiellidae sensu (Wunderlich, 2004). To infer the phylogenetic position of *Caerostris*, we included six of the 12 currently described species, which we sampled in SE Asia, continental Africa and Madagascar.

As outgroup taxa, we included several species formerly included in *Zygiella* s.l., such as the monotypic araneid *Yaginumia* (Archer, 1960) and several species of the tetragnathid genus *Guizygiella* (Zhu, 1997). Additionally, we included several species with web biology resembling “zygiellids”, e.g. the leaf-curling *Acusilas*, as well as the sector web building *Milonia*, *Araneus mitificus* and several other “araneids” and “tetragnathids”. Because the current phylogenetic placement of *Caerostris* hints at similar phylogenetic affinities of zygiellids and *Caerostris*, namely at the base of araneids, we also included taxa that previous phylogenetic studies recovered as basal araneids, e.g. *Oarces*, *Gnolus* and *Micrathena* (e.g. Sensenig *et al.*, 2010; Dimitrov *et al.*, 2012). As some former or potential zygiellids currently belong to Tetragnathidae, we also included several tetragnathid genera. In addition to our taxon choice that is biased towards families that build orb webs, we included all other orbicularian families, except Synaphridae, Sinopimoidae and Micropholcommatidae. Altogether, our data set included 114 species from 75 genera and 18 families (Appendix A). We rooted the trees with the non-araneoid *Deinopis* (Deinopidae).

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 two mitochondrial (COI, 16S) and four nuclear (H3, 18S, 28S, ITS2) gene fragments, which are among the standard genes of choice in spider phylogenetics. All PCR reactions had a total volume of 25 µl and consisted of 13.1 µl dd H_2O, 5 µl 5x PCR buffer “GoTaqFlexi” (Promega), 2.25 µl $MgCl_2$ (25 mM, Promega), 0.15 µl “5U GoTaqFlexi Polimerase” (Promega), 2.5 µl “dNTP Mix” (2µM each, Biotools), 0.5 µl of each forward and reverse 20 µM primers, and 1.5 µl of DNA. We included 0.15 µl of bovine serum albumin (Promega, Fitchburg, Wisconsin; 10mg/ml) to some reactions and accordingly decreased the H_2O volume. We performed the PCR amplifications using a "2720 Thermal Cycler" (Applied Biosystems) and a "Mastercycler® ep" (Eppendorf). We used different PCR protocols with varying annealing temperatures and cycle-settings for most genes.

We obtained ~ 1.2 kb fragments of the mitochondrial cytochrome c oxidase subunit I gene (COI) by using several primer combinations. We used the forward "LCO1490" (GGTCAACAAATCATAAAGATATTGG) (Folmer *et al.*, 1994) with the reverse "C1-N-2776" (also called "Maggy"; GGATAATCAGAATATCGTCGAGG) (Hedin & Maddison, 2001) primers to get the whole fragment. Alternatively, we used several combinations of the forward primers LCO1490, "degenerate LCO1490" (GGTCAACAAATCATAAAGAYATYGG) (Folmer *et al.*, 1994) and "Tom" (GATCGAAATTTTAATACTTCTTTTTTTGA) (Vidergar & Kuntner unpublished), with the reverse primers Maggy, "HCO2198" (TAAACTTCAGGGTGACCAAAAAATCA) (Folmer *et al.*, 1994), "degenerate HCO2198" (TAAACTTCAGGGTGACCAAARAAYCA) (Folmer *et al.*, 1994) and "Chelicerate-R2" (GGATGGCCAAAAAATCAAAATAAATG). 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 ~ 0.5 kb fragments of the mitochondrial large ribosomal subunit gene (16S) by using the forward "16Sar" (CGCCTGTTTATCAAAAAC) (Palumbi *et al.*, 1991) and reverse "16Sbr" (CTCCGGTTTGAACTCAGATCA) primers. We amplified the fragments using a touch up program with PCR cycling conditions 94°C for 5 min, followed by 20 cycles of 94°C for 1 min, 45°C – 55°C for 1 min 50 sec, 72°C for 2.5 min, followed by 17 cycles of 94°C for 1 min, 52°C for 1 min 50 sec, 72°C for 2.5 min, and a final extension period of 72°C for 10 min.

We obtained ~ 0.3 kb fragments of the nuclear protein coding histone 3 subunit A (H3) using the forward "H3aF1" (ATGGCTCGTACCAAGCAGACVGC) (Colgan *et al.*, 1998) and reverse "H3aR1" (ATATCCTTRGGCATRATRGTGAC) (Colgan *et al.*, 1998) primers. We amplified the fragments using a touch up program with PCR cycling conditions 94°C for 5 min, followed by 20 cycles of 94°C for 45 sec, 42°C – 50°C for 1.5 min, 72°C for 1.5 min, followed by 16 cycles of 94°C for 45 sec, 46°C for 1.5 min, 72°C for 1.5 min, and a final extension period of 72°C for 10 min.

We obtained ~ 0.7 kb and ~ 1.15 kb fragments of the nuclear small subunit ribosomal gene (18S). We obtained the ~ 0.7 kb fragments using the forward primer "18S-lev2" (CCTGAGAAACGGCTACCACATC) (Whiting *et al.*, 1997) with the reverse "18S-b0" (GTTTCAGCTTTGCAACCAT) (Whiting *et al.*, 1997). We obtained the ~ 1.15 kb fragments using two primer pair, the forward primer "18Sa" (ATTAAAGTTGTTGCGGTTA) with the reverse primer "18Sbi" (GAGTCTCGTTCGTTATCGGA), and the forward primer "5f" (GCGAAAGCATTTGCCAAGAA) with the reverse primer "9r" (GATCCTTCCGCAGGTTCACCTAC). We amplified the fragments using a touch up program with PCR cycling conditions 96ºC for 5 min, followed by 20 cycles of 94ºC for 55 sec, 49ºC – 59ºC for 1 min 50 sec, 72ºC for 2 min, followed by 15 cycles of 94ºC for 55 sec, 55ºC for 1.5 min, 72ºC for 2 min, and a final extension period of 72ºC for 10 min.

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

We obtained the ~ 0.4 kb fragments of the nuclear internal transcribed spacer 2 (ITS2) using the forward ITS4 (TCCTCCGCTTATTGATATGC) and reverse ITS5.8 (GGGACGATGAAGAACGCAGC) primers. We amplified the fragments using a touch up program with PCR cycling conditions 94ºC for 5 min, followed by 20 cycles of 94ºC for 45 sec, 42ºC – 50ºC for 1.5 min, 72ºC for 1.5 min, followed by 16 cycles of 94ºC for 45 sec, 46ºC for 1.5 min, 72ºC for 1.5 min, and a final extension period of 72ºC for 10 min.

Phylogenetic inference

We aligned protein coding genes (COI, H3) using ClustalW alignment and checked for stop codons to assure correct alignment. Because ribosomal gene fragments (16S, 18S, 28S, ITS2) show unequal lengths, we aligned them with the online version of the automatic aligner MAFFT v.6 (http://mafft.cbrc.jp/alignment/server/; Katoh & Standley, 2013), using secondary structure of RNA information during the alignment process (the Q-INS-i strategy) and other values set to default. The alignments of the ribosomal genes contained highly unequal distributions of indels. We thus used Gblocks 0.91b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) 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 smaller final blocks, gap positions within final blocks, and less strict flanking positions. Using Mesquite 2.75 (Maddison & Maddison, 2013), we concatenated gene fragments into two matrices, one with the full 5533 bp of data, and another containing ribosomal genes trimmed using Gblocks, summing up to 3875 bp of data.

We conducted both Bayesian and Maximum Likelihood (ML) analyses for four different partition schemes. We used unlinked models for each gene of both the full and Gblocks-trimmed data sets (the "full gene partition" and "gblocks gene partition", respectively), and we used unlinked models for each gene and codon position in protein coding genes, of both the full and Gblocks-trimmed data sets ("full codon partition" and "gblocks codon partition", respectively). 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). We performed two independent runs with four simultaneous Markov Chain Monte Carlo (MCMC) chains, each starting with random starting trees, running for a total of 35 million generations. We discarded 50% generations as burnin. We conducted ML analyses using Garli 2.0. For every partition scheme, we first ran two search replicates, used stepwise-addition starting topologies and used automated stopping criteria. Using the best trees obtained by these search replicates, we then ran 100 bootstrap replicates under same conditions.

For the species level phylogeny of *Caerostris*, we used COI, H3, ITS2 and 28S gene fragments. We calculated the difference in COI gene fragment between *Caerostris* individuals using Mega 5.1 (Tamura *et al.*, 2011). We combined the results of our molecular phylogenies with morphological evidence to delimit species. To avoid spliting populations into unnatural species, we regarded clades with more molecular structuring (e.g. *C. sumatrana* and *C. sexcuspidata*) as single species, but comment on that in the discussion. We plotted the presence of the sector web and web size on the preferred topology using Mesquite (Maddison & Maddison, 2013). We coded the sector web as absent (0), present (1) and inapplicable (2) for taxa that do not build the classical orb webs. To trace web size evolution among orb weavers, we coded web size as small (web size $< 0.1\ m^2$), large ($0.1\ m^2 <$ web size $< 0.5\ m^2$) and gigantic (web size $> 0.5\ m^2$). We set the large webs threshold to $0.1\ m^2$ following (Gregorič *et al.*, 2011b), and arbitrarily set the gigantic webs threshold to $0.5\ m^2$. To trace web size evolution among *Caerostris*, we plotted continuous web size data (Gregorič *et al.*, 2011b; own data).

Taxonomic methods

To differentially diagnose new species of *Caerostris* from the ones not sampled for our molecular phylogeny, we used Grasshoff's (1984) revision of *Caerostris*. We performed all measurements using a Leica M165 C stereomicroscope equipped with a Leica DFC 420C camera through the Leica Application Suite 3.8. We report all measurement in millimeters. We use the following anatomical abbreviations in text and figures: ALE, anterior lateral eyes; AME, anterior median eyes; BH, basal haematodocha; CB, cymbium; CD, copulatory duct; E, embolus; FD, fertilization duct; PME, posterior median eyes; S, spermatheca; SD, sperm duct; T, tegulum.

We captured images of external structures as well as palpal and epigynal anatomy using the Visionary Digital imaging system, equipped with a Canon 5D Mark II digital camera body and an Infinity K2 microscope with Olympus metallurgical lenses, and we captured the images for later stacking using Adobe Lightroom 4 (http://www.adobe.com/products/photoshop-lightroom.html?promoid=KAUCD). We "stacked" the images using Zerene Stacker (http://www.zerenesystems.com/) and Helicon Focus (http://www.heliconsoft.com/), and further manipulated them in Adobe Photoshop CS4 (http://www.adobe.com/products/photoshop.html?promoid=KAUCA).

Results

Bayesian and ML analyses, both under the four different partition schemes, yielded somewhat similar topologies regarding the position of Zygiellidae and *Caerostris* (Fig. 3). All analyses recovered a strongly supported monophyletic Zygiellidae, which includes *Zygiella* s.l. (*Zygiella*, *Leviellus*, *Parazygiella* and *Stroemiellus*) sister to *Phonognatha+Deliochus*, exclusive of other potential zygiellid genera. Both *Zygiella* s.l. and *Phonognatha+Deliochus* received strong support. However, *Zygiella*, *Parazygiella* and *Phonognatha* were not recovered monophyletic in the analyses. *Zygiella nearctica* from North America grouped with other *Parazygiella* instead of *Zygiella*, while *Parazygiella sp. A* from Taiwan was ambiguously placed between *Parazygiella* and *Zygiella*. The monotypic *Stroemiellus* was recovered sister to *Leviellus* and this clade was strongly supported. All analyses strongly supported a monophyletic Zygiellidae+Araneidae+Nephilidae. In the analyses under the "gblocks partition" schemes, the placement of Zygiellidae within this clade was poorly supported or unresolved, while the analyses under the "full partition" schemes strongly supported zygiellids as sister to all other araneids. None of the analyses recovered Zygiellidae sister to Nephilidae.

Other species that were associated with *Zygiella* in the past, or share certain web biology features "typical" of Zygiellidae (e.g. sector web, tubular silk retreat, leaf-retreat in web), were recovered within Araneidae or Tetragnathidae (Fig. 3, 5A). For example, *Chrysometa aureola*, placed within Zygiellidae sensu (Wunderlich, 2004), was recovered within Tetragnathidae in our analyses. Also supported by all analyses, all species of *Guizygiella*, some of them formerly in *Zygiella*, were recovered as monophyletic within Araneidae rather than the classical Tetragnathidae. The monotypic *Yaginumia*, also formerly *Zygiella*, was strongly supported as sister to all *Guizygiella*. The sector web building *Milonia* was strongly supported as sister to *Yaginumia*+*Guizygiella*. The leaf-retreat building *Acusilas* was closely associated with the Argiopinae subfamily in the analyses.

The monophyly of *Caerostris* received strong support and the genus was recovered within araneids in all analyses (Fig. 3). However, its exact placement is ambiguous. All analyses recovered *Caerostris* among basal araneids (excluding Zygiellidae), together with *Oarcis*+*Gnolus*, *Micrathena* and an undescribed araneid from Madagascar (code ORB019), but the relationships among them changed among the partition schemes. The clade of all other araneids also received strong support. Fig. 4 shows the *Caerostris* species level phylogeny (topology from BI for COI, 28S, H3 and ITS2) and the difference in the COI gene fragment between individuals. The phylogenetic relationship between species are poorly supported, and several species show a low difference in COI gene fragment between individuals (e.g. *C. darwini*, *C. extrusa* and *C. bojani* with a difference below 1.5%), but others show more structuring (e.g. *C. sumatrana* and *C. sexcuspidata* with a difference over 7% and 9%, respectively).

Based on our taxon choice, the sector web seems to have independently evolved twice in Tetragnathidae, in the zygiellid group *Zygiella* s.l. and at least five times in Araneidae (Fig. 5A). Large webs (web size > 0.1 m^2) evolved in Tetragnathidae, Nephilidae and several times in Araneidae, but gigantic webs (web size > 0.5 m^2) evolved only in nephilid genera *Nephila* and *Nephilingis*, and in *Caerostris* (Fig. 5B). Web size evolution within *Caerostris* does not show a clear pattern, and web gigantism within *Caerostris* seems to have evolved in the ancestor of *C. darwini* (Fig. 6).

Discussion

We provide the first robust test of the monophyly and phylogenetic placements of the controversial Zygiellidae and *Caerostris*. Our results support Zygiellidae as sister to all other araneids, and *Caerostris* among basal araneids. We further show that both Zygiellidae and *Caerostris* behaviorally differ from other araneids. Our results also indicate that Araneidae contains several non-typical groups, which hints at the need to redefine this diverse spider family. Additionally, we describe five new *Caerostris* species.

Phylogenetic placement of Zygiellidae

Recent molecular phylogenies of araneoid spiders consistently recover a zygiellid clade uniting *Zygiella* with *Phonognatha* and *Deliochus* (Alvarez-Padilla *et al.*, 2009; Blackledge *et al.*, 2009; Agnarsson *et al.*, 2012; Blackledge *et al.*, 2012a; Dimitrov *et al.*, 2012; Kuntner et al. 2013), but contradict each other in placing Zygiellidae sister to either Araneidae (Alvarez-Padilla *et al.*, 2009; Blackledge *et al.*, 2012a; Dimitrov *et al.*, 2012), Nephilidae (Blackledge *et al.*, 2009) or Araneidae+Nephilidae (Sensenig *et al.*, 2010; Agnarsson *et al.*, 2012; Kuntner et al. 2013). However, all mentioned studies strongly support the monophyly of a group containing Nephilidae, Zygiellidae and other Araneidae. As these recent phylogenetic studies each addressed a different question, the diversity of included focal taxa might have been too low to unambiguously resolve the relationships between zygiellids, araneids and nephilids. Namely, the mentioned studies mostly included one, at most two *Zygiella* species (*Z. x-notata* and/or *Z. atrica*), mostly one *Nephila* and one *Nephilengys* among nephilids, and very few non- araneines. On the other hand, besides an extensive outgroup sample, our analyses included all four *Zygiella* s.l. genera, several other former and potential zygiellids, all nephilid genera, several non-araneines, and tetragnathids that resemble zygiellids in web biology. Our results strongly support the clade consisting of Zygiellidae, Araneidae and Nephilidae, and also support the placement of Zygiellidae sister to other Araneidae, rather than Nephilidae or Nephilidae+Araneidae (Fig. 3).

The placement of Zygiellidae sensu Wunderlich (2004) as sister to Tetragnathidae is clearly refuted. Wunderlich (2004) also proposed Zygiellidae to include the sector web building *Chrysometa*, and the splitting of *Zygiella* s.l. into *Leviellus*, *Parazygiella*, *Stroemiellus* and *Zygiella*. While Zygiellidae might be considered a valid concept, it does not include *Chrysometa* that nested deep within tetragnathids in our analyses (Fig. 3). All of our analyses strongly support *Leviellus* as monophyletic and sister to the monotypic *Stroemiellus*. Thus, because monotypic genera do not contain grouping information and *Stroemiellus* forms a well-supported clade with *Leviellus*, we argue in favor of synonymizing *Stroemiellus* with *Leviellus*. All analyses recover *Zygiella* paraphyletic and most analyses recover *Parazygiella* paraphyletic, with *Z. nearctica* nested within *Parazygiella*, and an undescribed species (*sp. A* from Taiwan) that the authors identified as *Parazygiella* nested within *Zygiella*. As these two genera build a clade that contains all their species, perhaps *Parazygiella* should be synonymized with *Zygiella*. While *Leviellus*+*Stroeimiellus*, *Parazygiella* and *Zygiella* all seem to be diagnosable by genital morphology (Wunderlich, 2004), the relationships within *Zygiella*+*Parazygiella* are not well supported in our analyses and should be focused on in future studies. Furthermore, Zygiellidae seem to differ from other araneids in several details of their web building behavior (see *Orb web evolution and orb web features as taxonomic characters* for details).

Several former species of *Zygiella*, now belonging to *Guizygiella* and *Yaginumia*, were regarded as related to *Zygiella* by (Zhu *et al.*, 1997) and (Wunderlich, 2004), but none of these authors included them in any analysis. Our results strongly support *Guizygiella* as araneid rather than tetragnathid, with the monotypic *Yaginumia* its sister (Fig. 3). As monotypic genera in this form are unnecessary, *Guizygiella* Zhu et al. 1997 should probably be synonymized with *Yaginumia* Archer 1960. Our results further strongly support a group consisting of *Yaginumia*+*Guizygiella* and *Milonia*, all sector web building araneids, but the prevalance of the free sector in *Guizygiella* is variable (own data).

Phylogenetic placement of *Caerostris*

Our results support the basal position of *Caerostris* within Araneidae (excluding Zygiellidae), and stand in contrast with morphological phylogenies (Scharff & Coddington, 1997; Kuntner *et al.*, 2008). While these morphological analyses recovered *Caerostris* close to gasteracanthines (Scharff & Coddington, 1997) and argiopines (Kuntner *et al.*, 2008), molecular data strongly support *Caerostris* outside a group that includes argiopines (including *Gasteracantha*) and araneines (Sensenig *et al.*, 2010; this study). However, based on our results, the exact position of *Caerostris* is ambiguous: depending on the partition scheme used in our analyses, *Caerostris*, *Oarces*+*Gnolus*, *Micrathena* and an unidentified araneid from Madagascar mostly recovered as a grade leading to all other araneids, but the relationships among them are not resolved. These "basal araneids" are all morphologically distinct from other araneids (Scharff & Coddington, 1997), and with the exception of *Micrathena*, little is known about their biology. For example, virtually nothing is known about *Oarces* and *Gnolus*, which have been placed in Mimetidae based on several morphological features (Platnick & Shadab, 1993), but recent molecular studies place them at the base of Araneidae (Dimitrov *et al.*, 2012; this study). *Caerostris* morphologically differ from typical araneids, by e.g. the flattened tibiae and metatarsi, modified clypeus, abdominal sigillae and macrosetae on femur IV (Scharff & Coddington, 1997; Smith, 2006; Kuntner *et al.*, 2008), and *Caerostris* spiders also exhibit several peculiarities in their web building behavior (see *Orb web evolution and orb web features as taxonomic characters* for details).

Based on COI differences, *C. darwini*, *C. extrusa* and *C. bojani*, are well defined species, and all other species except *C. sexcuspidata* and *C. sumatrana* also seem to be well defined with differences between individuals at most around 3% (Fig. 6). Asian *Caerostris* only encompass the widely distributed *C. sumatrana* and *C. indica* from Myanmar (Grasshoff, 1984). As these two species resemble each other in genital morphology, we find it likely that most collected Asian *Caerostris* spiders have simply been identified as *C. sumatrana* based on genital morphology. However, the difference of up to 7.4% between our specimens from southern China and Laos indicates that throughout SE and S Asia, there is probably several more *Caerostris* species that morphologically resemble each other. Similarly, *C. sexcuspidata* shows a wide distribution based on morphological identifications, but our results show that within South African Republic alone, the difference of morphologically similar specimens ranges at least up to 9.1%. This strongly indicates that *C. sexcuspidata*, also collected from central and eastern Africa (Fig. 6), represents a species complex distributed across the whole southern half of Africa. Additionally to the five described species occurring in Madagascar (not counting *C. darwini* that was misidentified as *C. sexcuspidata* in past collections), we here describe an additional five new *Caerostris* species from Madagascar (Appendix B), which further hints at large *Caerostris* diversity in Madagascar and Africa.

Orb web evolution and orb web features as taxonomic characters

Phylogenies are essential in comparative biology, they can predict evolutionary pressures and outcomes, and can thus also be powerful predictors of traits in unstudied taxa (Felsenstein, 1985; Kiontke *et al.*, 2004). Phylogenies also seem to predict behavior in orb weaving spiders (Kuntner & Agnarsson, 2009; but see Eberhard *et al.*, 2008) for contradicting evidence in Theridiidae). For example, Eberhard (1982) investigated details in web architecture and web building behavior of at least 148 species from 55 genera, and concluded that some details are conservative enough to characterize families and genera. Although Eberhard (1982) established such behavioral synapomorphies more than three decades ago, many of them still seem to characterize major orb weaving lineages (Kuntner *et al.*, 2008). Among the studied traits, several details in construction of the web hub, the radial and spiral threads, and behavior when attacking prey were the most informative (Eberhard, 1982).

The sector web is a proposed synapomorphy and diagnostic character of Zygiellidae sensu (Wunderlich, 2004). We here show that first, the sector web building *Chrysometa* is a tetragnathid rather than a zygiellid, and second that the sector web clearly evolved several times across zygiellids, araneids and tetragnathids (Fig. 5A). Furthermore, the sector web is homologous for *Zygiella* s.l. rather than Zygiellidae, as the Australasian zygiellid genera *Deliochus* and *Phonognatha* do not build sector webs (Hormiga *et al.*, 1995). However, the combination of other orb web features seems to define Zygiellidae. *Zygiella* s.l. builds doubled radial threads in their orb webs (Hormiga *et al.*, 1995; unpublished data), a feature typical of the cribellate uloborid webs (Eberhard, 1982). The other orb weaving families, the nephilids, araneids and tetragnathids typically build single radial threads, but differ in details of their construction behavior (Eberhard, 1982; but see Kuntner *et al.*, 2008 who term the unique nephilid radii doubled). All zygiellids use a variation of the tubular silk retreat, be it a silken tube above the web in *Zygiella* s.l. (Gregorič *et al.*, 2010), a silken tube with a leaf next to the web in *Deliochus*, or a rolled leaf-retreat inside the web in *Phonognatha* (Kuntner *et al.*, 2008). Such retreats evolved convergently within araneids several times, and are found in *Acusilas* (leaf-retreat inside web; Kuntner *et al.*, 2008), *Milonia* and *Guizygiella* (silken tube and sector web in both genera; own data), and in *Singa* and *Perilla* (silken retreat inside a rolled grass stem; Kuntner *et al.*, 2008; own data), but silken tube retreats are also found in the nephilid Nephilengys and in several theridiids (Kuntner *et al.*, 2008). Although not documented for all genera, at least some zygiellids seem to exhibit several other non-typical araneid behaviors. *Phonognatha* leaves the web hub intact after orb construction, as do uloborids and nephilids (Eberhard, 1982; Hormiga *et al.*, 1995; Kuntner *et al.*, 2008), while tetragnathids bite it out and araneids bite it out and seal the hole back up (Eberhard, 1982). *Phonognatha* leaves the temporary spiral in the finished orb, as do nephilids, while most other orb weavers including *Zygiella* s.l. and *Deliochus* remove it when building the sticky spiral (Kuntner *et al.*, 2008). No zygiellids "decorate" their webs with stabilimenta or detritus, as do some nephilids, araneids and uloborids (e.g. *Nephila*, *Argiope*, *Cyclosa*, *Uloborus*; Eberhard, 1982; Kuntner *et al.*, 2008). All zygiellids attack their prey by biting first and then wrapping it, similar to nephilids and unlike most araneids (Kuntner *et al.*, 2008; Gregorič *et al.*, 2010). Zygiellids hide in the retreat during the day, and they do not shake their body or change the side of the web when threatened (Kuntner *et al.*, 2008; Gregorič *et al.*, 2010), as do some araneids (e.g. *Argiope* and *Azilia*) and nephilids (e.g. *Nephilengys*, *Nephila* and *Clitaetra*; Kuntner *et al.*, 2008), but rather run to the retreat or jump off web.

Similar to zygiellids, *Caerostris* also exhibits specialized orb web architecture and building behavior. *Caerostris* deviates in early orb web construction from all other known orb weavers: it employs almost no web site exploration, builds no secondary web frames, and constructs the entire orb below the initial bridge line, in contrast to other orb weavers that extensively explore their web sites, rarely do not build secondary web frames, and build the orb around the initial bridge line (Gregorič *et al.*, 2011a); unpublished data). Furthermore, *Caerostris* build orb webs that contain two types of radial threads, single radial threads in the upper and doubled radial threads in the lower orb half (Gregorič *et al.*, 2011a; unpublished data). To the best of the authors' knowledge, building the orb below the initial bridge and utilizing two types of radial threads in a single web, are both behaviors never observed in any other orb weaving genus. Moreover, *Caerostris* spiders sometimes build weak stabilimenta and never "decorate" webs with detritus (Gregorič *et al.*, 2011b). Some *Caerostris* species (e.g. *C. almae*, *C. bojani*) are nocturnal and hide mimicked as bark during the day, but do not build retreats when on web (Kuntner & Agnarsson, 2010; unpublished data). Other species utilize the web day and night, and never leave the web hub (e.g. *C. darwini*, *C. extrusa* and *C. pero*; Kuntner & Agnarsson, 2010; unpublished data). *Caerostris* do not shake their body or change the side of the web when threatened, but rather run to the edge of the web or jump off web (unpublished data). They attack their prey by biting first and then wrapping it, and carry all but the largest prey back to hub in their massive chelicerae or lift the largest prey to the hub still attached to other web parts (Gregorič *et al.*, 2011b). Other orb weavers typically hang all but the smallest prey to their spinnerets and this way carry the wrapped prey back to the hub (Foelix, 2011).

Large webs evolved several times in tetragnathids, nephilids and araneids (Fig. 5B), probably simply due to the fact that large spiders logically build larger webs. However, web gigantism seems to have evolved only twice, namely in the nephilids *Nephila* and *Nephilingis*, and in *Caerostris*. While this is somewhat expected for the two nephilid genera whose body sizes are the largest among orb weavers (Kuntner & Coddington, 2009), *Caerostris* are not exceptionally large spiders, but of size comparable or smaller to species of several prominent araneid genera like *Argiope*, *Araneus*, *Parawixia*, *Neoscona* etc. (Sensenig et al., 2010). Interestingly, the two nephilid genera and *Caerostris* represent two converse web architectures that also show converse strategies in compensatory evolution of web performance (Sensenig et al., 2010). Namely, *Nephila* and *Nephilingis* both build webs using silk threads of average quality (low quality corrected for spider size) while *Caerostris* uses the toughest silk (high quality even corrected for spider size) (Sensenig et al., 2010). However, *Nephila* and *Nephilingis* both build very dense webs (Kuntner et al., 2008; Sensenig et al., 2010), and their webs thus still exhibit a very high stopping potential, capable of stopping and retaining even vertebrate prey (Sensenig et al., 2010; Nyffeler & Knornschild, 2013). *Caerostris* on the other hand builds sparse webs (Sensenig et al., 2010; Gregorič et al., 2011b), but due to the extremely tough silk, the web's stopping potential is comparable to that of nephilid and best araneid webs (Sensenig et al., 2010). Both nephilids and *Caerostris* show web architecture and building behaviors different from araneids, zygiellids, and tetragnathids, and this begs the question whether these differences represented predispositions for the evolution of extremely large web architectures, and/or perhaps for the evolution of size gigantism in *Nephila* and the extreme material properties of silk in *Caerostris*.

Classification implications

The classification implications of our study delve into the definition of Araneidae. Araneidae is the largest orbicularian family and ranks third by species number among spiders in general (Platnick, 2013). While some groups are seemingly well defined, e.g. the Araneinae and Argiopinae (Scharff & Coddington, 1997), certain genera within Araneidae (e.g. *Araneus* and *Neoscona*) can probably be regarded as “waste baskets” for many species that have little in common. The “waste basket” phenomenon was also observed in other spider families, e.g. in *Theridion*, *Achaearanea* and *Anelosimus* among theridiids (Agnarsson, 2004) and *Leptyphantes* among linyphiids (Frick *et al.*, 2010; Platnick, 2013), and is characterized by heterogeneous and polyphyletic groups, with species even belonging to different families (Platnick, 2013). Furthermore, synapomorphies of the diverse Araneidae have been elusive and the composition of

Araneidae is a phylogenetic problem (Scharff & Coddington, 1997; Kuntner *et al.*, 2013). We thus argue in favor of redefining Araneidae, establishing several well defined families. For example, both Zygiellidae and Nephilidae, traditionally within Araneidae, were later transferred between Araneidae and Tetragnathidae, and could thus be regarded araneids in the broadest sense. However, Nephilidae is well defined morphologically and behaviorally, and remains to be ranked as family (Kuntner *et al.*, 2008; Kuntner *et al.*, 2013; Platnick, 2013). Similarly, molecular data strongly support Zygiellidae that characteristically differs from other araneids in behavior, although previous studies have failed to find solid morphological synapomorphies (Scharff & Coddington, 1997). Furthermore, our results continue to build evidence that there are several more distinct spider groups within Araneidae, especially lineages at basal nodes that are especially important for understanding ancestral traits. Our results thus highlight the need for further analyses that will include several more araneid genera, in order to resolve the phylogeny of Araneidae. A redefinition of Araneidae and resolved phylogenetic relationships among orb weavers would thus offer a platform for studying the mentioned diverse topics in evolutionary biology.

Conclusions

We here provide the first robust species level molecular phylogenies of the taxonomically controversial Zygiellidae and *Caerostris*. Using extensive ingroup and outgroup sampling, we show that first, the family Zygiellidae contains the Holarctic *Zygiella* s.l. and the Australasian *Phonognatha* and *Deliochus*, and might be considered a valid taxonomic concept, despite some ambiguity in its position in relation to nephilids and other araneids. Second, *Caerostris* is a basal araneid genus, not a member of Argiopinae or Araneinae, but its exact phylogenetic affinities remain ambiguous. Third, phylogenetic exclusivity seems to reflect behavioral differences, as both Zygiellidae and *Caerostris* are strongly supported clades that differ from other araneids and nephilids in the combination of behavioral traits. Despite somewhat ambiguously supported phylogenetic positions of Zygiellidae and *Caerostris*, we believe that our results represent substantial progress towards resolving phylogenetic relationships between major orb weaving lineages.

Acknowledgements

We thank Ren-Chung Cheng, Tjaša Lokovšek, Yadira Ortiz and Joel Duff for their laboratory and logistic help. We thank Jan Dolansky, Petr Dolejš, Peter Jäger, Daiqin Li, Wenjin Gan, “joker” from Xishuangbana, Yuri Marušik, Honore Rabarison, Sahondra Lalao Rahanitriniaina, Mark A. Townley, and MICET and ICTE crews for sending fresh material and/or helping in the field. 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

Agnarsson, I. 2003. Spider webs as habitat patches - The distribution of kleptoparasites (Argyrodes, theridiidae) among host webs (Nephila, tetragnathidae). *Journal of Arachnology* 31: 344-349.

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., Dhinojwala, A., Sahni, V. & Blackledge, T.A. 2009. Spider silk as a novel high performance biomimetic muscle driven by humidity. *Journal of Experimental Biology* 212: 1989-1993.

Agnarsson, I., Kuntner, M. & Blackledge, T.A. 2010. Bioprospecting finds the toughest biological material: Extraordinary silk from a giant riverine orb spider. *Plos One* 5: e11234.

Agnarsson, I. 2011. Habitat patch size and isolation as predictors of occupancy and number of argyrodine spider kleptoparasites in Nephila webs. *Naturwissenschaften* 98: 163-167.

Agnarsson, I., Gregorič, M., Blackledge, T.A. & Kuntner, M. 2012. The phylogenetic placement of Psechridae within Entelegynae and the convergent origin of orb-like spider webs. *Journal of Zoological Systematics and Evolutionary Research* 51: 100-106.

Agnarsson, I., Coddington, J.A. & Kuntner, M. 2013. Systematics: Progress in the study of spider diversity and evolution. In: *Spider Research in the 21st Century: Trends and Perspectives* (D. Penney, ed. Siri Scientific Press, Rochdale, UK.

Alvarez-Padilla, F., Dimitrov, D., Giribet, G. & Hormiga, G. 2009. Phylogenetic relationships of the spider family Tetragnathidae (Araneae, Araneoidea) based on morphological and DNA sequence data. *Cladistics* 25: 109-146.

Bel-Venner, M.C. & Venner, S. 2006. Mate-guarding strategies and male competitive ability in an orb-weaving spider: results from a field study. *Animal Behaviour* 71: 1315-1322.

Bel-Venner, M.C., Dray, S., Allaine, D., Menu, F. & Venner, S. 2008. Unexpected male choosiness for mates in a spider. *Proceedings of the Royal Society B-Biological Sciences* 275: 77-82.

Blackledge, T.A. & Gillespie, R.G. 2004. Convergent evolution of behavior in an adaptive radiation of Hawaiian web-building spiders. *Proceedings of the National Academy of Sciences of the United States of America* 101: 16228-16233.

Blackledge, T.A., Scharff, N., Coddington, J.A., Szuts, T., Wenzel, J.W., Hayashi, C.Y. & Agnarsson, I. 2009. Reconstructing web evolution and spider diversification in the molecular era. *Proceedings of the National Academy of Sciences of the United States of America* 106: 5229-5234.

Blackledge, T.A., Kuntner, M. & Agnarsson, I. 2011. The form and function of spider orb webs: Evolution from silk to ecosystems. In: *Advances in insect physiology, Vol 41: Spider physiology and behaviour - Behaviour*, Vol. 41 (J. Casas, ed., pp. 175-262.

Blackledge, T.A., Kuntner, M., Marhabaie, M., Leeper, T.C. & Agnarsson, I. 2012a. Biomaterial evolution parallels behavioral innovation in the origin of orb-like spider webs. *Scientific Reports* 2.

Blackledge, T.A., Perez-Rigueiro, J., Plaza, G.R., Perea, B., Navarro, A., Guinea, G.V. & Elices, M. 2012b. Sequential origin in the high performance properties of orb spider dragline silk. *Scientific Reports* 2.

Blamires, S.J. 2010. Plasticity in extended phenotypes: orb web architectural responses to variations in prey parameters. *Journal of Experimental Biology* 213: 3207-3212.

Bond, J.E. & Opell, B.D. 1998. Testing adaptive radiation and key innovation hypotheses in spiders. *Evolution* 52: 403-414.

Coddington, J.A. 1994. The roles of homology and convergence in studies of adaptation. In: *Phylogenetics and Ecology* (P. V. Eggleton & R. Vane-Wright, eds), pp. 53-78. The Linnean Society of London, London, UK.

Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G. & Gray, M.R. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology* 46: 419-437.

Craig, C.L., Weber, R.S. & Bernard, G.D. 1996. Evolution of predator-prey systems: Spider foraging plasticity in response to the visual ecology of prey. *American Naturalist* 147: 205-229.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772-772.

Dimitrov, D., Lopardo, L., Giribet, G., Arnedo, M.A., Alvarez-Padilla, F. & Hormiga, G. 2012. Tangled in a sparse spider web: single origin of orb weavers and their spinning work unravelled by denser taxonomic sampling. *Proceedings of the Royal Society B-Biological Sciences* 279: 1341-1350.

Eberhard, W.G. 1982. Behavioral characters for the higher classification of orb-weaving spiders. *Evolution* 36: 1067-1095.

Eberhard, W.G. 1986. Effects of orb-web geometry on prey interception and retention. In: *Spiders, webs, behavior and evolution* (W. A. Shear, ed., pp. 70-100. Stanford University Press, Stanford.

Eberhard, W.G. 2007. Miniaturized orb-weaving spiders: behavioural precision is not limited by small size. *Proceedings of the Royal Society B-Biological Sciences* 274: 2203-2209.

Eberhard, W.G., Agnarsson, I. & Levi, H.W. 2008. Web forms and the phylogeny of theridiid spiders (Araneae: Theridiidae): chaos from order. *Systematics and Biodiversity* 6: 415-475.

Felsenstein, J. 1985. Phylogenies and the comparative method. *American Naturalist* 125: 1-15.

Foelix, R.F. 2011. *Biology of Spiders*, 3 edn. Oxford University Press, Oxford.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294-299.

Frick, H., Nentwig, W. & Kropf, C. 2010. Progress in erigonine spider phylogeny-the Savignia-group is not monophyletic (Araneae: Linyphiidae). *Organisms Diversity & Evolution* 10: 297-310.

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., Kostanjšek, R. & Kuntner, M. 2010. Orb web features as taxonomic characters in *Zygiella* s.l. (Araneae, Araneidae). *Journal of Arachnology* 38: 319-327.

Gregorič, M., Agnarsson, I., Blackledge, T.A. & Kuntner, M. 2011a. How Did the Spider Cross the River? Behavioral Adaptations for River-Bridging Webs in Caerostris darwini (Araneae: Araneidae). *Plos One* 6.

Gregorič, M., Agnarsson, I., Blackledge, T.A. & Kuntner, M. 2011b. Darwin's bark spider: giant prey in giant orb webs (Caerostris darwini, Araneae: Araneidae)? *Journal of Arachnology* 39: 287-295.

Gregorič, M., Kiesbuy, H.C., Lebron, S.G.Q., Rozman, A., Agnarsson, I. & Kuntner, M. 2013. Optimal foraging, not biogenetic law, predicts spider orb web allometry. *Naturwissenschaften* 100: 263-268.

Griswold, C.E., Coddington, J.A., Hormiga, G. & Scharff, N. 1998. Phylogeny of the orb-web building spiders (Araneae, Orbiculariae : Deinopoidea, Araneoidea). *Zoological Journal of the Linnean Society* 123: 1-99.

Hayashi, C.Y. & Lewis, R.V. 2000. Molecular architecture and evolution of a modular spider silk protein gene. *Science* 287: 1477-1479.

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

Heimer, S. & Nentwig, W. 1991. *Spinnen Mitteleuropas. Ein Bestimmungsbuch*. Blackwell Wissenschafts-Verlag.

Herberstein, M. & Wignall, A. 2011. Introduction: spider biology. In: *Spider Behaviour: Flexibility and Versatility* (M. Herberstein, ed., pp. 1-30. Cambridge University Press, Cambridge.

Higgins, L.E. & Buskirk, R.E. 1998. Spider-web kleptoparasites as a model for studying producer-consumer interactions. *Behavioral Ecology* 9: 384-387.

Hormiga, G., Eberhard, W.G. & Coddington, J.A. 1995. Web-construction behavior in australian *Phonognatha* and the phylogeny of nephiline and tetragnathid spiders (Araneae, Tetragnathidae). *Australian Journal of Zoology* 43: 313-364.

Katoh, K. & Standley, D.M. 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution* 30: 772-780.

Kiontke, K., Gavin, N.P., Raynes, Y., Roehrig, C., Piano, F. & Fitch, D.H.A. 2004. *Caenorhabditis* phylogeny predicts convergence of hermaphroditism and extensive intron loss. *Proceedings of the National Academy of Sciences of the United States of America* 101: 9003-9008.

Kuntner, M., Coddington, J.A. & Hormiga, G. 2008. Phylogeny of extant nephilid orb-weaving spiders (Araneae, Nephilidae): testing morphological and ethological homologies. *Cladistics* 24: 147-217.

Kuntner, M. & Agnarsson, I. 2009. Phylogeny accurately predicts behaviour in Indian Ocean Clitaetra spiders (Araneae : Nephilidae). *Invertebrate Systematics* 23: 193-204.

Kuntner, M. & Coddington, J.A. 2009. Discovery of the largest orbweaving spider species: The evolution of gigantism in *Nephila*. *Plos One* 4: e7516.

Kuntner, M. & Agnarsson, I. 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., Gregorič, M. & Li, D.Q. 2010. Mass predicts web asymmetry in *Nephila* spiders. *Naturwissenschaften* 97: 1097-1105.

Kuntner, M., Arnedo, M.A., Trontelj, P., Lokovšek, T. & Agnarsson, I. 2013. Towards a molecular phylogeny of nephilid spiders: Evolutionary history of a model lineage. *Molecular Phylogenetics and Evolution*: in press.

Levi, H.W. 1974. The orb-weaver genus *Zygiella* (Araneae: Araneidae). *Bulletin of the Museum of Comparative Zoology* 146: 267–290.

Levi, H.W. 1980. The orb-weaver genus *Mecynogea*, the subfamily Metinae and the genera *Pachygnatha*, *Glenognatha* and *Azilia* of the subfamily Tetragnathinae north of Mexico (Araneae: Araneidae). *Bulletin of the Museum of Comparative Zoology* 149: 1–74.

Levy, G. 1987. Spiders of the genera *Araniella*, *Zygiella*, *Zilla* and *Mangora* (Araneae, Araneidae) from Israel, with notes on *Metellina* species from Lebanon. *Zoologica Scripta* 16: 243-257.

Maddison, W.P. & Maddison, D.R. 2013. Mesquite: a modular system for evolutionary analysis. *http://mesquiteproject.org*.

Mayntz, D., Toft, S. & Vollrath, F. 2009. Nutrient balance affects foraging behaviour of a trap-building predator. *Biology Letters* 5: 735-738.

Miller, M.A., Pfeiffer, W. & Schwartz, T. 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, LA*: 1-8.

Nyffeler, M. & Knornschild, M. 2013. Bat Predation by Spiders. *Plos One* 8.

Opell, B.D. & Schwend, H.S. 2007. The effect of insect surface features on the adhesion of viscous capture threads spun by orb-weaving spiders. *Journal of Experimental Biology* 210: 2352-2360.

Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L. & Grabowski, G. 1991. *The Simple Fool's Guide to PCR*. Department of Zoology and Kewalo Marine Laboratory, University of Hawaii, Honolulu, USA.

Peters, H.M. 1937. Studien am Netz der Kreuzspinne (*Aranea diadema*.). 1. Die Grundstruktur des Netzes und Beziehungen zum Bauplan des Spinnenkorpers. *Zeitschrift für Morphologie und Ökologie der Tiere* 33: 128-150.

Platnick, N.I. & Shadab, M.U. 1993. A review of the pirate spiders (Araneae, Mimetidae) of Chile. *American Museum Novitates* 3074: 1-30.

Platnick, N.I. 2013. The world spider catalog, version 13.5. from http://research.amnh.org/entomology/spiders/catalog81-87/index.html.

Roberts, M.J. 1995. *Spiders of Britain & Northern Europe*. HarperCollins Publishers, London.

Sahni, V., Blackledge, T.A. & Dhinojwala, A. 2011. A Review on Spider Silk Adhesion. *Journal of Adhesion* 87: 595-614.

Scharff, N. & Coddington, J.A. 1997. A phylogenetic analysis of the orb-weaving spider family Araneidae (Arachnida, Araneae). *Zoological Journal of the Linnean Society* 120: 355-434.

Sensenig, A., Agnarsson, I. & Blackledge, T.A. 2010. Behavioural and biomaterial coevolution in spider orb webs. *Journal of Evolutionary Biology* 23: 1839-1856.

Smith, H.M. 2006. A revision of the genus Poltys in Australasia (Araneae : Araneidae). *Records of the Australian Museum* 58: 43-96.

Swanson, B.O., Anderson, S.P., DiGiovine, C., Ross, R.N. & Dorsey, J.P. 2009. The evolution of complex biomaterial performance: The case of spider silk. *Integrative and Comparative Biology* 49: 21-31.

Talavera, G. & Castresana, J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* 56: 564-577.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731-2739.

Thevenard, L., Leborgne, R. & Pasquet, A. 2004. Web-building management in an orb-weaving spider, *Zygiella x-notata*: influence of prey and conspecifics. *Comptes Rendus Biologies* 327: 84-92.

Venner, S., Pasquet, A. & Leborgne, R. 2000. Web-building behaviour in the orb-weaving spider *Zygiella x-notata*: influence of experience. *Animal Behaviour* 59: 603-611.

Venner, S., Bel-Venner, M.C., Pasquet, A. & Leborgne, R. 2003. Body-mass-dependent cost of web-building behavior in an orb weaving spider, Zygiella x-notata. *Naturwissenschaften* 90: 269-272.

Venner, S. & Casas, J. 2005. Spider webs designed for rare but life-saving catches. *Proceedings of the Royal Society B-Biological Sciences* 272: 1587-1592.

Vollrath, F. & Selden, P. 2007. The role of behavior in the evolution of spiders, silks, and webs. *Annual Review of Ecology Evolution and Systematics* 38: 819-846.

Vollrath, F. & Porter, D. 2009. Silks as ancient models for modern polymers. *Polymer* 50: 5623-5632.

Watanabe, T. 2000. Web tuning of an orb-web spider, *Octonoba sybotides*, regulates prey-catching behaviour. *Proceedings of the Royal Society of London Series B-Biological Sciences* 267: 565-569.

Whiting, M.F., Carpenter, J.C., Wheeler, Q.D. & Wheeler, W.C. 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.

Wiehle, H. 1927. Beiträge zur Kenntnis des Radnetzbaues der Epeiriden, Tetragnathiden und Uloboriden. *Zeits. Morph. Ökol. Tiere* 8(3-4): 468-537.

Wiehle, H. 1929. Weitere Beiträge zur Biologie der Araneen, insbesondere zur Kenntnis des Radnetzbaues. *Zeits. Morph. Ökol. Tiere*.

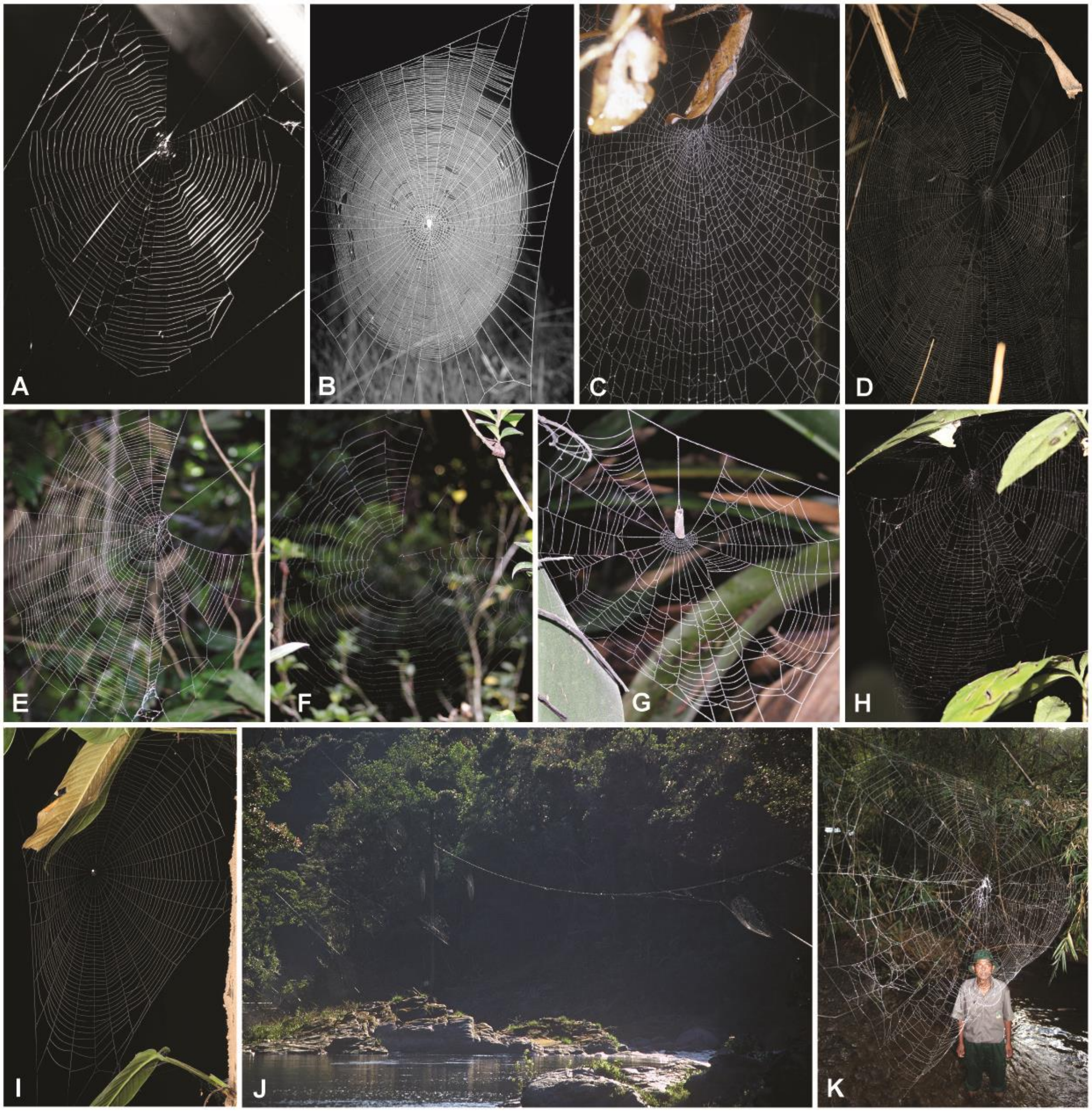
Witt, P.N., Reed, C.F. & Peakall, D.B. 1968. *A spider's web: Problems in regulatory biology*. Springer Verlag, New York.

Wunderlich, J. 2004. *Fossil spiders in amber and copal. Conclusions, revisions, new taxa and family diagnoses of fossil and extant taxa*.

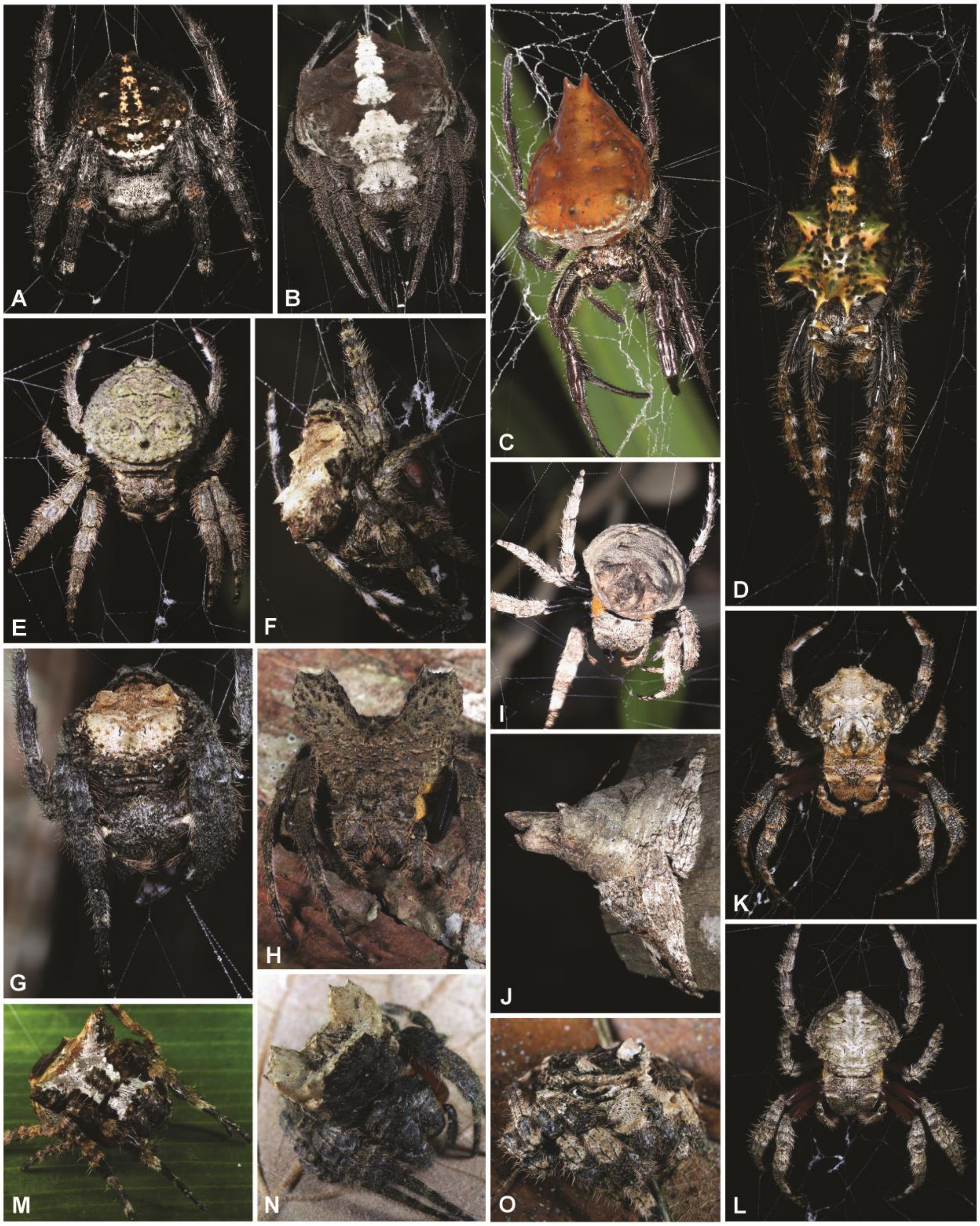
Zhu, M.S., Kim, J.P. & Song, D.X. 1997. On three new genera and four new species of the family Tetragnathidae (Araneae) from China. *Korean Arachnology* 13: 1-10.

Zschokke, S. & Vollrath, F. 1995. Web construction patterns in a range of orb-weaving spiders (Araneae). *European Journal of Entomology* 92: 523-541.

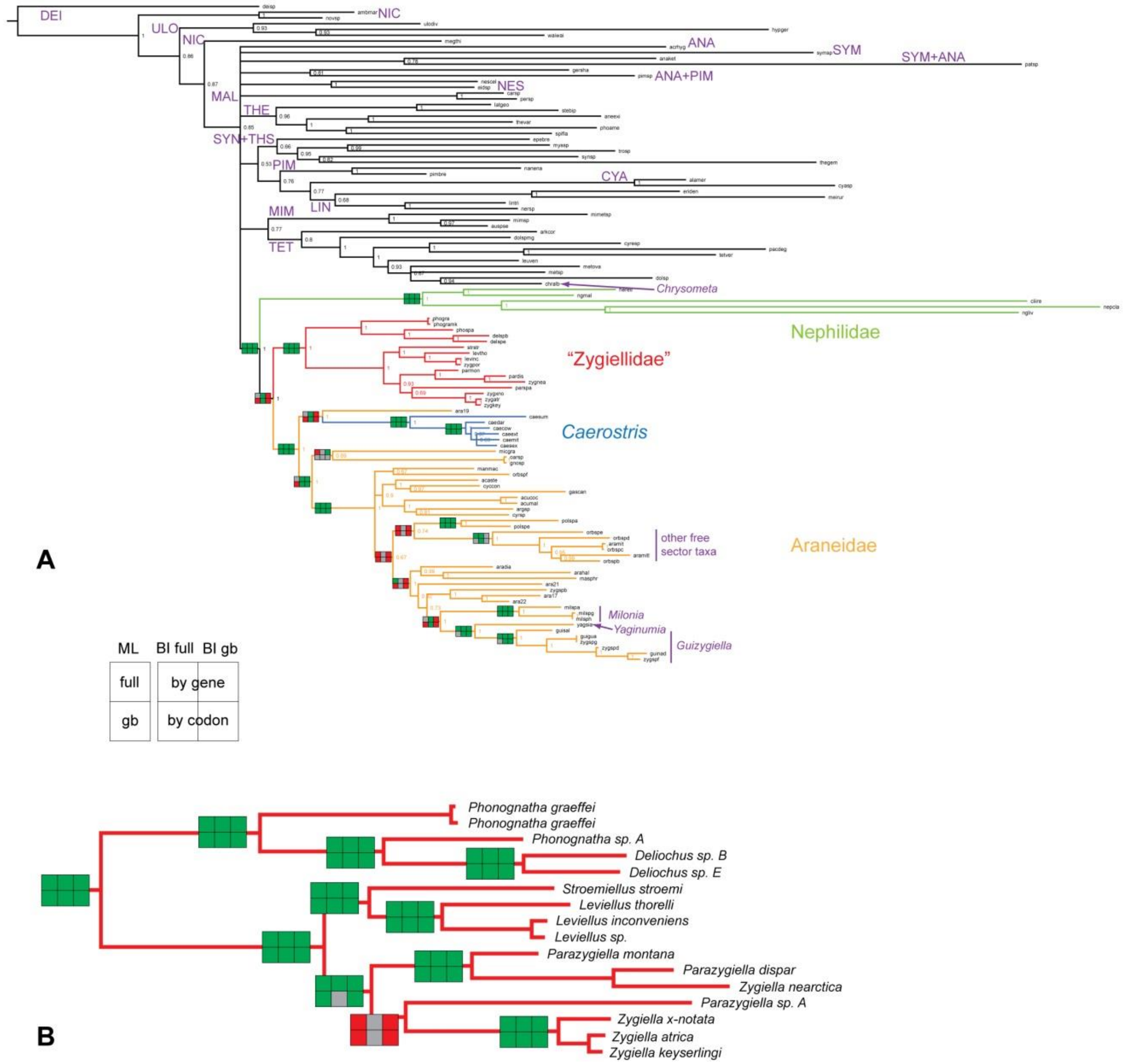
Figure legends



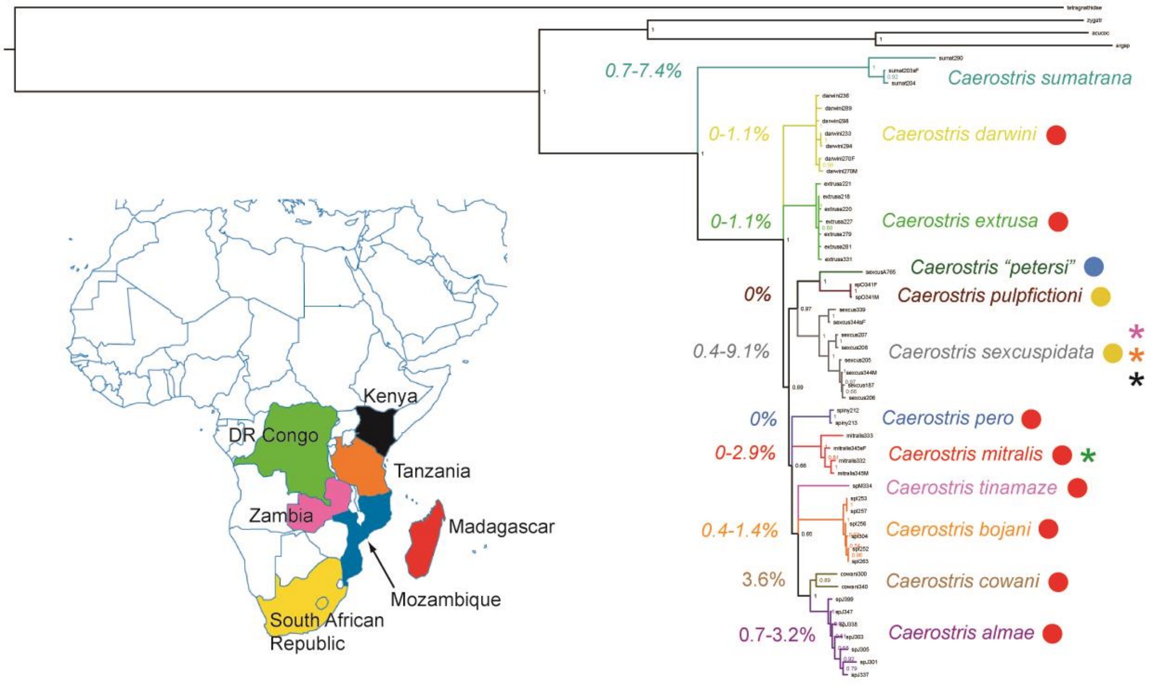
Slika 18: Figure 1: Web pictures of Zygiellidae, several other sector webs, and of *Caerostris*. A: *Zygiella x-notata*, Slovenia; B: *Deliochus sp.*, Brisbane, Australia; C: *Phonognatha graeffei*, Australia; D: Undetermined araneid from Halmahera, Indonesia; E: *Araneus mitificus*, Singapore; F: *Dolicognatha sp.*, Yunnan, China; G: *Acusilas coccineus*, Gombok, Malaysia; H: *Milonia sp.*, Yunnan, China; I: *Guizygiella sp.*, Yunnan, China; J: *Caerostris darwini* webs bridging river in Ranomafana, Madagascar; K: *C. darwini* web over small stream in Andasibe-Mantadia, Madagascar.



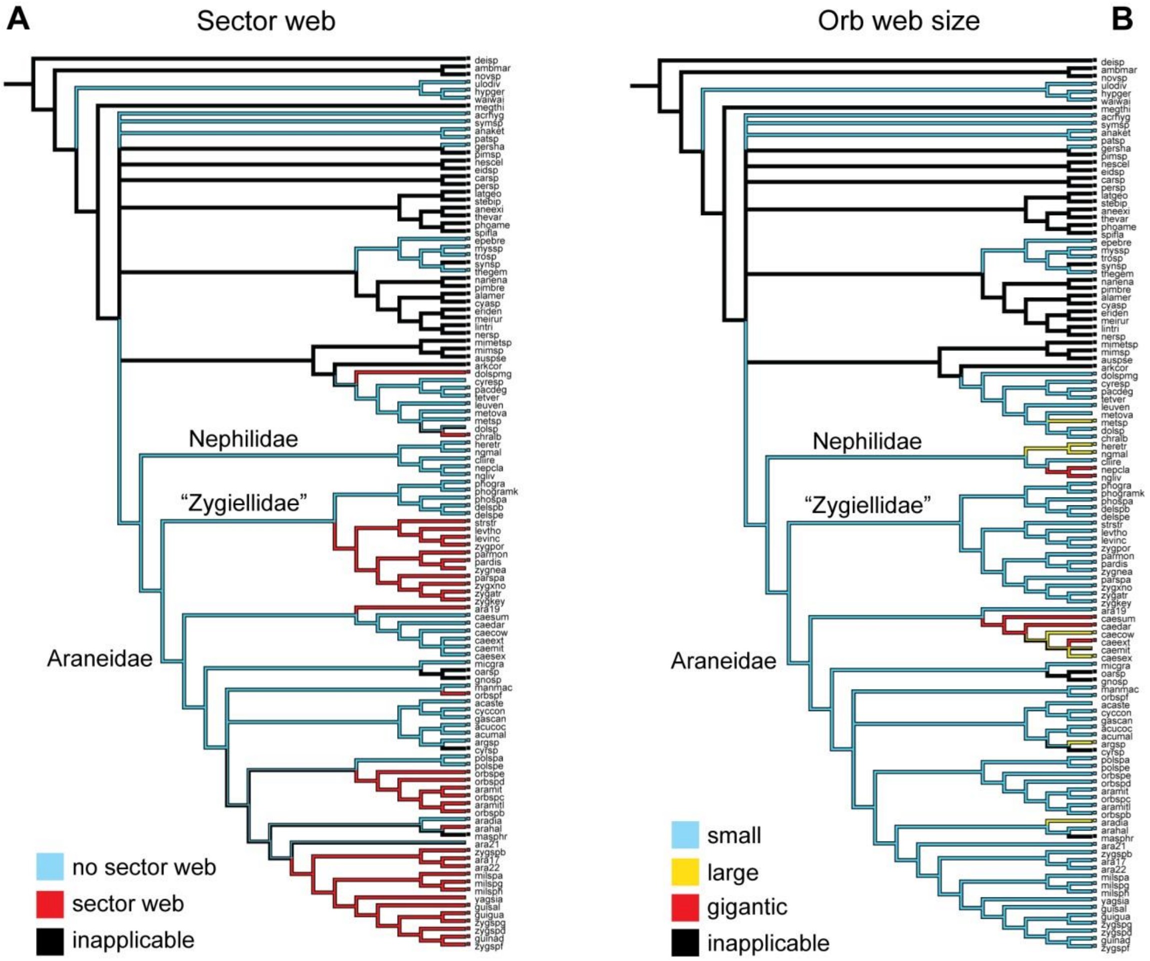
Slika 19: Figure 2: *Caerostris* diversity from Africa and Madagascar. A: *C. darwini*, Madagascar; B,C: *C. extrusa*, Madagascar; D: *C. pero*, Madagascar; E-H: *C. bojani*, Madagascar; I,J: *C. petersi*, Mozambique; K,L: *C. almae* from Madagascar; M: *C. cowani*, Madagascar; N,O: Undetermined subadult *Caerostris* females, Madagascar.



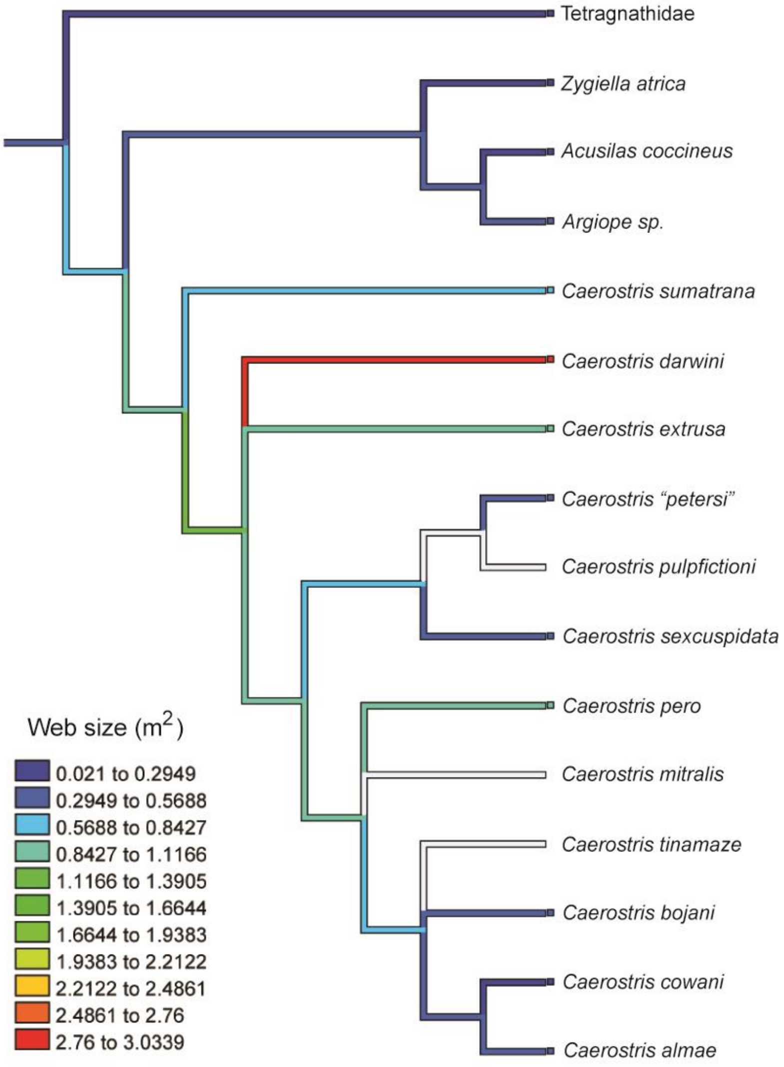
Slika 20: Figure 3: The preferred Orbicularian phylogeny. A. The topology is from the Bayesian analysis under the "full codon partition" scheme. B. Zygiellidae phylogeny with posterior probabilities and maximum likelihood bootstrap values at nodes. High support (PB > 0.95, ML boot > 0.75) is marked with green, a recovered clade but below high support threshold is marked with grey, and a clade that was not recovered is marked with red. Apart from Zygiellidae, Araneidae and Nephilidae, families are labeled using the following codes: ANA = Anapidae, CYA = Cyatholipidae, DEI = Deinopidae, HOL = Holarchaeidae, LIN = Linyphiidae, MAL = Malkaridae, MIC = Micropholcommatidae, MIM = Mimetidae, MYS = Mysmenidae, NES = Nesticidae, NIC = Nicodamidae, PIM = Pimoidae, SYM = Symphytognathidae, SYN = Synotaxidae, TET = Tetragnathidae, THD = Theridiidae, THS = Theridiosomatidae, ULO = Uloboridae.



Slika 21: Figure 4: The *Caerostris* phylogeny from Bayesian inference of COI, 28S, H3 and ITS2, and under the "full codon partition" scheme, with COI gene fragment differences for species clusters. The colored circles next to species names show the distribution of sequenced specimens, while the colored asterisks show the distribution of non-sequenced specimens found in museum collections.



Slika 22: Figure 5: The evolution of sector webs (A) and web size (B) plotted on the preferred topology (Bayesian inference under the "full codon partition" scheme). Web size is coded as small if smaller than 0.1 m^2, large if between 0.1 m^2 and 0.5 m^2 and gigantic larger than 0.5 m^2.



Slika 23: Figure 6: Web size evolution among the investigated *Caerostris* species, plotted on the trimmed preferred topology (Bayesian inference under the "full codon partition" scheme).

Taxonomy

Caerostris Thorell 1868 (Bark spiders)

(Fig. 3, 7-11)

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

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

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.

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

Caerostris almae new species

(Fig. 3K,L; 7)

Types. Female holotype deposited at USNM, and labeled: *Caerostris almae* CAE301; Andasibe-Mantadia NP, Madagascar; Gregorič, Agnarsson, Kuntner 2010.

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

Diagnosis. *Caerostris almae* similar in somatic morphology to *C. bojani* and *C. sexcuspidata*. *C. almae* differs from other *Caerostris* species by the large size of the epiginal hooks that are positioned median on the epiginal plate rather than anterior or posterior, and pointing laterally rather than posterior. *C. almae* differs from other *Caerostris* species by the posterior epigynal margin that circles around the copulatory openings.

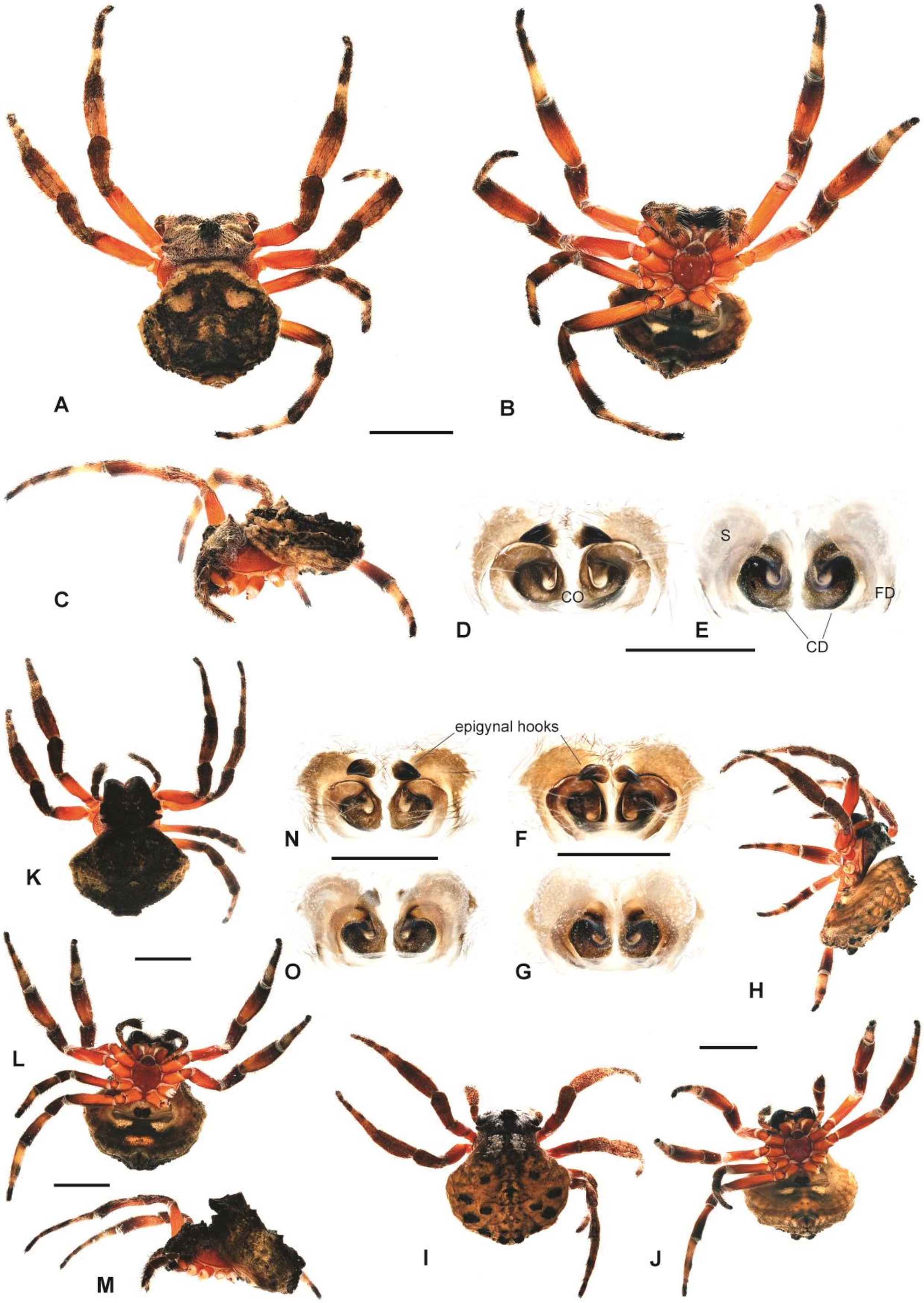
Description. *Female* (holotype, CAE301 from Ranomafana NP, Madagascar, Fig. 7A-C): Total length 10.1. *Prosoma* 4.8 long, 5.8 wide, 4.2 high. Carapace orange to brown, chelicerae dark reddish brown, both covered with white seatae. Sternum 2.5 long, 3.2 wide, widest between second leg coxae, light brownish red with white seatae 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 tubercules and several small tubercules. Venter brown, black in the middle, with two white transverse bands that end in bright white specks. *Epigynum* as diagnosed (Fig. 7D), spermathecae spheroid (Fig. 7E).

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 tubercules. Opisthosoma venter sometimes black with three pairs of white specks, sometimes one transverse white band, sometimes white speck anterior to spinnerets (Fig. 7).

Additional material examined. Five females collected by the authors in Andasibe-Mantadia NP and Ranomafana NP, Madagascar, to be deposited at USNM. One female collected by A. Pauly in 1995 at Razanaka, Toamasina, Madagascar, deposited at RMCA. Three females collected by C. Griswold, Fisher, A. Saucedo and H. Wood between 2003 and 2009 in Antsirakambiaty, Fianarantsoa and Analamazaotra, Toamasina, Madagascar, deposited at CASENT.

Distribution. Eastern Madagascar, known from Andasibe-Mantadia NP, Razanaka and Analamazaotra, all Toamasina province, and from Antsirakambiaty, Fianarantsoa, Madagascar.

Natural history. The species inhabits mountain rainforests of Eastern Madagascar. We always found the species at dawn or night, at forest edge close to water.



Slika 24: Fig. 7: *Caerostris almae*, female somatic and genital morphology, all from Andasibe-Mantadia, Madagascar. A-E: CAE301; F-J: CAE305; K-O: CAE303. Somatic scale bars = 5 mm, genital scale bars = 1 mm.

Caerostris bojani new species

(Fig. 3E-H, 8)

Types. Female holotype deposited at USNM, and labeled: *Caerostris bojani* and labeled 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. *Caerostris bojani* similar in somatic morphology to *C. almae* and *C. sexcuspidata*. The epiginal hooks are positioned anterior on the epiginal plate rather than median or posterior, and are pointing laterally rather than posterior, as in *C. pero* and different to other species. However, the epigynum differs from *C. pero* by the narrower, »U« shaped, posterior epigynal margins.

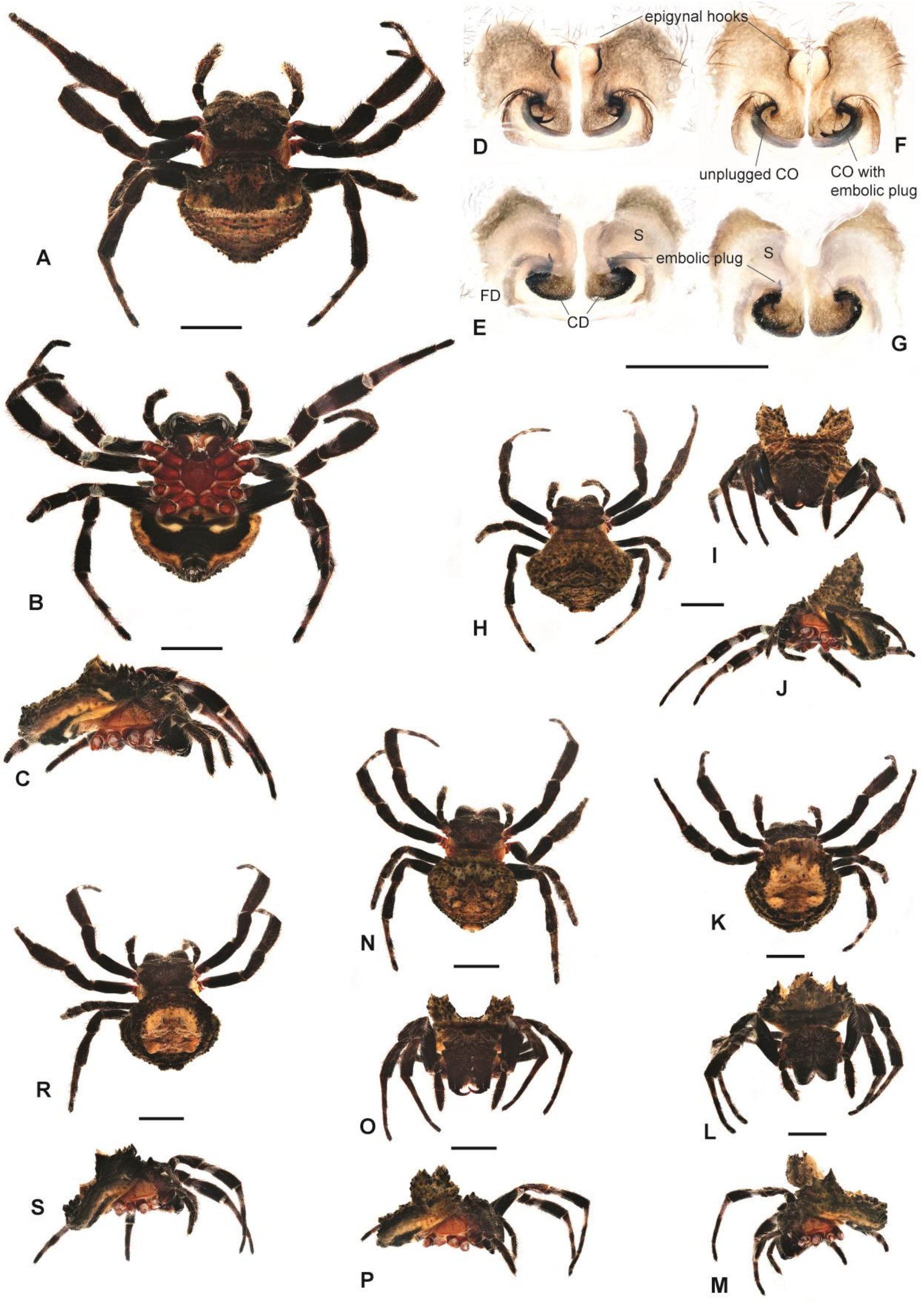
Description. *Female* (holotype, CAE254 from Andasibe-Mantadia NP, Madagascar, Fig. 8A-F): Total length 14.8. *Prosoma* 7.6 long, 7.8 wide, 6 high. Carapace and chelicerae dark reddish brown, covered with light brown seatae. Sternum 3.1 long, 3.1 wide, widest between second leg coxae, brownish red with white seatae 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 tubercules on anterior half. Venter black, outlined with a yellowish brown band, two white transverse bands. *Epigynum* as diagnosed (Fig. 8E), spermathecae kidney-shaped (Fig. 8F).

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 tubercules, or with a small to big pair of anterior tubercules (Fig. 3E-H, 8).

Additional material examined. 15 females collected by the authors in Andasibe-Mantadia NP, Madagascar, to be deposited at USNM.

Distribution. Eastern Madagascar, 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.



Slika 25: Fig. 8: *Caerostris bojani*, female somatic and genital morphology, all from Andasibe-Mantadia, Madagascar. A-E: CAE254; F-J: CAE255; K-M: CAE252; N-P: CAE262; R-S: CAE263. Somatic scale bars = 5 mm, genital scale bar = 1 mm.

Caerostris pero **new species**

(Fig. 2D, 9)

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

Etymology. The species epithet honors the first author's brother Peter "Pero" Gregorič.

Diagnosis. *Caerostris pero* differs in somatic morphology from all other *Caerostris* species by the 11 pointy tubercules on the opisthosoma dorsum. The epiginal hooks are positioned anterior on the epiginal plate rather than median or posterior, and are pointing laterally rather than posterior, as in *C. bojani* and different to other species. However, the epigynum differs from *C. bojani* by the wider, »U« shaped, posterior epigynal margins.

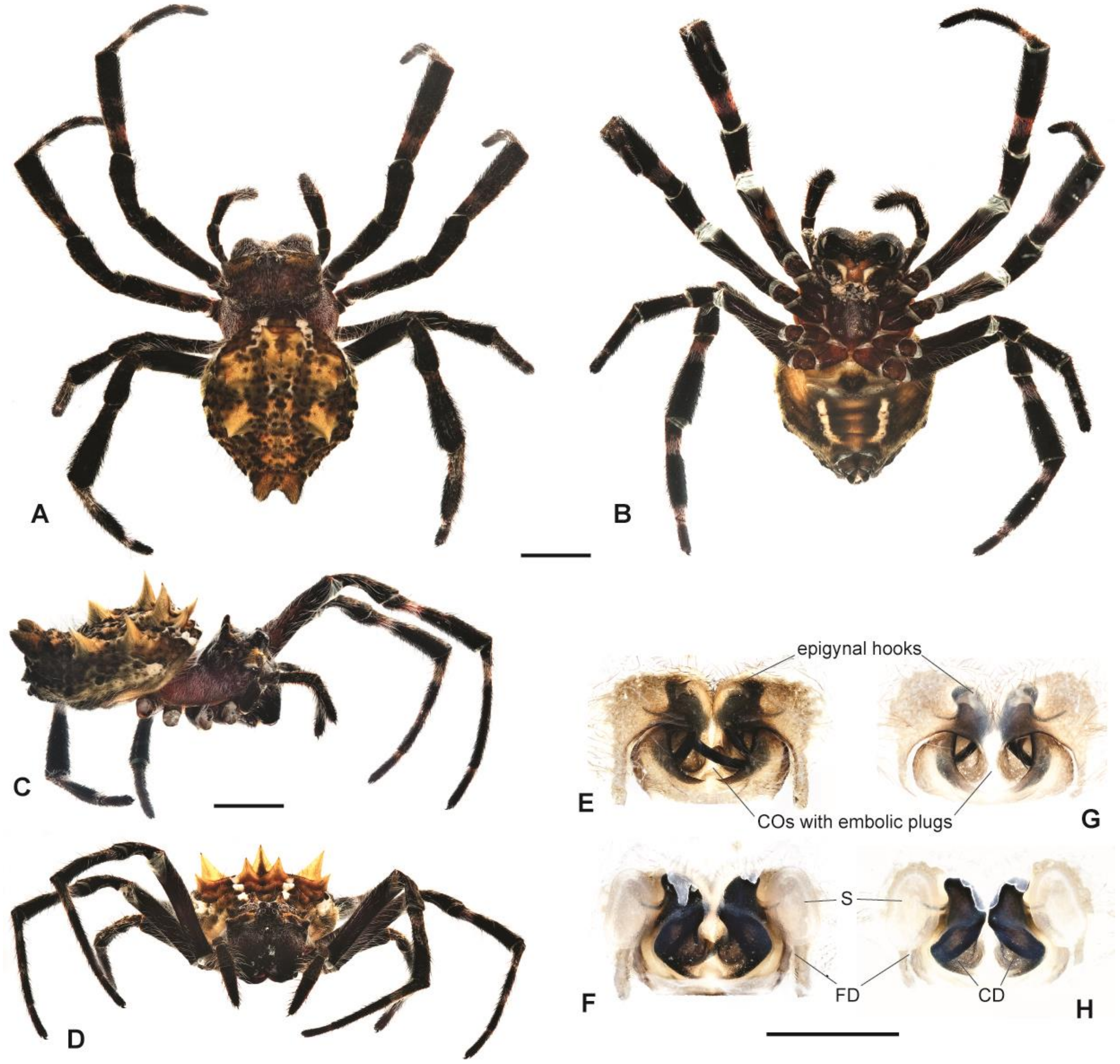
Description. *Female* (holotype, CAE215 from Andasibe-Mantadia NP, Madagascar, Fig. 9D-F): Total length 16.4. *Prosoma* 6.6 long, 6.9 wide, 3.1 high. Carapace and chelicerae dark reddish brown, covered with white seatae. Sternum 2.5 long, 3.2 wide, widest between second leg coxae, dark reddish brown with white seatae 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 . 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 tubercules. Venter brown with two narrow, white median longitudinal bands. *Epigynum* as diagnosed (Fig. 9E), spermathecae spheroid (Fig. 9F).

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

Additional material examined. Numerous females collected by the authors between 2008 and 2012 in Andasibe-Mantadia NP, Madagascar.

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

Natural history. The species inhabits mountain rainforests of Eastern Madagascar. They suspend their large orb webs in the air column over forest streams under closed canopy.



Slika 26: Fig. 9: *Caerostris pero*, female somatic and genital morphology, Andasibe-Mantadia NP, Madagascar. A-C, G-H: CAE216. D-F: CAE215. A-D scale bar = 5 mm, E-H scale bar = 1 mm.

Caerostris pulpfictioni new species

(Fig. 10A)

Types. Female holotype deposited at CASENT, and labeled: *Caerostris pulpfictioni* CAE334, have to check label xx.

Etymology. The species epithet, a noun in genitive case, honors the Quentin Tarantino movie Pulp Fiction.

Diagnosis. *Caerostris pulpfictioni* similar in somatic morphology to *C. mitralis* and *C. sexcuspidata*. Epigynal hooks on the epigynal plate positioned median rather than anterior or posterior, large and pointing lateral, similar to *C. almae*. However, the epigynum differs from *C. almae* by the posterior epigynal margin that does not circle around copulatory openings.

Description. *Female* (holotype, CAE334 from Kirindy, Toliara, Madagascar, Fig. 10A): 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 bown. 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 tubercules and sclerotized dots. Venter brown. *Epigynum* as diagnosed (Fig. 10A).

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.

Caerostris tinamaze new species

(Fig. 10B)

Types. Female and male holotype deposited at CASENT, and labeled: *Caerostris tinamaze* CAE341.

Etymology. The species was described in the all-time record-breaking season of the Slovenian alpine skiing champion Tina Maze. The species epithet thus honors Tina Maze and her achievements in alpine skiing.

Diagnosis. *Caerostris tinamaze* similar in somatic morphology to *C. almae*, *C. bojani* and *C. sexcuspidata*. The posterior epigynal margin circles around the copulatory openings, similar to *C. bojani*. However, epigynum differs from *C. bojani* by the epigynal hooks on the epigynal plate positioned median rather than anterior. However, the epigynum differs from *C. almae*.

Description. *Female* (holotype, CAE341 from Entabeni NR, Limpopo province, South African Republic, Fig. 10B): Total length 9. *Prosoma* 4.3 long, 4.6 wide, 3.8 high. Carapace and chelicerae brown, covered with light brown seate. 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 seatae. 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 tubercules. Venter outlined with light brown, median black with two pairs of white specks. *Epigynum* as diagnosed (Fig. 10B).

Variation. Unknown.

Additional material examined. None.

Distribution. Madagascar, known only from the type locality.

Natural history. The examined specimens inhabited an afromontane forest fragment in pine plantation.

Slika 27: Fig. 10: A: *C. pulpfictioni* somatic and genital morphology; B: *C. tinamaze* somatic and genital morphology. Somatic scale bars = 5 mm, genital scale bars = 0.5 mm.

3 RAZPRAVA IN SKLEPI

Kot smo omenili že v uvodu, so pajki mrežarji iz naddružine Araneoidea pomembni in pogosto celo modelni organizmi v mnogih evolucijskih študijah, še posebej v raziskavah adaptivne selekcije, evolucijske ekologije in evolucije vedenja v najširšem smislu (Coddington, 1994; Herberstein in Wignall, 2011). V raziskovanju evolucije lastnosti, ki vplivajo na delovanje mrež in vrstno pestrost pajkov, potrebujemo jasno sliko filogenetskih odnosov med sorodnimi linijami pajkov mrežarjev. Pa vendar so kljub napredku filogenetskih metod sorodstveni odnosi med družinami in celo znotraj njih slabo poznani (Agnarsson s sod., 2013). S ciljem, da naredimo korak proti poznavanju sorodstvenih odnosov pajkov mrežarjev, predvsem velike družine Araneidae in z njo povezanimi družinami Nephilidae in Tetragnathidae, smo testirali monofilijo in obseg potencialne družine Zygiellidae in rodu *Caerostris* ter ugotavljali njun filogenetski položaj. Na podlagi dobljenih filogenetskih odnosov smo testirali tudi evolucijo nekaterih lastnosti pajčjih mrež in pokazali, da sorodstvene linije kažejo značilne vedenjske posebnosti. Rezultati raziskav v doktorski disertaciji prav tako nakazujejo, da: 1) Arhitektura mrež in mehanske lastnosti koevoluirajo z ekološkimi lastnostmi mrež in vedenjem ob njihovi gradnji. 2) Pajčje mreže tekom ontogenetskega razvoja ne sledijo splošnemu biogenetskemu pravilu, ampak se spreminjajo tako, da osebku omogočajo čim učikovitejšo rabo svojega mikrookolja, v našem primeru učinkovito plenjenje. 3) Energijski vložek v plenjenje je verjetno pod močnim selekcijskim pritiskom, pajki pa ta vložek spreminjajo tako skozi arhitekturo mrež, kot z manipulacijo svilenih niti in lepila, in tako je za natančno oceno materialnega vložka v plenjenje potrebna kvantifikacija vsega naštetega.

3.1 FILOGENETSKA SISTEMATIKA IN EVOLUCIJA MREŽ RODOV *Zygiella* S.L. IN *Caerostris*

3.1.1 Filogenetski položaj in obseg skupine Zygiellidae in rodu *Caerostris*

Monofilija pajkov mrežarjev (Orbiculariae) je bila dolgo sporna in znatno testirana, z napredkom filogenetske sistematike pa je postajal monofiletski status skupine čedalje bolje podprt (Coddington, 1986b; Scharff in Coddington, 1997; Griswold s sod., 1998),čeprav prve molekularne analize tega niso podpirale. Tako je študija (Blackledge s sod., 2009) v analizi, ki je obsegala morfološke in molekularne podatke, prva podprla monofilijo pajkov mrežarjev tudi v dobi molekularnih podatkov. Sledile so ji druge študije, ki so z vključitvijo večih terminalov in uporabo več molekularnih podatkov, podpirale monofilijo pajkov mrežarjev izključno z molekularnimi podatki (npr. Sensenig s sod. (2010); Agnarsson s sod. (2012); Blackledge s sod. (2012a); Dimitrov s sod. (2012)). Kljub temu, da zgoraj omenjene študije podpirajo določene sorodstvene odnose med skupinami pajkov mrežarjev, sorodstveni odnosi med večino družin še vedno niso zadostno podprti (Agnarsson s sod., 2013). Običajen nabor genetskih markerjev v filogenetski sistematiki pajkov mrežarjev obsega markerje, imenovane tudi osumljenih pet (angl. »the usual suspects«), to so geni za COI (podenota I citokrom c oksidaze), 16S (16S ribosomska RNA), 18S (18S ribosomska RNA), 28S (28S ribosomska RNA), H3 (histon H3). Kljub dokaj velikim naborom podatkov očitno omenjeni podatki niso dovolj, da bi nedvoumno razrešili filogenetske odnose med skupinami pajkov mrežarjev, tudi študije, ki so dodale druge molekulske markerje (npr. Wnt signalni protein wtn1 ali 12S ribosomska RNA) pa pri tem niso bile mnogo uspešnejše. Pa vendar je ostalim študijam skupno to, da večje število dodanih terminalov (t.j. najmanjših skupin, vključenih v analizo) izboljša ločljivost rezultatov filogenetskih analiz vsaj na ravni ožjega izbora skupin (npr. Alvarez-Padilla s sod., 2009; Dimitrov s sod., 2012; Kuntner s sod., 2013).

Cilj filogenetske analize v doktorski disertaciji je bil najprej raziskati sorodstvene odnose dveh netipičnih skupin družine Araneidae, torej raziskati obseg potencialne družine Zygiellidae, testirati monofilijo rodu *Caerostris* in določiti njun filogenetski položaj. V te namene smo sestavili nabor podatkov, ki je obsegal 114 terminalov iz skoraj vseh družin pajkov mrežarjev, uporabili pa smo dva mitohondrijska (COI, 16S) in štiri jedrne (H3, 18S, 28S, ITS2) dele genov. Pri izbiri taksonov smo se ob potencialnih predstavnikih skupine Zygiellidae in rodu *Caerostris* osredotočili predvsem na družine, ki so jih pretekle študije z njimi povezovale – družine Araneidae, Nephilidae in Tetragnathidae. Za razliko od prejšnjih študij, ki so vključevale zgolj eno ali dve vrsti rodu *Zygiella* (*Z. atrica* in/ali *Z. x-notata*; Blackledge s sod., 2009, 2012a; Sensenig s sod., 2010; Agnarsson s sod., 2012; Dimitrov s sod., 2012; Kuntner s sod., 2013), smo v analizo z vsaj dvema vrstama na rod vključili vse štiri rodove *Zygiella* s.l. (razen za monotipski rod *Stroemiellus*), kar skupaj znaša 9 od 17 opisanih vrst *Zygiella* s.l. in dve še neopisani vrsti. V analizo smo vključili tudi druge rodove, ki so jih predhodne študije uvrstile v družino Zygiellidae oz. so jih združevale z rodom *Zygiella* (*Deliochus*, *Phonognatha*, *Chrysometa*; Wunderlich, 2004; Blackledge s sod., 2009; Sensenig s sod., 2010; Dimitrov s sod., 2012), rodove, ki vsebujejo vrste, včasih uvrščene v rod *Zygiella* s.l. (*Guizygiella*, *Yaginumia*; Platnick, 2013), in rodove, ki po biologiji mrež spominjajo na predstavnike potencialne družine Zygiellidae (npr. *Acusilas* in *Milonia*). V analizo smo vključili tudi veliko število zunanjikov (angl. »outgroup«): vključili smo večje število predstavnikov Araneidae, tudi tistih, ki morfološko niso značilni za družino in tistih, ki so jih predhodne študije uvrščale med bazalna razvejišča v družini (Dimitrov s sod., 2012). Za razliko od predhodnjih študij, ki so iz družine Nephilidae večinoma vsebovale le enega predstavnika rodov *Nephila* in *Nephilengys* (Blackledge s sod., 2009, 2012a; Sensenig s sod., 2010; Agnarsson s sod., 2012), smo vključili vseh pet rodov (Kuntner s sod., 2013). Poleg že omenjenih rodov *Chrysometa* in *Guizygiella* iz družine Tetragnathidae, smo iz te družine vključili še vsaj osem rodov. Za test filogenetskega položaja rodu *Caerostris* smo vključili šest od 12 do sedaj opisanih vrst, za analize na nivoju rodu pa še dodatnih 6 vrst. Ker so predhodne analize, ki so vključevale zgolj eno do dve vrsti rodu *Caerostris*, ta rod uvrstile na bazalno razvejišče v družino Araneidae (Sensenig s sod., 2010), je bil zgoraj opisan nabor zunanjikov primeren tudi za filogenetsko uvrščanje rodu *Caerostris*.

V doktorski disertaciji smo predstavili prvi test monofilije in filogenetskega položaja problematične družine Zygiellidae in rodu *Caerostris*. Čeprav odnosi med večino družin pajkov mrežarjev niso dobro podprti, je bil velik vzorec tarčnih taksonov verjetno ključen za dobro podprte filogenetske odnose med skupinami Zygiellidae, Araneidae, Nephilidae in Tetragnathidae. Tako moji rezultati potrjujejo rezultate predhodnih študij v tem, da tvorijo skupine Zygiellidae, Araneidae in Nephilidae monofiletsko skupino (Blackledge s sod., 2009, 2012a; Sensenig s sod., 2010; Agnarsson s sod., 2012; Dimitrov s sod., 2012; Kuntner s sod., 2013). Rezultati mojih analiz tako z dobro podporo umeščajo skupino Zygiellidae kot sestrsko vsem ostalim predstavnikom Araneidae. Moji rezultati dobro podpirajo monofilijo rodu *Zygiella* s.l., znotraj katerega rodovi po študiji Wunderlich-a (2004) niso monofiletski, v Zygiellidae pa uvrščajo še rodova *Deliochus* in *Phonognatha*. Družina Zygiellidae po študiji Wunderlich-a (2004) v naši analizi ni podprta, saj ni sestrska družini Tetragnathidae, ne vključuje rodu *Chrysometa*, ki je umeščen globoko v družino Tetragnathidae, prav tako pa mreža z manjkajočim sektorjem ni podprta kot sinapomorfija družine. Analiza v doktorski disertaciji je tudi prva, ki vključuje nekdanje vrste iz rodu *Zygiella*, danes umeščene v rodova *Guizygiella* (Tetragnathidae) in *Yaginumia* (Araneidae). Moji podatki nedvoumno uvrščajo rod *Guizygiella* v družino Araneidae in ta rod celo združujejo z rodovoma *Yaginumia* in *Milonia* v monofiletsko skupino, katere predstavniki gradijo mreže z manjkajočim sektorjem.

Moji rezultati prav tako dobro podpirajo monofilijo rodu *Caerostris* in ga uvrščajo med sorodstvene linije, ki se nahajajo med bazalnimi razvejišči v družini Araneidae, natančen filogenetski položaj rodu pa je slabše podprt. Rodovi *Caerostris*, *Oarces*+*Gnolus* in *Micrathena* tvorijo parafiletsko skupino (t.i. »grade«) izven dobro podrpte skupine tipičnih predstavnikov družine Araneidae, vendar filogenetski odnosi med temi evolucijsko izvornimi predstavniki niso popolnoma jasni. Vsi omenjeni rodovi se morfološko razlikujejo od ostalih predstavnikov družine Araneidae (Scharff in Coddington, 1997), z razliko rodu *Micrathena* pa je o njihovi biologiji malo znanega. Tako sta bila rodova *Oarces* in *Gnolus* še do nedavnega po nekaterih morfoloških znakih uvrščena v ne tako sorodno družino Mimetidae (Platnick in Shadab, 1993; Dimitrov s sod., 2012). Kot smo pokazali v doktorski disertaciji, se rod *Caerostris* zraven morfoloških značilnostih od ostalih predstavnikov družine Araneidae razlikuje tudi po vedenju in biologiji mrež. Vrstna filogenija rodu *Caerostris* kaže na uporabnost molekularnih metod v filogenetski sistematiki. Glede na razliko v delu gena COI med osebki tako vidimo, da so nekatere vrste dobro opisane, medtem ko to za druge ne drži. Predvsem je to očitno za široko razširjeni vrsti *C. sumatrana* iz južne in jugovzhodne Azije in *C. sexcuspidata* iz srednje, vzhodne in južne Afrike. Čeprav so vrste rodu *Caerostris* v splošnem morfološko pestre, so si številne po morfologiji spolovil precej podobne (Grasshoff, 1984). Tako so bili številni vzorci, nabrani v Aziji in Afriki, po morfologiji spolovil preprosto določeni kot *C. sumatrana* oz. *C. sexcuspidata*. Moji podatki kažejo, da gre v primeru obeh vrst verjetno za komplekse večih vrst. Razlika v COI med populacijami *C. sumatrana* iz sosednjih Yunnana in Laosa namreč presega 7%, pri *C. sexcuspidata* pa zgolj znotraj Južnoarfiške Republike razlika presega 9%. Pri ostalih vrstah namreč razlika v COI med osebki sega do 3%, večinoma pa celo pod 1%.

3.1.2 Evolucija mrež in lastnosti mrež kot taksonomski znaki

Eberhard (1982) je na izredno velikem naboru taksonov raziskoval arhitekturo kolesastih mrež, njihovo gradnjo in vedenje povezano z mrežami ter ugotovil, da so nekatere podrobnosti v njihovi evoluciji dovolj konzervativne, da opisujejo družine in rodove. Čeprav je Eberhard (1982) omenjene vedenjske sinapomorfije postavil že pred več kot tremi desetletji, mnoge od njih še danes opisujejo glavne sorodstvene linije pajkov mrežarjev (Kuntner s sod., 2008). Med omenjenimi vedenjskimi lastnostmi so se kot najuporabnejše izkazale podrobnosti v gradnji središča mreže, gradnji žarkastih in spiralnih niti ter vedenje ob plenjenju (Eberhard, 1982). Moji rezultati podpirajo vedenjske posebnosti sorodnih linij in kažejo, da se predstavniki družin Zygiellidae in *Caerostris* od ostalih pajkov mrežarjev ločijo po kombinaciji določenih vedenjskih lastnosti. Tako se zdi, da je predstavnikom skupine Zygiellidae skupna gradnja podvojenih radialnih niti, ki jih med drugimi pajki mrežarji gradijo zgolj predstavniki bazalne družine Uloboridae, ki gradijo kribelatne mreže (Eberhard, 1982). Vsi predstavniki Zygiellidae izdelujejo določeno obliko svilenega zatočišča, bodisi je to preprosta svilena cev ali zatočišče v listu ob/v mreži, vendar je do evolucije takšnih zatočišč prišlo še v vsaj štirih drugih družinah pajkov mrežarjev (Kuntner s sod., 2008). Sicer pa za razliko od drugih predstavnikov družine Araneidae predstavniki Zygiellidae napadejo svoj plen z ugrizom namesto zapredanjem, ne spreminjajo središča mreže po končani gradnji in ne tresejo mreže ali menjajo njene strani ob nevarnosti (Eberhard, 1982; Hormiga s sod., 1995; Scharff in Coddington, 1997; Kuntner s sod., 2008). Mreža z manjkajočim sektorjem je homologna za rod *Zygiella* s.l., vendar se je glede na naše podatke razvila še vsaj petkrat v družini Araneidae in dvakrat v družini Tetragnathidae. Rod *Caerostris* je edini rod pajkov mrežarjev, ki v mreži gradi tako podvojene kot enojne žarkaste niti, in edini, ki celotno lovilno ploskev mreže zgradi pod izvorno nitjo mostu. Predstavniki rodu *Caerostris* tudi skoraj ne raziskujejo okolja, kjer zgradijo mrežo (ang. »web site exploration«), mreža pa je preprosta, brez sekundarnih okvirjev in z le tremi sidrnimi nitmi. Tudi predstavniki rodu *Caerostris* napadejo plen z ugrizom namesto zapredanjem, zraven tega pa že obvladan plen v središče mreže nesejo na edinstven način.

Moji podatki kažejo, da je do evolucije velikih kolesastih mrež prišlo večkrat, in sicer v družinah Araneidae, Nephilidae in Tetragnathidae. To seveda ni presenetljivo, saj je pričakovano, da večje vrste pajkov gradijo večje mreže, vendar je do evolucije izjemno velikih mrež prišlo zgolj v družini Nephilidae v rodovih *Nephila* in *Nephilingis,* in prav v družini Araneidae v rodu *Caerostris,* kjer pri vrsti *C. darwini* najdemo tudi izreden gigantizem mrež. Ob tem je zanimivo, da oba nephilidska rodova in *Caerostris* kažejo nasprotni strategiji v evoluciji zmogljivosti mrež (Sensenig s sod., 2010). Zmogljivost mreže, da ustavi določeno kinetično energijo plena, nato pa maso plena tudi zadrži, je namreč pogojena tako z mehanskimi lastnostmi samega prediva in količino lepila, kot tudi s tem koliko in katere niti pridejo v kontakt s plenom (Blackledge s sod., 2011; Harmer s sod., 2012; Sensenig s sod., 2012). Velikost in gostota mreže je na drugi strani pogojena s količino prediva, ki ga ima pajek na voljo (Eberhard, 1988), zato je v evoluciji zmogljivosti kolesastih mrež prišlo do nadomestne evolucije (angl. »compensatory evolution«) (Sensenig s sod., 2010). To pomeni, da je splošen trend v evoluciji večje telesne velikosti pri pajkih mrežarjih tudi prehod na predivo z večjo natezno trdnostjo, ki omogoča večjim pajkom uporabo relativno manjše količine prediva oz. gradnjo manj gostih, a še vedno učinkovitih mrež (Sensenig s sod., 2010). Vrsta *C. darwini* je v tem pogledu ekstrem, saj gradi redke vendar največje mreže, narejene iz najkvalitetnejšega prediva (Agnarsson s sod., 2010; Sensenig s sod., 2010). Na drugi strani rodova *Nephila* in *Nephilingis* uporabljata predivo povprečne kvalitete (popravljeno na pajčjo velikost celo slabe kvalitete), vendar gradita izredno goste mreže, tako da lahko njune mreže ustavijo najtežje leteče žuželke in celo manjše vretenčarje (Sensenig s sod., 2010; Nyffeler in Knornschild, 2013). Vse našteto zastavlja dodatna vprašanja o vzrokih in posledicah evolucije gigantizma telesne velikosti in mrež. Predstavniki rodov *Nephila* in *Nephilingis* so namreč največji pajki mrežarji sploh, medtem ko je *C. darwini* sicer velika vrsta, vendar primerljive ali manjše velikosti od drugih velikih križevcev (Sensenig s sod., 2010). Ker predstavniki obeh skupin gradijo mreže na edinstven način (Eberhard, 1982; Kuntner s sod., 2008; Gregorič s sod., 2011b), se zastavlja vprašanje, ali so morda posebnosti v gradnji mrež predstavljale predpogoj za njun gigantizem mrež in/ali so predstavljale predpogoj za gigantizem telesne velikosti v družini Nephilidae in evolucijo izjemnih mehanskih lastnosti prediva pri rodu *Caerostris*.

3.2 KOEVOLUCIJA VEDENJA, EKOLOGIJE IN BIOMATERIALA

Kot smo omenili zgoraj, je bilo nedavno pokazano, da evolucijo večje telesne velikosti pri pajkih mrežarjih spremlja tudi prehod na bolj kvalitetno predivo, ki omogoča večjim pajkom uporabo relativno manjše količine prediva oz. gradnjo manj gostih, a še vedno učinkovitih mrež (Sensenig s sod., 2010). Ker smo pričakovali, da bo evolucijo novih arhitektur kolesaste mreže in evolucijo ekstremnih mehanskih lastnosti spremljala tudi evolucija novih vedenj v gradnji mrež, smo za objekt študije izbrali nedavno odkrito vrsto *C. darwini*. Ta vrsta gradi največje znane mreže, ki so narejene iz najmočnejšega poznanega prediva in postavljene v edinstveno mikrookolje – razpete so čez potoke ter manjše reke in jezera (Agnarsson s sod., 2010; Kuntner in Agnarsson, 2010). V doktorski disertaciji smo ugotovili, da evolucijo ekoloških (njihovo mikrokolje) in biomehanskih lastnosti mrež (materialne lastnosti prediva), spremlja tudi evolucija vedenjskih lastnosti (gradnja mreže).

Pajki mrežarji tipično namenijo veliko časa raziskovanju okolja, kjer bodo zgradili mrežo, kar verjetno služi izogibanju preprek, ki bi lahko bile v napoto lovilni ploski (Eberhard, 1972; 1990a; Zschokke, 1996). To storijo tako, da najprej zgradijo svilen most, nato iz njega do tal spletejo novo nit na način, da obe niti tvorita konstrukcijo v obliki črke »Y«. Od tukaj naprej raziskovanje ni ustaljeno, saj je okolje, ki je pajkom na voljo, precej raznoliko (Eberhard, 1972). Rezultat raziskovalnega vedenja so t.i. proto-mreže (ang. »proto-orb«), ki so podobne naključnemu prepletu niti, nekateri deli proto-mrež pa niso del končanih kolesastih mrež (Eberhard, 1990a; Zschokke, 1996). Pajki se o tem, kateri del proto-mreže bo postal središče končane kolesaste mreže, odločijo komaj po končanem raziskovanju (Coddington in Eberhard, osebno). Za razliko od značilnih pajkov mrežarjev pri vrsti *C. darwini* ne srečamo raziskovanja okolja, kjer bodo zgradili mrežo, proto-mreže osebkov pa so vedno enake. Prvi niti, ki ju zgradijo, po obliki ne spominjata na črko »Y«, temveč na črko »T« (slika 7), končan lovilni del mreže pa je vedno postavljen pod prvotni most. Prvi niti, ki tvorita omenjen »T« tudi predstavljata edine tri sidrne niti – najmanjše število niti, ki je potrebno za gradnjo ploščate mreže. Tudi sicer so mreže enostavne in ne vsebujejo sekundarnih okvirjev. Dodatno posebnost mrež vrste *C. darwini* pomenita dva tipa žarkastih niti v isti mreži – *C. darwini* uporabi podvojene žarkaste niti v zgornji in enotne žarkaste niti v spodnji polovici mreže.

Takšni rezultati nakazujejo, da je resnično šlo za koevolucijo arhitekture mreže in njene gradnje, osvajanja novega mikrookolja in materialnih lastnosti prediva. Raziskovanje okolja, kjer bo zgrajena mreža, je eden od energetsko bolj potratnih procesov gradnje kolesaste mreže (Zschokke, 1996), moji rezultati pa nakazujejo, da je pri *C. darwini* prečkanje potokov in rek energijsko precej bolj potraten proces, predvsem zaradi velike količine prediva, ki je potrebno za prečkanje tako izjemno velikih razdalj. Pajki mrežarji tipično obnavljajo lovilni del mreže vsak dan in ob tem menjajo tudi okvir mreže, medtem ko *C. darwini* dnevno menja vse dele lovilne ploskve, razen mostu, katerega zgolj dodatno ojača. Slednje nakazuje, da je energijski vložek v most pri tej vrsti dejansko večja kot pri pajkih, ki gradijo običajne mreže s precej krajšimi mostovi. Razlaga podvojenih žarkastih niti v zgornji polovici ni tako očitna kot odsotnost raziskovalnega vedenja. Znano je, da so žarkaste niti v zgornjem delu mreže izpostavljene večji napetosti (Wirth in Barth, 1992), tako da bi gradnja podvojenih žarkastih niti lahko služila razporejanju sile na večjo količino prediva, še posebej ker *C. darwini* po gradnji mreže ne spremeni središča – le-to bi naj služilo prav prilagajanju napetosti žarkastih niti (Eberhard, 1981). Pa vendar smo nedavno ugotovili, da tudi druge vrste rodu *Caerostris* gradijo podvojene žarkaste niti v zgornjem delu mreže. To pomeni, da je takšna arhitekturna posebnost morda predstavljala predpogoj za osvajanje edinstvenega mikrookolja vrste *C. darwini*, ali po drugi strani, da v njej ni igrala nikakršne vloge.

Poleg zgoraj omenjenih vprašanj me je v doktorski disertaciji zanimalo tudi, ali so izjemno velike mreže *C. darwini* adaptirane na lov specifičnega plena, npr. ptičev ali netopirjev, ki uporabljajo reke kot letalne poti, in ali te mreže morda izkoristijo množično pojavljanje vodnih žuželk, npr. enodnevnic (Kuntner in Agnarsson, 2010). Čeprav mreže *C. darwini* teoretično lahko razprši kinetično energijo majhnih vrst ptičev ali netopirjev, rezultati v doktorski disertaciji nakazujejo, da mreže *C. darwini* niso prilagojene na vretenčarski plen. Največji običajen plen so verjetno velike žuželke, predvsem kačji pastirji, ki glede na velikost pajkov ustrezajo definiciji redkega, velikega plena (Blackledge, 2011). Prav tako v času terenskega dela nismo opazili niti enega primera izkoriščanja množičnega pojavljanja vodnih insektov (Kuntner in Agnarsson, 2010), čeprav bi takšni množični ulovi sicer na pajkov fitnes (prispevek gen(fen)otipa h genetskemu skladu naslednje generacije) lahko delovali podobno velikemu, redkemu plenu. Sedanji podatki o plenu *C. darwini* torej ne potrjujejo povezave med evolucijo ekstremnih mehanskih lastnosti prediva in tipom plena, ampak nakazujejo, da ima izjemno predivo dejansko druge funkcije, povezane z velikostjo in mikrookoljem mreže.

3.3 ONTOGENETSKE SPREMEMBE V ARHITEKTURI MREŽ

Biogenetsko pravilo predvideva, da ontogenija organizma sledi vzorcem evolucijskih sprememb v sorodstveni liniji tega organizma (Olsson s sod., 2010). Kljub temu, da večina morfoloških študij hipotezo zavrača kot splošno biološko pravilo (Richardson in Keuck, 2002), ostaja biogenetsko pravilo na nivoju vedenja slabo raziskano (Nakata, 2010). Pajčje mreže so idealen objekt raziskav ontogenije vedenja, saj so fizičen odraz vedenja, pajki pa jih gradijo skozi celotno obdobje odraščanja (Benjamin in Zschokke, 2004). Tukaj smo raziskali alometrijo mrež pri vrsti *Leucauge venusta* in tako preverili, ali opaženi vzorci ustrezajo napovedim biogenetskega pravila ali napovedim alternativne hipoteze optimizacije plenjenja. Vrsta *L. venusta* gradi mreže, ki se v naklonu raztezajo od vodoravnih, preko poševnih, do navpičnih: biogenetsko pravilo predvideva, da alometrija ustreza ontogenetskemu stadiju (Eberhard, 2008), medtem ko optimizacija plenjenja predvideva, da alometrija sledi vplivom gravitacije (Nakata, 2010). Natančneje, optimizacija plenjenja predvideva, da bomo tekom ontogenetskega razvoja opazili povečano asimetričnost pri navpičnih mrežah, ne pa tudi pri vodoravnih. V študiji smo izmerili 98 mrež *L. venusta* in pokazali, da navpične mreže tekom ontogenetskega razvoja postajajo vse bolj asimetrične, medtem ko to ne drži za poševne in vodoravne mreže.

Takšni rezultati ne ustrezajo napovedim biogenetskega pravila in podpirajo hipotezo optimizacije plena. Pajčje mreže so t.i. »razširjen fenotip«, so torej tisto, s čimer so pajki v neposrednem stiku z okoljem, zato neposredno vplivajo na njihov fitnes (Eberhard, 1990; Herberstein in Tso, 2011). Zato je pričakovano, da je v evolucijskem smislu boljša tista arhitektura mreže, ki skozi osebkovo življenje veča učinkovitost njegovega plenjenja. Temu v prid govorijo tako naši podatki, kot tudi druge študije. Tako npr. pajki rodu *Cyclosa* povečajo tiste dele mreže proti katerim so sami obrnjeni (Nakata in Zschokke, 2010), pajki rodu *Telaprocera* gradijo za plenjenje slabše, lestvičaste mreže v horizontalno omejenem prostoru in za plenjenje boljše, okrogle mreže v neomejenem prostoru (Harmer in Herberstein, 2009), težji osebki mnogih vrst pa prilagodijo simetrijo mreže (Herberstein in Heiling, 1999; Kuntner s sod., 2010a). Čeprav vse zgoraj omenjeno govori močno v prid optimizaciji plenjenja namesto hipotezi biogenetskega pravila, lahko boljši test takšne hipoteze ponudi zgolj filogenetska informacija. Ker filogenija rodu *Leucauge* ni znana, rekonstrukcija evolucije njihovih mrež trenutno ni mogoča. Naslednjo težavo predstavlja vrednotenje predniške oblike kolesaste mreže, ki še ni bila zabeležena in lahko zgolj predvidevamo, da je resnično šlo za okroglo in simetrično mrežo (Eberhard, 2008; Hesselberg, 2010).

3.4 ENERGIJSKI VLOŽEK V PLENJENJE IN VEDENJSKA PLASTIČNOST

Vedenjska plastičnost je pomembna za večanje fitnesa v spreminjajočih se okoljih in je dokumentirana v večini živalskih skupinah (Hazlett, 1987; Garland in Kelly, 2006). Pri pajkih mrežarjih je vedenjska plastičnost dobro dokumentirana in verjetno definirana predvsem s spremembami v delovanju mrež, do nje pa lahko pride zaradi različnih razlogov, med katerimi sta med pomembnejšimi prehranjevanje in telesna velikost (Sherman, 1994; Herberstein s sod., 1999, 2000; Blamires, 2010; Harmer s sod., 2011). V doktorski disertaciji smo pri petih ozkosorodnih vrstah rodu *Zygiella* s.l. raziskovali materialni vložek v plenjenje (mreže) v odvisnosti od telesne velikosti. Kot smo omenili v uvodu, se rezultati preteklih študij ne ujemajo in si včasih celo nasprotujejo (Witt s sod., 1968; Sherman, 1994; Venner s sod., 2000; Nakata, 2007; Mayntz s sod., 2009; Blamires, 2010).

V doktorski disertaciji smo pokazali na vsaj dve pomembni pomanjkljivosti pravkar omenjenih študij. Prvič, mnoge med njimi raziskujejo zgolj razlike v binarnih primerjavah, npr. lačni proti sitim pajkom. Drugič, zgoraj omenjene študije v glavnem obravnavajo zgolj eno mero vlaganja v plenjenje, npr. velikost mreže ali število niti, zanemarijo pa dejstvo, da lahko pajki nadzorujejo debelino niti in količino lepila, ki ga nanesejo nanje (Blackledge s sod., 2011). Moji podatki za zgolj tiste lastnosti mrež, ki so jih obravnale zgoraj naštete študije, tako podpirajo sklepe, da večji in bolj nahranjeni pajki vlagajo enako ali več v plenjenje. Pa vendar izračuni skupnega volumna prediva in lepila kažejo drugo sliko in kažejo, da je merjenje zgolj nekaterih lastnosti mreže preveč poenostavljeno. Hkrati je merjenje premera niti tudi ključno za razumevanje zmogljivosti mrež, ki jih gradijo različno veliki osebki. Tako bi npr. nek osebek lahko zgradil dvakrat večjo mrežo, narejeno iz enake količine prediva, če bi upoabil daljše in tanjše niti. Takšni mreži bi imeli posledično precej drugačne lastnosti prestrezanja in zadržanja plena.

V doktorski disertaciji smo pokazali tudi, da telesna velikost in nahranjenost nista edina, ki pri pajkih mrežarjih vplivata na vložek v plenjenje, saj se razmerja med telesno velikostjo in vsemi tremi tipi prediva razlikujejo med vrstami, hkrati pa tudi podrobnosti v arhitekturi mreže, ki privedejo do teh razmerij, med vrstami niso iste. Tako večji osebki nekaterih vrst gradijo večje mreže, ki so enako goste in narejene iz svilenih niti večjega premera, pri drugih vrstah večji osebki gradijo večje in bolj goste mreže, vendar narejene iz svilenih niti enakega premera, pri tretjih vrstah pa je vzorec lahko spet drugačen. Možna razlaga teh vzorcev je, da se je telesna rast pajkov alometrična in favorizira nekoliko drugačne lastnosti mrež, ali da pajki v določenih stopnjah svojega življenja preusmerijo energijo iz nadaljnega plenjenja drugam, npr. v levitev ali odlaganje jajc. Medvrstne razlike v lastnostih mrež bi lahko zaznali tudi zaradi drugačnih selekcijskih pritiskov med vrstami. V primeru kolesastih mrež, ki so predvsem adaptacije na plen (Blackledge s sod., 2011), bi do takšnih razlik lahko prišlo zaradi prilagojenosti na različne kinetične lastnosti plena, ki je specifičen za določen mikrookoljem.

4 POVZETEK

4.1 POVZETEK

Pajki mrežarji iz naddružine Araneoidea so pomembni in pogosto celo modelni organizmi v mnogih evolucijskih študijah, npr. v raziskovanju adaptivne evolucije, evolucijske ekologije in evolucije vedenja v najširšem smislu. V doktorski disertaciji smo tako raziskovali ali različne lastnosti pajčjih mrež, ki vplivajo na vrstno pestrost pajkov, med seboj koevoluirajo, ali ontogenetske spremembe v vedenju resnično sledijo biogenetskemu pravilu in kako individualni pajki glede na svojo velikost spreminjajo energijski vložek v mreže. Večino študij smo izvajali na rodovih *Zygiella* in *Caerostris*, oba iz družine križevcev (Araneidae), ki sta filogenetsko problematična in sta zanimiva tudi po biologiji mrež. Ta rodova smo v disertaciji filogenetsko umestili in raziskali evolucijo njunih mrež.

Koevolucija na nivoju medvrstnih interakcij je dobro dokumentirana, slabo pojasnjeno pa ostaja kako koevoluirajo lastnosti znotraj vrste, predvsem vedenjske in biomehanske lastnosti živalskih »razširjenih fenotipov«. Pri pajkih mrežarjih je pričakovana koevolucija vedenja z ekološkimi in fizičnimi lastnostmi njihovih mrež. Darwinov drevesni pajek (*Caerostris darwini*) je znan po gradnji največjih mrež, ki so narejene iz najmočnejšega poznanega prediva in postavljene v unikatno mikrookolje – razpete so čez potoke ter manjše reke in jezera. Tukaj testiramo domneve, da unikatna ekologija in arhitektura mrež te vrste koevoluira z vedenjem ob gradnjih mrež. Raziskali smo tudi ali so ogromne mreže *C. darwini* prilagojene na lov specifičnega plena, npr. ptičev ali netopirjev, ki uporabljajo reke kot letalne poti, in ali te mreže morda izkoristijo množično pojavljanje vodnih insektov. *C. darwini* smo opazovali v njihovem naravnem habitatu, kjer smo raziskovali tako arhitekturo in ekologijo njihovih mrež, kot tudi interakcije s plenom, posneli pa smo tudi gradnjo njihovih mrež. Ovrednotili smo 90 dogodkov ob gradnji mrež in ta vedenja primerjali z drugimi vrstami pajkov mrežarjev. Ugotovili smo, da *C. darwini* predstavlja najbolj ekstremen primer gigantizma mrež, kjer največje lovilne ploskve merijo do 2,76 m^2, najdaljši most pa do 25,5 m. Trenutni podatki kažejo, da so mreže *C. darwini* učinkovite pasti za vodne žuželke, npr. enodnevnice in kačje pastirje, medtem vretenčarskega plena nismo opazili. *C. darwini* za gradnjo mrež uporablja edinstveno kombinacijo vedenj, nekaj neznanih pri drugih pajkih. To edinstveno vedenje rezultira v izjemno velikih, vendar precej preprostih mrežah. Naši rezultati nadalje podpirajo koevolucijo vedenjskih (arhitektura mrež), ekoloških (mikrookolje mreže) in biomaterialnih (mehanske lastnosti prediva) lastnosti, ki skupaj omogočajo *C. darwini*, da je zasedel to med pajki edinstveno ekološko nišo.

Biogenetsko pravilo predvideva, da ontogenija organizma sledi vzorcem evolucijskih sprememb v sorodstveni liniji tega organizma. Nekateri morfološki dokazi to hipotezo podpirajo, vendar jo precej dokazov tudi zavrača. Vseeno ostaja biogenetsko pravilo na nivoju vedenja slabo raziskano. Pajčje mreže so idealen objekt raziskav ontogenije vedenja, saj predstavljajo fizično manifestacijo vedenja. Pri pajkih mrežarjih se simetrija njihovih mrež pogosto spreminja tekom ontogenetskega razvoja, nekateri avtorji pa menijo, da takšne spremembe odsevajo biogenetsko pravilo. Tukaj smo raziskali alometrijo mrež pri vrsti *Leucauge venusta* in tako testirali, ali opaženi vzorci ustrezajo napovedim biogenetskega pravila ali napovedim alternativne hipoteze optimizacije plenjenja. Vrsta *L. venusta* gradi mreže, ki se v naklonu raztezajo od vodoravnih, preko poševnih, do navpičnih; biogenetsko pravilo predvideva, da alometrija ustreza ontogenetskemu stadiju, medtem ko optimizacija plenjenja predvideva, da alometrija sledi vplivom gravitacije. Natančneje, optimizacija plenjenja predvideva, da bomo skozi ontogenijo opazili povečano asimetričnost zgolj pri navpičnih mrežah. V študiji smo izmerili 98 mrež *L. venusta* in pokazali, da navpične mreže skozi ontogenijo postajajo vse bolj asimetrične, medtem ko to ne drži za poševne in vodoravne mreže. Takšni rezultati ne ustrezajo napovedim biogenetskega pravila, temveč podpirajo hipotezo optimizacije plena.

Vedenjska plastičnost omogoča živalim, da spremenijo svoje vedenje kot odziv na različne okoljske ali fiziološke pogoje. Živali povečajo svoj fitnes (prispevek gen(fen)otipa h genetskemu skladu naslednje generacije) z vložkom različne količine časa in energije v vedenja, kot so iskanje hrane, reprodukcijo in obramba pred plenilci. Pajki mrežarji in njihove mreže so dobri modeli za preučevanje takšnih odzivov na okoljske spremembe, saj kolesaste mreže verjetno odsevajo »trade-off« (nasprotujoče selekcijske pritiske) med visokimi materialnimi stroški produkcije prediva in učinkovitostjo plenjenja. Predhodne študije so pokazale nasprotujoče si rezultate, kako pajki spreminjajo lastnosti svojih mrež kot odgovor na nahranjenost, telesno velikost in plen. Vendar pa so te študije večinoma dokumentirale le spremembe v arhitekturi mrež, zanemarile pa so spremembe v rabi prediva, čeprav bi bil pristop, ki bi ocenil kombinacijo vseh teh informacij, ključnega pomena za razumevanje pajkovega energijskega vložka v mrežo. Da bi bolje razumel »trade-off« tega tipa, smo pri petih ozkosorodnih vrstah rodu *Zygiella* s.l. raziskovali materialni vložek v plenjenje (mreže) v odvisnosti od telesne velikosti. Izmerili smo skupen volumen treh izločkov predilnih žlez, ki jih pajki večinoma uporabljajo za gradjo kolesastih mrež, raziskali pa smo tudi več podrobnosti v arhitekturi mrež in rabi prediva pri samicah različne mase. Ugotovili smo, da med vrstami *Zygiella* s.l. količina materialov s telesno maso narašča izometrično in da v odvisnosti od telesne mase pajki spreminjajo svoje mreže skozi i) arhitekturo mrež, ii) vložek v svilene niti, iii) vložek v lepilo, ter da je za oceno skupnega vložka v plenjenje potrebna kvantifikacija vseh treh parametrov. Za sklep predvidevam, da se vložek v plenjenje spreminja tudi glede na del življenjskega cikla, v katerem se nahaja osebek in da je vedenjska plastičnost, ki veča osebkov fitnes, pod močnim selekcijskim pritiskom, kar med vrstami verjetno privede do različnih vzorcev vlaganja v plenjenje.

Vrstna pestrost pajkov mrežarjev je tesno povezana z evolucijo delovanja mrež. Za razumevanje evolucije lastnosti, ki vplivajo na delovanje mrež, bi v idealnem primeru imeli na voljo jasno sliko o filogenetskih odnosih med sorodnimi linijami pajkov mrežarjev. Kljub napredku filogenetskih metod so filogenetski odnosi med družinami in celo znotraj njih slabo poznani. Večina pajkov, ki gradi kolesaste mreže, pripada družini Araneidae. Z več kot 3000 opisanimi vrstami ta družina po vrstni pestrosti med pajki zaseda tretje mesto in zajema veliko raznolikost pajkov, ki sicer izvorno gradijo kolesaste mreže, vendar razen tega nimajo nujno veliko skupnih lastnosti. Na poti k močnemu orodju za evolucijske raziskave je torej nujno boljše poznavanje odnosov med skupinami pajkov mrežarjev, morda pa bi bilo celo potrebno na novo definirati tradicionalno družino Araneidae. V doktorski nalogi se zato osredotočamo na dva rodova družine Araneidae, ki sta tako filogenetsko problematična, kot tudi zanimiva modela mnogih evolucijskih raziskav: to sta rod *Zygiella* Pickard-Cambridge 1902 v širšem smislu (*Zygiella* s.l.) in rod *Caerostris* Thorell 1868. Na poti k učinkovitemu orodju za evolucijske raziskave, tukaj ponujamo prvi test filogenetskega položaja filogenetsko spornih skupin »Zygiellidae« in *Caerostris*. Rod *Zygiella* je pomemben raziskovalni objekti v študijah odnosov med plenom in plenilcem, spolne selekcije, vedenjske plastičnosti ter vedenja in fiziologije gradnje mrež, vendar je filogenetsko problematičen. Čeprav trenutno umeščen v družino Araneidae, je ta rod v preteklosti bil večkrat premeščen v različne družine, nedavno pa celo razdeljen v štiri rodove, ki bi naj pripadali svoji družini Zygiellidae. Drevesni pajki iz rodu *Caerostris* so precej neraziskani tako ekološko, vedenjsko, kot tudi taksonomsko, vendar postajajo pomembni objekti v raziskavah evolucije mrež in biomaterialov: predstavniki rodu namreč gradijo največje znane kolesaste mreže, zgrajene iz najmočnejšega znanega biomateriala. Z obsežnim izborom notranjih in zunanjih taksonov smo sestavili nabor podatkov, ki temelji na delih mitohondrijskih (COI, 16S) in jedrnih (H3, 18S, 28S, ITS2) genov ter obsega 114 terminalov pajkov mrežarjev, s poudarkom na skupinah, ki gradijo kolesaste mreže. Dodatno smo testirali ali podrobnosti v arhitekturi in gradnji mrež odsevajo filogenetske odnose med skupinami in se posebej osredotočili na evolucijo gradnje mrež in gigantizma mrež. Pokazali smo, da Zygiellidae vsebujejo holarktični rod *Zygiella* s.l. ter rodova *Deliochus* in *Phonognatha*, ki sta razširjena v Avstraliji in Aziji. Skupina Zygiellidae je verjetno sestrska ostalim predstavnikom Araneidae in jo lahko smatramo za veljaven taksonomski pojem. Rod *Caerostris* se uvršča med bazalna razvejišča družine Araneidae, vendar je natančen filogenetski položaj rodu nejasen. Predstavljamo tudi prvo vrstno filogenijo rodu *Caerostris* vključno z opisi petih novih vrst. Naši rezultati podpirajo vedenjske posebnosti filogenetskih linij in kažejo, da se tako Zygiellidae kot *Caerostris* od ostalih pajkov mrežarjev ločijo po kombinaciji določenih vedenjskih lastnosti.

4.2 SUMMARY

The orb weaving araneoid spiders are considered important or even model organisms in several topics of evolutionary research, like adaptive evolution, evolutionary ecology and evolution of behavior in the broadest sense. In my doctoral thesis, I investigated whether spider webs coevolve with other traits that influence spider diversification, whether ontogenetic changes in spider webs really follow the biogenetic law, and how individual spiders alter their foraging investment in relation to body size. I conducted most studies on the genera *Zygiella* s.l. and *Caerostris* from the family Araneidae. Both genera are taxonomically controversial and exhibit interesting web biology. Thus, I phylogenetically placed both genera and investigated the evolution of their webs.

Interspecific coevolution is well described, but we know significantly less about how multiple traits coevolve within a species, particularly between behavioral traits and biomechanical properties of animals' »extended phenotypes«. In orb weaving spiders, coevolution of spider behavior with ecological and physical traits of their webs is expected. Darwin's bark spider (*Caerostris darwini*) bridges large water bodies, building the largest known orb webs utilizing the toughest known silk. Here, we test the prediction that this spider's unique web ecology and architecture coevolved with new web building behaviors. We also investigate *C. darwini* web architecture and provide data to begin to answer whether *C. darwini* webs are specialized to subdue unusually large, perhaps even vertebrate, prey, and whether these large, riverine webs allow the spiders to capitalize on catching numerous small semi-aquatic insects? We observed *C. darwini* in its natural habitat, studied their web architecture and ecology, as well as interactions with prey, and filmed web building. We observed 90 web building events, and compared web building behaviors to other species of orb web spiders. We found that *C. darwini* represents the most extreme case of web gigantism, with the largest orbs up to 2.76 m^2 and longest bridge lines reaching 25.5 m. Current data suggest that *C. darwini* webs are effective snares for semi-aquatic insects such as mayflies and dragonflies, while vertebrate prey were never observed. *C. darwini* uses a unique set of behaviors, some unknown in other spiders, to construct its enormous webs. These unique behaviors result in a giant, yet rather simplified web. Our results continue to build evidence for the coevolution of behavioral (web building), ecological (web microhabitat) and biomaterial (silk biomechanics) traits that combined allow *C. darwini* to occupy a unique niche among spiders.

The biogenetic law posits that the ontogeny of an organism recapitulates the pattern of evolutionary changes. Morphological evidence has offered some support for, but also considerable evidence against, the hypothesis. However, biogenetic law in behavior remains underexplored. As physical manifestation of behavior, spider webs offer an interesting model for the study of ontogenetic behavioral changes. In orb-weaving spiders, web symmetry often gets distorted through ontogeny, and these changes have been interpreted to reflect the biogenetic law. Here, we test the biogenetic law hypothesis against the alternative, the optimal foraging hypothesis, by studying the allometry in *Leucauge venusta* orb webs. These webs range in inclination from vertical through tilted to horizontal; biogenetic law predicts that allometry relates to ontogenetic stage, whereas optimal foraging predicts that allometry relates to gravity. Specifically, pronounced asymmetry should only be seen in vertical webs under optimal foraging theory. We measured 98 webs of *L. venusta* and show that, through ontogeny, vertical webs in *L. venusta* become more asymmetrical in contrast to tilted and horizontal webs. Biogenetic law thus cannot explain *L. venusta* web allometry, but our results instead support optimization of foraging area in response to spider size.

Behavioral plasticity allows animals to change their behavior in response to varying environmental or physiological conditions. Animals maximize their fitness by investing different amounts of time and energy into behavioral efforts such as foraging, reproduction and predator defense. Spiders and their webs are good models for studying such animal responses to environmental cues because an orb web likely reflects a trade-off between the high material costs of producing silk and glue versus prey-catching efficiency. Previous studies showed conflicting results of how spiders alter their webs in response to body condition and prey. However, these studies have mostly documented only changes in web architecture, not changes in the use of silk and glue, although a combined approach assessing all such information is critical for understanding foraging investment. To investigate this trade-off, we examined the material investment in relation to body mass in five closely related species of orb web spiders *Zygiella* s.l. (Araneidae). We measured the total volumes of three spinning gland secretions that spiders use for building orb webs, and explored differences in several web architecture measures and silk use between females of different mass. We found that orb web materials scale isometrically with body mass across *Zygiella* s.l. species, and that in relation to body mass, spiders alter their webs through i) web architecture, ii) investment in silk, and iii) investment in glue, and that quantifying all three parameters is necessary for meaningful estimates of total foraging investment. We conclude by suggesting that foraging investment likely scales differently to body mass according to the stage of spider life cycles, and that plasticity to maximize foraging is under strong selection, and thus differs between species.

The diversification of orb weavers is closely linked with the evolution of traits associated with orb web biology. To understand the evolution of these traits, ideally, one would rely on well resolved phylogenetic relationships among orb weaving lineages. However, the phylogenetic relationships among orb weaving families and even within them are poorly understood. Most spiders building classical orb webs are grouped into the family Araneidae, which is the third largest spider family with more than 3000 species. It

contains a large diversity of orb weavers that do not necessarily have much in common except primitively building orb webs . For building towards a robust tool for evolutionary research, better resolved relationships among orb weaving groups, and even a redefinition of the classical Araneidae might be needed. We here focus on two araneid groups that are both taxonomically controversial, as well as interesting models for several evolutionary questions, including web biology: the genera *Zygiella* Pickard-Cambridge 1902 in the broad sense (from here on "*Zygiella* s.l.") and *Caerostris* Thorell 1868. To build towards a robust tool for evolutionary research, we here provide the first robust tests of the phylogenetic placement of the taxonomically controversial "Zygiellidae" and *Caerostris*. *Zygiella* is an important object in studies of orb web biology, predator-prey interactions, sexual selection, behavioral plasticity and web-building behavior and physiology, but is taxonomically controversial. Although currently in Araneidae, *Zygiella* was transferred between families in the past, and was recently split into four genera and proposed to belong to its own family Zygiellidae. The enigmatic araneid bark spiders of the genus *Caerostris* are understudied taxonomically, ecologically and behaviorally, but are becoming important objects in research of web evolution and biomaterials: they build the largest orb webs known that are made of nature's toughest material. Using extensive ingroup and outgroup sampling, we here gathered a molecular data set using mitochondrial (COI, 16S) and nuclear (H3, 18S, 28S, ITS2) gene fragments for 114 orbicularian taxa, focusing on orb weaving representatives. We additionally test whether phylogenetic relationships among orb weaving lineages are reflected in behavior connected to orb web biology, and we specifically discuss the evolution of web building behavior and web gigantism. We show that Zygiellidae contains the Holarctic *Zygiella* s.l., and the Australasian *Phonognatha* and *Deliochus*. Zygiellidae is probably sister to other araneids, and might be considered a valid taxonomic concept. We recover *Caerostris* as basal araneid, but its exact phylogenetic affinities are ambiguous. We provide the first species level phylogeny of *Caerostris* and describe five new *Caerostris* species. We conclude that phylogenetic exclusivity reflects behavioral differences, as both Zygiellidae and *Caerostris* behavior differs from other orb weavers.

5 VIRI

Agnarsson I. 2003. Spider webs as habitat patches - The distribution of kleptoparasites (*Argyrodes*, Theridiidae) among host webs (*Nephila*, Tetragnathidae). Journal of Arachnology, 31: 344-349

Agnarsson I. 2011. Habitat patch size and isolation as predictors of occupancy and number of argyrodine spider kleptoparasites in *Nephila* webs. Naturwissenschaften, 98: 163-167

Agnarsson I., Aviles L., Coddington J.A., Maddison W.P. 2006. Sociality in theridiid spiders: Repeated origins of an evolutionary dead end. Evolution, 60: 2342-2351

Agnarsson I., Boutry C., Wong S.C., Baji A., Dhinojwala A., Sensenig A.T., Blackledge T.A. 2009a. Supercontraction forces in spider dragline silk depend on hydration rate. Zoology, 112: 325-331

Agnarsson I., Dhinojwala A., Sahni V., Blackledge T.A. 2009b. Spider silk as a novel high performance biomimetic muscle driven by humidity. Journal of Experimental Biology, 212: 1989-1993

Agnarsson I., Kuntner M., Blackledge T.A. 2010. Bioprospecting finds the toughest biological material: Extraordinary silk from a giant riverine orb spider. Plos One, 5: e11234

Agnarsson I., Gregorič M., Blackledge T.A., Kuntner M. 2012. The phylogenetic placement of Psechridae within Entelegynae and the convergent origin of orb-like spider webs. Journal of Zoological Systematics and Evolutionary Research, 51: 100-106

Agnarsson I., Kuntner M. 2012. The generation of a biodiversity hotspot: Biogeography and phylogeography of the western Indian ocean islands. V: Anamthawat-Jonsson K., ur. Current topics in phylogenetics and phylogeography of terrestrial and aquatic systems. Rijeka: In Tech Publishers. 33-82

Agnarsson I., Coddington J.A., Kuntner M. 2013. Systematics: Progress in the study of spider diversity and evolution. V: Penney D., ur. Spider Research in the 21st century: Trends and perspectives. Rochdale, UK: Siri Scientific Press.

Alvarez-Padilla F., Dimitrov D., Giribet G., Hormiga G. 2009. Phylogenetic relationships of the spider family Tetragnathidae (Araneae, Araneoidea) based on morphological and DNA sequence data. Cladistics, 25: 109-146

Andrade M.C.B. 1996. Sexual selection for male sacrifice in the Australian redback spider. Science, 271: 70-72

Arnqvist G., Rowe L. 2002. Antagonistic coevolution between the sexes in a group of insects. Nature, 415: 787-789

Bel-Venner M.C., Venner S. 2006. Mate-guarding strategies and male competitive ability in an orb-weaving spider: results from a field study. Animal Behaviour, 71: 1315-1322

Bel-Venner M.C., Dray S., Allaine D., Menu F., Venner S. 2008. Unexpected male choosiness for mates in a spider. Proceedings of the Royal Society B-Biological Sciences, 275: 77-82

Benjamin S.P., Zschokke S. 2004. Homology, behaviour and spider webs: web construction behaviour of *Linyphia hortensis* and *L. triangularis* (Araneae : Linyphiidae) and its evolutionary significance. Journal of Evolutionary Biology, 17: 120-130

Bidegaray-Batista L., Arnedo M.A. 2011. Gone with the plate: the opening of the Western Mediterranean basin drove the diversification of ground-dweller spiders. Bmc Evolutionary Biology, 11

Blackledge T.A. 2011. Prey capture in orb weaving spiders: are we using the best metric? Journal of Arachnology, 39: 205-210

Blackledge T.A., Binford G.J., Gillespie R.G. 2003. Resource use within a community of Hawaiian spiders (Araneae : Tetragnathidae). Annales Zoologici Fennici, 40: 293-303

Blackledge T.A., Gillespie R.G. 2004. Convergent evolution of behavior in an adaptive radiation of Hawaiian web-building spiders. Proceedings of the National Academy of Sciences of the United States of America, 101: 16228-16233

Blackledge T.A., Eliason C.M. 2007. Functionally independent components of prey capture are architecturally constrained in spider orb webs. Biology Letters, 3: 456-458

Blackledge T.A., Zevenbergen J.M. 2007. Condition-dependent spider web architecture in the western black widow, *Latrodectus hesperus*. Animal Behaviour, 73: 855-864

Blackledge T.A., Scharff N., Coddington J.A., Szuts T., Wenzel J.W., Hayashi C.Y., Agnarsson I. 2009. Reconstructing web evolution and spider diversification in the molecular era. Proceedings of the National Academy of Sciences of the United States of America, 106: 5229-5234

Blackledge T.A., Kuntner M., Agnarsson I. 2011. The form and function of spider orb webs: Evolution from silk to ecosystems. V: Casas J., ur. Advances in insect physiology, Vol 41: Spider physiology and behaviour - Behaviour. 175-262.

Blackledge T.A., Kuntner M., Marhabaie M., Leeper T.C., Agnarsson I. 2012a. Biomaterial evolution parallels behavioral innovation in the origin of orb-like spider webs. Scientific Reports, 2: 833

Blackledge T.A., Perez-Rigueiro J., Plaza G.R., Perea B., Navarro A., Guinea G.V., Elices M. 2012b. Sequential origin in the high performance properties of orb spider dragline silk. Scientific Reports, 2: 782

Blamires S.J. 2010. Plasticity in extended phenotypes: orb web architectural responses to variations in prey parameters. Journal of Experimental Biology, 213: 3207-3212

Blanckenhorn W.U., Dixon A.F.G., Fairbairn D.J., Foellmer M.W., Gibert P., van der Linde K., Meier R., Nylin S., Pitnick S., Schoff C., Signorelli M., Teder T., Wiklund C. 2007. Proximate causes of Rensch's rule: Does sexual size dimorphism in arthropods result from sex differences in development time? American Naturalist, 169: 245-257

Bond J.E., Opell B.D. 1998. Testing adaptive radiation and key innovation hypotheses in spiders. Evolution, 52: 403-414

Brown K.M. 1981. Foragin ecology and niche partitioning in orb-weaving spiders. Oecologia, 50: 380-385

Coddington J.A. 1986a. The genera of the spider family Theridiosomatidae. Smithsonian Contributions to Zoology: 1-96

Coddington J.A. 1986b. The monophyletic origin of the orb web. V: Shear W.A., ur. Spiders: Webs, Behavior,and Evolution. Stanford, CA: Stanford Univ. Press. 319-363

Coddington J.A. 1994. The roles of homology and convergence in studies of adaptation. V: Eggleton P.V. in Vane-Wright R., ur. Phylogenetics and Ecology. London, UK: The Linnean Society of London. 53-78

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

Coddington J.A., Hormiga G., Scharff N. 1997. Giant female or dwarf male spiders? Nature, 385: 687-688

Coddington J.A., Colwell R.K. 2000. Arachnids Encyclopedia of Biodiversity. Waltham, Massachusetts: Academic Press, Elsevier. 199-218

Craig C.L. 1987. The significance of spider size to the diversification of spider-web architectures and spider reproductive modes. American Naturalist, 129: 47-68

Craig C.L., Weber R.S., Bernard G.D. 1996. Evolution of predator-prey systems: Spider foraging plasticity in response to the visual ecology of prey. American Naturalist, 147: 205-229

Dechant H.E., Hossl B., Rammerstorfer F.G., Barth F.G. 2006. Arthropod mechanoreceptive hairs: modeling the directionality of the joint. Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology, 192: 1271-1278

Denny M. 1976. Physical properties of spider silk and their role in design of orb-webs. Journal of Experimental Biology, 65: 483-506

Dimitrov D., Lopardo L., Giribet G., Arnedo M.A., Alvarez-Padilla F., Hormiga G. 2012. Tangled in a sparse spider web: single origin of orb weavers and their spinning work unravelled by denser taxonomic sampling. Proceedings of the Royal Society B-Biological Sciences, 279: 1341-1350

Eberhard W.G. 1972. Web of *Uloborus diversus* (Araneae-Uloboridae). Journal of Zoology, 66: 417-465

Eberhard W.G. 1975. Inverted ladder orb web of *Scoloderus* sp. and intermediate orb of *Eustala* sp. (Araneae - Araneidae). Journal of Natural History, 9: 93-106

Eberhard W.G. 1981. Construction behaviour and the distribution of tensions in orb webs. Bull. Brit. Arachnol. Soc., 5: 189-204

Eberhard W.G. 1982. Behavioral characters for the higher classification of orb-weaving spiders. Evolution, 36: 1067-1095

Eberhard W.G. 1986. Effects of orb-web geometry on prey interception and retention. V: Shear W.A., ur. Spiders, webs, behavior and evolution. Stanford: Stanford University Press. 70-100.

Eberhard W.G. 1988. Behavioral flexibility in orb web construction - effects of supplies in different silk glands and spider size and weight. Journal of Arachnology, 16: 295-302

Eberhard W.G. 1990a. Early stages of orb construction by *Philoponella vicina*, *Leucauge mariana*, and *Nephila clavipes* (Araneae, Uloboridae and Tetragnathidae), and their phylogenetic implications. Journal of Arachnology, 18: 205-234

Eberhard W.G. 1990b. Function and phylogeny of spider webs. Annual Review of Ecology and Systematics, 21: 341-372

Eberhard W.G. 1995. The web and building behavior of *Synotaxus ecuadorensis* (Araneae, Synotaxidae). Journal of Arachnology, 23: 25-30

Eberhard W.G. 2000. Spider manipulation by a wasp larva. Nature, 406: 255-256

Eberhard W.G. 2007. Miniaturized orb-weaving spiders: behavioural precision is not limited by small size. Proceedings of the Royal Society B-Biological Sciences, 274: 2203-2209

Eberhard W.G. 2013. The rare large prey hypothesis for orb web evolution: a critique. Journal of Arachnology, 41: 76-80

Eberhard W.G., Barrantes G., Madrigal-Brenes R. 2008. Vestiges of an orb-weaving ancestor? The "biogenetic law" and ontogenetic changes in the webs and building behavior of the black widow spider *Latrodectus geometricus* (Araneae Theridiidae). Ethology Ecology & Evolution, 20: 211-244

Elgar M.A., Allan R.A., Evans T.A. 1996. Foraging strategies in orb-spinning spiders: Ambient light and silk decorations in Argiope aetherea Walckenaer (Araneae: Araneoidea). Australian Journal of Ecology, 21: 464-467

Felsenstein J. 1985. Phylogenies and the comparative method. American Naturalist, 125: 1-15

Ferrari M.C.O., Sih A., Chivers D.P. 2009. The paradox of risk allocation: a review and prospectus. Animal Behaviour, 78: 579-585

Foelix R.F. 2011. Biology of Spiders. Oxford, Oxford University Press 432.

Fratzl P., Barth F.G. 2009. Biomaterial systems for mechanosensing and actuation. Nature, 462: 442-448

Freudenstein J.V. 2005. Character, States and Homology. Systematic Biology, 54: 965-973

Futuyma D.J. 1997. Evolutionary Biology. Sunderland, Sinauer Publications 763.

Garb J.E., Gillespie R.G. 2009. Diversity despite dispersal: colonization history and phylogeography of Hawaiian crab spiders inferred from multilocus genetic data. Molecular Ecology, 18: 1746-1764

Garland T., Kelly S.A. 2006. Phenotypic plasticity and experimental evolution. Journal of Experimental Biology, 209: 2344-2361

Gillespie R.G. 2002. Biogeography of spiders on remote oceanic islands of the Pacific: archipelagoes as stepping stones? Journal of Biogeography, 29: 655-662

Gillespie R.G. 2004. Community assembly through adaptive radiation in Hawaiian spiders. Science, 303: 356-359

Gould S.J. 1992. Ontogeny and phylogeny - revisited and reunited. Bioessays, 14: 275-279

Grasshoff M. 1984. Die Radnetzspinnen-Gattung *Caerostris* (Arachnida: Araneae). Revue Zoologique Africaine, 98: 725-765

Gregorič M., Kostanjšek R., Kuntner M. 2010. Orb web features as taxonomic characters in *Zygiella* s.l. (Araneae, Araneidae). Journal of Arachnology, 38: 319-327

Gregorič M., Agnarsson I., Blackledge T.A., Kuntner M. 2011a. Darwin's bark spider: giant prey in giant orb webs (Caerostris darwini, Araneae: Araneidae)? Journal of Arachnology, 39: 287-295

Gregorič M., Agnarsson I., Blackledge T.A., Kuntner M. 2011b. How did the spider cross the river? Behavioral adaptations for river-bridging webs in *Caerostris darwini* (Araneae: Araneidae). Plos One, 6: e26847

Griswold C.E., Coddington J.A., Hormiga G., Scharff N. 1998. Phylogeny of the orb-web building spiders (Araneae, Orbiculariae : Deinopoidea, Araneoidea). Zoological Journal of the Linnean Society, 123: 1-99

Harmer A.M.T., Herberstein M.E. 2009. Taking it to extremes: what drives extreme web elongation in Australian ladder web spiders (Araneidae: Telaprocera maudae)? Animal Behaviour, 78: 499-504

Harmer A.M.T., Blackledge T.A., Madin J.S., Herberstein M.E. 2011. High-performance spider webs: integrating biomechanics, ecology and behaviour. Journal of the Royal Society Interface, 8: 457-471

Harmer A.M.T., Kokko H., Herberstein M., Madin J.S. 2012. Optimal web investment in sub-optimal foraging conditions. Naturwissenschaften, 99: 65-70

Harvey M.S. 2002. The neglected cousins: What do we know about the smaller Arachnid orders? Journal of Arachnology, 30: 357-372

Hayashi C.Y., Lewis R.V. 2000. Molecular architecture and evolution of a modular spider silk protein gene. Science, 287: 1477-1479

Hazlett B.A. 1987. Behavioural plasticity as an adaptation to a variable environment. V: Chelazzi G., Vannini M. (ur.), Behavioral adaptation to intertidal life. Plenum Press, New York, 317–332

Heiling A.M., Herberstein M.E. 1998. The web of *Nuctenea sclopetaria* (Araneae, Araneidae): Relationship between body size and web design. Journal of Arachnology, 26: 91-96

Heiling A.M., Herberstein M.E. 1999. The role of experience in web-building spiders (Araneidae). Animal Cognition, 2: 171-177

Heimer S., Nentwig W. 1991. Spinnen Mitteleuropas. Ein Bestimmungsbuch, Blackwell Wissenschafts-Verlag 543.

Herberstein M.E., Heiling A.M. 1999. Asymmetry in spider orb webs: a result of physical constraints? Animal Behaviour, 58: 1241-1246

Herberstein M.E., Craig C.L., Elgar M.A. 2000. Foraging strategies and feeding regimes: Web and decoration investment in *Argiope keyserlingi* Karsch (Araneae : Araneidae). Evolutionary Ecology Research, 2: 69-80

Herberstein M.E., Wignall A. 2011. Introduction: spider biology. V: Herberstein M., ur. Spider behaviour: Flexibility and versatility. Cambridge: Cambridge University Press. 1-30

Herberstein M.E., Tso I.M. 2011. Spider webs: evolution, diversity and plasticity. V: Herberstein M.E., ur. Spider behaviour: Flexibility and versatility. Cambridge: Cambridge University Press. 57-98.

Hesselberg T. 2010. Ontogenetic changes in web design in two orb-web spiders. Ethology, 116: 535-545

Higgins L.E. 1992. Developmental changes in barrier web structure under different levels of predation risk in *Nephila clavipes* (Araneae, Tetragnathidae). Journal of Insect Behavior, 5: 635-655

Higgins L.E. 2000. The interaction of season length and development time alters size at maturity. Oecologia, 122: 51-59

Higgins L.E., Buskirk R.E. 1998. Spider-web kleptoparasites as a model for studying producer-consumer interactions. Behavioral Ecology, 9: 384-387

Hormiga G., Eberhard W.G., Coddington J.A. 1995. Web-construction behavior in australian *Phonognatha* and the phylogeny of nephiline and tetragnathid spiders (Araneae, Tetragnathidae). Australian Journal of Zoology, 43: 313-364

Kiontke K., Gavin N.P., Raynes Y., Roehrig C., Piano F., Fitch D.H.A. 2004. *Caenorhabditis* phylogeny predicts convergence of hermaphroditism and extensive intron loss. Proceedings of the National Academy of Sciences of the United States of America, 101: 9003-9008

Kraut B. 2001. Krautov strojniški priročnik. Založba Litostroj, Ljubljana

Kuhn-Nentwig L., Fedorova I.M., Luscher B.P., Kopp L.S., Trachsel C., Schaller J., Vu X.L., Seebeck T., Streitberger K., Nentwig W., Sigel E., Magazanik L.G. 2012. A venom-derived neurotoxin, CsTx-1, from the spider *Cupiennius salei* exhibits cytolytic activities. Journal of Biological Chemistry, 287: 25640-25649

Kuntner M., Coddington J.A., Hormiga G. 2008. Phylogeny of extant nephilid orb-weaving spiders (Araneae, Nephilidae): testing morphological and ethological homologies. Cladistics, 24: 147-217

Kuntner M., Agnarsson I. 2009. Phylogeny accurately predicts behaviour in Indian Ocean *Clitaetra* spiders (Araneae : Nephilidae). Invertebrate Systematics, 23: 193-204

Kuntner M., Coddington J.A. 2009. Discovery of the largest orbweaving spider species: The evolution of gigantism in *Nephila*. Plos One, 4: e7516

Kuntner M., Agnarsson I. 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., Gregorič M., Li D.Q. 2010a. Mass predicts web asymmetry in *Nephila* spiders. Naturwissenschaften, 97: 1097-1105

Kuntner M., Kralj-Fišer S., Gregorič M. 2010b. Ladder webs in orb-web spiders: ontogenetic and evolutionary patterns in Nephilidae. Biological Journal of the Linnean Society, 99: 849-866

Kuntner M., Arnedo M.A., Trontelj P., Lokovšek T., Agnarsson I. 2013. Towards a molecular phylogeny of nephilid spiders: Evolutionary history of a model lineage. Molecular Phylogenetics and Evolution: in press

Levi H.W. 1974. The orb-weaver genus *Zygiella* (Araneae: Araneidae). Bulletin of the Museum of Comparative Zoology, 146: 267–290

Levi H.W. 1980. The orb-weaver genus *Mecynogea*, the subfamily Metinae and the genera *Pachygnatha*, *Glenognatha* and *Azilia* of the subfamily Tetragnathinae north of Mexico (Araneae: Araneidae). Bulletin of the Museum of Comparative Zoology, 149: 1–74

Levi H.W. 1993. The neotropical orb-weaving spiders of the genera *Wixia*, *Pozonia*, and *Ocrepeira* (Araneae: Araneidae). Bull. Mus. Comp. Zool., 153: 47-141

Levy G. 1987. Spiders of the genera *Araniella*, *Zygiella*, *Zilla* and *Mangora* (Araneae, Araneidae) from Israel, with notes on *Metellina* species from Lebanon. Zoologica Scripta, 16: 243-257

Liao C.P., Chi K.J., Tso I.M. 2009. The effects of wind on trap structural and material properties of a sit-and-wait predator. Behavioral Ecology, 20: 1194-1203

Lima S.L., Dill L.M. 1990. Behavioral decisions made under the risk of predation - a review and prospectus. Canadian Journal of Zoology-Revue Canadienne De Zoologie, 68: 619-640

Lin L.H., Edmonds D.T., Vollrath F. 1995. Structural-engineering of an orb-spiders web. Nature, 373: 146-148

Lubin Y.D. 1986. Web building and prey capture in the Uloboridae. V: Shear W.A., ur. Spiders: webs, behavior, and evolution: Stanford University Press, Stanford, California, USA. 132-171.

Lubin Y.D., Bilde T. 2007. The evolution of sociality in spiders. V: Brockmann H.J., Roper T.J., Naguib M., WynneEdwards K.E., Barnard C. in Mitani J., ur. Advances in the Study of Behavior, Vol 37. 83-145.

Mayntz D., Toft S., Vollrath F. 2009. Nutrient balance affects foraging behaviour of a trap-building predator. Biology Letters, 5: 735-738

Mitter C., Farrell B., Wiegmann B. 1988. The phylogenetic study of adaptive zones - has phytophagy promoted insect diversification. American Naturalist, 132: 107-128

Nakata K. 2007. Prey detection without successful capture affects spider's orb-web building behaviour. Naturwissenschaften, 94: 853-857

Nakata K. 2008. Spiders use airborne cues to respond to flying insect predators by building orb-web with fewer silk thread and larger silk decorations. Ethology, 114: 686-692

Nakata K. 2010. Does ontogenetic change in orb web asymmetry reflect biogenetic law? Naturwissenschaften, 97: 1029-1032

Nakata K., Zschokke S. 2010. Upside-down spiders build upside-down orb webs: web asymmetry, spider orientation and running speed in *Cyclosa*. Proceedings of the Royal Society B-Biological Sciences, 277: 3019-3025

Nelson G. 1978. Ontogeny, phylogeny, paleontology, and the biogenetic law. Systematic Zoology, 27: 324-345

Nentwig W. 1983. The non-filter function of orb webs in spiders. Oecologia, 58: 418-420

Nentwig W. 1985. Prey analysis of 4 species of tropical orb-weaving spiders (Araneae, Araneidae) and a comparison with araneids of the temperate zone. Oecologia, 66: 580-594

Nyffeler M., Knornschild M. 2013. Bat Predation by Spiders. Plos One, 8

Olsson L., Levit G.S., Hossfeld U. 2010. Evolutionary developmental biology: its concepts and history with a focus on Russian and German contributions. Naturwissenschaften, 97: 951-969

Opell B.D. 1997a. A comparison of capture thread and architectural features of deinopoid and araneoid orb-webs. Journal of Arachnology, 25: 295-306

Opell B.D. 1997b. The material cost and stickiness of capture threads and the evolution of orb-weaving spiders. Biological Journal of the Linnean Society, 62: 443-458

Opell B.D. 1999. Redesigning spider webs: Stickiness, capture area and the evolution of modern orb-webs. Evolutionary Ecology Research, 1: 503-516

Opell B.D., Schwend H.S. 2007. The effect of insect surface features on the adhesion of viscous capture threads spun by orb-weaving spiders. Journal of Experimental Biology, 210: 2352-2360

Opell B.D., Markley B.J., Hannum C.D., Hendricks M.L. 2008. The contribution of axial fiber extensibility to the adhesion of viscous capture threads spun by orb-weaving spiders. Journal of Experimental Biology, 211: 2243-2251

Pekar S., Coddington J.A., Blackledge T.A. 2012. Evolution of stenophagy in spiders (Araneae): Evidence based on the comparative analysis of spider diets. Evolution, 66: 776-806

Pellegrini M., Marcotte E.M., Thompson M.J., Eisenberg D., Yeates T.O. 1999. Assigning protein functions by comparative genome analysis: Protein phylogenetic profiles. Proceedings of the National Academy of Sciences of the United States of America, 96: 4285-4288

Peters H.M. 1937. Studien am Netz der Kreuzspinne (*Aranea diadema*.). 1. Die Grundstruktur des Netzes und Beziehungen zum Bauplan des Spinnenkorpers. Zeitschrift für Morphologie und Ökologie der Tiere, 33: 128-150

Platnick N.I. 2013. The world spider catalog, version 13.5: American Museum of Natural History.

Platnick N.I., Shadab M.U. 1993. A review of the pirate spiders (Araneae, Mimetidae) of Chile. American Museum Novitates, 3074: 1-30

Richardson M.K., Keuck G. 2002. Haeckel's ABC of evolution and development. Biological Reviews, 77: 495-528

Richardson M.L., Hanks L.M. 2009. Partitioning of niches among four species of orb-weaving spiders in a grassland habitat. Environmental Entomology, 38: 651-656

Ridley M. 1993. Evolution. Oxford, Blackwell Scientific Publications 670.

Roberts M.J. 1995. Spiders of Britain & Northern Europe. London, HarperCollins Publishers

Roderick G.K., Gillespie R.G. 1998. Speciation and phylogeography of Hawaiian terrestrial arthropods. Molecular Ecology, 7: 519-531

Sahni V., Blackledge T.A., Dhinojwala A. 2011. A review on spider silk adhesion. Journal of Adhesion, 87: 595-614

Scharff N., Coddington J.A. 1997. A phylogenetic analysis of the orb-weaving spider family Araneidae (Arachnida, Araneae). Zoological Journal of the Linnean Society, 120: 355-434

Schneider J.M., Lubin Y. 1996. Infanticidal male eresid spiders. Nature, 381: 655-656

Sensenig A.T., Agnarsson I., Blackledge T.A. 2010. Behavioural and biomaterial coevolution in spider orb webs. Journal of Evolutionary Biology, 23: 1839-1856

Sensenig A.T., Lorentz K.A., Kelly S.P., Blackledge T.A. 2012. Spider orb webs rely on radial threads to absorb prey kinetic energy. Journal of the Royal Society Interface, 9: 1880-1891

Sherman P.M. 1994. The orb-web - an energetic and behavioral estimator of a spiders dynamic foraging and reproductive strategies. Animal Behaviour, 48: 19-34

Stowe M.K. 1986. Prey specialization in the Araneidae. V: Shear W.A., ur. Spiders, Webs, Behavior, and Evolution. Stanford: Stanford University Press. 101-131.

Swanson B.O., Blackledge T.A., Summers A.P., Hayashi C.Y. 2006. Spider dragline silk: Correlated and mosaic evolution in high-performance biological materials. Evolution, 60: 2539-2551

Swanson B.O., Anderson S.P., DiGiovine C., Ross R.N., Dorsey J.P. 2009. The evolution of complex biomaterial performance: The case of spider silk. Integrative and Comparative Biology, 49: 21-31

Thevenard L., Leborgne R., Pasquet A. 2004. Web-building management in an orb-weaving spider, *Zygiella x-notata*: influence of prey and conspecifics. Comptes Rendus Biologies, 327: 84-92

van Veelen M., Garcia J., Aviles L. 2010. It takes grouping and cooperation to get sociality. Journal of Theoretical Biology, 264: 1240-1253

Venner S., Bel-Venner M.C., Pasquet A., Leborgne R. 2003. Body-mass-dependent cost of web-building behavior in an orb weaving spider, *Zygiella x-notata*. Naturwissenschaften, 90: 269-272

Venner S., Pasquet A., Leborgne R. 2000. Web-building behaviour in the orb-weaving spider *Zygiella x-notata*: influence of experience. Animal Behaviour, 59: 603-611

Venner S., Casas J. 2005. Spider webs designed for rare but life-saving catches. Proceedings of the Royal Society B-Biological Sciences, 272: 1587-1592

Vollrath F., Samu F. 1997. The effect of starvation on web geometry in an orbweaving spider. Bulletin of the British Arachnological Society, 10: 295-298

Vollrath F., Selden P. 2007. The role of behavior in the evolution of spiders, silks, and webs. Annual Review of Ecology Evolution and Systematics, 38: 819-846

Vollrath F., Porter D. 2009. Silks as ancient models for modern polymers. Polymer, 50: 5623-5632

Watanabe T. 2000. Web tuning of an orb-web spider, *Octonoba sybotides*, regulates prey-catching behaviour. Proceedings of the Royal Society of London Series B-Biological Sciences, 267: 565-569

West-Eberhard M.J. 1989. Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics, 20: 249-278

Wiehle H. 1927. Beiträge zur Kenntnis des Radnetzbaues der Epeiriden, Tetragnathiden und Uloboriden. Zeits. Morph. Ökol. Tiere, 8(3-4): 468-537

Wiehle H. 1929. Weitere Beiträge zur Biologie der Araneen, insbesondere zur Kenntnis des Radnetzbaues. Zeits. Morph. Ökol. Tiere

Wirth E., Barth F.G. 1992. Forces in the spider orb web. Journal of Comparative Physiology A, 171: 359-371

Wise D.H. 1993. Spiders in ecological webs. New York, Cambridge University Press

Witt P.N., Reed C.F., Peakall D.B. 1968. A spider's web: Problems in regulatory biology. New York, Springer Verlag 107.

Wunderlich J. 2004. Fossil spiders in amber and copal. Conclusions, revisions, new taxa and family diagnoses of fossil and extant taxa,

Yip K.Y., Patel P., Kim P.M., Engelman D.M., McDermott D., Gerstein M. 2008. An integrated system for studying residue coevolution in proteins. Bioinformatics, 24: 290-292

Zschokke S. 1996. Early stages of orb web construction in *Araneus diadematus* Clerck. Revue Suisse de Zoologie hors série, 2: 709-720

Zschokke S. 2002. Form and function of the orb web. European Arachnology, 2000: 99-106

Zschokke S., Vollrath F. 1995a. Unfreezing the behavior of 2 orb spiders. Physiology & Behavior, 58: 1167-1173

Zschokke S., Vollrath F. 1995b. Web construction patterns in a range of orb-weaving spiders (Araneae). European Journal of Entomology, 92: 523-541

Zschokke S., Nakata K. 2010. Spider orientation and hub position in orb webs. Naturwissenschaften, 97: 43-52

ZAHVALA

Zahvaljujem se mentorju višjemu znan. sod. doc. dr. Matjažu Kuntnerju za odlično vodenje, vsestranske nasvete, dobre ideje, podporo in potrpljenje. Zahvaljujem se Ingiju Agnarssonu in Toddu Blackledgu, da sta mi omogočila obisk njunih raziskovalnih skupin in bistveno prispevala h kvaliteti mojega raziskovalnega dela. Za logistično pomoč se zahvaljujem tudi njunim študentom, predvsem Heineju Kiesbüyu, Shakiri Quiñones Lebrón, Seanu Kellyju in Andrewju Sensenigu. Doktorski študij je omogočila finančna podpora Javne agencije za raziskovalno dejavnost Republike Slovenije v okviru programa Mladi raziskovalci.

Na Biološkem inštitutu Jovana Hadžija ZRC SAZU se zahvaljujem sodelavcem, predvsem Simoni Kralj-Fišer in Tatjani Čelik za pomoč pri statistični obdelavi podatkov, Tjaši Lokovšek, Nini Vidergar in Ren-Chung Chengu pa za pomoč pri molekularnih metodah dela. Posebej se zahvaljujem Simoni, da mi je omogočila sodelovanje na več projektih, ki sicer niso bili del moje doktorske disertacije.

Zahvaljujem se Matjažu, Simoni in Ingiju, da so mi omogočili obilico zanimivega terenskega dela, ki ne bi bilo mogoče brez dodatne finančne podpore društev National Geographic in The Explorers Club ter vseh, ki so pomagali neposredno na terenu. Še posebej se zahvaljujem sledečim: Tea Knapič, Alenka in Lovro Rozman, Martin Turjak, Melita Vamberger, Rebeka Šiling, Ren-Chung Cheng, Klavdija Šuen, Daiqin Li, Shichang Zhang, Wenjin Gan, osebju društva MICET, Sahondra Lalao Rahanitriniaina, Honore Rabarison, Christian Kropf, Holger Frick, Wolfgang Nentwig in drugim.

Jan Dolansky, Peter Dolejš, Peter Jäger, Yurij Marušik in Mark Townley so prispevali sveže nabran material za moje analize, Jonathan Coddington, William Eberhard in Samuel Zschokke pa so bili ob pivu na voljo za plodne debate o temah, ki v splošnem sicer zanimajo bolj malo ljudi.

Članoma komisije za zagovor prof. dr. Roku Kostanjšku in doc. dr. Cenetu Fišerju se zahvaljujem za strokoven pregled doktorske disertacije in nadvse koristne komentarje, ki so vzpodbudili moje nadaljne razmišljanje.

Vesni Janežič Ješe se zahvaljujem za pomoč pri reševanju mnogih birokratskih in logističnih zagonetk v času podiplomskega študija ter za neprestano dobro razpoloženje in pozitivno naravnanost.

Veliko zahvalo sem dolžan tudi staršem, bratu in prijateljem za stalno podporo na moji življenski poti, obilo dobre zabave in drugo.

PRILOGE

Priloga 1: Dovoljenje založnika za objavo članka How did the spider cross the river? Behavioral adaptations for river-bridging webs in *Caerostris darwini* (Araneae: Araneidae), v tiskani in elektronski verziji doktorske disertacije.

OPEN ACCESS Freely available online

PLoS one

How Did the Spider Cross the River? Behavioral Adaptations for River-Bridging Webs in *Caerostris darwini* (Araneae: Araneidae)

Matjaž Gregorič[1]*, Ingi Agnarsson[2,4], Todd A. Blackledge[3], Matjaž Kuntner[1,4,5]

1 Scientific Research Centre, Institute of Biology, Slovenian Academy of Sciences and Arts, Ljubljana, Slovenia, **2** Department of Biology, University of Puerto Rico, San Juan, Puerto Rico, United States of America, **3** Integrated Bioscience Program, Department of Biology, University of Akron, Akron, Ohio, United States of America, **4** Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC, United States of America, **5** College of Life Sciences, Hubei University, Wuhan, Hubei, China

Abstract

Background: Interspecific coevolution is well described, but we know significantly less about how multiple traits coevolve within a species, particularly between behavioral traits and biomechanical properties of animals' "extended phenotypes". In orb weaving spiders, coevolution of spider behavior with ecological and physical traits of their webs is expected. Darwin's bark spider (*Caerostris darwini*) bridges large water bodies, building the largest known orb webs utilizing the toughest known silk. Here, we examine *C. darwini* web building behaviors to establish how bridge lines are formed over water. We also test the prediction that this spider's unique web ecology and architecture coevolved with new web building behaviors.

Methodology: We observed *C. darwini* in its natural habitat and filmed web building. We observed 90 web building events, and compared web building behaviors to other species of orb web spiders.

Conclusions: *Caerostris darwini* uses a unique set of behaviors, some unknown in other spiders, to construct its enormous webs. First, the spiders release unusually large amounts of bridging silk into the air, which is then carried downwind, across the water body, establishing bridge lines. Second, the spiders perform almost no web site exploration. Third, they construct the orb capture area below the initial bridge line. In contrast to all known orb-weavers, the web hub is therefore not part of the initial bridge line but is instead built *de novo*. Fourth, the orb contains two types of radial threads, with those in the upper half of the web doubled. These unique behaviors result in a giant, yet rather simplified web. Our results continue to build evidence for the coevolution of behavioral (web building), ecological (web microhabitat) and biomaterial (silk biomechanics) traits that combined allow *C. darwini* to occupy a unique niche among spiders.

Citation: Gregorič M, Agnarsson I, Blackledge TA, Kuntner M (2011) How Did the Spider Cross the River? Behavioral Adaptations for River-Bridging Webs in *Caerostris darwini* (Araneae: Araneidae). PLoS ONE 6(10): e26847. doi:10.1371/journal.pone.0026847

Editor: Stephen Pratt, Arizona State University, United States of America

Received August 11, 2011; **Accepted** October 4, 2011; **Published** October 26, 2011

Funding: This is the second contribution resulting from the 2010 Madagascar expedition, funded by National Geographic (grant 8655-09 to Dr. Agnarsson, Dr. Kuntner and Dr. Blackledge). Additional funding came from the Slovenian Research Agency (grants P1-0236, J1-2063 to Dr. Kuntner) and National Science Foundation (IOS-0745379 to Dr. Blackledge). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: matjaz.gregoric@gmail.com

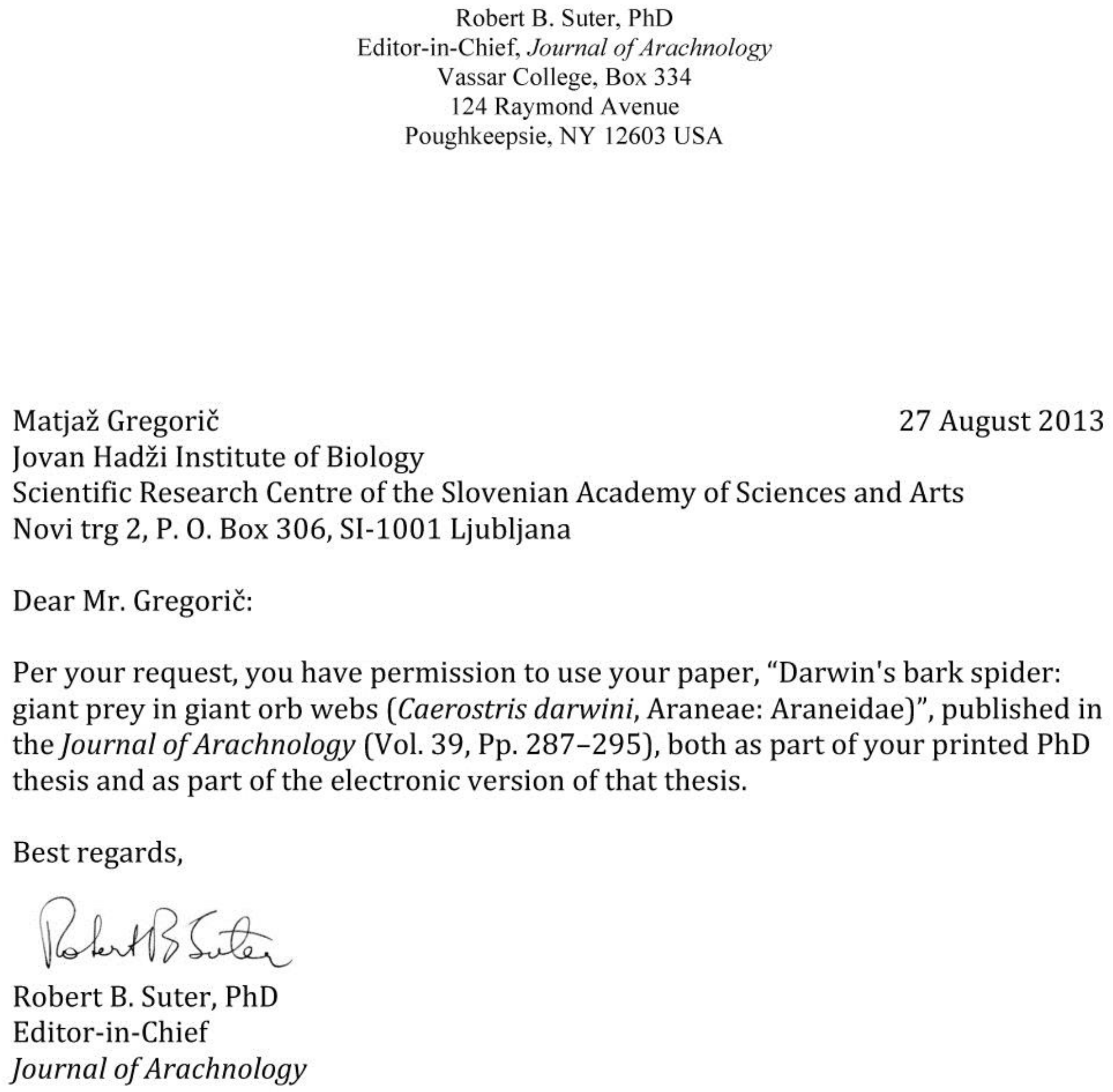
Introduction

Coevolution, change of one trait triggered by shifts in a related trait [1], can occur at many hierarchical levels from amino acids to interspecific arms races [2–4]. While species coevolution is well documented, we lack a broad understanding of how multiple traits coevolve to enable resource use within a species. This particularly holds true for the potential coevolution of traits that lack obvious genetic linkage, such as ecological "extended phenotypic" (e.g. spider webs and their microhabitat), behavioral (e.g. web building behaviors), and biomechanical (e.g. intrinsic properties of silk) traits [5].

Spider webs are physical manifestations of web building behaviors and are built using some of the world's "highest performance" biomaterials – spider silks. Spider webs are thus ideal for studying coevolution between behaviors, ecology, and performance of biomaterials [6–8]. The orb web's evolutionary origin defines a single clade, Orbiculariae, a large and diverse group with more than 12.000 species [9–12]. Architectural evolution of orb webs through time has resulted in novel web types [9,13,14], such as the linyphiid sheetwebs and theridiid cobwebs [10,15,16], the deinopid casting web [17], as well as many modifications of the classical orb web [7,9,18–20]. Because spiders build orb webs using highly stereotypical behaviors that are evolutionarily conserved and phylogenetically informative [13,20], the evolution of new web architectures are expected to coincide with novel behaviors.

The impressive range of web designs within the Orbiculariae represents adaptations to a large range of prey types in diverse habitats [7,8]. Two major components in spider web evolution are the changes in quality (intrinsic material properties) of the different types of spider silk composing webs and the changes in behaviors

Priloga 2: Dovoljenje založnika za objavo članka Darwin's bark spider: Giant prey in giant orb webs (*Caerostris darwini*, Araneae: Araneidae)?, v tiskani in elektronski verziji doktorske disertacije.



Robert B. Suter, PhD
Editor-in-Chief, *Journal of Arachnology*
Vassar College, Box 334
124 Raymond Avenue
Poughkeepsie, NY 12603 USA

Matjaž Gregorič
Jovan Hadži Institute of Biology
Scientific Research Centre of the Slovenian Academy of Sciences and Arts
Novi trg 2, P. O. Box 306, SI-1001 Ljubljana

27 August 2013

Dear Mr. Gregorič:

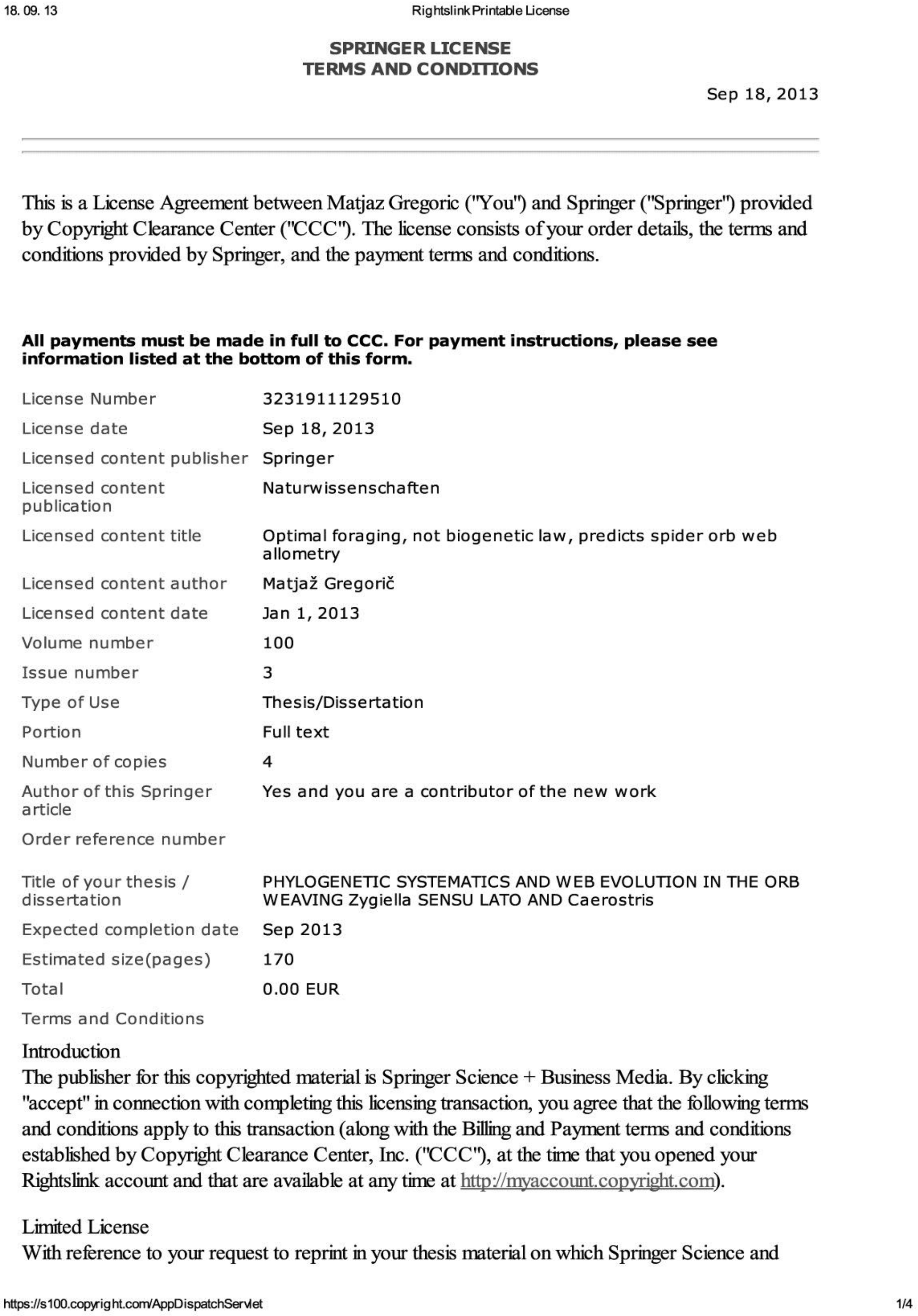
Per your request, you have permission to use your paper, "Darwin's bark spider: giant prey in giant orb webs (*Caerostris darwini*, Araneae: Araneidae)", published in the *Journal of Arachnology* (Vol. 39, Pp. 287–295), both as part of your printed PhD thesis and as part of the electronic version of that thesis.

Best regards,

Robert B Suter

Robert B. Suter, PhD
Editor-in-Chief
Journal of Arachnology

Priloga 3: Dovoljenje založnika za objavo članka Optimal foraging, not biogenetic law, predicts spider orb web allometry, v tiskani in elektronski verziji doktorske disertacije.



18. 09. 13 Rightslink Printable License

SPRINGER LICENSE
TERMS AND CONDITIONS

Sep 18, 2013

This is a License Agreement between Matjaz Gregoric ("You") and Springer ("Springer") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Springer, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

License Number	3231911129510
License date	Sep 18, 2013
Licensed content publisher	Springer
Licensed content publication	Naturwissenschaften
Licensed content title	Optimal foraging, not biogenetic law, predicts spider orb web allometry
Licensed content author	Matjaž Gregorič
Licensed content date	Jan 1, 2013
Volume number	100
Issue number	3
Type of Use	Thesis/Dissertation
Portion	Full text
Number of copies	4
Author of this Springer article	Yes and you are a contributor of the new work
Order reference number	
Title of your thesis / dissertation	PHYLOGENETIC SYSTEMATICS AND WEB EVOLUTION IN THE ORB WEAVING Zygiella SENSU LATO AND Caerostris
Expected completion date	Sep 2013
Estimated size(pages)	170
Total	0.00 EUR
Terms and Conditions	

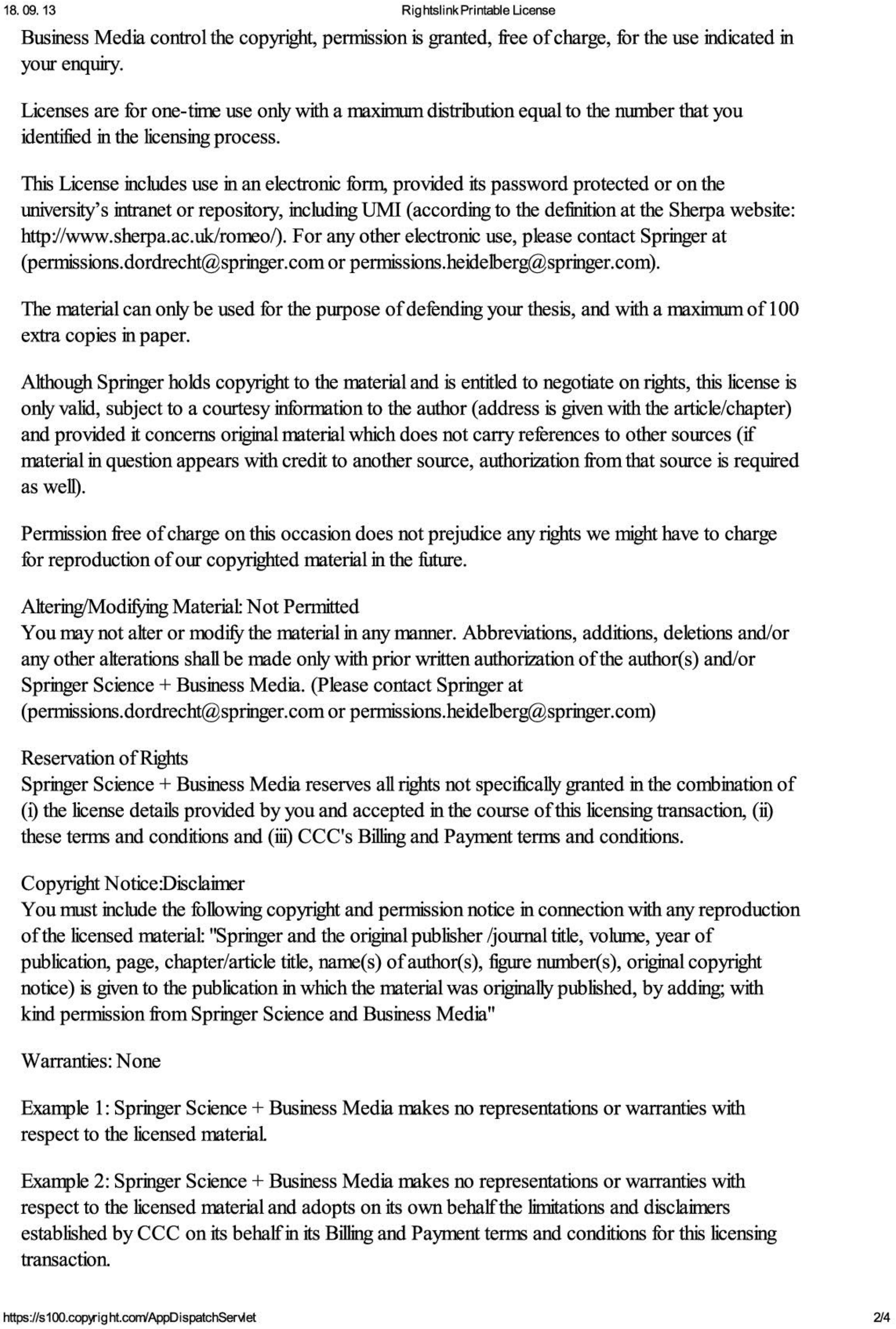
Introduction

The publisher for this copyrighted material is Springer Science + Business Media. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at http://myaccount.copyright.com).

Limited License

With reference to your request to reprint in your thesis material on which Springer Science and

https://s100.copyright.com/AppDispatchServlet 1/4



Business Media control the copyright, permission is granted, free of charge, for the use indicated in your enquiry.

Licenses are for one-time use only with a maximum distribution equal to the number that you identified in the licensing process.

This License includes use in an electronic form, provided its password protected or on the university's intranet or repository, including UMI (according to the definition at the Sherpa website: http://www.sherpa.ac.uk/romeo/). For any other electronic use, please contact Springer at (permissions.dordrecht@springer.com or permissions.heidelberg@springer.com).

The material can only be used for the purpose of defending your thesis, and with a maximum of 100 extra copies in paper.

Although Springer holds copyright to the material and is entitled to negotiate on rights, this license is only valid, subject to a courtesy information to the author (address is given with the article/chapter) and provided it concerns original material which does not carry references to other sources (if material in question appears with credit to another source, authorization from that source is required as well).

Permission free of charge on this occasion does not prejudice any rights we might have to charge for reproduction of our copyrighted material in the future.

Altering/Modifying Material: Not Permitted
You may not alter or modify the material in any manner. Abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of the author(s) and/or Springer Science + Business Media. (Please contact Springer at (permissions.dordrecht@springer.com or permissions.heidelberg@springer.com)

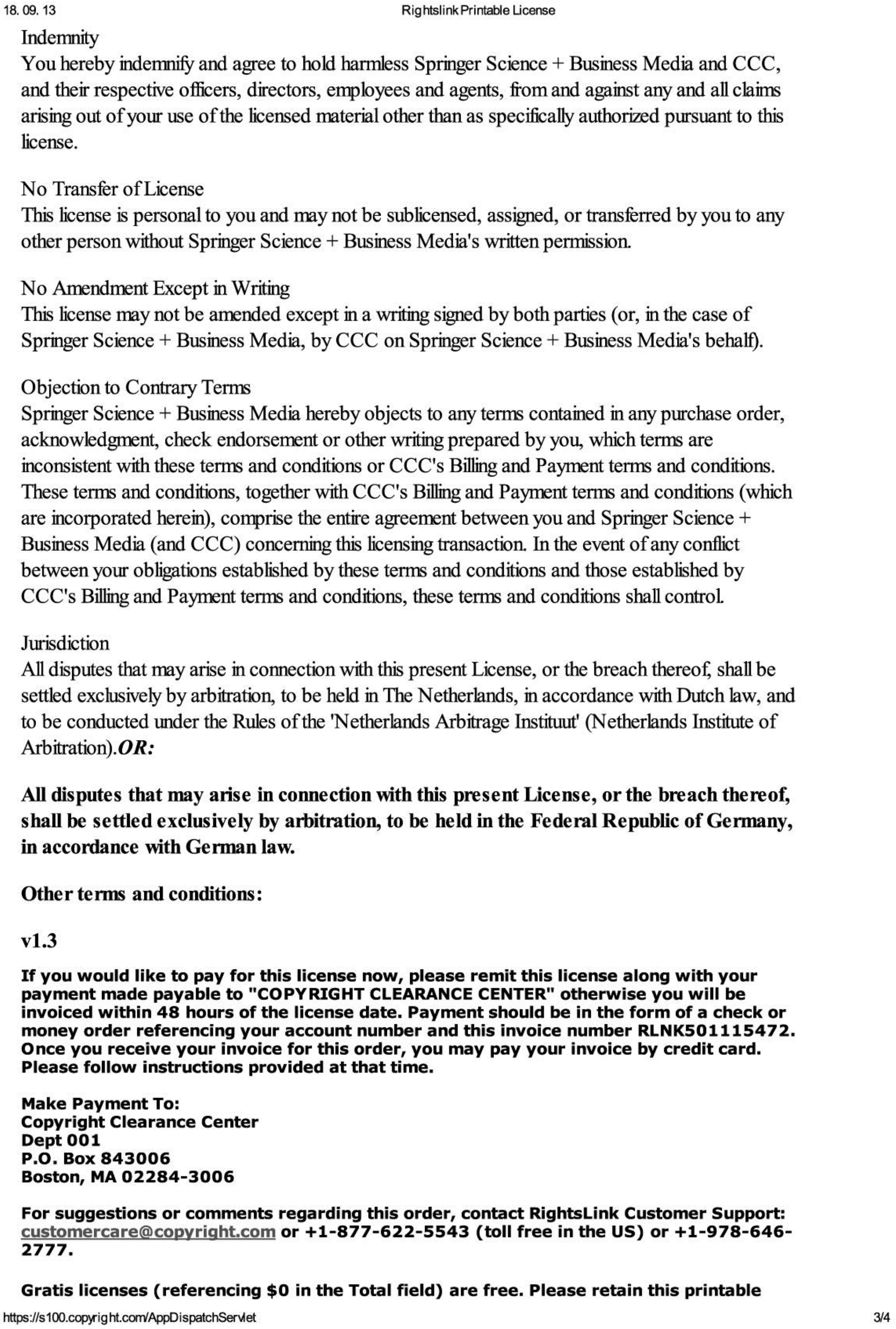
Reservation of Rights
Springer Science + Business Media reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

Copyright Notice:Disclaimer
You must include the following copyright and permission notice in connection with any reproduction of the licensed material: "Springer and the original publisher /journal title, volume, year of publication, page, chapter/article title, name(s) of author(s), figure number(s), original copyright notice) is given to the publication in which the material was originally published, by adding; with kind permission from Springer Science and Business Media"

Warranties: None

Example 1: Springer Science + Business Media makes no representations or warranties with respect to the licensed material.

Example 2: Springer Science + Business Media makes no representations or warranties with respect to the licensed material and adopts on its own behalf the limitations and disclaimers established by CCC on its behalf in its Billing and Payment terms and conditions for this licensing transaction.



18. 09. 13 Rightslink Printable License

Indemnity
You hereby indemnify and agree to hold harmless Springer Science + Business Media and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

No Transfer of License
This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without Springer Science + Business Media's written permission.

No Amendment Except in Writing
This license may not be amended except in a writing signed by both parties (or, in the case of Springer Science + Business Media, by CCC on Springer Science + Business Media's behalf).

Objection to Contrary Terms
Springer Science + Business Media hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and Springer Science + Business Media (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

Jurisdiction
All disputes that may arise in connection with this present License, or the breach thereof, shall be settled exclusively by arbitration, to be held in The Netherlands, in accordance with Dutch law, and to be conducted under the Rules of the 'Netherlands Arbitrage Instituut' (Netherlands Institute of Arbitration).***OR:***

All disputes that may arise in connection with this present License, or the breach thereof, shall be settled exclusively by arbitration, to be held in the Federal Republic of Germany, in accordance with German law.

Other terms and conditions:

v1.3

If you would like to pay for this license now, please remit this license along with your payment made payable to "COPYRIGHT CLEARANCE CENTER" otherwise you will be invoiced within 48 hours of the license date. Payment should be in the form of a check or money order referencing your account number and this invoice number RLNK501115472. Once you receive your invoice for this order, you may pay your invoice by credit card. Please follow instructions provided at that time.

**Make Payment To:
Copyright Clearance Center
Dept 001
P.O. Box 843006
Boston, MA 02284-3006**

For suggestions or comments regarding this order, contact RightsLink Customer Support: customercare@copyright.com or +1-877-622-5543 (toll free in the US) or +1-978-646-2777.

Gratis licenses (referencing $0 in the Total field) are free. Please retain this printable

https://s100.copyright.com/AppDispatchServlet 3/4