

UNIVERZA V LJUBLJANI
BIOTEHNIŠKA FAKULTETA

Ren-Chung Cheng

**EVOLUTIONARY PATTERNS AND PROCESSES
RESULTING IN EXTREME SEXUAL DIMORPHISM IN
THE SPIDER GROUP ARGIOPINAE**

DOCTORAL DISSERTATION

Ljubljana, 2016

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ZA EKSTREMNI SPOLNI DIMORFIZEM PRI
PAJKIH SKUPINE ARGIOPINAE**

DOKTORSKA DISERTACIJA

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This doctoral dissertation work is a completion of Interdisciplinary Doctoral Programme in Biosciences. The work was conducted at Jovan Hadži institute of Biology, Scientific Research Centre of the Slovenian Academy of Sciences and Arts, Slovenia.

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AI Predstavljam prvo primerjalno študijo spolnega velikostnega dimorfizma (SSD) pri pajkih skupine Argiopinae s prvo vrstno molekulske filogenijo poddružine. V disertaciji podajam štiri zaključene raziskave. V prvi podajam primerjalno analizo, ki na molekulske filogenije argiopinov preverja usmerjenost in alometrične vzorce SSD. Splošna hipoteza SSD v korist samic predvideva, da selekcija za večjo plodnost samic sproži povečevanje velikosti samic, drugačni selekcijski pritiski, ki so povezani z iskanjem partnerjev, pa ohranjajo majhne velikosti samcev. Moji rezultati vzorcev neusmerjene in izometrične evolucije SSD ne podpirajo splošne hipoteze evolucije SSD pri tej skupini organizmov. V drugi študiji sem raziskoval razmerja med SSD in spolnim dimorfizmom oblik/morfologij (SShD) v skupini Argiopinae, za katere se pogosto domneva, da korelirata. Čeprav sem pokazal na njuno povezavo na nivoju družin vseh pajkov mrežarjev, ta korelacija ni veljala za Argiopinae, kjer je bila evolucija velikosti samic filogenetsko omejena ter delno povezana z geografsko zgodovino skupine, ne pa s selekcijo plodnosti. V tretji raziskavi sem preučeval potencialne geografske dejavnike, ki bi lahko vplivali na intraspecifične vzorce SSD pri ameriški vrsti *Argiope argentata*. Rezultati kažejo na spolno specifični vzorec velikostne variacije, kjer se velikost samic manjša z geografsko širino ter z distribucijo populacij preko kontinenta oziroma otokov, velikost samcev pa je neodvisna od geografskih dejavnikov. Zaključil sem, da spolno specifični odgovori na geografske dejavnike lahko pojasnijo dele prepoznane intraspecifične variacije SSD. V četrti študiji sem na nivoju poddružine raziskoval, ali in kako je evolucija velikosti genitalij povezana s telesno velikostjo pri obeh spolih. V novejši literaturi so prikazani negativno alometrični vzorci evolucije velikosti genitalij pri obeh spolih, kar kaže na primat spolne selekcije z mehanizmom kriptične izbire samic. Čeprav bi pričakovali podobne vzorce tudi na interspecifičnem nivoju, v svojih rezultatih nisem našel alometričnih vzorcev evolucije genitalij v skupini Argiopinae, taki nepričakovani rezultati pa niso v podporo glavni vlogi spolne selekcije v evoluciji genitalne velikosti. Moji rezultati kažejo tudi, da spolni velikostni dimorfizem genitalij ter SSD ne korelirata. Iz vseh rezultatov zaključujem, da komponente spolnega dimorfizma niso nujno med seboj povezane ter da potrebujemo nove primerjalne študije vzrokov in vzdrževanja spolnega dimorfizma pri pajkih.

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AB We included four research works in this dissertation. In study 1, we performed comparative analyses on a molecular phylogeny to explore directionality and allometric patterns of sexual size dimorphism (SSD) in argiopine spiders. The general hypothesis of female biased SSD posits that fecundity selection drives female size increases and that mate-searching related selection pressures maintain small male sizes. By demonstrating patterns of non-directional and isometric evolution of SSD, my results provide no support for this general hypothesis of SSD evolution in this particular clade. In study 2, we explored the relationships between SSD and sexual shape dimorphism (SShD) in argiopines, which are often assumed to be correlated. While we demonstrated their relatedness at the family level in all orbweb spiders, this correlation did not hold true in argiopines, where the evolution of female body shapes was phylogenetically constrained and partially related to geographic history rather than fecundity. In study 3, we explored potential geographic factors that may affect intraspecific patterns of SSD in the American *Argiope argentata*. We found a sex-specific pattern of size variation, with female body size decreasing with latitude and with distribution over mainland versus islands, but male size not relating to any geographical factor. I concluded that sex-specific body size responses to geographical factors may explain parts of the detected intraspecific variation in SSD. In study 4, we explored over the subfamily scale if and how genital size evolution relates to somatic size in both sexes. Recent work at the intraspecific levels has demonstrated negative allometric patterns of genital size evolution in both sexes, implying the primacy of sexual selection via cryptic female choice. Although similar patterns would be predicted also at the interspecific levels, we detected no allometric patterns of genital size evolution in argiopines, and this surprising result fails to support sexual selection as the driver of male and female genital size evolution. In addition, I found that sexual genital size dimorphism and SSD were not related. From all our results, we conclude that different components of sexual dimorphism are not necessarily related and that there is a need for research to investigate the correlation between sexual dimorphism and geographical or ecological factors.

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GLOSSARY

SSD	Sexual size dimorphism
SShD	Sexual shape dimorphism
SGD	Sexual genital size dimorphism
CO1/ <i>cox1</i>	Cytochrome c oxidase subunit I
CO2/ <i>cox2</i>	Cytochrome c oxidase subunit II
K2P	Kimura 2-parameter
BL	Total body length
TPL	first leg tibia-patella length
CW	Carapace width
CL	Carapace length
AbdL	Abdominal length
OTU	molecular operational taxonomic units
GMYPs	the General Mixed Yule-Coalescent model with single threshold
PTP	the Poisson tree processes model
BM	Brownian motion model
Trend	Brownian motion with a directional trend
OU	single optimum Ornstein-Uhlenbeck model

1 INTRODUCTION

1.1 SEXUAL DIMORPHISM IN SPIDERS

Most organisms are sexually dimorphic, i.e. they exhibit phenotypic differences in morphology, physiology, and behavior between the sexes within a species. How these sexual differences evolve has attracted attention since before the time of Darwin. Darwin was of the opinion that most sexually dimorphic traits arise through sexual selection, their evolution increasing mating success, but potentially reducing their survival rate (Darwin, 1871). Although Darwin's ideas have stood the test of time, some cases of sexual dimorphism seem to be best explained by natural selection (Fairbairn et al., 2007). Nonetheless, the developing view is that the degree of sexual dimorphism in a species is the result of the difference between the sums of different evolutionary pressures affecting the males and the females.

Spiders resemble many other animals by exhibiting a wide range of intersexual differences in morphology, color, size, shape and other characteristics. For instance, many jumping spiders (family Salticidae) have bright colors and complex decoration, and show sexual color dimorphism in which males are generally brighter than females (Huber, 2005). The sexually dimorphic coloration may have evolved by sexual selection (Oxford and Gillespie, 1998), in which female jumping spiders prefer the prominent males, which are those with brighter coloration (Li et al., 2008; Lim et al., 2008). This preference likely relates to salticid spider extraordinary visual abilities, i.e. high resolution and color vision (Forster, 1982). Sexual ornamental dimorphism is also known in wolf spiders (family Lycosidae), a group of spiders with fairly good visual resolution, but limited color perception. However, sexual color dimorphism is also widespread in some families without advanced vision (Land, 1985), such as crab spiders (family Thomisidae). In these spiders, males and females may live in different microhabitats and acquire different adaptive colors (Oxford and Gillespie, 1998). In many orb weavers, such as *Nephila* (family Nephilidae) and *Argiope* (family Araneidae), females show conspicuous color patterns, but males are monotonous. Again, such difference between the sexes is likely due to different life strategies. While females are sit-and-wait predators throughout their lives and thus benefit

from remarkable coloration that functions as luring signals to attract prey (Cheng and Tso, 2007; Tso et al., 2004), roving males with short foraging periods remain inconspicuous to avoid the possibility of predatory attacks.

Among all animal morphological traits, body size is the most obvious and the most extensively studied character. Spiders may be considered as a model animal group for studying the origin and maintenance of sexual size dimorphism (SSD) since they exhibit a wide range of SSD values, from moderately male-biased to extremely female-biased (Head, 1995; Vollrath and Parker, 1992). Among all terrestrial animals, spiders exhibit the most extreme cases of SSD with females being up to 10 times larger and 125 times heavier than the males (Elgar, 1991; Kuntner et al., 2012). Extreme SSD, in which the body length of female is more than twice as that of male (Elgar, 1991; Hormiga et al., 2000), is taxonomically limited to Nephilidae, Araneidae, Tetragnathidae, Theridiidae and Thomisidae. Extreme SSD has evolved several times independently in orb-weavers (Coddington et al., 1997; Hormiga et al., 2000), and has been shown to arise either solely through female gigantism or both female gigantism and male dwarfism processes simultaneously.

Recent studies on the extremely sexual size dimorphic spiders, family Nephilidae, demonstrated a gradual evolutionary SSD increase due to significant female size increases (Kuntner and Coddington, 2009; Kuntner and Elgar, 2014). However, although this pattern is consistent with the conventional arachnological literature, SSD does not always increase in all sexual size dimorphic clades and most lineages remain sexually size monomorphic. Therefore, to understand the occurrence and maintenance of SSD, more comparative and experimental studies are needed.

In the recent decades, different hypotheses have been proposed to explain the evolution and maintenance of SSD. 'Fecundity selection', which predicts selection for larger females due to their higher reproductive output, has been well supported by experimental research and is thus strongly favored as the best explanation for the evolution of extreme SSD, especially for those due to female size increases (Head 1995; Prenter et al. 1999). An alternative hypothesis, named here 'predatory selection', also invokes selection for larger

females, and posits that big spiders have lower predatory risks from their main predators (Higgins, 2002; Hormiga et al., 2000). Cheng and Tso (2007) showed that, in *Argiope aemula*, medium-sized spiders were more easily attacked by wasps than large ones. Apart from this, the evidence for this hypothesis is weak. It is important to note that both hypotheses that focus on the females, relate to natural, not sexual selection.

Other hypotheses explain the small male advantage, and except for one, are also linked with natural, not sexual selection. The ‘gravity hypothesis’ posits that small males have an advantage in scramble competition by reaching females faster while climbing (Brandt and Andrade, 2007; Moya-Laraño et al., 2002; Moya-Laraño et al., 2007a; Moya-Laraño et al., 2009). The ‘differential mortality model’ posits that higher male mortality rates should select for early-maturation at small male size (Prenter et al., 1997; Vollrath and Parker, 1992). As the only hypothesis that invokes sexual selection, ‘sexual cannibalism’ predicts that small males have an advantage because they are nimbler and faster, and thereby more easily evade female attacks (Elgar, 1992). On the whole, the female biased SSD in spiders is usually interpreted as the sum of selection for large females and for small males. However, the relative importance of these selection pressures is unknown, and contentious (Kuntner and Elgar, 2014).

Spiders are found worldwide in all terrestrial habitats. However, compared to the SSD literature that focused on the effects of fecundity and/or sexual selection, very few studies have considered whether and how geographical factors, such as latitude, elevation, and island size, affect body size. The mean body size of European spider assemblages was consistent with converse Bergmann’s rule, where body size was negatively correlated with latitude (Entling et al., 2010). In the wolf spider *Pardosa lapponica*, body size decreased with the increase of altitude and sexual size differences only persisted in coastal populations, and not at higher elevations (Bowden et al., 2013). A previous study furthermore found a positive correlation between variation in female size and latitude in nephilid spiders (Higgins et al., 2011). However, any relationships between body size and SSD variation on the one hand, and geographical variables including island biogeography on the other, are unknown.

In spiders and most other animals, females have similar shaped, but bigger abdomens than males, which is likely due to fecundity selection (Bonnet et al., 2010). However, orb weaving spiders also exhibit considerable sexual dimorphism in body shape, with female abdominal shapes, ranging from egg shape and cylindrical shape to leaf- or twig-mimicry, being much more diverse than those of males (Elgar and Jebb, 1999; Pekár, 2014). The phenomenon of sexual shape dimorphism (SShD) has received surprisingly limited attention, and it remains unclear whether and how its evolution relates to SSD.

The araneid subfamily Argiopinae (Simon, 1890) including three genera *Argiope*, *Gea* and *Neogea* has approximately 100 species. Argiopine spiders are known for the strong size and color dimorphism and their spectacular mating behaviors, such as sexual cannibalism and mate plugging as well as male self-sacrifice (Miller, 2007; Uhl et al., 2010). Male argiopine males only mate once or twice in their lifetime. These males commonly lodge a piece of pedipalp in the female genital opening as plug to avoid the female remating with other males (Uhl et al., 2007). The efficiency of plugs limits the copulating numbers of females, enforcing low female mating rates. Their specific mating systems lend themselves for studying the evolution of sexual behaviors and sexual selection mechanisms, e.g. cryptic female choice, and sexual conflict (Elgar and Schneider, 2004; Schneider, 2014). Therefore, Argiopinae is a good model system to test the existing hypotheses of evolution of sexually related traits, and this may include sexual dimorphism.

This dissertation is a broad investigation into the sexual dimorphic argiopine spiders. In it, We address several major aspects of the evolution of sexual dimorphism. First, we take a phylogenetic approach to examining the evolutionary patterns of sexual dimorphism in body size, body shape and genital size. we then test if the current hypotheses of sexual dimorphism can explain the evolutionary patterns detected in Argiopinae. Finally, we investigate the correlation of sexual genital size dimorphism, and of SShD with SSD.

1.2 PHYLOGENY AND TAXONOMY OF ARGIOPINAE

Due to large body size and strikingly colored female abdomens, species of the genus *Argiope* Audouin, 1827 are among the most instantly recognized spiders, and among the

earliest ones described (Pallas, 1772; Scopoli, 1772). The subfamily Argiopinae occurs worldwide and is represented by three genera and 99 valid species (World Spider Catalog, 2015). Simon (1895) erected the subfamily “Argiopea” with two genera, *Argiope* and *Gea*. C. L. Koch, 1843. Levi (1983) revised the Western Pacific species and added the genus *Neogea* Levi, 1983 into the group. Scharff and Coddington (1997) carried out a cladistic analysis of Araneidae using morphological characters and provided preliminary support for the monophyly of the three genera.

The taxonomy of argiopine spiders has been extensively studied: Western Pacific species were revised by Levi (1983), American species by Levi (2004), African *Argiope* species by Bjørn (1997), Chinese species by Yin et al. (1997), Indian species by Sebastian & Peter (2009), Philippine species by Barrion & Litsinger (1995), and the revision on global *Argiope* species by Jäger (2012). After a long history of more than two centuries on argiopines descriptions, the first phylogenetic relationship using the molecular data was published recently on few *Argiope* species focusing on the evolution of web decoration (Cheng et al., 2010). However, the latter included only 21 *Argiope* species and two mitochondrial genes. For the phylogenetic comparative analyses, a solid phylogeny including more species, especially *Gea* and *Neogea* is needed. Therefore, the first necessary step of our study was to investigate the phylogenetic relationship of Argiopinae. In this dissertation, two different phylogenies of argiopine spiders were provided, one phylogeny using four molecular markers was presented in the study 1, and the other phylogeny including more taxa with six molecular markers was presented in the study 4.

1.3 SEXUAL DIMORPHISM IN ARGIOPINE SPIDERS

In Argiopinae, females and males differ not only in appearance but also in behaviors. The abdomens of females have diverse shapes and contain bright spots of silver or gold coloration, and commonly show paired conspicuous ventral paraxial lines (Levi, 1983). Female argiopines usually decorate their webs by an extra silk structure on web, called decoration or stabilimentum, in species-specific linear or x-shape (Cheng et al., 2010). Compared to females, male argiopines are small, monotonous in color, and their webs lack silk decorations.

The argiopine spiders exhibit much interspecific variation in body size and SSD (female size: 4,4 – 28,5 mm, male size: 2,4 – 7,4 mm, SSD: Female / male ratio: 1,36 - 5,67). The remarkable size variation among argiopine species provides a convenient case to test the general explanation of female-biased SSD in spiders, that the female body size is affected by fecundity selection but an intermix of sexual and natural selection pressures influence male body size evolution. In other words, different evolutionary pressures drive size evolution in each of the sexes. In the study 1, we test if the general explanation of female-biased SSD can explain SSD evolution of argiopine spiders by reconstructing the size and SSD changes, by comparing the evolutionary signals and by detecting the allometry pattern.

Spider body consists of the anterior prosoma and the posterior opisthosoma. The prosoma contains carapace, eyes, chelicerae with fangs, palps and legs. In contrast, the opisthosoma, the spider's abdomen, includes most of its visceral organs, the gonads and silk glands (Foelix, 2011). In argiopines, the female's abdominal shape is morphologically very diverse, being either egg-shaped (*Gea*, *Neogea* and *A. aurantia*), long cylindrical (*Argiope ocula*), pentagonal (*A. aetherea* and *A. appensa*) or lobed (*A. lobata* and *A. blanda*) (Bjørn, 1997; Levi, 1983; Levi, 2004). The abdominal shape of the male, on the other hand, is more conserved being either egg-shaped or long cylindrical. No research has been conducted on the evolution of different morphotypes and the biological meaning of abdominal shape diversity. One possibility is that fecundity selection not only affects female body size but also abdominal shape. Spiders may increase total abdomen volume via altering the abdomen shape (i.e. lobed structure appearance) to respond to fecundity selection. In chapter 3, we first examine the evolutionary pattern of sexual shape dimorphism. Then we test whether the different morphotypes have different fecundity by comparing the abdominal weight difference among them.

Since argiopine spiders are good dispersers, many species exhibit wide ranges, some being broadly distributed over the tropical and temperate areas. For example, *A. aemula* can be found north from Japan and south to Australia, *A. trifaciata* shows global distribution, *A. bruennichi* inhabits the Palaearctics, *A. lobata* the Old World and *A. argentata* extends

over the Americas (Levi, 1983; Levi, 2004). These widespread species are appropriate models to examine the influence of geographical factors on body size and SSD. In chapter 4, we test whether the converse Bergmann's rule and the island rule can explain the intra-specific size and SSD variation in *Argiope argentata*.

Since male argiopines practice genital breakage and plugging, the relative size of the male pedipalp compared with the female epigynum must be adaptive, and may be sexually selected. In argiopines, the SSD ratios may be driven largely by natural selection, and hence it is interesting to know if and how the evolution of genital size and degrees of SSD are related. In chapter 5, we test if evolution of the genital size was driven by sexual selection by comparing the allometry slope of somatic and genital size. In addition, we focus on the correlation between genital size of both sexes and between the genital size dimorphism and SSD.

Four approaches were made to comprehensively understand the evolution of sexual dimorphism in argiopines: (1) we tested if the general explanation of female-biased SSD can explain the evolution of SSD in argiopines using several comparative analyses; (2) we examined the evolution of sexual shape dimorphism and tested if sexual shape dimorphism was driven by fecundity selection. In addition, we tested the correlation between SSD and SShD in two hierarchical levels: Argiopinae and Araneoidea; (3) we investigated the effect of geographical factors on size and SSD variation; (4) we used comparative analyses to test whether the genital size evolution is under sexual selection and if sexual genital size dimorphism is associated with sexual size dimorphism. The four approaches used in this study are presented in four research chapters, and their implications are summarized in a general discussion.

2 LITERATURE REVIEW

2.1 STUDY 1: NON-DIRECTIONAL AND ISOMETRIC EVOLUTION OF SEXUAL SIZE DIMORPHISM IN ARGIOPINE SPIDERS

Ever since Darwin (1871), evolutionary biologists have attempted to find general explanations for sexual dimorphism, a phenomenon describing substantial phenotypic differences between males and females within a species. The sexual differences may encompass morphology, size, coloration, behavior, chemical pathways, as well as any other intrinsic organismal features. Similar to other animals, discussions of sexual dimorphism in spiders almost exclusively revolve around their size. Spiders are the terrestrial group with the most extremely female-biased SSD; females are up to 10 times larger or 125 times heavier than males (Kuntner et al., 2012; Robinson and Robinson, 1976). Some of sexually dimorphic clades are becoming model organisms for studying the evolution and maintenance of SSD, since they exhibit whole ranges of SSD from moderately male-biased to extremely female-biased (Foellmer and Moya-Laraño, 2007).

Highly sexually size dimorphic spider clades often exhibit peculiar sexual behaviors or mating strategies, which have been proposed to arise as sexually selected adaptations to extreme SSD. For example, studies showed that the occurrences of sexual cannibalism and male genital emasculation were phylogenetically positively correlated with extreme female biased SSD (Kuntner et al., 2015; Miller, 2007; Wilder and Rypstra, 2008; Wilder et al., 2009). In some highly size dimorphic genera (*Nephila*, *Herennia* and *Caerostris*), males perform mate binding (Robinson and Robinson, 1980) by winding fine silk around their mates before copulation in order to reduce the aggressiveness of giant females (Zhang et al., 2011). The courtship location in orb-weaving spiders has also been suggested to relate with degrees of SSD (Elgar, 1991) with highly dimorphic spiders mating at the hub of their webs, and less dimorphic taxa constructing a mating thread. To understand the evolution and maintenance of these unusual behaviors and their relationship with SSD, more focused and integrated phylogenetic, comparative and experimental studies are needed.

In spiders, extreme SSD (females more than twice the male size) is taxonomically mostly confined to a handful of web-building lineages (Nephilidae, Araneidae, Tetragnathidae and Theridiidae) and, among cursorial lineages, to crab spiders (Thomisidae). Previous studies, using genus-level morphological phylogenies (Coddington et al., 1997; Hormiga et al., 2000), concluded that extreme SSD in orb-weaving spiders arose through female evolutionary increase (a.k.a. female gigantism) or both female increase and male decrease (female gigantism and male dwarfism combined). It is generally believed that fecundity selection, favoring large females, represents the major evolutionary force shaping extreme SSD in orb-web spiders (Head, 1995; Prenter et al., 1999). Fecundity selection, however, cannot explain why male size does not follow the females' evolutionary size increase, and thus other selection pressures seem to affect males (Blanckenhorn, 2000). Hypotheses related to mate searching have been proposed as explanations for why male size does not increase along with females. Gravity hypothesis proposes that small size is selected for in the searching sex (males) due to better climbing ability (Moya-Laraño et al., 2002; Moya-Laraño et al., 2007b). Moya-Laraño et al. (2009) discovered a curvilinear pattern of SSD in high-habitat spiders, thus demonstrating a decoupled evolution of male and female size, and estimated an optimal size (about 43 mg or 7.4 mm) for climbing on vertical surfaces. Although the gravity hypothesis has been supported by results of Brandt and Andrade (2007) as well as Foellmer and Fairbairn (2005), other research has contradicted its predictions (Foellmer and Fairbairn, 2005; Prenter et al., 1995). Therefore, the general validity of the gravity hypothesis remains questionable. The differential mortality model (Vollrath and Parker, 1992), on the other hand, predicted that higher male mortality rates should select for early maturation of males at small sizes. Morse (2007), however, has failed to confirm this model in a size dimorphic crab spider *Misumena*. The remaining explanations favoring small male advantage, such as sexual cannibalism (Elgar, 1991) and protandry (Danielson-François et al., 2012; Elwood and Prenter, 2013), likewise remain only weakly supported or are directly disputed.

Since different taxa may show different processes leading to the evolution of extreme SSD (Hormiga et al., 2000), our understanding of SSD evolution would benefit from more comparative research. Most studies related to extreme SSD have been conducted either with selected single species or across families (Coddington et al., 1997; Higgins, 2002;

Hormiga et al., 2000), while studies that investigate the evolution and maintenance of extreme SSD within a certain lineage are arguably better at detecting clade-wide patterns. As I show below, however, only one spider clade has been thoroughly researched for phylogenetic patterns resulting in extreme SSD, which has precluded any generalizations across spiders. This dissertation adds a second such lineage for a species-level comparison.

Kuntner and Coddington (2009) examined the evolution of body size in nephilid spiders based on a species level phylogeny generated from morphological and behavioral characters (Kuntner et al., 2008), and tested the coevolution between female and male size. They found that the sizes in both sexes were phylogenetically independent, that ancestral sizes of both sexes were small, that female size steadily increased through evolution, but that male sizes showed no clear pattern of change. Their study reinforced the view that extreme SSD in nephilids was explained by female gigantism, not male dwarfism (Coddington et al. 1997). A new study on an updated, molecular phylogeny (Kuntner et al. 2013) only changed the evolutionary picture in the ancestral sizes being moderate, and male evolutionary size showing a slight increase (Kuntner and Elgar, 2014). Since no other detailed species level, clade-wide comparisons exist, female size increase and decoupled evolution in gender sizes are currently the two strongest emerging patterns in orb-web spiders.

In this dissertation, we focus on another conspicuously dimorphic spider clade, the araneid subfamily Argiopinae (Levi, 1983), a global lineage of orb weaving spiders that contains three genera and 99 species showing a range of sizes and SSD, and includes the commonly encountered wasp-, garden-, and cross-spiders (Fig. 5 inset). We devised an original molecular phylogeny to reconstruct size and SSD evolution, to test potential direction of size changes, and to test male and female correlated size evolution. We also examined the validity of different models of size evolution for each sex and allometry in SSD evolution, to establish if size related traits are under significant selection, and to test Rensch's rule, an allometric law predicting that SSD increases with body size when males are the larger sex, and decreases with body size when females are the larger sex. Following the above conventional knowledge, we predicted that low SSD level or even size monomorphism would be ancestral in Argiopinae, and that SSD would increase gradually through the

phylogeny. We predicted that female size would increase but that male size would be phylogenetically independent and remain at small, ancestral values. Finally, We predicted to find negative allometry for Argiopininae SSD in support of the converse pattern of Rensch's rule (Abouheif and Fairbairn, 1997; Fairbairn, 1997).

2.2 STUDY 2: DISENTANGLING THE *SIZE* AND *SHAPE* COMPONENTS OF SEXUAL DIMORPHISM

Sexual dimorphism is among the most conspicuous phenotypic qualities of those organisms that reproduce sexually, arising as a logical result of the interactions between natural and sexual selection. While in most animal groups, male and female phenotypes differ in a variety of size-related, morphological and functional traits, most evolutionary studies on sexual dimorphism almost exclusively focus on sexual *size* dimorphism (Blanckenhorn, 2000; Fairbairn, 1997; Vollrath and Parker, 1992), thereby neglecting sexual *shape* dimorphism (SShD).

Similar to size evolution, the evolution of body shape may also be driven by different evolutionary pressures on each sex. Common hypotheses explaining sex-specific evolution in body size and shapes relate to female fecundity, intraspecific niche divergence and to sexual selection (Badyaev and Martin, 2000; Butler and Losos, 2002; Greenberg and Olsen, 2010; Head, 1995; Olsson et al., 2002; Shine, 1989). These varying selection pressures are expected to be reflected in sex-specific phenotypes. In birds, for example, beak shapes and sizes have been shown to evolve in response to intersexual foraging differences (Price, 1984; Shine, 1989; Temeles et al., 2010). Male-male competition, on the other hand, is responsible for the occurrence of exaggerated secondary sexual characters, such as horn-like outgrowths on head or thorax in many male beetles (Andersson, 1994; Eberhard, 1982; Emlen et al., 2005). However, it is the female abdomen in both vertebrates and invertebrates that is particularly prone to evolutionary changes since it contains reproductive organs. In turtles, fecundity selects for relatively larger internal abdominal volumes in females to provide space for carrying more eggs, whereas sexual selection drives males towards larger shell openings, thereby facilitating mobility (Bonnet et al., 2010; Bonnet et al., 2001; Kaddour et al., 2008). Lizards are another

example of sexual shape dimorphism, where fecundity favors longer female trunks for holding eggs, whereas males develop greater head size used in male-male combat (Cox et al., 2003; Olsson et al., 2002; Scharf and Meiri, 2013). Looking at interspecific differences, ecological factors, e.g. habitat type, also play a role in directing body shape evolution. Aquatic turtles, for example, have flatter and more streamlined shells compared with terrestrial ones (Bonnet et al., 2010), and rock-dwelling lizards exhibit relatively flatter heads and bodies, adaptations that enhance locomotion (Goodman et al., 2009; Revell et al., 2007). These examples of vertebrate SShD, however, are not as extreme as in many invertebrates, yet invertebrates are rarely subject of comparative SShD study (Gidaszewski et al., 2009).

Among invertebrates, spiders provide a good opportunity to disentangle the shape and size components that together contribute to their extreme sexual dimorphism for several reasons. First, compared to other animals, spiders show extreme abdominal shape differences between males and females, especially in orb weavers (Fig. 1a-b). This difference may relate to sex-specific life histories; orb web spider males have shorter life spans and they cease web building after reaching maturity. In contrast to males, longer lived females face more challenges such as higher predation risk due to long exposure on the web, as well as higher energetic demands related to development and reproduction. One may logically expect female spiders to respond to such sex-specific challenges through adaptive morphological changes that contribute to sexual shape, as well as size dimorphism. Spiders offer numerous such examples (Fig. 1), their abdominal morphological modifications presumably enhancing female survival, e.g. through leaf- or twig-mimicry (*Polys*, *Deinopis*, *Arachnura*) or spinal adornments (*Micrathena*, *Gasteracantha*, *Polys* and others) (Elgar and Jebb, 1999; Pekár, 2014). These highly modified female spider morphologies may be accompanied by extreme sexual size dimorphism, but comparative research has never tested for a direct connection between SSD and SShD. Second, as in other invertebrates, fecundity selection is expected to be the most prominent selective force promoting female gigantism (Head, 1995), but since a large percentage of spiders are web dwellers, gravity may constitute a critical counterforce. Considering all these factors that may contribute to sexual size and shape dimorphism, I

expected that shape and size components of spider sexual dimorphism may be only weakly related, or not related.

In the orb web spider subfamily Argiopinae, the sexes differ not only in body size (Cheng and Kuntner, 2014) but also in abdomen shape with females exhibiting greater diversity. The female abdomens may be oval (*Gea*, *Neogea* and some *Argiope*), long and cylindrical (*Argiope ocula*), pentagonal (*A. aetherea* and *A. appensa*) or lobed (*A. lobata* and *A. blanda*) (Bjørn, 1997; Levi, 1983; Levi, 2004). The male abdominal shape, on the other hand, is more conserved being either oval or cylindrical. The evolution of different morphotypes in argiopines and their biological significance remain unknown. Since the spider's abdomen includes reproductive organs (Foelix, 2011), the female abdominal shape may influence their fecundity via the changes of total abdomen volume, thus the abdominal shape evolution would be as important to females as their body size.

Here, we examined argiopine intra-specific sexual shape dimorphism and inter-specific abdominal morphotypes, reconstructed the evolution of abdominal shape in both sexes using a recent species level phylogeny (Cheng and Kuntner, 2014), investigated the relationship between body size and different morphotypes and tested the correlation between sexual size and shape dimorphism. We also investigated whether female abdominal morphotypes and sizes reflect differences in abdominal weight, used as a proxy for abdomen volume (that, in turn, indicative of fecundity). Because SShD is a common phenomenon in other orb-weaving clades, especially those known for significant size dimorphism, we further explored the correlation of SShD and SSD at higher hierarchical levels, i.e. at the genus level in all araneoid spiders (Bond et al., 2014). These analyses combined may help us disentangle the shape and size components of sexual dimorphism, and inform us on their association.



Figure 1. **Spolni dimorfizem v korist samic ter raznolikost somatskih morfologij pajkov mrežarjev.** Female biased sexual dimorphism and diversity of orb weaving spider somatic morphologies: a-b, sexual size and shape dimorphism in *Argiope pulchella* (a) and *Herennia etruscilla* (b); c-d, sexual size but not shape dimorphism in *Nephila pilipes* (c) and *Argiope bruennichi* (d); e, female araneids with modified abdominal shapes. All images by M. Kuntner (2015).

2.3 STUDY 3: GEOGRAPHICAL FACTORS PREDICT FEMALE SIZE VARIATION IN A SEXUALLY DIMORPHIC SPIDER

How morphological variables are affected by environment is one of the fundamental and most studied topics in biology. Body size is among the most conspicuous organismal traits since it is easy to quantify and at the same time represents a salient trait that affects individual's physiology and fitness. An organism's body size could be constrained by evolutionary history of its lineage (Cope's rule), or could be affected more directly by geographical factors (Bergmann's and island rule) and development history (Bergmann, 1847; Blackburn and Gaston, 1994; Foster, 1964; Rensch, 1948). Our study focuses on the importance of geographical factors shaping intraspecific variation in body size.

Body size of endothermic vertebrates tends to increase from low to high latitude (Bergmann, 1847; Mayr, 1963), a pattern termed Bergmann's rule. Bergmann's rule was first formulated for endothermic vertebrates, but similar trends have been documented for numerous ectothermic taxa (Partridge and Coyne, 1997; Ray, 1960), including invertebrates (Chown and Gaston, 2010; Shelomi, 2012). However, the opposite tendency, i.e. converse Bergmann's rule, predicting size decrease with latitude, is also frequently observed in animals. Although Bergmann's rule was originally defined between closely related species, it may also predict variation between populations within a single species (Blackburn et al., 1999; Meiri, 2011; Shelomi, 2012). More recently, the rule has been broadened to encompass size trends according to temperature changes, and thus may apply to environmental latitude or altitude gradients (Meiri, 2011).

Insular populations and their continental relatives commonly show body size differences consistent with Foster's, also known as the island rule (Foster, 1964; Van Valen, 1973), a general tendency for small individuals or species to evolve larger island sizes, and conversely, for large founders to give rise to smaller descendants. Such tendencies has been documented in mammals, birds and reptiles (Clegg and Owens, 2002; Lomolino, 1985; Lomolino et al., 2006; Meiri et al., 2006), much less so in invertebrates with only preliminary reports of island gigantism (Hormiga, 2002; Hormiga et al., 2003; Wesener and VandenSpiegel, 2009). As an extension, the island rule has also been applied to body

size changes among populations on different sized islands, where body size decreases or increases relate to island size (Heaney, 1978).

Orb weaving spiders (Orbiculariae) exhibit the most extreme sexual size dimorphism (SSD) among terrestrial animals (Elgar, 1991; Fairbairn et al., 2007; Kuntner et al., 2012) and are becoming a model animal group for studying size evolution emphasizing the origin and maintenance of SSD. Extreme SSD, in which females are at least twice the body length of males, evolved several times independently in orb weaving spiders through evolutionary trajectories termed female gigantism or female gigantism combined with male dwarfism (Coddington et al., 1997; Hormiga et al., 2000). Fecundity selection favoring large females (Head, 1995; Prenter et al., 1999), and selection for small males as explained by the gravity hypothesis (Moya-Laraño et al., 2002; Moya-Laraño et al., 2009), the sexual cannibalism hypothesis (Elgar, 1991), and the differential mortality model (Vollrath and Parker, 1992), have all been hypothesized as selective regimes favoring extreme SSD. Recent comparative studies on the extremely sexually size dimorphic spiders of the family Nephilidae have broadly confirmed these predictions by demonstrating an evolutionary increase in SSD and female size, and to a lesser degree, also male size (Kuntner and Coddington, 2009; Kuntner and Elgar, 2014). However, in another female-biased size dimorphic spider lineage, Argiopinae, comparative work found no clear trends in evolutionary size changes in either sex, and a pattern of interspecific SSD evolution consistent with Brownian motion (Cheng and Kuntner, 2014). In argiopine spiders, a clade estimated to be 40 million years old, current evidence therefore cannot confirm the validity of general explanations of the origin and maintenance of SSD. It is possible that in this particular clade intraspecific patterns of size variation contribute to SSD, among which ecological and/or geographical factors may be at work (Cheng and Kuntner, 2015).

When one sex expresses stronger response to geographical factors than the other, levels of sexual size dimorphism change. For example, in a meta-analysis of latitudinal variation in SSD of 98 animal species, Blanckenhorn et al. (2006) found that males showed stronger size responses to latitude than females in two-thirds of species. In a seed-feeding beetle, *Stator limbatus*, populations at lower latitudes were smaller but more dimorphic due to a steeper latitudinal cline in body size cline (Stillwell et al., 2007). In the wolf spider

Pardosa lapponica, body size decreased with both altitude and latitude, with notably size dimorphic populations being confined to low altitudes (Bowden et al., 2013). However, contrary to predictions from the converse Bergmann's rule, a global study on nephilid spiders found a positive correlation between variation in female size and latitude (Higgins et al., 2011). These studies underline the difficulty of predicting latitude and altitude related size patterns in sexually dimorphic invertebrates. Moreover, the relationships between size and SSD variation with other geographical variables, such as island versus continental range, or island size factors, have so far not been considered.

Here, we aim to fill this gap by investigating intraspecific body size variation and potential geographical factors affecting it, in an extremely sexually size dimorphic argiopine spider that ranges widely across the Caribbean islands and the American mainland. First, we used molecular data for species delimitation in *Argiope argentata*, in order to establish intraspecific population ranges, and thus units for comparison. We then examined latitudinal and altitudinal body size variation in each sex, and tested if these patterns conform to Bergmann's rule or its converse. Finally, we tested the validity of the island rule by comparing the sizes of continental and insular populations. With all evidence combined, I discuss the effects of geographical factors on spider SSD evolution.

2.4 STUDY 4: INDEPENDENT EVOLUTION OF GENITAL AND SEXUAL SIZE DIMORPHISM

Male genitalia of animals with internal fertilization are among the most complex and divergent morphological structures (Eberhard, 1985) and several hypotheses have been proposed to explain their evolution. Although mechanisms unrelated to sexual selection, such as the lock-and-key and the pleiotropy hypothesis, may plausibly explain genital diversity (Dufour, 1844; Mayr, 1963), there is increasing support for the possibility that sexual selection acts as the primary force driving the evolution of genitalia (Eberhard, 2009; Hosken and Stockley, 2004; Rowe and Arnqvist, 2012; Simmons, 2014). Four main sexual selection mechanisms have been proposed for genital evolution: Fisherian selection, good gene, sperm competition and sexual conflict (also called sexually antagonistic

coevolution); Among these hypotheses, the first two presume that male genitalia are driven by cryptic female choice (Hosken and Stockley, 2004).

Examining allometric patterns of animal genitalia relative to somatic features provides a way of understanding the role of sexual selection in genital size evolution and may potentially identify the roles of differing selective pressures. In both vertebrates and some invertebrates, many sexually selected structures often exhibit positive allometry, and relatively high variation, both as outcomes of directional sexual selection (Green, 1992; Kodric-Brown et al., 2006; Pomiankowski and Moller, 1995; Simmons and Tomkins, 1996). In insects and spiders, however, genital size evolution tends to be negatively allometric (Arnqvist, 1998; Eberhard, 2009; Eberhard et al., 1998). One explanation for such negative allometric pattern of genital size evolution is that those males with genitalia that fit average-sized female genitalia are favored, a hypothesis known as “one size fits all” (Eberhard et al., 1998).

Despite an increasing intensity of research on male genitalia in recent years, the lack of studies of female genital properties has seriously hampered our understanding of genital evolution (Ah-King et al., 2014). In effect, although the general consensus is that sexual selection is responsible for male genital evolution (see above), its effect on female genital morphology is poorly understood. In insects and spiders, female genitalia size have been shown to exhibit patterns comparable with males, that is low allometric slopes, and their evolutionary trends are thought to importantly influence the evolution of male size genitals (Eberhard, 2009). Although the exact mechanism is still unclear, sexual selection may thus play an important role also in female genital evolution. In addition, while comparative analyses have demonstrated the evolution of genitalia between sexes to be correlated (Arnqvist and Rowe, 2002; Kuntner et al., 2009; Rönn et al., 2007; Sota and Tanabe, 2010), it is difficult to speculate whether it is the female or the male genital features that drive these coevolutionary patterns. Several studies on spiders have shown that male and female genitalia exhibit low allometric values at intra-specific levels (Eberhard, 2009; Eberhard et al., 1998; Uhl and Vollrath, 2000), however, inter-specific patterns remain unknown. Therefore, phylogenetically based comparative work is needed to begin to understand the evolution of male and female genitalia as well as their coevolution.

Another question that has not been adequately addressed by prior research is if and how genital size variation relates with sexually dimorphic somatic features. As shown in our previous chapters, some orb-weaving spiders exhibit extreme female-biased sexual size dimorphism (SSD) with females on average up to 10 times larger and 125 times heavier than the males (Elgar, 1991; Kuntner et al., 2012). It is generally thought that such female-biased extreme SSD results from female and male size evolution responding to differing selection pressures, although this hypothesis may not hold true in some lineages (Cheng and Kuntner, 2014; Kuntner et al., 2013). Considering that most morphological features positively correlate with body size, genital size evolution in animals with extreme SSD may likewise be restricted by body size evolution.

Ramos et al. (2005) allowed for three different evolutionary scenarios (Fig. 2) of how sexual genital dimorphism (SGD) may relate to female-biased SSD: 1) SGD increases with increasing SSD; this is the **positive genital divergence model** that predicts that female genital size changes faster than male genital size when SSD gradually increases. 2) SGD remains constant as SSD increases; this is the **constant genital dimorphism model** that predicts that female and male genital sizes change at the same rates as SSD gradually increases. 3) SGD decreases as SSD increases; this is the **negative genital divergence model** predicting a slower female genital size increase compared with male genital size when SSD gradually increases, a pattern that has never been observed.

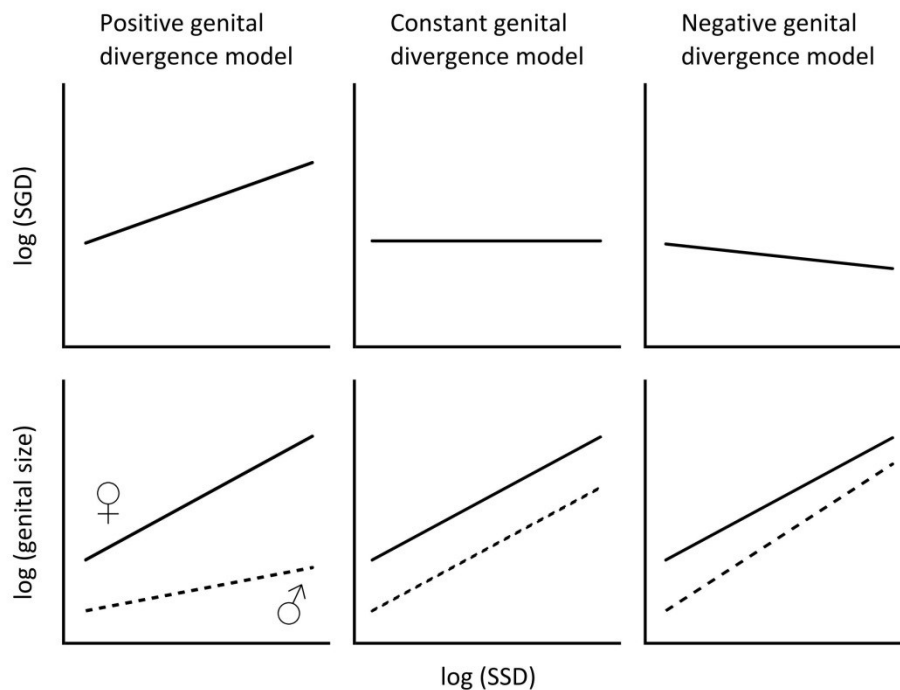


Figure 2. **Trije potencialni scenariji povezanosti SGD ter SSD v korist samic.** The three potential scenarios of how SGD may relate to female-biased SSD: the positive genital divergence model, the constant genital dimorphism model and the negative genital divergence model. The upper figures are the expected relationships of SGD to SSD, the lower figures are the predicted patterns of female (solid line) and male (dashed line) genital size on SSD.

In study 4, we study how genital size evolution in argiopine spiders, known for varying degrees of SSD, relates to the evolution of sex-specific body sizes. We first examined the allometric patterns of body and genital sizes in both sexes at the inter-specific level, building on the updated argiopine phylogeny. Specifically, we tested for negative allometric patterns of genital versus body size in each sex, which would strongly point towards the role of sexual selection in the evolution of genitalia. We were also interested in whether female external and internal genital sizes were any different in allometric patterns. We then analyzed whether the sexes show any coevolutionary patterns in both genital and body size. Finally, we looked for support for any of the proposed models on how SGD and SSD relate.

3 MATERIAL AND METHODS

3.1 STUDY 1: PHYLOGENY SUGGESTS NON-DIRECTIONAL AND ISOMETRIC EVOLUTION OF SEXUAL SIZE DIMORPHISM IN ARGIOPINE SPIDERS

3.1.1 Taxon sampling

Argiopinae spiders are distributed worldwide in diverse habitats, and exhibit different mating behaviors (Levi, 1983; Robinson and Robinson, 1980). Although their biology is relatively well-studied, their phylogenetic relationships remain unclear. In this part of dissertation, we included as ingroups 47 species—approximately 50 % of described Argiopinae diversity—from all three genera (42 *Argiope*, 4 *Gea* and 1 *Neogea*), selected sixteen outgroups based on the only comprehensive araneid genus-level phylogeny (Scharff and Coddington, 1997) (*Cyrtophora*, *Arachnura*, *Witica*, *Araneus*, *Caerostris*, *Cyclosa*, *Gasteracantha*, and *Zygiella*), and rooted the trees with *Nephila* from the family Nephilidae, which is sister to Araneidae (Kuntner et al., 2013).

3.1.2 Molecular procedures

Genomic DNA was extracted from one leg of each specimen using MagMAXTM DNA Multi-sample kit (Applied Biosystems, Foster City, CA, USA). Two mitochondrial, cytochrome oxidase I (*cox1*) and cytochrome oxidase II (*cox2*) and two nuclear genes (the protein coding histone 3 subunit A (H3) and the ribosomal 28S rRNA (28S)) were used for phylogenetic analyses. Partial sequences of *cox1* were amplified using several primer combinations: the forward “LCO-1490” (5'-GGTCAACAAATCATAAAGATATTGG-3') with the reverse “HCO-2198” (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994) or the reverse “Chelicerate-R2” (5'-GGATGGCCAAAAATCAAATAAATG -3') (Barrett and Hebert, 2005), and the unpublished forward “Tom” (5'-CAACATTTATTTTGATTTTTTGG-3') with the reverse “Maggie” (5'-GGATAATCAGAATATCGTCGAGG-3') (Hedin and Maddison, 2001). Partial sequences of *cox2* were amplified using the primer combination COII-FWD (5'-CCTCGACGATATTCTGATTATC-3') and “C2-N-3661r”

(5'-CCACCAATTTTCAGAACATTGACC-3') (Simon et al., 1994). Partial sequences of H3 were amplified by two primer combinations (Colgan et al., 1998): the forward "H3aF1" (5'-ATGGCTCGTACCAAGCAGACVGC-3') with the reverse "H3aR1" (5'-ATATCCTTRGGCATRATRGTGAC-3'), and the forward primer "H3aF2" (5'-ATGGCTCGGTACCAAGCAGAC-3') with the reverse "H3aR2" (5'-ATRTCCTTGGGCATGATTGTTAC-3'). Finally, partial sequences of 28S were amplified using the forward primer "28S-B1f" (5'-GACCGATAGCAAACAAGTACCG-3') with the reverse "28S-B2r" (5'-GATTAGTCTTTCGCCCTATA-3') (Bruvo-Madarić et al., 2005).

To amplify *cox1*, *cox2* and H3, two PCR protocols were used. In the first protocol, one cycle of DNA denaturation at 94 °C for 2 min was followed by 35 cycles of sequence amplification (DNA denaturation at 94 °C for 50 s, primer annealing at 46 or 48 °C to for 40 s, and sequence extension at 72 °C for 50 s), with a final extension period of 72 °C for 5 min. Unsuccessful samples were processed by a touch-up protocol: one cycle of DNA denaturation at 94 °C for 2 min followed by 20 cycles of touch-up sequence amplification (DNA denaturation at 94 °C for 50 s, primer annealing at 46 to 52 °C to for 1 min, and sequence extension at 72 °C for 50 s), followed by 15 cycles of sequence amplification (DNA denaturation at 94 °C for 50 s, primer annealing at 52 °C to for 1 min, and sequence extension at 72 °C for 50 s) and a final extension period of 72 °C for 7 min. To amplify 28S, the cycling profile of PCR amplifications began with one cycle of DNA denaturation at 94 °C for 2 min followed by 35 cycles of sequence amplification (DNA denaturation at 94 °C for 30 s, primer annealing at 50 °C to for 40 s, and sequence extension at 72 °C for 30 s), and a final extension period of 72 °C for 5 min.

The PCR products were assayed on 1 % ethidium bromide staining agarose gel and photographed under a UV light. Purifying and sequencing was done at Macrogen Inc. (Amsterdam, Netherlands). DNA sequences and their chromatograms were assembled, manually edited and checked for internal stop codons using Geneious Pro v5.6.6 (Biomatters, USA).

3.1.3 Phylogenetic analyses

Multiple alignments of *cox1*, *cox2* and H3 were implemented by ClustalW (Thompson et al., 1994) in Geneious v.5.6.6 with gap opening/gap extension penalty ratio (24 / 8), and sequences of 28S were aligned using the online version of MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/>) with Q-INS-I strategy and other default settings. Alignments were concatenated in Mesquite v.2.75 (Maddison and Maddison 2011). The nucleotide substitution models of each gene were evaluated using jModelTest v.2.1.3 (Posada, 2008) with 24 models tested. The best-fit model for each gene was selected by Akaike Information Criterion (AIC) and used in all phylogenetic analyses.

Using the concatenated matrix, we performed maximum likelihood (ML) analyses in GARLI v.2.0 (Zwickl 2006) and Bayesian inference (BI) analyses in MrBayes v.3.2.1 (Ronquist et al., 2012). For ML analyses, the best-fit substitution model (selected by jModelTest) for each gene was assigned by setting the ratematrix, nucleotide equilibrium state frequencies, alpha shape parameter, and proportion of invariable sites. A ML bootstrapping was run in GARLI with 2 search replicates and 100 bootstrap replicates. Bayesian analysis was performed on the concatenated data with four partitions, each with the best-fit model. Two independent runs, each with four Markov chain Monte Carlo (MCMC) chains, were performed simultaneously with random starting trees, and the MCMC process was run for 10.000.000 generations, with a sampling frequency of 100 and a burn-in of the first 25% generations. The standard deviation of split frequencies between two runs (< 0,01) and the effective sample size were confirmed in TRACER v.1.5 (Rambaut and Drummond, 2007).

An ultrametric phylogeny with branch lengths based on clock-like assumption, needed for comparative analyses, was constructed in BEAST v1.8.0 (Drummond et al., 2012). In the BEAST analysis, we limited the outgroup selection to only include the closest relative, *Cyrtophora*. Four partitions according to gene with the best-fit substitution model (selected by jModelTest) were analyzed. In BEAST we constrained the monophyly of *Cyrtophora* and the Argiopininae, given the BI and ML analyses on the complete taxon selection. The time-calibrated node leading to Argiopininae was set a normal distribution (mean = 30, SD =

5, ranging from 21,78 to 38,22 Mya) (Dimitrov et al., 2012; Kuntner et al., 2013). The uncorrelated lognormal relaxed clock model with fixed estimated substitution rate (mean = 0,0112, SD = 0,001) (Bidegaray-Batista and Arnedo, 2011; Kuntner et al., 2013) was used in *coxI* partition and the strict model with uniform prior distribution was used in other partitions. We used the Yule speciation process (Gernhard, 2008) option in BEAST. The chain length was set to 50.000.000 with tree parameters sampled every 10.000 generations. The burn-in was set to 25 %, and thus 3751 trees were used to infer the consensus tree of the BEAST analysis.

3.1.4 Ancestral size reconstruction

To obtain size data, we collected argiopine specimens through field work, or obtained them via museum loans and international collaborators. I measured the total body length of 442 specimens of 51 species, and used size dimorphism index [SDI: (female body length / male body length) - 1] (Lovich and Gibbons 1992) to quantify SSD (Annex A). In the cases of species with no available specimens for measuring, the data sources were marked in Annex A. We reconstructed ancestral states of continuous characters (male size, female size and SDI) on the best Bayesian tree pruned for most outgroups (not *Cyrtophora*) using squared change parsimony in Mesquite v.2.75. Although other character reconstructions based on maximum likelihood and Bayesian inference are available, our aims were to understand the evolutionary changes of characters rather than the probability of particular ancestral states on the phylogeny.

3.1.5 Phylogenetic comparative analyses

To examine the evolutionary tendency of size for each sex, and of SSD, we tested size evolutionary patterns against three evolutionary models: Brownian motion (BM), Brownian motion with a directional trend (Trend) and single optimum Ornstein-Uhlenbeck (OU). Under the BM model, the trait evolutionary changes are regarded as outcomes of genetic drift or selection randomly fluctuating through time (Felsenstein, 1973). The Trend model has a Brownian motion-like dynamic with a linear trend in rate through time (Hunt, 2006). Under the OU model, the trait evolution is selected towards an optimal value

(Butler and King, 2004; Hansen, 1997). If male and female sizes are under selection (either to be maintained or to increase, as predicted), the Trend or OU model will be chosen and the Brownian motion rejected. In contrast, no specific evolutionary tendency is postulated if the BM model best fits the data. Specifically, we expected male size to be maintained at optimal values, and hence to fit the OU model, while females would likely show a trend towards larger sizes and would thus fit the Trend model. I tested these models using the function “fitContinuous” in the R package “Geiger” (Harmon et al., 2008). Since the program does not allow using taxa with missing values, we implemented two schemes differing in our handling of missing data. First, the taxa with missing values were pruned from the phylogeny. Second, the ancestral value was used for each missing taxon, an approach assuming parsimonious change. Likelihood ratio test helped us select the best fit models.

To investigate the coevolution between female and male size, we performed phylogenetically independent contrasts analyses (Felsenstein, 1985) using the PDAP:PDTREE module (Garland Jr et al., 1999) in Mesquite v.2.75 on both Bayesian and BEAST topologies. In addition, we used major-axis regression (Model II regression) to test for allometry of male and female body sizes; for this test, we \log_{10} -transformed the size data since both variables were expressed in the same physical units and were expected to have similar measurement errors (Fairbairn, 1997). The analyses were conducted in R environment using the package “lmodel2” (Legendre 2014) for both species tip data (Annex A) and phylogenetically corrected data (the independent contrasts dataset from PDAP). Statistical significance of the allometric pattern was determined based on the 95% confidence intervals of the slope of the model (β); isometry is implied when the slope $\beta = 1$ falls within the confidence interval.

3.2 DISENTANGLING THE *SIZE* AND *SHAPE* COMPONENTS OF SEXUAL DIMORPHISM

3.2.1 Species level analyses: Argiopinae

The araneid spider subfamily Argiopinae is one of the most remarkable lineages of orb-weavers that has been widely used in diverse research, e.g. of silk biology (Albertson et al., 2014; Blamires et al., 2012; Tso, 2004; Zhang et al., 2013b), sexual selection (Elgar et al., 1996; Fromhage, 2012; Herberstein et al., 2012; Nessler et al., 2009; Welke and Schneider, 2012), visual ecology and signal evolution (Bruce et al., 2005; Cheng and Tso, 2007; Cheng et al., 2010; Rao et al., 2009; Walter and Elgar, 2012), and climatic change (Kumschick et al., 2011). This lineage consists of 99 described (ca. 83 in the genus *Argiope*, 13 *Gea* and 3 *Neogea*) and these have been shown to differ dramatically in their sizes and in SSD (Cheng and Kuntner, 2014). In study 2, we examined 656 specimens of 47 species from all three genera for sizes and shapes (Annex B).

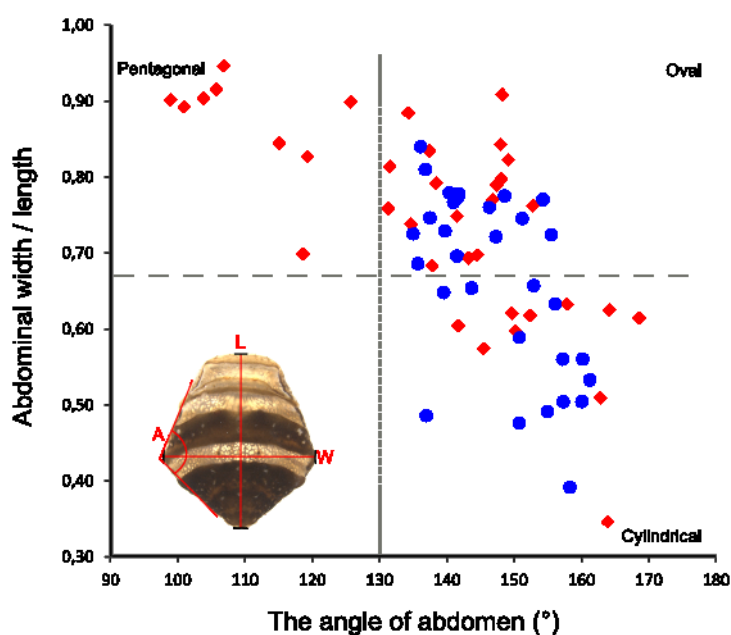


Figure 3. **Parametri uporabljeni pri kvatifikaciji morfotipov zadkov.** Measurement data to delimit three abdominal morphotypes (cylindrical, oval and pentagonal shape; for lobed shape, see Fig. 9b). Data points are species averages, blue circles are male values, red diamonds are female values.

To categorize abdominal shapes, we measured the angle of abdomen and abdominal length as well as abdominal width (Fig. 3) and counted the number of tubercles, i.e. protruding, lobed or pyramid-shaped abdominal structures (Fig. 9b). All the size measurements were

performed using Leica Application Suite software (Leica, Bannockburn, IL) and the angles were measured using Measure v2.0.1 (C-Thing, Mountain View, CA). We categorized abdominal shapes of male and female argiopinines into four morphotypes (Fig. 3; Fig. 9b), defined as follows: (i) Oval, the angle of abdomen was larger than 130° and abdominal width/length $\geq 0,67$; (ii) Cylindrical, the angle of abdomen was larger than 130° and abdominal width/length $< 0,67$; (iii) Lobed, the abdomen had 5 to 9 tubercles; (iv) Pentagonal, the angle of abdomen was less than 130° . Studies often use multivariate and morphometric analysis to quantify sexual shape dimorphism in fish (Brooks, 1991; Spoljaric and Reimchen, 2008), reptiles (Kaliontzopoulou et al., 2007; Kaliontzopoulou et al., 2008; Valenzuela et al., 2004) and invertebrates (Gidaszewski et al., 2009). However, morphometrics may be more useful in taxa with less clear morphotype boundaries than shown in our four categories (Fig. 3), while our simpler approach clearly categorized species- and sex-specific shape variation typical of argiopinines (Fig. 3; Fig. 9b).

The best Bayesian inference tree based on 4 molecular markers (from study 1) was used as reference phylogeny in the comparative methods. To examine the evolution of abdominal shape, we reconstructed ancestral states of male and female morphotypes using the Mk1 maximum likelihood method in Mesquite v.3.0 (Maddison and Maddison, 2014).

We used four parameters as indexes of body size: total body length, first leg tibia-patella length (TPL), carapace width and abdominal length. The understanding of phylogenetic signal is a key prerequisite for studies of character evolution (Cubo et al., 2005). To measure the strength of phylogenetic signal in size data, we determined Pagel's λ (Pagel, 1997; Pagel, 1999) using the function "fitContinuous" in R package "Geiger" (Harmon et al., 2008), and Blomberg's K (Blomberg et al., 2003) using the function "phylosignal" in R package "picante" (Kembel et al., 2010). We tested whether these two indices of phylogenetic signal that were estimated from the observed data were significantly different from values estimated from the null hypothesis (without phylogenetic signal). We also tested the phylogenetic signal of abdominal shape (as discrete character) by estimating Pagel's λ using the function "fitDiscrete" in R package "geiger". Since most size data showed phylogenetic signal (Table 1), we performed phylogenetic analysis of variance (Garland et al., 1993) using the function "phylANOVA" in R package "phytools" (Revell,

2011) to compare body sizes among different morphotypes. We then performed a generalized estimating equations (Paradis and Claude, 2002) to test the correlation of sexual size (as a continuous trait) and shape dimorphism (as a categorical trait) using the function “compar.gee” in R package “ape” (Paradis et al., 2004). In all comparative analyses, the phylogenetic trees were trimmed to include only those taxa for which both size index and shape data were available.

Table 1. **Filogenetski signal kontinuiranih podatkov (meritve velikosti) ter njihova/njegova primerjava z ničelno hipotezo.** Phylogenetic signal of the continuous characters (size measurements) and comparison with null hypothesis. The Pagel’s λ here refers to the signal in the residuals from the generalized linear model, and not individual traits. The λ value of null hypothesis was derived from $\lambda = 0$ and the K value of null hypotheses were obtained from 1.000 simulations of tip shuffling randomization. Abbreviations: BL= Total body length, TPL= Tibia-patella length of the first leg, CW= Carapace width, AbdL= Abdominal length.

	Pagel's λ		Blomberg's K	
Female				
BL	0,96	$P < 0,001$	0,65	$P < 0,001$
TPL	0,86	$P = 0,015$	0,47	$P = 0,003$
CW	0,79	$P = 0,006$	0,49	$P < 0,001$
AbdL	0,87	$P < 0,001$	0,63	$P < 0,001$
Male				
BL	0,92	$P < 0,001$	0,58	$P = 0,002$
TPL	0,74	$P = 0,035$	0,40	$P = 0,151$
CW	1,00	$P = 0,137$	0,34	$P = 0,614$
AbdL	0,87	$P < 0,001$	0,55	$P < 0,001$

Invertebrate female abdominal volume positively correlates with fecundity (Preziosi et al., 1996), and thus an examination of abdominal volume differences between female morphotypes would be highly informative. However, abdominal volume in argiopines is difficult to estimate given their irregular shapes. As a proxy for abdominal volume I thus measured abdominal weight in females of all four morphotypes. I performed an analysis of covariance (ANCOVA), with abdominal weight as dependent variable, abdominal shape as independent variable and abdominal length as covariance. Since the original data violated the assumption of the general linear model, the linearity and homogeneity, the cube root of abdominal weight was used in the analysis in R.

3.2.2 Genus level analyses: Araneoidea

Compared with the four easily scored argiopine morphotypes, morphological diversity at higher phylogenetic levels precludes such precise quantification, and we therefore had to simplify the shape dimorphism scores. We scored araneoid spider shape dimorphism from the taxonomic literature as a simple presence/absence character (Annex C) and obtained SSD indices from recent SSD studies (Cheng and Kuntner, 2014; Kuntner et al., 2015). We followed a recent study of emasculation evolution (Kuntner et al., 2015) in devising a genus level phylogeny from Dimitrov et al. (2012), but we modified the familial relationships according to the newest phylogenomic topology (Bond et al., 2014). We also added two additional genera known for SShD, *Arachnura* as sister to Argiopine + Cyrtophorinae (Cheng and Kuntner, 2014) and *Poltys* as sister to *Araneus* + *Neoscona* (Gregorič et al., 2015). Because this reference araneoid phylogeny was patched from several sources, it lacked branch length information and we therefore transformed them to equal 1. Shape dimorphic genera are confined to the families Araneidae and Nephilidae, while size dimorphic ones appear throughout Araneoidea. To investigate the correlation between SShD and SSD, We therefore performed generalized estimating equations analyses using the function “compar.gee” in R package “ape” with two different datasets: at the less inclusive level we analyzed araneids and nephilids (28 genera) and at the more inclusive level we used all representatives of Araneoidea (120 genera) (Annex C).

3.3 GEOGRAPHICAL FACTORS PREDICT FEMALE SIZE VARIATION IN A SEXUALLY DIMORPHIC SPIDER

3.3.1 Specimen sampling

We studied the relation of gender specific body size with geographical factors in the Neotropical silver argiope, *Argiope argentata* (Fabricius, 1775), a common and widespread spider (Fig. 12) with a conspicuous size variation within and between sexes (Levi, 2004). Specimens derive from the collections of the Smithsonian Institution’s National Museum of Natural History and from the specimens collected by the CARBIO team (the Caribbean island biogeography project).

3.3.2 Species delimitation

Although biology of *A. argentata* is relatively well-known, the species limits of this species widespread over Americas have not been rigorously tested with molecular data. In order to establish which populations can be considered as conspecific, and thus merit intraspecific comparisons, I first tested *A. argentata* species limits by using DNA barcodes (standard region of *cox1*) (Hebert et al., 2003) obtained from Caribbean and mainland American samples, and from African argiopines that represented outgroups. DNA extraction and sequencing followed established protocols for *Argiope* (Cheng and Kuntner, 2014). Sequence alignment was implemented by ClustalW in Geneious (Biomatters, Auckland, New Zealand). The appropriate substitution model was selected by jModelTest version 2.1.4 (Posada, 2008) with 24 models tested. The GTR+I+G models was selected by Akaike information criterion (AIC) and used in all phylogenetic analyses. We employed a Bayesian approach to phylogenetic reconstruction implemented in MrBayes 3.1.2 (Ronquist et al., 2012). Two independent runs, each with four Markov chain Monte Carlo (MCMC) chains, were performed simultaneously with random starting trees, and the MCMC process was run for 10.000.000 generations, with a sampling frequency of 100 and a burn-in of the first 25% generations. We also reconstructed an ultrametric tree in BEAST 1.8.0 (Drummond et al., 2012). The BEAST run comprised 50.000.000 generations, sampled every 1000 generations, using a lognormal relaxed clock with fixed estimated substitution rate (mean = 0,0112, SD = 0,001)(Bidegaray-Batista and Arnedo, 2011), assuming a birth-death speciation model for the tree prior, with the best fit substitution models, and default options for all other prior and operator settings. To ensure convergence and correct effective sample size (ESS), two chains were run (one for 40.000.000 and one for 10.000.000) then combined using LogCombiner v1.8.0. The final consensus tree was produced in TreeAnnotator v1.8.0, with 25 % burn-in.

To test for cryptic species in *A. argentata*, we used a combination of tree-based species delimited methods and the DNA barcode gap. We first explored the molecular operational taxonomic units (OTUs), and then created a final set of OTUs by their genetic

distance. In addition, we calculated the genetic distance of OTUs using Kimura 2-parameter (K2P) (Kimura, 1980) in Mega 6.06 (Tamura et al., 2013).

Two phylogenetic species delimitation approaches were used: the General Mixed Yule-Coalescent model with single threshold (GMYCs) (Pons et al., 2006), and the Poisson tree processes (PTP) model (Zhang et al., 2013a), both requiring a previously generated phylogenetic hypothesis to delimitate species boundaries. The GMYC model infers species boundaries on time calibrated phylogenies by detecting the threshold between the species-level (i.e., speciation and extinction) and the population-level (i.e., coalescence). We used the GMYC with single threshold because recent literature (Brewer et al., 2012; Kekkonen and Hebert, 2014) favors it over the multiple thresholds approach. On the contrary, PTP does not require an ultrametric tree; it models speciation by inferring substitutions from branch lengths assuming Poisson distribution (Zhang et al., 2013a). The single threshold GMYC model was applied to the BEAST tree in R 3.0.3 (R Development Core Team, 2014) with the Splits package (<http://splits.r-forge.r-project.org/>). The PTP analysis based on the Bayesian tree was performed on the bPTP web server (<http://species.h-its.org/ptp/>) with 500.000 MCMC generations, with a thinning value of 100 and a burn-in of 10%. Finally, we also tested species limits through DNA barcoding gap analysis, showed in recent literature to be effective in spiders (Čandek and Kuntner, 2014).

3.3.3 Size and geographic comparison

We measured the carapace width of all females and males, as prior research has suggested it as appropriate index of body size (Foellmer and Moya-Laraño, 2007). To explore the correlation between body size and geographical factors, in other word to test the Bergmann's and island rules, we first tested whether spider body size related with altitude (m above sea level) and latitude (absolute degrees), as well as distribution (mainland versus islands) in all females and males, then tested these relationships in the island and mainland populations, respectively. Multiple linear regression analyses with stepwise backwards elimination were used, and in all analyses, body size and island size were log-transformed. Since multiple regression models were evaluated by examining the

distribution of residuals, we tested for normality of residuals using Shapiro–Wilk test. All tests were conducted using R 3.0.3 (R Development Core Team, 2014) in RStudio (R Studio, 2014).

3.4 INDEPENDENT EVOLUTION OF GENITAL AND SEXUAL SIZE DIMORPHISM

3.4.1 Molecular phylogeny

In order to infer on the phylogenetic relationships of argiopines, we added two molecular markers (16S and 18S) and 11 species (9 *Argiope*, 1 *Gea* and 1 *Neogea*, see Annex D.) to the existing argiopine phylogeny (Cheng and Kuntner, 2014). In this study, a total of fifty-eight ingroup species—approximately 60% of described Argiopinae diversity—from all three genera (51 *Argiope*, 5 *Gea* and 2 *Neogea*) and thirty-two outgroups (*Cyrtophora*, *Mecynogea*, *Manogea*, *Arachnura*, *Witica*, *Araneus*, *Caerostris*, *Cyclosa*, *Gasteracantha*, and *Zygiella*) were included.

The DNA extraction and PCR reactions of COI, COII, 28S and H3 followed established protocols for argiopine spiders (Cheng and Kuntner, 2014). Partial sequences of 16S were amplified using the primer combination 16Sa (5'- CGCCTGTTTACCAAAAACAT -3') and 16Sb (5'- ATGATCATCCAATTGAT -3'). Partial sequences of 18S were amplified using the primer combination 18Sa (5'- ATTAAAGTTGTTGCGGTTA -3') and 18Sb (5'- GAGTCTCGTTCGTTATCGGA -3'). The amplification of 16S and 18S also followed the argiopine PCR protocols for other genes. Sequencing was done in China. The sequences of COI and COII as well as H3 were aligned automatically using ClustalW in Geneious Pro v5.6.6 (Biomatters, Auckland, New Zealand) with default gap opening/gap extension penalty ratio (24 / 3). For 16S, 18S, and 28S sequences, multiple alignments were done online in Mafft v. 7 (<http://mafft.cbrc.jp/alignment/server/>) using the Q-INS-i iterative refinement method and default settings. The appropriate substitution model of each gene was selected by jModelTest version 2.1.4 (Posada, 2008) with 24 models tested. We employed a Bayesian approach to phylogenetic reconstruction implemented in MrBayes 3.1.2 (Ronquist et al., 2012) using the combined sequence data matrix. The combined data

matrix was partitioned and the best-fit model of each gene was assigned (SYM+I+G for 18S and GTR+I+G for others). Two independent runs, each with four Markov chain Monte Carlo (MCMC) chains, were performed simultaneously with random starting trees, and the MCMC process was run for 10.000.000 generations, with a sampling frequency of 1000 and a burn-in of the first 25 % generations. Since the consensus tree was not bifurcated, the best Bayesian tree pruned for all outgroups was used in all comparative analyses.

3.4.2 Measurement of somatic and genital characters

We examined genital and body morphology of 774 individuals of 58 argiopininae species (Annex D). We recorded four somatic characters: total body length, the tibia-patella length of first leg, carapace width and carapace length. Among these somatic characters, we used carapace width as the indicator of body size. For genital characters, male pedipalp volume and female spermathecal and epigynal volume were calculated from size measurements (Fig. 4). The male genital characters were measured from the ventral and anterior view of either the right or left pedipalp. For the female genital characters, we removed and cleared the epigynum with 10 % KOH (15 mins at 55 °C), and measured from the interior, dorsal and ventral view. All the measurements were taken using the software LAS v. 2.5.0 (Leica Application Suite). The genital characters were regarded as ellipsoids, which facilitated calculation of the ellipsoid volume: $V = \frac{4}{3}\pi abc$.



Figure 4. **Meritve genitalnih znakov.** The measurements of genital characters. In both sexes, the genital characters were regarded as ellipsoid. In males, the pedipalp volume was the combination of genital bulb and embolus plus conductor, which means the pedipalp volume = $(4\pi/3) \cdot \text{Palp_D} \cdot \text{Palp_W} \cdot \text{Palp_H} + (4\pi/3) \cdot \text{Embolic_H} \cdot \text{Embolic_W} \cdot \text{Embolic_L}$. The female internal genital character, spermathecal volume = $(4\pi/3) \cdot \text{Sp_D} \cdot \text{Sp_W} \cdot \text{Sp_L}$; the female external genital character, epigynal volume = $(4\pi/3) \cdot \text{Ep_D} \cdot \text{Ep_W} \cdot \text{Ep_L}$.

3.4.3 Allometric pattern

To examine the allometric patterns, we used standardized major-axis (Model II) regression with log₁₀ transformed data using the R package “smatr” (Warton et al., 2012), since there is no cause-effect relationship between two observations (Sokal and Rohlf, 1995). We used both raw measurement and volume data for each species (Tips) and phylogenetically corrected data (the phylogenetically independent contrasts (PICs)). The PICs data were estimated using the PDAP:PDTREE module (Garland Jr et al., 1999) in Mesquite version 2.75 on a tree with exponentially transformed branch lengths due to the fact that contrasts of some somatic and genital characters were not normally distributed on the best BI tree.

To test whether genital size evolution is under sexual selection, we first analyzed the allometric patterns of somatic and genital characters on the indicator of body size (carapace width) in both sexes. To convert both somatic (in mm) and genital (in mm³)

variables to the same biological scale, the female epigynum and spermatheca as well as male pedipalp volume were cube-root transformed. We then tested if the genital allometric slope values were significantly different from the somatic character allometric slopes. The slope comparisons were done using the function “slope.com” in R package “smatr”. In addition, we tested if female internal and external genital structure showed comparable evolutionary patterns by comparing their allometric slope values.

To investigate coevolutionary patterns between female and male genital sizes, we performed a phylogenetically independent contrasts regression using the PDAP:PDTREE module in Mesquite.

To test the relationship of SGD (defined as female to male genital size ratios) and SSD (defined as female to male carapace width ratio), we performed model II regressions of female and male genital size as well as SGD on SSD.

4 RESULTS

4.1 STUDY 1: NON-DIRECTIONAL AND ISOMETRIC EVOLUTION OF SEXUAL SIZE DIMORPHISM IN ARGIOPINE SPIDERS

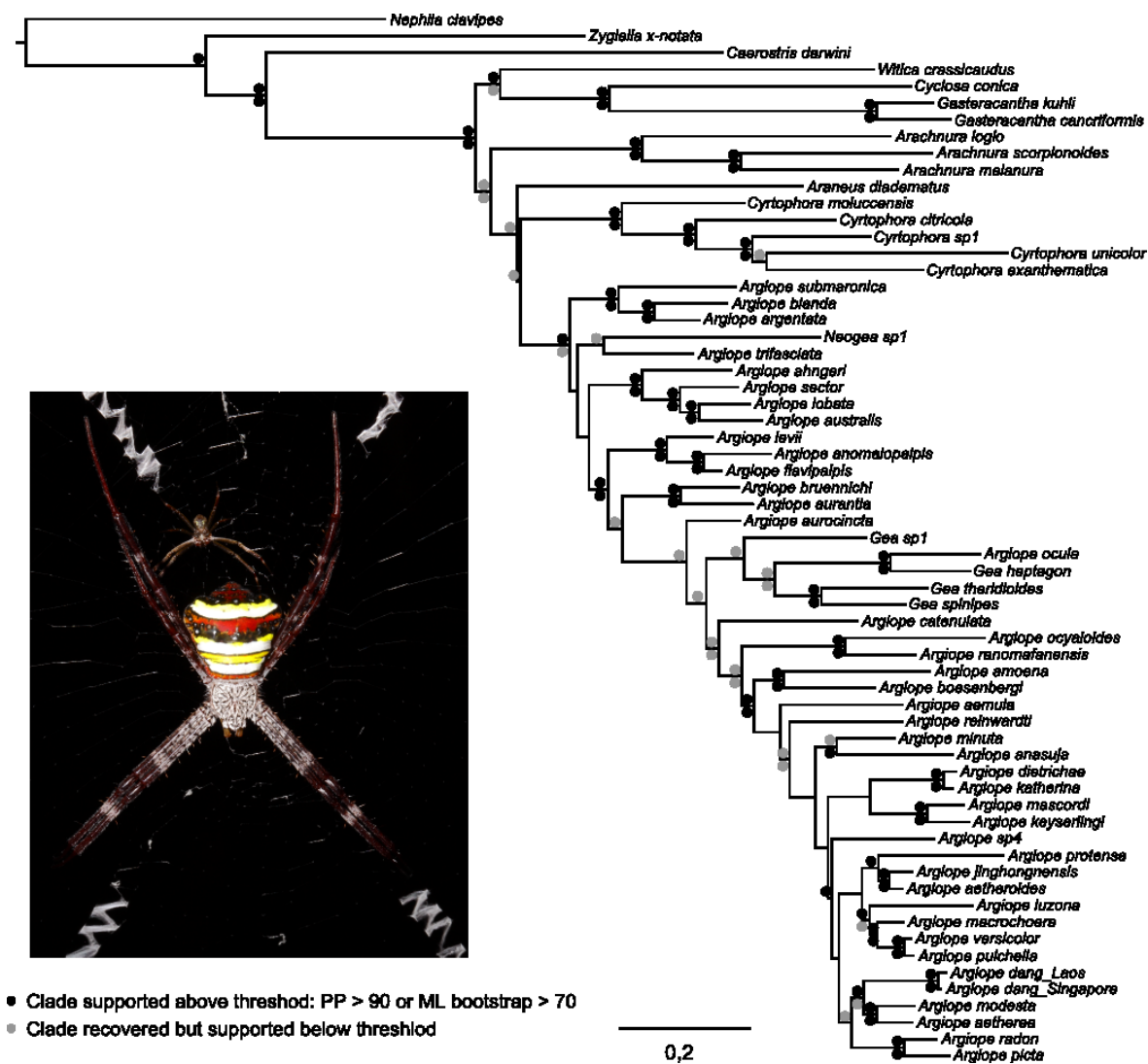


Figure 5. Filogenija pajkov skupine Argiopinae, najboljša Bayesovo drevo. Phylogeny of argiopine spiders based on the best Bayesian tree. The circles above each branch indicate Bayesian posterior probabilities ($PP \leq 50$ are not reported), whereas circles below each branch indicate bootstrap supports from maximum likelihood analysis (ML bootstrap ≤ 50 are not reported). The inset shows pronounced sexual size dimorphism typical of the clade; a male *Argiopoidea pulchella* above a female.

The concatenated matrix consisted of 3062 base pairs, including the mitochondrial *cox1* (1264 bps) and *cox2* (576 bps), and the nuclear genes H3 (345 bps) and 28S (987 bps). The ML and BI analyses of the combined data generated best trees with slightly different

topologies, but they agree on argiopine monophyly (Fig. 5). The phylogenetic topologies do not, however, recover *Gea* and *Neogea* as monophyletic; these lineages nest within *Argiope*. Within Argiopinae, most clades are strongly supported with some exceptions, notably among deeper nodes (Fig. 5). The genus *Cyrtophora* is sister to Argiopinae and this relationship was moderately supported.

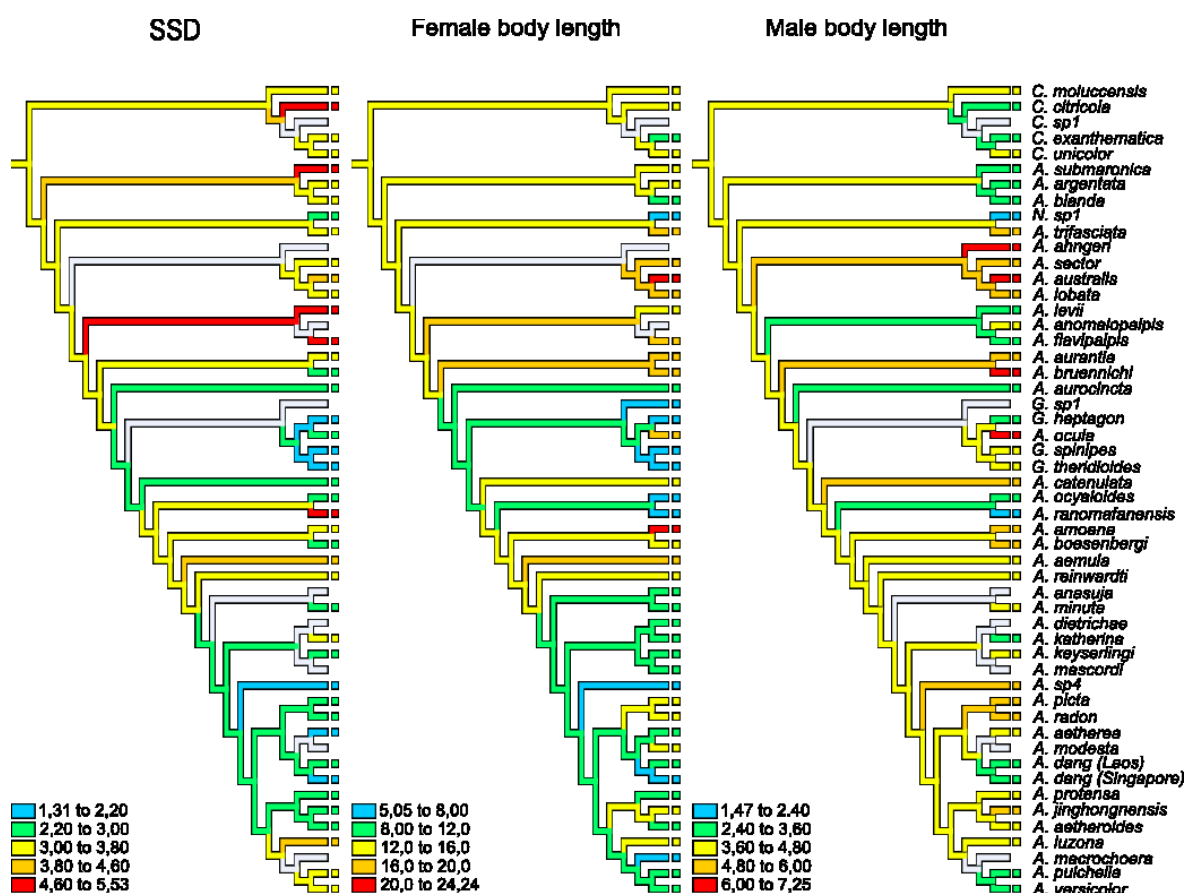


Figure 6. Rekonstrukcija evolucije telesne velikosti samic in samcev na podlagi Bayseove filogenije in SSD razmerij recentnih predstavnikov skupine Argiopinae. Reconstructed evolution of female body length (mm), male body length (mm) and sexual size dimorphism (SDI) on the best Bayesian tree. Colors denote size classes (legends).

Tracing the evolution of body size and of SSD on a pruned Bayesian phylogeny unequivocally optimizes median size as ancestral in Argiopinae (female size = 14,76 mm; male size = 4,33 mm; SDI = 2,61). Size evolution is then inferred to have proceeded through repeated increases and decreases (Fig. 6) and the size of both sexes as well as SSD exhibit substantial differences among species (Annex A, female size range: 5,05 – 24,07 mm; male size: 1,47 – 6,63 mm; SDI: 0,51 – 4,67).

Fig. 6 visually traces the evolution of size for each sex, and of SSD, on the BEAST consensus tree estimated to date back to 41 ($40,78 \pm 5,48$) Mya. Contrary to our predictions, SSD and female size do not show any phylogenetic increase (Fig. 7); their changes do not follow any specific direction. According to our predictions, we also detected no pattern in male size evolution (Fig. 7).

The results of model fitting revealed no specific evolutionary tendency in SSD and gender sizes (Table 2). The two evolutionary models implying selection for size (the Trend model and the OU model) did not better fit the data than the BM model in any of the three characters and under two different optimization schemes.

Both female size and male size conformed to the assumptions required to calculate the phylogenetically independent contrasts (female size: $P_{\text{Beast}} = 0,112$, $P_{\text{BI}} = 0,340$, male size: $P_{\text{Beast}} = 0,484$, $P_{\text{BI}} = 0,308$) (Díaz-Uriarte and Garland Jr 1996, 1998). Female and male size changes were significantly positively correlated on both topologies ($P_{\text{Beast}} < 0,001$, $P_{\text{BI}} = 0,008$).

In the model II regression analyses, the MA slopes did not differ significantly from 1 (Table 3) when using tips as data points (Fig. 8a), and also when using phylogenetically independent contrasts on the BEAST (Fig. 8b) and on the Bayesian tree (Fig. 8c). Although phylogenetically uncorrected analysis comes close to negative allometry, the phylogenetically informed datasets clearly reject it. These results suggest that body size evolution is isometric and is inconsistent with Rensch's rule and its converse.

Table 2. Log-likelihood vrednosti (InL), Akaike informacijski kriterij (AIC) ter rezultati testa 'likelihood ratio', za izbiro med evolucijskimi modeli za razlago SSD in velikosti spolov na dveh topologijah, ki se razlikujeta v načinu obravnavanja taksonov z manjkajočimi vrednostmi. Log-likelihood values (InL), the Akaike information criterion (AIC) and results of likelihood ratio test choosing among evolutionary models explaining SSD and gender size on two topologies that differ in the treatment of the taxa with missing values. The *P* values refer to the likelihood ratio comparison with BM model. Their non-significance means that in no case the BM model can be rejected. BM=Brownian motion; trend= Brownian motion with a directional trend; OU= single optimum Ornstein-Uhlenbeck model.

Model	Pruned			Presumed		
	BM	Trend	OU	BM	Trend	OU
FBL						
InL	-141,084	-139,505	-139,349	-145,349	-143,788	-143,692
AIC	286,168	285,011	284,698	294,698	293,577	293,385
<i>P</i> value		0,076	0,062		0,077	0,069
MBL						
InL	-67,333	-66,828	-67,138	-72,383	-71,906	-72,174
AIC	138,665	139,657	140,276	148,765	149,813	150,348
<i>P</i> value		0,315	0,532		0,329	0,518
SDI						
InL	-53,808	-53,347	-53,540	-61,911	-60,570	-60,495
AIC	111,617	112,694	113,080	127,821	127,140	126,990
<i>P</i> value		0,337	0,464		0,102	0,092

Table 3. Rezultati analize 'Major-axis regression' velikosti spolov (log10-transformirana dolžina telesa) za surove podatke (Tips) ter za filogenetsko neodvisne kontraste (PICs) dveh različnih topologij. Major-axis regression results of male size on female size (log10-transformed body length) for uncorrected data (Tips) and for phylogenetically independent contrasts (PICs) from two different topologies.

	Intercept	95% CI of intercept	Slope (β)	95% CI of slope	R^2
Tips	-0,201	-0,548 – 0,065	0,740	0,496 – 1,058	0,418
PICs (BEAST tree)	-0,014	-0,033 – -0,003	0,996	0,562 – 1,761	0,258
PICs (Bayesian tree)	-0,166	-0,396 – -0,042	1,051	0,591 – 1,899	0,250

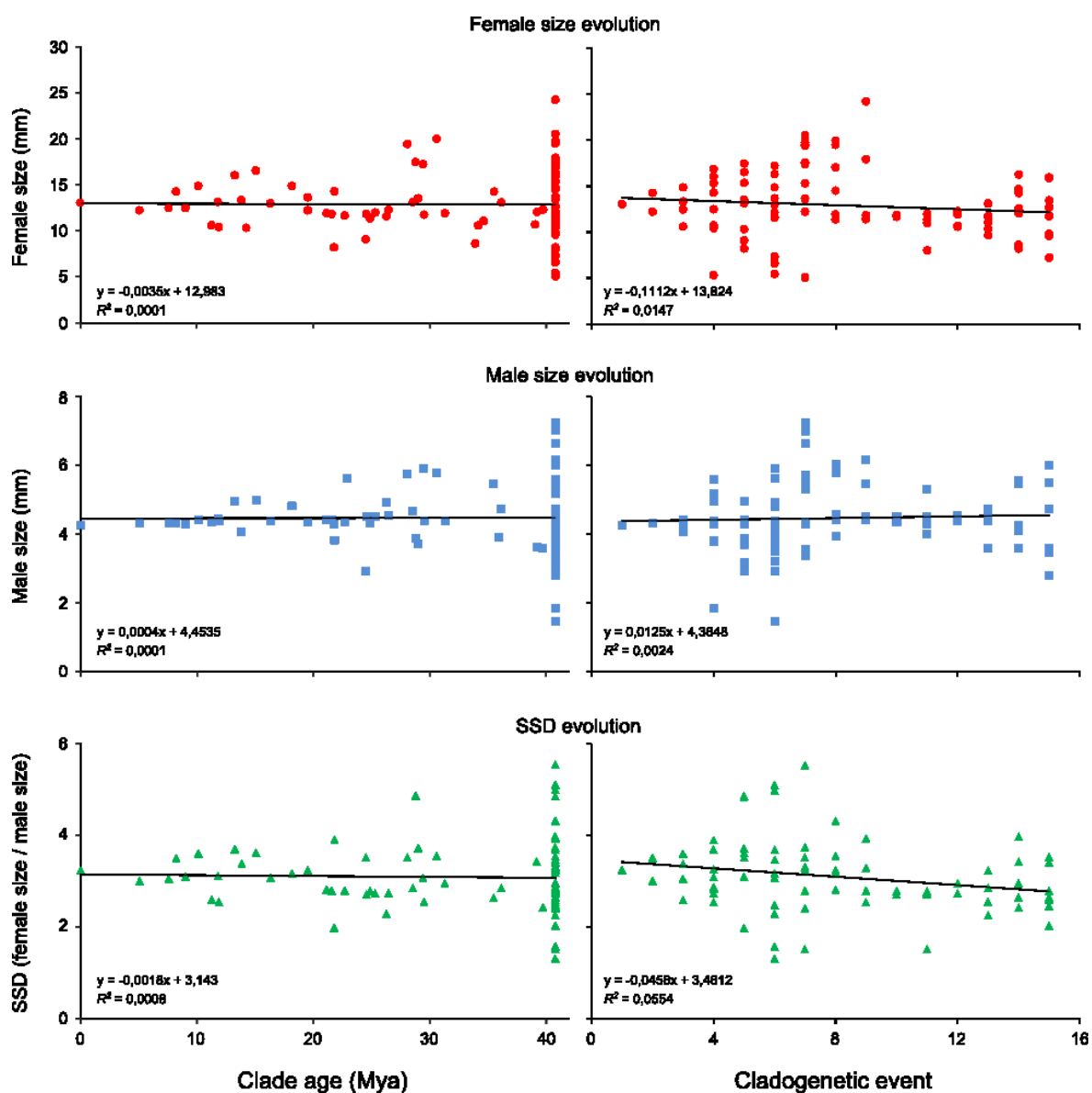


Figure 7. **Evolucijske spremembe velikosti samic, samcev in SDI.** Evolutionary changes of female and male size and SDI. As a perspective of time, the graphs have reconstructed clade age (left) or number of cladogenetic events (right) on the x axis. At this phylogenetic scale, linear regression statistics reveal no significant size changes.

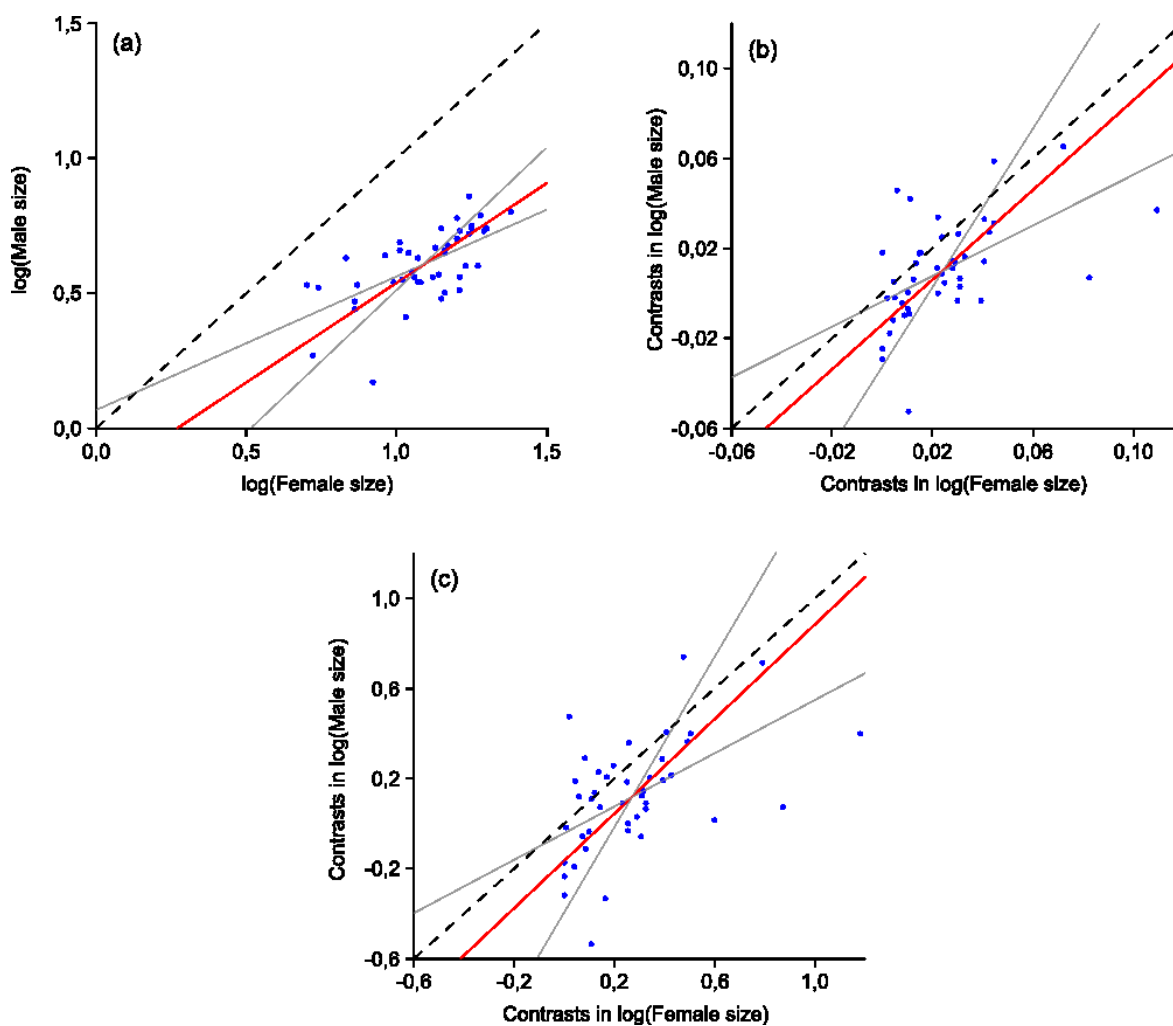


Figure 8. Alometrični vzorci SSD pri pajkih skupine Argiopinae. Isometry for sexual size dimorphism in argiopine spider using major-axis regression between \log_{10} -transformed male and female body sizes from (a) phylogenetically uncorrected data (tips), (b) phylogenetically independent contrasts data on the Beast tree and (c) phylogenetically independent contrasts data on the best Bayesian tree. Red lines: major-axis regression line; grey lines: confidence intervals of the major axis regression; black dashed lines: slope = 1.

4.2 STUDY 2: DISENTANGLING THE *SIZE* AND *SHAPE* COMPONENTS OF SEXUAL DIMORPHISM

4.2.1 Species level analyses: Argiopinae

In argiopine spiders, sexual shape dimorphism only occurs in those species with lobed or pentagonal shaped females (Fig. 9a); in contrast, the species with oval or cylindrical females are always shape monomorphic, i.e. also having oval or cylindrical males.

Both female and male shapes exhibited phylogenetic signal (female: $P < 0.001$; male: $P = 0.018$). The ancestral abdominal shape reconstruction postulates a single origin of lobed shaped females and a switch to oval shape that persists through the large part of the phylogeny (but with two additional, independent origins of oval female shape; Fig. 9a). On the other hand, cylindrical female abdomen evolved eight times, and pentagonal forms evolved five times (Fig. 9a). In males, cylindrical shape is reconstructed as ancestral, and this form shifted to oval shape at least twice, although precise evolution is ambiguous due to unknowns (Fig. 9a). The evolutionary switch to oval shaped abdomen, reconstructed at the node subtending *A. aurocineta* plus a large Old World clade, is clearly synchronized between males and females (Fig. 9a). The switches from oval female abdomen to pentagonal shape are accompanied by the retention of oval males except in the case of *A. reinwardti* (Fig. 9a). Conversely, all switches from oval to cylindrical female abdomen shape are also accompanied by the same morphological shifts in the males (Fig. 9a). Figure 9b shows the evolution of four morphotypes as well as female and male sizes on a time calibrated phylogeny. The results from phylogenetic ANOVA indicated no significant differences in female size among different morphotypes (Table 4). On the other hand, males of the oval shaped species were significantly smaller than those of cylindrical shaped species considering body length and abdominal length, but did not significantly differ in carapace widths (Table 4).

Results from the generalized estimating equations analyses revealed no significant correlation between sexual *size*- and *shape* dimorphism in argiopine spiders (BL: $t =$

0,0859, $d.f. = 11,883$, $P = 0,410$; TPL: $t = 2,062$, $d.f. = 11,218$, $P = 0,068$; CW: $t = 2,085$, $d.f. = 11,883$, $P = 0,063$).

Table 4. **Velikostne primerjave med različnimi morfotipi z uporabo filogenetske analize varianc (phylANOVA).** Size comparisons among different morphotypes using phylogenetic analysis of variance (phylANOVA). For abbreviations see Table 1.

	F value	P value	
Female			
Shape vs BL	4,943	$P = 0,213$	
Shape vs TPL	2,727	$P = 0,405$	
Shape vs CW	2,027	$P = 0,536$	
Shape vs AbdL	5,681	$P = 0,156$	
Male			
Shape vs BL	14,879	$P = 0,003$	Oval < Cylindrical
Shape vs TPL	6,040	$P = 0,087$	
Shape vs CW	1,414	$P = 0,449$	
Shape vs AbdL	18,892	$P = 0,003$	Oval < Cylindrical

Table 5. **Primerjava teže zadka med samicami različnih morfotipov (primerjano s cilindrično obliko) z uporabo analize ANCOVA, dolžina zadka kot kovarianca.** The abdominal weight comparison among females of different morphotypes (compared with cylindrical shape) using ANCOVA and abdomen length as covariance.

	Estimate	SE	t value	P value
(Intercept)	0,099	0,037	2,690	0,008
AbdL	0,042	0,004	11,662	< 0,001
ShapeLobed	-0,019	0,066	-0,285	0,776
ShapeOval	-0,073	0,046	-1,576	0,118
ShapePentagonal	-0,085	0,075	-1,138	0,258
AbdL:ShapeLobed	0,011	0,006	1,846	0,068
AbdL:ShapeOval	0,017	0,005	3,488	0,001
AbdL:ShapePentagonal	0,020	0,008	2,497	0,014

ANCOVA analyses indicated that female abdomen length had a significant effect on abdomen weight (Table 5). There were no differences in female abdominal weight among morphotypes, however, considering the interaction of length and shape, females with

cylindrical shaped abdomens were lighter than those with oval and pentagonal shapes, but only at maximal abdominal lengths (Table 4 and Fig 9, 10).

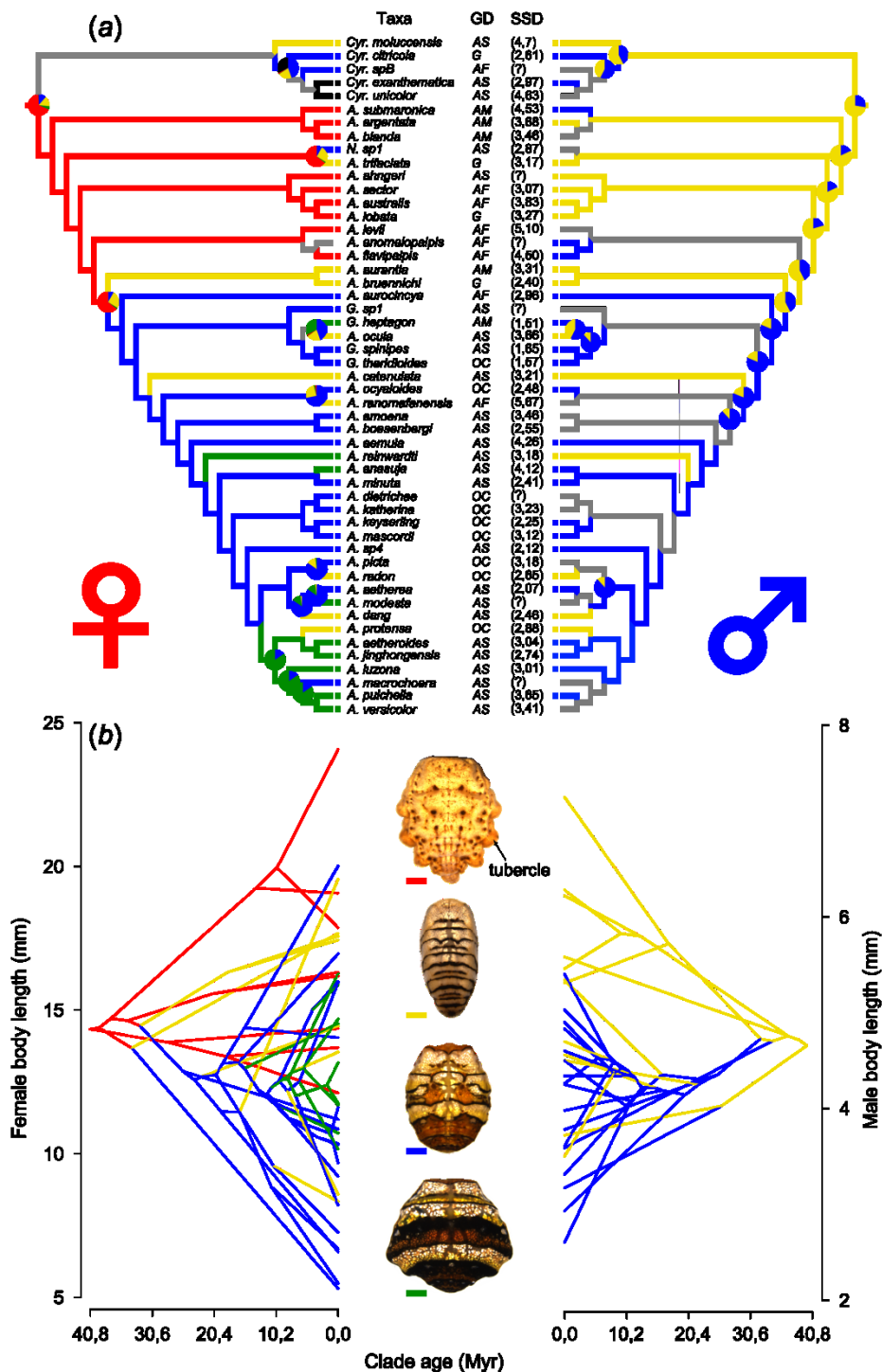


Figure 9. Rekonstrukcija evolucije oblik in velikosti pajkov skupine Argiopinae na podlagi morfotipov zadkov recentnih taksonov in sorodstvenih odnosov skupine. Reconstructed evolution of argiopine spider shape and size: a, Maximum-likelihood ancestral reconstruction of male and female abdominal shapes on a

species level argiopine phylogeny. The geographical distribution (GD; AS = Asia, AF = Africa, AM = Americas, OC = Oceania and G = Global) and sexual size dimorphism index (SSD = female body length/male body length) are provided. Pie charts denote the relative likelihood that an ancestor had a particular abdominal shape, but are omitted for clarity at likelihoods over 90%; b, Phenogram relating female (left) and male (right) body size and body shape of argiopine spiders over evolutionary time scale. The phenogram was reconstructed using R-package “phytools” by transforming a time calibrated phylogeny (Cheng and Kuntner 2014) using the function “chronos” in R package “Ape” with a known argiopine age (40,2 Myr). Colors denote abdominal shapes (red: lobed, yellow: cylindrical, blue: oval, green: pentagonal, black: triangular, only in outgroup, grey: unknown).

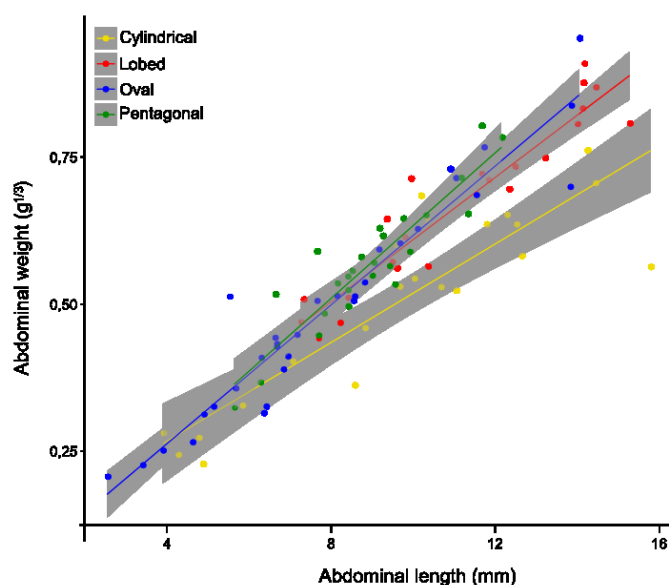


Figure 10. **Primerjava teže in dolžine zadkov med različnimi morfotipi.** Abdominal weight and length comparison among different morphotypes. Trend lines with 95% confidence interval (shadow) shown.

4.2.2 Genus level analyses: Araneoidea

At higher hierarchical levels, the reconstruction of SShD evolution in araneoid spiders suggested that SShD evolved six or seven times independently (Fig. 11). Our result showed a significant association between SShD and SSD (Araneoidea: slope = $0,073 \pm 0,007$, $t = 10,757$, $d.f. = 25,07$, $P < 0,001$; Nephilidae and Araneidae: slope = $0,083 \pm 0,017$, $t = 4,812$, $d.f. = 7,485$, $P = 0,004$) with sexually shape dimorphic lineages exhibiting higher SSD levels (Fig. 11, Annex C). Running these analyses without *Arachnura* and *Poltys* did not change the significance of the results (Araneoidea: slope = $0,051 \pm 0,009$, t

= 5,463, $d.f. = 24,992$, $P < 0,001$; Nephilidae and Araneidae: slope = $0,096 \pm 0,028$, $t = 3,479$, $d.f. = 7,191$, $P = 0,017$).

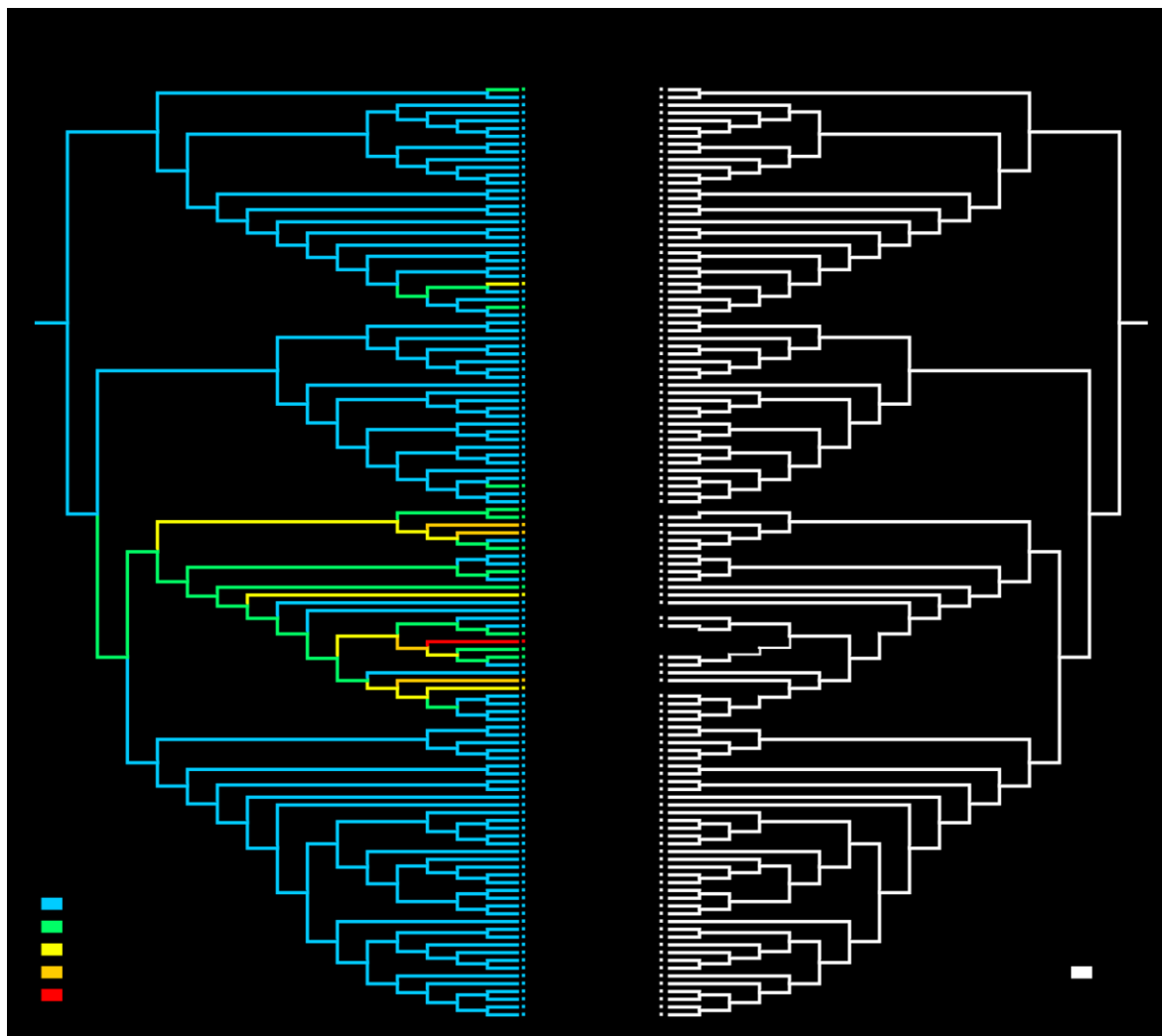


Figure 11. **Rekonstrukcija evolucije SSD in SShD na podlagi rodovne filogenije pajkov mrežarjev in razmerij SSD recentnih predstavnikov.** Reconstructed evolution of sexual size dimorphism (SSD) and sexual shape dimorphism (SShD) on a genus level phylogeny of orb-web spiders.

4.3 STUDY 3: GEOGRAPHICAL FACTORS PREDICT FEMALE SIZE VARIATION IN A SEXUALLY DIMORPHIC SPIDER

4.3.1 Species delimitation

A total of 118 sequences were used for phylogenetic reconstructions, including outgroups and 85 individuals morphologically identified as *A. argentata*; these 85 were also used in species delimitation and genetic distance testing. The GMYC method and the PTP model (Fig. 12) identified a total of 3 OTUs in the samples of *A. argentata*: *A. argentata* from California (argentataUS), *A. argentata* from Cuba (argentataCU) and *A. argentata* from the Caribbean (argentataCAR). The genetic distance test revealed very low K2P values within the OTUs (Table 6). In contrast, K2P values between the OTUs were all above 3%, but were particularly high between the Cuban OTU and the others (Table 6): While the Caribbean plus mainland OTUs comfortably fall within the intraspecific range for spiders, the average genetic distances between the Cuban OTU and the remaining two (roughly between 6 and 7%) were higher than the interspecific boundary in spiders (Čandek and Kuntner, 2014). These results indicated that the “*A. argentata*” from Cuba is genetically different from those from Americas and the other Caribbean islands, and can be labeled as a cryptic species. Therefore, we omitted the specimens from Cuba from size comparisons.

Table 6. Descriptive statistics for K2P (Kimura 2-parameter) distances within and between the molecular operational taxonomic units (OTUs), which were identified by molecular species delimitation methods

Within OTUs			
OTU	N	K2P	
		Mean	Std. Err.
argentataCAR	74	0,0093	0,0019
argentataUS	2	0,0016	0,0015
argentataCU	9	0,0059	0,0018
Between OTUs			
OTU 1	OTU 2	K2P	
		Mean	Std. Err.
argentataCAR	argentataUS	0,0352	0,0071
argentataCAR	argentataCU	0,0613	0,0102
argentataUS	argentataCU	0,0691	0,0114

4.3.2 Size comparison

Within the above delimited *A. argentata*, we measured a total of 74 females and 42 males from the Caribbean islands and 48 females and 4 males from the American mainland. Due to small sample sizes, we omitted from the analyses the males from the mainland. The results of multiple regression analyses suggest that female body size significantly decreases with latitude, but not with altitude or island size, however, this relationship breaks down when considering only island populations (Table 7, Fig. 13). The results also show that mainland females are larger than those on islands (Table 7). On the other hand, we detect no correlation between male body size and any of the geographic factors (Table 7). Combined, these results support a sex-specific response of body size to latitude in *A. argentata*.

Table 7. **Rezultati multiple regresije z metodo 'stepwise back elimination'**. The results of multiple regression analyses with stepwise back elimination method. The Shapiro-Wilk normality tests showed that all selected models conform to the assumption of linear regression.

Dataset	Selected variables	Estimate	SE	<i>t</i> value	<i>P</i> value	<i>d. f.</i>	<i>R</i> ²	Shapiro-Wilk test	
								<i>w</i> value	<i>P</i> value
All females	(Intercept)	1,454	0,040	36,781	< 0,001	119	0,119	0,982	0,117
	Latitude	-0,006	0,002	-3,639	< 0,001				
	Distribution	-0,068	0,030	-2,291	0,024				
Females on islands	(Intercept)	0,635	0,037	17,157	< 0,001	69	0,062	0,980	0,302
	Latitude	-0,003	0,002	-1,455	0,150				
	Altitude	0,000	0,000	-0,152	0,879				
Females in mainland	(Intercept)	0,609	0,014	42,580	< 0,001	46	0,080	0,987	0,339
	Latitude	-0,002	0,001	-3,241	0,001				
	(Intercept)	0,136	0,036	3,758	< 0,001				
Latitude	0,002	0,002	1,165	0,250					
All males	Distribution	0,136	0,069	1,969	0,056				
	Latitude *	-0,007	0,004	-1,718	0,093				
	Distribution								
Males on islands	(Intercept)	0,271	0,064	4,251	< 0,001	38	0,047	0,986	0,887
	Latitude	-0,005	0,004	-1,352	0,184				
	Altitude	0,000	0,000	-0,383	0,704				
	Island size	0,004	0,009	0,508	0,614				

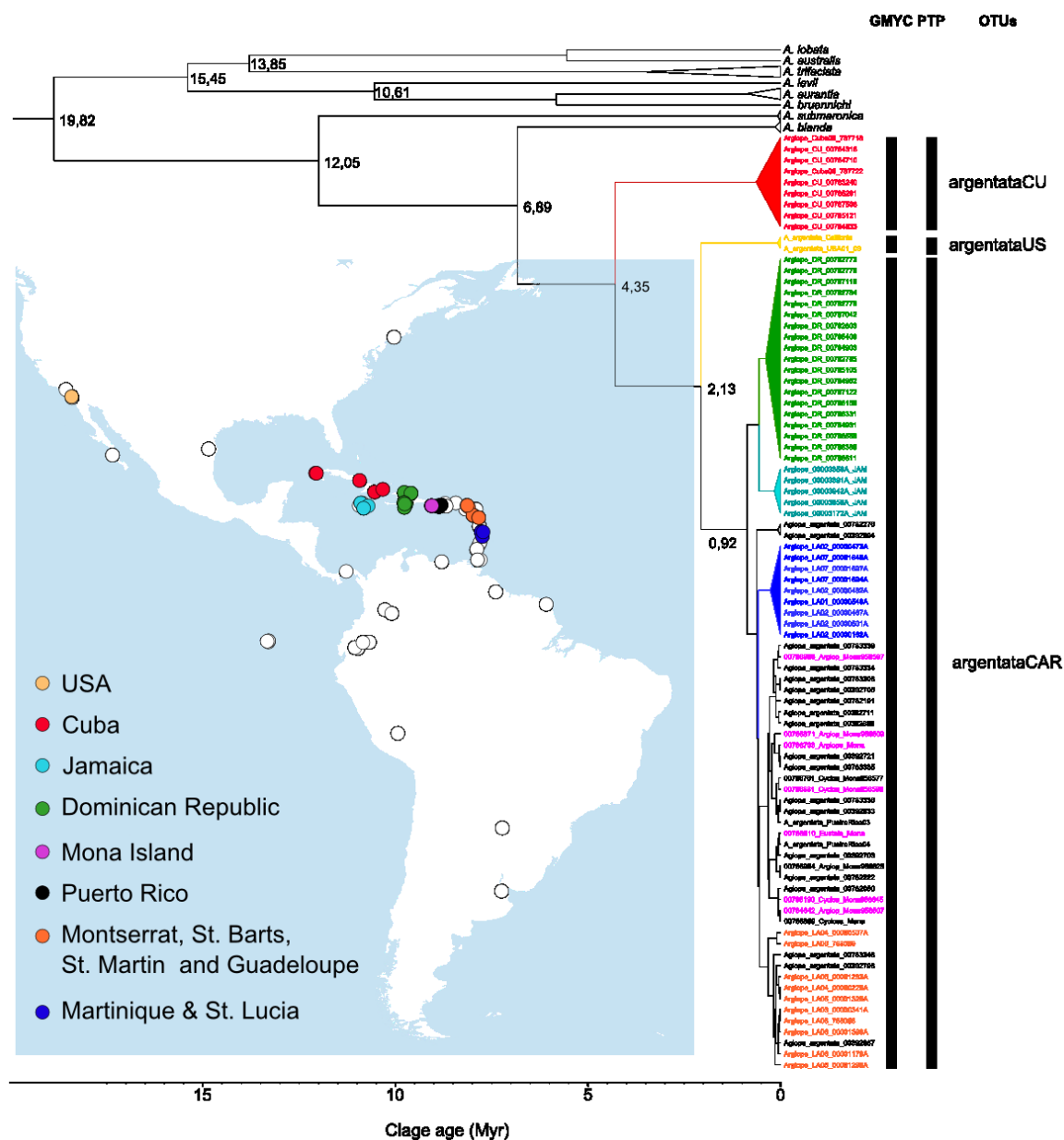


Figure 12. Časovno kalibrirana filogenija rodu *Argiopa* s poudarkom na populacijah vrste poznane kot *A. argentata*. A time calibrated phylogeny of *Argiopa* focusing on the populations commonly known as *A. argentata*. Shown are the results of tree based species delimitation analyses (GMYC method and the PTP model) on a BEAST phylogeny (node ages in million years) and the location of spiders used in this study (inset picture). White circles refer to source populations for size measurement, while colored circles represent source populations for DNA barcoded specimens, colors matching the terminals on phylogeny (see legend). The OTUs (operational taxonomic units) correspond to a cryptic species from Cuba (argentataCU) and populations from other Caribbean islands (argentataCAR) plus mainland (argentataUS) treated as conspecific (*A. argentata*).

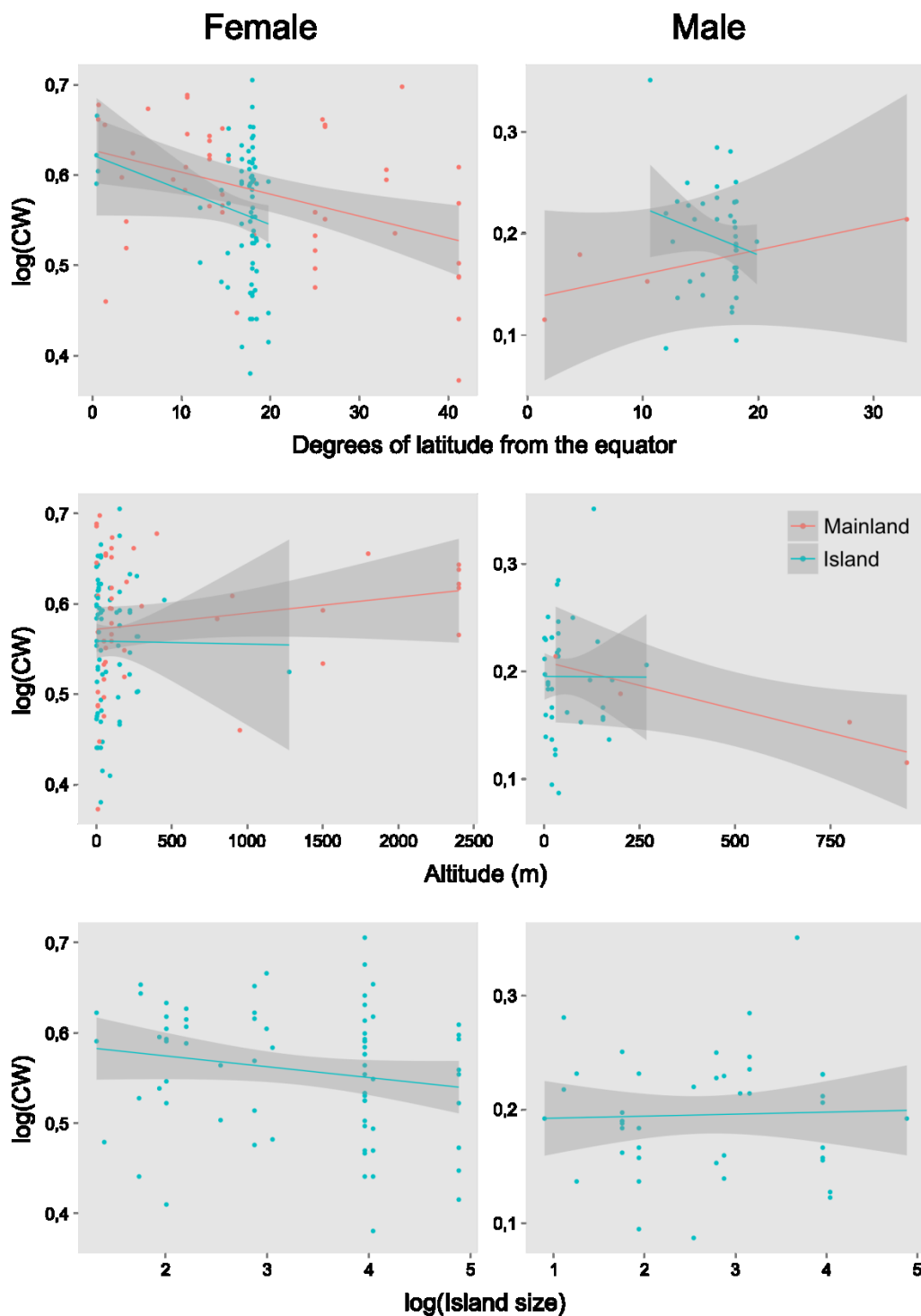


Figure 13. **Spolno specifične spremembe velikosti glede na geografske dejavnike.** Sex specific size changes with geographical factors. Body size versus latitude and elevation as well as island size for mainland/insular *Argiopa argentata*. The tendency line with 95 % confidence interval shown.

4.4 STUDY 4: INDEPENDENT EVOLUTION OF GENITAL AND SEXUAL SIZE DIMORPHISM

4.4.1 Male and female genital allometry

The model II regressions did not detect the predicted negative allometric patterns for male and female genitalia (Table 8a, Fig. 14). Instead, the results showed that the allometric slope value for female spermathecal and male pedipalp size did not significantly differ from the values of the somatic characters (Table 8a and 3, Fig. 14). Using tip data, the allometric value for the female epigynal size was significantly larger than the slope for a somatic character (carapace length) and spermathecae (Table 8a and 9, Fig. 14). However, the analysis of the contrasts revealed the allometric value for the female epigynal size not significantly departing from zero (Table 8a).

4.4.2 Coevolution of male and female genitalia

The phylogenetically independent contrasts regression revealed that male pedipalp size was significantly correlated to female epigynal size ($P < 0,001$, $\text{slope}_{\text{MA}} = 0,252$, $R^2 = 0,419$), but not to female spermathecal size ($P = 0,468$). However, spermathecal size was significantly correlated to epigynal size ($P < 0,001$, $\text{slope}_{\text{MA}} = 0,028$, $R^2 = 0,271$).

4.4.3 SGD vs. SSD

Using tip data, we detected significantly positively allometric slope values for both versions of the SGD ratio (ones with spermathecal, then epigynal volume over palpal volume), and relatively higher allometric slopes in females (Table 8b). However, in phylogenetically corrected analyses neither female genital allometric slopes, nor the two versions of SGD significantly departed from zero, but the male genital allometric slope was positively correlated with SSD (Table 8b).

Table 8. **Rezultati standardizirane 'major-axis regression' z analizo surovih podatkov (TIPs) ter filogenetsko popravljenih podatkov (PICs).** The results of standardized major-axis regression analyzing tip data (TIPs) and phylogenetically corrected data (PICs). (a) Allometric slopes of somatic and genital characters regressed on body size. (b) Allometric slopes of SGD (first ratio using spermathecal, second epigynal volume) and genital sizes regressed on SSD (ratio based on carapace width). The *P* value of the slope compared to 0 is provided.

(a)

	TIPs				PICs				
	Slope	95% CI	R^2	<i>P</i> value	Slope	95% CI	R^2	<i>P</i> value	
Female									
Somatic character									
BL	1,07	0,98 - 1,16	0,87	< 0,001	1,09	0,88 - 1,35	0,43	< 0,001	
TPL	1,11	1,00 - 1,23	0,85	< 0,001	1,05	0,84 - 1,30	0,28	< 0,001	
CL	1,00	0,96 - 1,04	0,97	< 0,001	0,95	0,84 - 1,06	0,85	< 0,001	
Genital character									
SV	0,99	0,86 - 1,14	0,76	< 0,001	1,11	0,83 - 1,47	0,14	0,006	
EV	1,22	1,06 - 1,40	0,68	< 0,001	1,34	1,02 - 1,75	0,06	0,078	
Male									
Somatic character									
BL	1,05	0,87 - 1,28	0,75	< 0,001	1,08	0,83 - 1,40	0,26	< 0,001	
TPL	1,21	1,02 - 1,43	0,70	< 0,001	0,94	0,40 - 1,25	0,27	< 0,001	
CL	0,95	0,85 - 1,06	0,89	< 0,001	1,15	0,96 - 1,39	0,44	< 0,001	
Genital character									
PV	1,17	0,97 - 1,42	0,78	< 0,001	1,25	0,92 - 1,69	0,21	0,005	

(b)

	TIPs				PICs			
	Slope	95% CI	R^2	<i>P</i> value	Slope	95% CI	R^2	<i>P</i> value
SGDSVPV vs. SSDCW	2,54	1,89 - 3,42	0,48	< 0,001	1,18	0,78 - 1,77	0,10	0,065
SGDEVPV vs. SSDCW	2,46	1,75 - 3,47	0,34	< 0,001	1,41	0,95 - 2,09	0,01	0,557
SV vs. SSDCW	1,23	0,91 - 1,67	0,28	< 0,001	1,31	0,92 - 1,89	0,00	0,923
EV vs. SSDCW	1,48	1,07 - 2,06	0,10	0,040	1,43	1,02 - 1,99	0,01	0,551
PV vs. SSDCW	0,95	0,64 - 1,40	0,02	0,411	1,21	0,85 - 1,72	0,17	0,015

Table 9. **Rezultat primerjave krivulj.** Allometric slope comparisons between genital and somatic characters. For abbreviations, see Tables 1.

		TIPs	PICs
FSV	FBL	0,358	0,938
	FTPL	0,207	0,750
	FCL	0,912	0,306
FEV	FBL	0,115	0,236
	FTPL	0,281	0,157
	FCL	0,009	0,019
	SV	0,042	0,334
MPV	MBL	0,437	0,475
	MTPL	0,806	0,175
	MCL	0,062	0,672

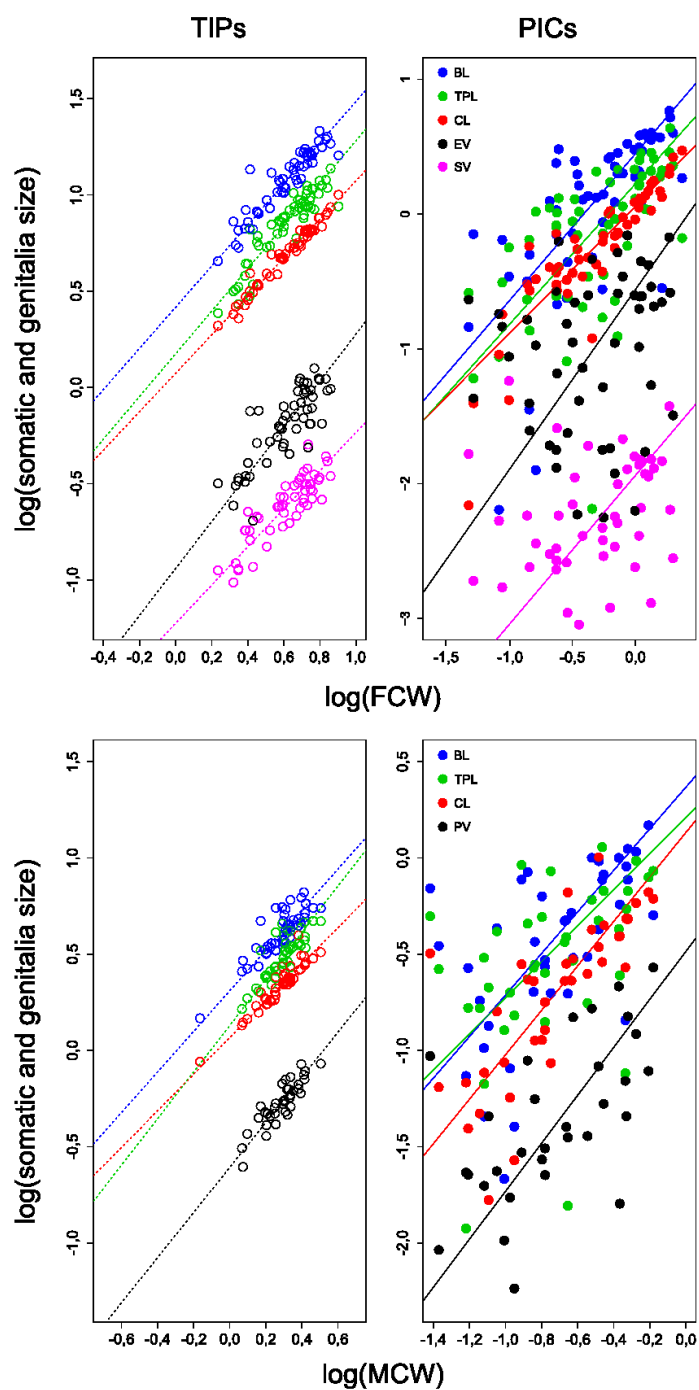


Figure 14. Analize regresij telesnih in genitalnih velikosti na pokazatelj telesne velikosti pri obeh spolih. Regressions analyses for somatic and genital size on body size indicator in both sexes. The raw data (TIPs) is presented by open circle and phylogenetically corrected contrast data (PICs) is presented by solid cycle. For abbreviations, see Table 1.

5 DISCUSSION

5.1 STUDY 1: INDEPENDENT EVOLUTION OF GENITAL AND SEXUAL SIZE DIMORPHISM

Theory predicts that a female biased SSD arises and is maintained by strong fecundity selection for large female size (Head, 1995; Prenter et al., 1999), and a strong tendency for males to remain small, either due to gravity (Moya-Laraño et al., 2002), mortality (Vollrath and Parker, 1992), or scramble competition (Danielson-François et al., 2012). At species level phylogenetic scales, we would thus predict to find one or more of the following patterns: 1) a female size increase across the phylogeny in combination with maintenance of a small, optimal male size, 2) support for an evolutionary model other than Brownian motion which would suggest lack of selection, and 3) a negative allometric SSD pattern with female size contributing to SSD; such patterns would be consistent with a converse Rensch's rule. Our study, surprisingly, detected none of these patterns. At this phylogenetic scale, I therefore find no obvious support for classical explanations of the evolution of female biased SSD in this spider clade.

We tested general hypotheses about the evolution of sexual size dimorphism in spiders using a combination of evolutionary analyses on an original molecular phylogeny of the Argiopinae. We found that moderate levels of SSD and medium sizes in both sexes were ancestral in this 41 million year old clade, and that the evolution of SSD as well as female and male sizes showed no specific trend, hence SSD evolution seems to be non-directional. Phylogenetically, the inferred size changes in both sexes were significantly positively correlated. Furthermore, we found that the evolution of SSD in argiopines was isometric. Against the predictions from the spider literature (Abouheif and Fairbairn, 1997; Fairbairn, 1997), the female biased SSD in this clade is not due to allometric patterns, and thus does not follow Rensch's rule or its converse. This pattern is reinforced by our evaluation of three models of size evolution. Since they detected no trend or direction in the size of either sex, our results failed to reject the Brownian motion evolutionary model. This means that in argiopines we failed to detect strong selection for increased female size or for the maintenance of small male size.

Our results thus largely contradict the conventional understanding of spider SSD evolution by not confirming the expected decoupling of male and female size evolution. We expected to recover a pattern more similar to nephilids (Kuntner and Coddington, 2009), where the correlation of size evolution between the sexes is broken. The sexually decoupled size changes elegantly explain extremely female-biased SSD in nephilids, but a different explanation is required in the case of argiopines.

A reconstructed history of sex-specific size changes at a higher taxonomic level phylogeny of orb-weaving spiders implied multiple origins of female-biased SSD (Coddington et al., 1997; Hormiga et al., 2000). According to these authors, female-biased SSD evolved early in the evolutionary history of a large “argiopoid clade” including argiopines and several other araneid lineages that we sampled as outgroups. Our results corroborate this interpretation by suggesting moderate levels of ancestral SSD levels in argiopine spiders and their immediate outgroups. The degree of SSD, however, did not significantly increase through argiopine evolution, as is the case in nephilid spiders. In contrast with other extremely size dimorphic spiders, where the evolution of female and male size is usually decoupled (Kuntner and Coddington, 2009), the reconstructed size changes of females and males in argiopines are phylogenetically positively correlated. Such a result may suggest that fluctuations in SSD in this clade should be limited (Reeve and Fairbairn, 1996). Following this logic, we would expect to detect directional SSD evolution towards optimum values, or alternatively retention of ancestral SSD levels. Contrary to these predictions, our analyses detected no evolutionary trends in any size measure. This negative finding suggests non-directional evolution of argiopine SSD, as well as of female and male size; all these traits exhibit a random walk-like pattern on the phylogeny. The converse pattern of Rensch’s rule predicts a negative allometry in female-biased SSD (Fairbairn, 1997), however, the analyses of tips and phylogenetically independent contrasts data had a slope not different from one, thus following a pattern of isometry. Hence, at the macroevolutionary scale, our results cannot point towards any particular selection pressure shaping the evolution of SSD and the size of both sexes in argiopines. Nevertheless, argiopine species do show considerable size differences, raising the question of which selection forces contribute to such interspecific variation in the size of both sexes and SSD.

We interpret the results from all analyses to question the primacy of fecundity selection hypothesis and mate search related hypotheses as general explanations for the evolution of female-biased SSD in spiders. Plausibly, the intra- and inter-specific variation in size and SSD observed in argiopines could arise through different gender- or species-specific responses to ecological or geographical factors. Both intra- and interspecific differences in SSD and size can be influenced by geographic variation (Blanckenhorn et al., 2006; Loison et al., 1999), habitat types (Butler et al., 2000; Ceballos et al., 2013), mating systems (Pérez-Barbería et al., 2002), life-history traits (Blanckenhorn, 1994; Blanckenhorn and Perner, 1996), or other abiotic and biotic factors (Castillo-Guerrero and Mellink, 2011; Cox et al., 2003; Isaac, 2005). In an ant-eating spider, *Zodarion rubidium*, higher levels of SSD were found in warmer regions due to a gender-specific response, where only female and not male body size changed in response to temperature (Pekár and Vaňhara, 2006). Gender- and species-specific responses to elevation have been discovered in Arctic wolf spiders (Bowden et al., 2013; Høye and Hammel, 2010). There, female size declined with increased elevation while male size did not change; this pattern, however, did not hold true for all species. Inkpen and Foellmer (2010) argued that sex-specific foraging behaviors and growth rates resulted in the extreme female-biased SSD in the black and yellow garden spider, *Argiope aurantia*. The authors demonstrated different foraging behaviors and hence prey availabilities for females, building larger webs, and for males, constructing more conspicuous web decorations, so called stabilimenta. Considering that argiopines exhibit diverse web architectures and three distinct types of stabilimenta that differ in function and prey/predator attractiveness (Cheng et al., 2010; Walter and Elgar, 2012), an interesting open question remains if and how different foraging behaviors relate to the evolution of SSD.

Argiopine spiders occupy many different habitats on all continents, show the whole range of sexual size dimorphism from moderate to extreme, and exhibit various foraging and mating behaviors. They are thus an excellent model system for testing a range of hypotheses relating to the evolution of body size and shape. Sexual size dimorphism is the outcome of an interplay between natural and sexual selection on both sexes. At the investigated macroevolutionary scale, our study failed to find the expected patterns in

argiopines and cannot directly attribute the evolution of female biased SSD to fecundity, gravity, and differential mortality selection, so perhaps future studies should focus on how size changes are affected by ecological or geographical factors such as latitude, altitude, landmass size and isolation, habitat and prey diversity and many others. There may also be numerous behavioral traits that correlate with the evolution of SSD (Blanckenhorn, 2005): sexual cannibalism, emasculation and other sexually conflicted behaviors (Kuntner et al., 2015). At this point, our understanding of how geographic, behavioral and ecological factors affect spider size is too limited. Finally, future work should unravel to what extent female phenotypes other than size contribute to fecundity.

In conclusion, extreme female biased SSD continues to puzzle and elude general patterns. However, we here provide a test case of how, amongst numerous analytical approaches, macroevolutionary patterns in SSD could be detected. By combining species level phylogenetic analysis with tests of evolutionary models implying no versus pronounced selection pressures, with parsimony or other optimization methods reconstructing size and SSD ratios, and by model II regression analysis that establishes allometric versus isometric trends, I can test similarity of patterns between unrelated clades that inform us of selection mechanisms responsible for SSD. We emphasize the phylogenetic basis of the phenomenon (Coddington et al., 1997); although we additionally perform more classical correlational analyses to illustrate the differences, we question the validity of using species as data points.

5.2 STUDY 2: DISENTANGLING THE *SIZE* AND *SHAPE* COMPONENTS OF SEXUAL DIMORPHISM

We predicted that shape and size components of spider sexual dimorphism would show only weak or no association due to different selection pressures. In accordance with these predictions, our contrasting results at the species and higher hierarchical levels suggest that sexual dimorphism in spiders consists of two individual components, shape and size, that may or may not exhibit dependency.

5.2.1 Species level patterns in Argiopinae

Studies of invertebrate sexual dimorphism have so far failed to disentangle size evolution (SSD) and abdominal shape evolution (SShD) (Benítez et al., 2013; Bonduriansky, 2006; Gidaszewski et al., 2009; Kitano et al., 2008; Minton and Wang, 2011), while our study shows their disassociation in argiopine spiders. We showed that SShD in argiopine spiders is confined to those species and clades where females are lobed or pentagonal, whereas those species and clades with oval and cylindrical animals are sexually shape-monomorphic (Fig. 9). We detected a single phylogenetic origin of lobed shaped females, a repeated evolution of cylindrical (eight origins) and pentagonal (five origins) female shapes, and six independent reversals to cylindrical shape in males. Our analyses failed to detect any significant differences in female size between morphotypes. On the other hand, using body length and abdominal length (but not other size measures), cylindrical males were larger than oval males. In addition, we discovered that cylindrical shaped females have lighter abdomens than those with oval or pentagonal shapes, but only when females are large, while small females do not show significant weight differences among morphotypes.

Argiopine males only show two alternate (but species specific) abdominal morphologies, while female argiopines are morphologically more diverse with four (Fig. 9). These sexually specific differences imply that abdominal shape evolution is largely driven by evolutionary pressures on females, among which literature consistently singles out fecundity selection (Coddington et al., 1997; Head, 1995; Higgins, 1992; Hormiga et al., 2000). One common evolutionary outcome of fecundity selection is female gigantism as is the case in another orb web clade, the nephilid spiders (Head, 1995; Hormiga et al., 2000; Kuntner and Coddington, 2009; Kuntner and Elgar, 2014). An important difference between these clades is that in nephilids, size evolution between the sexes runs independently (Kuntner and Coddington, 2009; Kuntner and Elgar, 2014), whereas in argiopines it is highly positively correlated (Cheng and Kuntner, 2014). The correlated evolution of argiopine male and female size implies that female size changes also depend on evolutionary pressures on males (Elgar, 1991; Moya-Laraño et al., 2009; Vollrath and Parker, 1992). However, even if females are restricted in evolutionary size increase, their

fecundity may plausibly increase via morphological evolution, such as adding additional abdominal volume through shape changes.

Following this logic, one may expect to detect differences in abdomen volume between similarly sized females of different morphotypes. More specifically, derived morphotypes may be expected to exhibit superior fecundity. Contrary to this prediction, our weight comparison—assuming that weight and fecundity are related (Table 4; Fig. 10)—showed no significant difference among different morphotypes in small females, implying comparable fecundity of morphotypes. However, at longer abdomen lengths, cylindrical females had lighter abdomens than those with oval or pentagonal abdomens, implying inferior fecundity in large, cylindrical females. In theory this would mean increased fecundity of large females evolving from cylindrical to oval or pentagonal shape, however, on the phylogeny such shifts never occur. Instead, six repeated shifts from oval or pentagonal to cylindrical shape in females imply the possibility of repeated risk of decreased fecundity. Because this is entirely counterintuitive from the female's perspective, we explore the idea below that these shifts may be driven by the males.

The phylogenetic reconstruction of male body shapes (Fig. 9a) indicate six shifts from oval to cylindrical shape, and in five of these cases these male shape changes are also accompanied by female transformation to cylindrical shape. Although our prior phylogenetic reconstruction of male argiopine size did not detect general size increases (study 1, see Fig. 7), by bringing male shape into the picture, we now see a significant size difference between larger cylindrical and smaller oval shaped males. The repeated shifts from oval to cylindrical males detect a phylogenetic pattern towards larger, not smaller males (Fig. 9b). It therefore seems that cylindrical shape evolution is driven by males, with the female shape following through genetic constraints.

We also detected no abdominal weight differences between oval and lobed as well as pentagonal female shapes. This may imply that morphotype shifts towards sexual shape dimorphism may likewise not relate to female fecundity. Instead some of these evolutionary transformations may relate to species ecology or geographic history. Species with lobed morphotypes are confined to Americas and to Africa, and those with pentagonal

abdomens only live in Southeast Asia (Bjørn, 1997; Levi, 1983; Levi, 2004), and abdominal shapes show strong phylogenetic inertia.

5.2.2 Genus level patterns in Araneoidea

While the species level correlation comparisons in argiopines rejected a clear association between shape and size dimorphism evolution, our genus level analyses revealed that at two hierarchical levels of araneoid spiders, the evolution of SShD and SSD were significantly correlated. Highly sexually shape dimorphic forms in orb web spiders seem to evolve only in certain lineages where females are several times larger than the males (Fig. 11). In other words, SShD is found in a subset of all sexually size dimorphic orb weavers. This detected phylogenetic pattern implies a greater evolutionary plasticity in size compared with shape, and suggests that a highly female biased SSD is a prerequisite for shape dimorphism to evolve.

The comparative analysis of Prenter et al. (1997) found no association between levels of SSD and spider life history such as web versus non web building groups. However, Prenter et al. (1997) used only northern European regional faunas as examples, while most extreme cases of sexual dimorphic spiders are found in the tropics. Our general understanding of both *shape* and *size* dimorphism in spiders suggests that extremes are more typical of tropical than temperate spiders, and of orb web rather than cursorial hunters. All specialized female abdominal shapes in Fig. 1, such as bark-, leaf-, twig-mimicking forms, and those with varying thorny projections, are araneids, the archetypical orb web builders (Scharff and Coddington, 1997). As sit-and-wait hunters limited to their web space, orb-weaving spiders are exposed to predators, and may be under substantial ecological pressure to increase their survival rates through abdomen shape changes. Our study implies that these morphological changes evolve more frequently in those lineages that are highly female size biased, perhaps because of already disassociated male and female phenotypic evolution (Kuntner and Coddington, 2009; Kuntner and Elgar, 2014). Although speculative, we suggest that responses to predation pressure may trigger a sex-specific response affecting morphology in those lineages with giant females. Argiopines may be an exception; because the evolution of male and female sizes in this lineage is not

independent (Cheng and Kuntner, 2014), there is also no clear association between SSD and SShD.

5.2.3 Broader implications

Different components of sexual dimorphism such as size and shape may evolve indirectly, perhaps resulting from selection on other traits. In general, sexual size and shape dimorphism often co-occur (Schwarzkopf, 2005). As we show at higher level phylogenetic scales in spiders, shape dimorphism is confined to those clades that are already size dimorphic, implying that the sex-specific trait changes are more likely when other traits already evolve under differing evolutionary pressures on each sex. Sexual dimorphism research should test whether such pattern is universal in animals, or symptomatic of spiders, and whether the (dis)association between SSD and SShD in other animal groups also depends on the phylogenetic level of investigation.

5.3 STUDY 3: GEOGRAPHICAL FACTORS PREDICT FEMALE SIZE VARIATION IN A SEXUALLY DIMORPHIC SPIDER

We used an integrative approach to examining factors affecting body size variation in a widespread American web building spider. We discovered that a cryptic, likely undescribed, species inhabits Cuba, and we thus omitted this sample from intraspecific comparisons of *A. argentata*. Among all remaining populations of *A. argentata*, males did not exhibit any latitudinal cline in body size, while in females we found a pattern consistent with converse Bergmann's rule. In addition, mainland females were significantly larger than those on islands, hinting at some general validity of the island rule, although island size did not significantly explain female size. These findings demonstrate pronounced sex specific responses to some of the investigated geographical factors, providing a novel explanation of intraspecific body size variation in a sexually dimorphic spider. However, too sparse sampling of continental males precluded a clearer understanding of the relationship between latitude and body size in *A. argentata* and therefore, our understanding of geographic variation of SSD remains preliminary.

5.3.1 Species delimitation

Our tree-based species delimitation analyses revealed three OTUs within *A. argentata* species complex (Fig. 12), which we tentatively name *argentata*CU (specimens from Cuba), *argentata*US (from mainland Americas) and *argentata*CAR (from the Caribbean islands except Cuba). Despite these three units suitable for comparison, the calculated genetic distances within and among them strongly suggested that the American mainland populations were conspecific with the Caribbean ones (not from Cuba), and we therefore recognize two species within our sample (*A. argentata* and an unnamed species from Cuba, see Table 5). Our interpretation, however, could be inaccurate due to several reasons. First, some studies have doubted the appropriateness of using *cox1* as molecular marker for species delimitation in spiders since it could potentially overestimate the numbers of species (Astrin et al., 2006; Lopardo and Uhl, 2014). In addition, considering that any method of species delimitation is sensitive to sampling (Papadopoulou et al., 2009; Puillandre et al., 2012), the evenness of the sampling may have led our species delimitation approaches to underestimate or overestimate the number of species. Nonetheless, in the case of the wasp spider *Argiope bruennichi*, a widespread Palearctic species, the maximum intraspecific K2P distance among the pairwise comparisons of nearly two thousand *cox1* sequences was only 0,023 (Kreihenwinkel and Tautz, 2013). Such a conserved evolutionary rate of *cox1* in *A. bruennichi* could support the suitability of using *cox1* as marker and for the validity of the general spider barcoding gap (Čandek and Kuntner, 2014) in *Argiope* species delimitation.

5.3.2 Converse Bergmann's rule in female *A. argentata*

As in other ectothermic animals, especially insects, the converse Bergmann's rule is believed to also apply to spiders (Shelomi, 2012). Our analyses revealed that in *A. argentata* female size decreased with latitude, but this only held true for the continental dataset and for the combined dataset (Table 6). Our result was consistent with the study of the Palearctic wasp spider, *A. bruennichi*, which showed that the Mediterranean populations were significantly larger than those at higher latitudes (Kreihenwinkel and

Tautz, 2013). This negative relationship between female body size and latitude has been found in several other web-building, as well as cursorial spiders (Entling et al., 2010; Higgins, 1992; Puzin et al., 2014). While temperature (Atkinson and Sibly, 1997) and moisture, i.e. rainfall and humidity (Kennington et al., 2003; Stillwell et al., 2007), have been presumed as the major selective mediators responsible for the Bergmann clines in animals, it is the season length that is considered to be the critical factor affecting the converse Bergmann clines. At higher latitudes and altitudes shorter growing seasons pose stricter limits to the time available for growth (Blanckenhorn and Demont, 2004; Telfer and Hassall, 1999). In addition to longer seasons available for growth, tropical populations may also exhibit faster growth rates than temperate populations. In *Nephila clavipes* for example, a combination of higher growth per molt and higher number of juvenile molts resulted in large tropical and small temperate females (Higgins, 1992), a pattern that may also hold in *A. argentata*.

In contrast to continental females, the insular females of *A. argentata* did not follow the converse Bergmann's rule. This discrepancy probably relates to the relatively smaller latitudinal distribution in the Caribbean (from 0 ° to 18 °) compared with the continental populations (from 0 ° to 42 °). A meta-analysis exploring the Bergmann pattern in insects (Shelomi, 2012) demonstrated that the studies of species with large latitudinal ranges were more likely to show Bergmann or converse Bergmann clines than those with smaller ranges. The pattern is not surprising, as all the Caribbean island populations of *A. argentata* are distributed in the tropical belt with high temperatures year round. Therefore, the difference in growing season length between lowest and highest latitude Caribbean islands may be negligible, and therefore any size differences undetected.

Although we predicted that altitudinal variation in size would also follow the converse Bergmann's rule, we did not confirm this. In our study, female *A. argentata* changes in body size appear not to be affected by altitude. Although our sampling included a wide range of altitudes (from 0 to 2500 m), these localities were again mostly confined to low

latitudes. A geographically broader sampling is needed to test more rigorously any relationship between female body size and altitude.

5.3.3 Island rule in female *A. argentata*

Our result revealed that the females from continental *A. argentata* populations were significantly larger than the island ones, which may be interpreted as a case of moderate insular dwarfism. However, in our data we did not detect a size decrease with the decrease in island size. Explanations for size difference between mainland and island populations in vertebrates usually relate to island-specific food availability, predator pressures, or inter-species competition (Foster, 1964; Heaney, 1978). However, the causes for patterns in size that relate to the island rule are less clear in invertebrates. Perhaps the sole evidence for the existence of island giants is found in a linyphiid spider clade native to Hawaii, although it is unclear what precisely its closest mainland relatives are (Hormiga, 2002; Hormiga et al., 2003). In beetles, smaller islands may harbor smaller sized animals (Palmer, 2002). To the best of our knowledge, *Argiope* may be the only intraspecific case in spiders that may relate to the island rule.

5.3.4 No patterns in males

Our results fail to relate male size variation to any geographical factors. Any influence of latitude on male size, however, was unlikely to be detected due to insufficient sample sizes, as most of the available museum male specimens were from the Caribbean islands with limited variation in seasonality, and too few were from the mainland. Nonetheless, our result may show at least preliminary implications that size responses to latitude in both sexes differ. This may be a common phenomenon in spiders, as for example in wolf spiders, females show stronger responses to altitude than males (Bowden et al., 2013; Høye and Hammel, 2010; Høye et al., 2009). Whether *A. argentata* exhibits sex-specific responses to latitude and altitude remains to be further studied.

5.3.5 Broader implications

Sexual difference in responses of body size to geographical factors may result in intraspecific, geographical variation of sexual size dimorphism. While Blanckenhorn et al. (2006) showed that males commonly exhibit steeper latitudinal body size clines than females, it seems that it is the opposite in spiders, where females respond more strongly to the environment than males. More precisely, female spiders follow the converse Bergmann's rule, whereas the males, it seems, do not. Female body size may be more sensitive to geographical factors than male body size, since different selection pressures on each sex result in sexual specific growth strategies (Legrand and Morse, 2000; Uhl et al., 2004). In female-biased size dimorphic taxa, the selective advantage of protandry dictates earlier and shorter male development times and higher growth rates (Blanckenhorn et al., 2007).

In some wolf spiders (*Pardosa glacialis*, *P. groenlandica* and *P. lapponica*), the male body size does not change in response to increased altitude but female body size exhibits a significant decline (Bowden et al., 2013; Høye and Hammel, 2010). This sex specific response to altitude results in SSD declines with altitude. On the other hand, in another wolf spider, *P. uintana*, SSD does not change in response to altitude, since both female and male body sizes decline with altitude (Bowden et al., 2013). These sex-specific and species-specific responses to geographical factors may lead to SSD variation between population and species. Whether such patterns are also present in argiopine spiders remains to be further explored. Regardless, our study, discovering a sex-specific response to latitude and a size difference between mainland and island populations provides a novel explanation of SSD variation in a sexually size dimorphic spider.

5.4 STUDY 4: INDEPENDENT EVOLUTION OF GENITAL AND SEXUAL SIZE DIMORPHISM

In arthropods, theory predicts that if low allometric slope values are detected for male and female genital size evolution, such patterns would imply them being driven by sexual selection via cryptic female choice (Eberhard, 2009; Eberhard, 2010). We tested whether this hypotheses is still valid at inter-species levels by examining the genital size

evolutionary patterns in argiopine spiders. Against the predictions from the theory, however, the phylogenetically comparative analyses failed to detect any negative allometric patterns for genital size evolution. Female spermathecal size and male pedipalp size exhibited isometric slope values compared to somatic characters. In female epigynal size evolution, however, a positive allometric slope was detected in tip data, but the slope was not significantly allometric when using contrasts data. Although the female epigynal (external genital character) size evolution was significantly positively correlated with spermathecal (internal genital character), male genital size only significantly positively correlated with the former. In addition, the comparative analyses found no support for the positive, the constant, or the negative genital divergence model.

Low allometric slope values of genital size have been detected in several species at intra-specific levels (Eberhard, 2009; Eberhard et al., 1998; Uhl and Vollrath, 2000). Using the length of median apophysis and conductor as male genital size characters and epigynal length and width as female genital size characters, Eberhard et al. (1998) showed that in *A. trifasciata*, the slope values of genital size in both sexes were significantly lower than those of somatic characters. If genital size evolution in argiopine spiders was under sexual selection via cryptic female choice as found in previous studies (Eberhard et al., 1998), one might also expect to detect similarly low slope values of genital size evolution at the interspecific level. Instead, we only detected isometric patterns for genital size at the subfamily level, resembling recent findings in several species of damselfly (Nava-Bolaños et al., 2014). These results suggest that cryptic female choice does not provide a general explanation for genital size evolution in these two clades. However, the rarity of tests for allometric patterns of genitalia at inter-specific levels precludes generalizations from our study, but rather calls for further comparative exploration.

In argiopine spiders, male genital size coevolves with female external but not internal genital size. The primary function of the female external genitalia, the epigynum, is to receive and direct the male pedipalp during copulation, while the primary function of the female internal genitalia, the spermathecae, is to receive and store sperm from the male (Foelix, 2011). Since male pedipalps directly interact with the epigynum, it is only logical that their sizes are positively correlated. This pattern provides new support for the sexually

correlated genital evolution theory (Kuntner et al., 2009). Most argiopine males are known to break off the embolus (partially or whole) during copulation, and these genital leftover acts as mating plug (Uhl et al., 2010). Since argiopine males only get to mate with one or at the most two females in their lifetime, mating plugs likely function as to prevent female remating with subsequent males. Our results are consistent with this sperm competition scenario, as males must be selected to maintain pedipalp sizes that closely match the female external genital size. However, this scenario would imply sexual selection, but the detected isometry of genital and somatic sizes do not. Such contradictory results are puzzling.

Ramos et al. (2005) tested the three potential evolutionary scenarios of relationship between SGD and SSD using phylogenetically comparative analyses with 16 species of orb-weaving spiders, but their SGD analyses only considered the male genital size on female internal genital size. Because their study revealed no correlation between male pedipalp size and female spermathecal size, it provided support for the positive genital divergence model where SGD increases as SSD increases. They also found that the male to female genital ratio was larger in size monomorphic species compared with size dimorphic ones. They concluded that such SGD divergence may limit the change of SSD. Although the results of our comparative analyses are consistent with a non-correlated male genital and female internal genital size evolution, they nevertheless support a correlated genital size evolution between male and female external organs. Our results also failed to find a significantly positive or negative allometric slope of SGD on SSD using two versions of SGD ratio. However, we argue below that these results cannot be used to support the positive genital divergence model, nor any of the remaining two models.

The constant genital divergence model predicts that SGD remains constant as SSD increases, which means the male and female genitalia increase equally as SSD increases. If this condition was met, the allometric slope value for SGD on SSD would not depart from zero, and this is the pattern found here (Fig. 2). A reasonable expectation of this model is that when SSD increases, genital size increasing rate of one sex is constrained by the other sex, since selection favors a constant ratio of female to male genital size as SSD increases. Following this logic, we would predict detecting a positively correlated genital size

evolution between both sexes and a significant relationship between the genital and body size in one of the sexes, depending on which drives the genital evolutionary trends. However, we detected only in part that male and female genital sizes were indeed positively correlated on the phylogeny. However, against the predictions, non-significantly correlated patterns for male and female genital size over somatic size, as well as SGD on SSD indicate that the genital size evolution in argiopines is independent from SSD evolution.

We conclude that none of the three proposed models that explain the relationships of the genital versus sexual size dimorphism, the positive, the negative, and the constant divergence, seem to hold in argiopine spiders. Theoretical and empirical research of genital evolution needs to redefine these models or find alternatives. What is clear, however, is that genital divergence is independent of the size difference between the sexes.

5.5 GENERAL DISCUSSION ON PHYLOGENY AND TAXONOMY OF ARGIOPINAE

Although this dissertation does not focus on the systematics and taxonomy of argiopines, there are clear implications stemming from the phylogenetic results (Fig. 15). These support three major argiopine clades: 1) *A. trifaciata* + *A. lobata* + African lobed shaped *Argiope*; 2) *A. bruennichi* + *A. aurantia* + *Neogea* + American lobed shaped *Argiope*; 3) *Gea* + other *Argiope*. However, the phylogeny did not unequivocally resolve the relationships among these three clades. Future research on argiopine phylogeny should focus on resolving this problem using next generation sequencing, which will produce vast quantities of nucleotide data. However, although preliminary, these results indicate that *Gea* and *Neogea* are not monophyletic, but rather nest as distinct clades within *Argiope*, and should therefore be synonymized with *Argiope*. In addition, since *A. ocula* does not topologically fit into Argiopinae, the species may need to be removed from the genus *Argiope*. During this dissertation research, we were able to accumulate the material representing 72 species of argiopines through field work, museum work, and international collaborators. Of these, 58 species were included in the molecular analyses, and for others we have been unable to amplify the selected genes. New approaches and resources are

needed to include all argiopine species to form the ultimate phylogeny. In addition, some undescribed species were discovered, such as the cryptic species from Cuba, two *Argiope* and one *Neogea* from Southeast Asia, and two *Gea* from Africa.

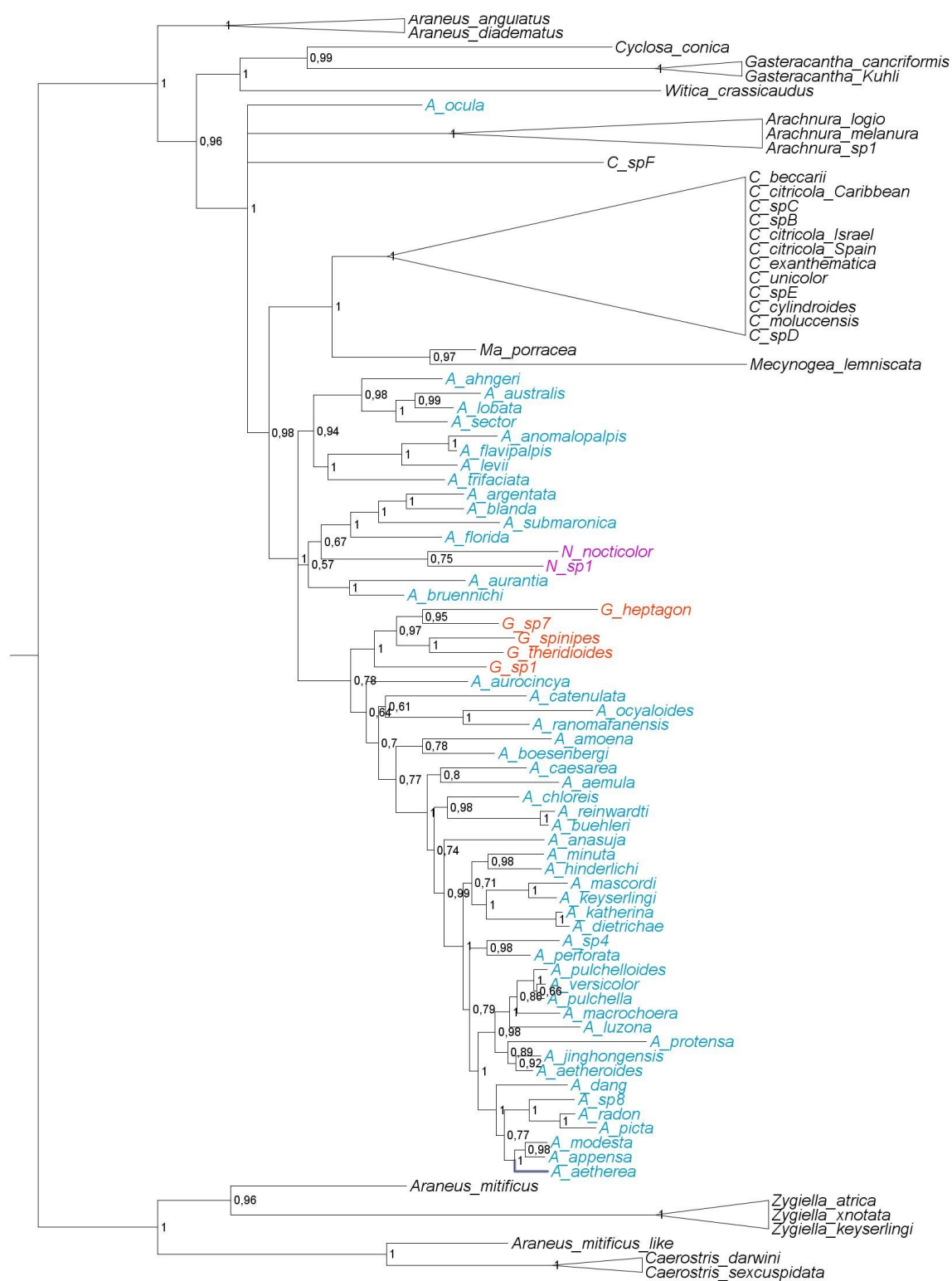


Figure 15. **Konsenzno drevo Bayesovih analiz.** The consensus tree of Bayesian analysis. The number of node indicate Bayesian posterior probabilities. *Argiope* species are colored in blue, *Gea* species are colored in orange and *Neogea* species are colored in purple.

6 CONCLUSION

In this first detailed study of the evolution of sexual dimorphism in Argiopinae, and the first molecular phylogenetic analysis including all three genera, we (1) inferred a robust molecular phylogeny of Argiopinae, (2) examined macroevolutionary patterns of body size and SSD, (3) reconstructed the evolution of sexual shape dimorphism and tested the correlation between SShD and SSD at two hierarchical levels, (4) tested the Bergmann's and the island rule on Neotropical silver argiope, *Argiope argentata*, and (5) compared the allometry patterns of somatic and genital size evolution, and tested the correlation between sexual genital size dimorphism and SSD. The combined results of these different approaches make it possible to address some of the key questions in the evolution of sexual dimorphism in argiopine spiders.

One of the key questions is whether fecundity selection represents the major evolutionary force of SSD and SShD. The female biased SSD in spiders has been believed to result from the interaction of different evolutionary pressures on each sex, and the predicted a pattern of change is a gradual increase in both female size and SSD, and a decoupled size evolution between genders. While such pattern has been demonstrated in nephilid spiders (Kuntner et al., 2009; Kuntner and Elgar, 2014), the comparative analyses in this study in contrast show a non-directional SSD evolution and a correlated size evolution of both sexes. Our results suggest that the ancestor of argiopines was already size dimorphic, and thus push the origin of SSD in this clade to a much earlier point. The subfamily Cyrtophorinae is sister to Argiopinae, and this clade includes both sexually size dimorphic and monomorphic genera. In order to understand the origin and maintenance of SSD in this group, it would be beneficial to clarify which evolutionary forces drive the evolution of female biased SSD.

Counter to predictions from the SSD literature, our study failed to support fecundity selection as a general explanation for argiopine SSD evolution. Instead, detected evolutionary size changes may respond to ecological or geographical factors. The latter can be tested by focusing on size variation within a selected widespread species. We documented a pattern of female body size decreasing with latitude (converse Bergmann's

rule) and with distribution (females on islands were smaller) in *A. argentata*. As in wolf spiders (Bowden et al., 2013; Entling et al., 2010), where previous studies showed the influence of geographic factors on size was sex- and species-specific, sex-specific response in *A. argentata* may also explain a part of intraspecific SSD variation. However, how geographic factors shape the evolution and maintenance of SSD in argiopine spiders needs to be explored in a comparative framework. For example, future studies may focus on the consistency of a particular geographical factor (such as latitude) affecting different species, on how species distribution ranges affect SSD ratios, and on how climatic effects in tropical versus temperate zones affect SSD.

Furthermore, the analyses of sexual shape dimorphism in argiopines suggested that evolution of SShD was related to geographic history rather than fecundity selection and that the selection for male size (or shape) may shape the sexually shape monomorphic cases. Although two specific female abdominal shapes relate to their geographical distribution (lobed shape is confined to Africa and Americas, pentagonal shape to SE Asia), biological significance of different abdominal morphotypes is still unclear. Research demonstrates that web design of argiopine spiders is shaped by the prey visual preference (Cheng et al., 2010), and it may be plausible that different abdominal morphotypes likewise relate to the prey visual preference. This seems a worthwhile, yet unexplored research topic. Considering that cylindrical males were significantly larger than oval ones, future work should address potential benefits of evolutionary shifts from oval to cylindrical abdomen shape in the males.

Another key question that our research addressed is whether sexual selection alone drives genital evolution. Although sexual selection is considered to be the major evolutionary force in genital evolution, our results fail to support this scenario when considering genital size evolution in argiopines. The detected pattern of similar allometric slope values of genital and somatic sizes suggest that genital size is directly related to body size. This implies that selection on body size also drives genital size evolution. While this scenario of genital evolution via natural selection may explain genital size per se, sexual selection may nevertheless play an important role in the evolution of genital complexity and divergence. Although not discussed in this dissertation, our preliminary data suggest that argiopine

male genital complexity varies more than the females', which would imply levels of sexual selection.

Finally, a key question addressed in our dissertation was if different components of sexual dimorphism relate to SSD. In animals, the degrees of SSD may extend to other morphological traits and to behavior. For example, the different mating positions in orb weavers are reported to relate to degrees of SSD (Elgar, 1991). Other examples are extreme mating behaviors in highly size dimorphic lineages, such as mate binding and genital emasculation (Kuntner et al., 2015; Zhang et al., 2011). In orb weavers, it has been demonstrated that sexual genital size dimorphism was phylogenetically positively correlated to SSD (Ramos et al., 2005). At the level of the subfamily Argiopinae, however, our analyses failed to detect any significant correlation between SSD and sexual shape or genital size dimorphism. The detected pattern suggests that different components of sexual dimorphism respond to different evolutionary pressures. Unlike in other extremely sexually size dimorphic spiders, the size evolution between sexes in argiopines is correlated, which indicates that female and male body size may be under the same evolutionary pressure. If other, non-size related components of sexual dimorphism arise through sex-specific selection, the finding that these traits are independent from SSD is logical. Mine is the first study of the correlation between the different components of sexual dimorphism in spiders, and similar research on other clades would benefit our understanding of the general rules governing the evolution of sexual dimorphism.

7 SUMMARY

7.1 SUMMARY

Sexual dimorphism describes substantial differences between male and female phenotypes. How sexual dimorphism evolves, what constitutes it, and what the relationships between its components are has remained elusive. In this dissertation, we investigated the macroevolutionary patterns of sexual dimorphism in Argiopinae spiders by concluding four units of research. In study 1, we examined the evolutionary patterns of sexual size dimorphism (SSD) and tested the validity of general explanations of female biased SSD. In study 2, we explored the relationships between SSD and sexual shape dimorphism (SShD). In study 3, we explored potential geographic factors that may affect intraspecific patterns of SSD in the American *Argiope argentata*. In study 4, we explored over the subfamily scale if and how genital size evolution relates to somatic size in both sexes.

In spiders, sexual dimorphism research almost exclusively focuses on size, and recent studies have recovered steady evolutionary size increases in females, and independent evolutionary size changes in males. Their discordance is due to negative allometric size patterns caused by different selection pressures on male and female size (converse Rensch's rule). In study 1, we investigated macroevolutionary patterns of SSD in Argiopinae, a global lineage of orb weaving spiders with varying degrees of SSD. We devised a Bayesian and maximum likelihood molecular species level phylogeny, then used it to reconstruct sex specific size evolution, to examine general hypotheses and different models of size evolution, to test for sexual size coevolution, and to examine allometric patterns of SSD. Our results, revealing ancestral moderate sizes and SSD, failed to reject the Brownian motion model, which suggests a non-directional size evolution. Contrary to predictions, male and female sizes were phylogenetically correlated, and SSD evolution was isometric. We interpret these results to question the classical explanations of female-biased SSD via fecundity, gravity, and differential mortality. In argiopines, SSD evolution may be driven by these or additional selection mechanisms, but perhaps at different phylogenetic scales.

Many organisms are sexually dimorphic, reflecting sex-specific selection pressures. But although sexual dimorphism may consist of different variables from size to shape and physiology, most research emphasizes a single aspect of sexual dimorphism, notably size, without specifying its components and their relationship. Among terrestrial animals, spiders exhibit most extreme sex-specific differences in size and abdominal shape, and therefore represent ideal models to address this question. In study 2, we dissected sexual dimorphism in spiders at two phylogenetic hierarchical levels. At the species level, we employed comparative phylogenetic tests to explore the association between SShD and SSD in the orbweb clade Argiopinae. At the genus level, we then explored such patterns on a phylogeny of orb weavers. Female argiopines had more diverse abdominal morphotypes than the males and the abdominal shape evolution was only poorly correlated between the sexes. Phylogenetic and comparative data suggested that evolution of sexual shape dimorphism in argiopines was related to geographic history, but that sexually shape monomorphic cases arose through selection for male size, perhaps acting against fecundity selection. While in argiopines there was no clear association between SShD and SSD, we detected a significant correlation in all orb weavers at the genus level. The *shape* and the *size* components of sexual dimorphism may thus respond independently to selection pressures, but at certain phylogenetic levels SSD may be a prerequisite for SShD. Research on other animal groups is needed to establish whether the here detected patterns on spiders are general.

Body size is among the most conspicuous organismal traits, yet in many invertebrates our understanding of inter and intraspecific patterns in body size evolution, and their causes, are poor. Body size variation in ectotherms is thought to follow a converse Bergmann's rule, predicting that individuals of a species or a lineage at higher altitudes or latitudes will be smaller. The island rule, on the other hand, predicts island-related, inter- or intraspecific size shifts. In spiders, patterns of size variation in relation to geographical factors are virtually unknown, and patterns in size evolution are often further complicated by extreme sexual size dimorphism. In study 3, we investigated geographic variation of body size in a widespread, sexually size dimorphic spider, *Argiope argentata*, and tested the above biological rules. Because we were interested in intraspecific patterns of size variation as they relate to geography, we first established the species limits of American populations

using DNA barcodes and tree-based species delimitation methods. These analyses suggested that the Cuban populations belong to a cryptic species distinct from *A. argentata*, and we therefore omitted that population from size analyses. We then examined the effects of latitude, altitude, distribution (continent versus islands) and island size on female and male body size variation in *A. argentata* spanning the Caribbean archipelago and the American mainland. We found a sex-specific pattern of size variation, with female body size decreasing with latitude (converse Bergmann's rule) and distribution, but male size not relating to any geographical factor. However, the negative relationship between female size and latitude did not hold for the island populations. We concluded that sex-specific body size responses to geographical factors may explain parts of the detected intraspecific variation in SSD.

Literature suggests that genital evolution in animals with internal fertilization is under sexual selection and that it is correlated between the sexes. Prior studies on selected spiders, for example, demonstrated that both male and female genital sizes exhibit low allometric slopes, which is strongly indicative of their evolution being driven by cryptic female choice. However, it is unclear whether such pattern also holds at inter-specific levels. Furthermore, while spiders exhibit a wide range of SSD, its relationship with genital size evolution is unknown. In study 4, we tested these hypotheses by performing comparative analyses in Argiopinae. We first examined and compared the allometric patterns of somatic and genital size characters regressed on body size in both sexes, then tested for genital size coevolutionary pattern between sexes, and finally explored the relationship between sexual size and genital size dimorphism. The analyses failed to detect any negative allometry in either male or female genital size evolution. While the slopes of male pedipalp and female spermathecal size evolution were isometric and similar to those of somatic size characters, no significant allometric pattern was found in female epigynal size evolution. Male genital size was only correlated to female external, and not internal genital size, a pattern partially consistent with the sexually correlated genital evolution scenario. The comparative analyses did not find a significant relationship between sexual size and genital size dimorphism. These results fail to support any of the three existing models of how genital divergence relates to somatic size. Although genital and somatic

size evolution are independent in argiopine spiders, genital size changes at the interspecific levels may surprisingly not be driven by sexual selection.

7.2 POVZETEK

Spolni dimorfizem pomeni, da obstajajo znatne razlike med fenotipom samcev in samic. Evolucija spolnega dimorfizma, njegove komponente ter razmerja med njimi so slabo poznana. V svoji disertaciji sem raziskoval makroevolucijske vzorce spolnega dimorfizma pri pajkih skupine Argiopinae in jih združil v štiri sklope. V prvi študiji sem rekonstruiral evolucijske vzorce s spolom pogojenega velikostnega dimorfizma (SSD) ter preverjal ustreznost splošnih razlag za nastanek SSD v korist samic. V drugi študiji sem raziskoval razmerja med SSD in s spolom pogojenega morfološkega dimorfizmoma (SShD). V tretji študiji sem raziskoval geografske dejavnike, ki bi lahko vplivali na intraspecifične vzorce SSD znotraj ameriške vrste *Argiope argentata*. V četrti študiji pa sem raziskoval, če in kako se na nivoju poddružine Argiopinae evolucija velikosti genitalij ujema z velikostjo osebkov obeh spolov.

Raziskave spolnega dimorfizma pri pajkih temeljijo skoraj izključno na velikosti telesa, njihovi rezultati, pa kažejo na vzorce vztrajnega evolucijskega naraščanja velikosti samic ter neodvisno evolucijo velikosti samcev. Njuna neodvisnost je posledica negativnih alometričnih vzorcev velikosti, ki sta odraz različnih selekcijskih pritiskov na velikost samcev in samic (obratno Renschevo pravilo). V prvi študiji sem raziskoval makroevolucijske vzorce SSD v skupini Argiopinae, globalno razširjeni poddružini pajkov z raznolikimi stopnjami SSD. Na podlagi nukleotidnih zaporedij sem izdelal izvirno filogenijo skupine po Bayesovi metodi in metodi največjega verjetja ter jo uporabil za rekonstrukcijo velikosti obeh spolov z namenom preverjanja splošnih hipotez in različnih modelov evolucije velikosti, preverjanja koevolucije velikosti med spoloma ter preverjanja alometričnih vzorcev SSD. Rezultati so pokazali srednje vrednosti velikosti in SSD pri prednikih skupine ter skozi podporo Brownovega modela evolucije nakazali na neusmerjeno evolucijo velikosti. V nasprotju s pričakovanji so bile velikosti samcev in samic filogenetsko korelirane, evolucija SSD pa je bila izometrična. Moja interpretacija rezultatov podaja dvome o klasičnih razlagah evolucije SSD v korist samic skozi selekcijo

povezano s plodnostjo, gravitacijo in spolno specifično smrtnostjo. V skupini Argiopinae morda evolucijo SSD vodijo ti ali dodatni, v študijah nereziskani selekcijski pritiski, vendar verjetno na drugih filogenetskih nivojih.

Mnogi organizmi so spolno dimorfni, kar odraža spolno specifične selekcijske pritiske. Kljub temu, da spolni dimorfizem vključuje raznolike značilnosti, od telesne velikosti do oblike in fiziologije, pa se večina obstoječih raziskav osredotoča predvsem na velikost, medtem ko so druge komponente in njihove povezave v tem pogledu zanemarjene. Med kopenskimi organizmi so največje razlike v obliki in velikosti zadka poznane prav pri pajkih, zato so ustrezni modeli za tovrstne raziskave. V drugi študiji sem razčlenil spolni dimorfizem pajkov na dveh filogenetskih hierarhičnih nivojih. Na vrstnem nivoju sem s pomočjo primerjalnih filogenetskih pristopov testiral povezanost med SShD in SSD v skupini pajkov mrežarjev Argiopinae. Na nivoju rodov sem nato enako povezanost testiral na filogeniji vseh pajkov mrežarjev. Rezultati so pokazali, da imajo samice argiopinov bolj raznolike oblike zadkov kot samci, evolucija teh pa le šibko korelira s spolom. Filogenetski in primerjalni podatki kažejo na povezavo evolucije spolnega dimorfizma oblik z geografsko zgodovino, primere monomorfizma oblikovanosti zadka pa lahko najverjetneje pripišemo selekciji velikosti samcev, ki verjetno deluje v nasprotju s selekcijo na podlagi plodnosti značilni za samice. Znotraj argiopinov ni bilo jasne povezave med SShD in SSD, korelacija pa je bila statistično značilna na rodovnem nivoju. Oblika in velikost kot komponenti spolnega dimorfizma se lahko torej različno odzivata na selekcijske pritiske, vendar je na določenih filogenetskih nivojih SSD lahko predpogoj za SShD. Za splošnost teh vzorcev na pajkih potrebujemo raziskave tudi na drugih organizmih.

Kljub temu, da je telesna velikost med najbolj očitnimi lastnostmi organizmov, je naše razumevanje intra in interspecifičnih vzorcev evolucije velikosti nevretenčarjev, ter njihovih vzrokov, pomanjkljivo. Pri ektotermnih živalih naj bi variacija v velikosti sledila obratnemu Bergmannovemu pravilu, ki predvideva, da bodo osebki alivrste manjši na višjih geografskih širinah oziroma višjih nadmorskih višinah. Pravilo otokov, pa po drugi strani predvideva, da se bo variacija velikosti znotraj vrst in med vrstami prilagajala topografskim, površinskim in klimaskim lastnostim otokov. Pri pajkih so povezave med variacijo v velikosti in geografskimi dejavniki praktično nepoznane, vzorci evolucije

velikosti pa so še posebej prikriti zaradi ekstremnega spolnega velikostnega dimorfizma. V tretji študiji sem raziskoval geografsko variacijo v telesni velikosti pri splošno razširjeni vrsti *Argiope argentata*, z namenom preverjanja zgornjih bioloških pravil. Ker so me zanimali znotrajvrstni vzorci variacije velikosti ter njihova povezava z geografijo, sem najprej analiziral medvrstne meje med ameriškimi populacijami z uporabo DNA črtnih kod in filogenij. Te analize so pokazale, da populacije, ki poseljujejo Kubo, pripadajo kriptični vrsti, ki ne sodi med *A. argentata*, zato sem to populacijo izločil iz nadaljnjih analiz. Na preostalih populacijah *A. argentata*, ki segajo preko Karibov na ameriško celino, sem nato preverjal vpliv geografske širine, nadmorske višine, razširjenosti (kontinent proti otokom) ter velikosti otokov na variacijo telesne velikosti obeh spolov. Rezultat je spolno specifičen vzorec variacije velikosti, ki se pri samicah zmanjšuje z geografsko širino (obratno Bergmannovo pravilo) in razširjenostjo, pri samcih pa ne kaže odvisnosti od geografskih dejavnikov. Vendar pa negativni odnos med velikostjo samic in geografsko širino ne velja za otoške populacije. Sklepamo, da lahko spolno specifični odgovori na geografske dejavnike razložijo del prepoznane znotrajvrstne variacije SSD.

Literatura predvideva, da je evolucija genitalij pri živalih z notranjim oplojevanjem podvržena spolni selekciji ter, da korelira med spoloma. Predhodne študije na izbranih vrstah pajkov so tako pokazale nizke alometrične vrednosti krivulje za genitalij samcev in samic, kar nakazuje na njihovo evolucijo preko mehanizma kriptične izbire spolnih partnerjev s strani samic. Ostaja pa nejasno, ali enak vzorec velja tudi na medvrstnem nivoju. Prav tako ni znano, ali so variacije v SSD pri pajkih povezane z evolucijo velikosti genitalij. V četrti študiji sem preverjal te hipoteze z uporabo primerjalnih metod na skupini Argiopinae. Najprej sem preverjal in primerjal alometrične vzorce znakov, ki se tičejo telesnih in genitalnih velikosti, regresiranih na velikost obeh spolov, nato preverjal koevolucijske vzorce velikosti genitalij med spoloma, nazadnje pa preveril razmerje med SSD in velikostnim dimorfizmom genitalij. Analize niso potrdile negativnih alometričnih vzorcev v evoluciji genitalij obeh spolov. Izračunani krivulji evolucije velikosti samčevega pedipalpa ter samičnih spermatek sta izometrični in podobni krivuljam evolucije telesne velikosti, evolucija velikosti samičine epigine pa ni kazala značilnih alometriji. Velikost samčevih genitalij je korelirala z velikostjo samičnih zunanjih spolnih organov, ne pa tudi notranjih, kar je deloma skladno s scenarijem spolno korelirane evolucije genitalij.

Primerjalne analize niso našle značilnih razmerij med SSD in velikostnim dimorfizmom genitalij. Rezultati tako ne podpirajo nobenega od treh obstoječih modelov o povezavah med evolucijo genitalij s telesno velikostjo. Evolucija velikosti genitalij in teles sta v skupini Argiopinae neodvisni, v nasprotju s pričakovanji pa na medvrstnem nivoju spolna selekcija morda ne vodi sprememb v velikosti genitalij.

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Annex A

Average size (BL) of each sex and size dimorphism index (SDI) used in study 1. The data derived from the literature are marked with superscripted data source. Female and male body length measurements are in millimeters.

Species	Female			Male			SDI
	N	BL	log(BL)	N	BL	log(BL)	
<i>A. aemula</i>	19	16,95	1,23	9	3,98	0,60	3,26
<i>A. aetherea</i>	30	10,16	1,01	9	4,90	0,69	1,07
<i>A. aetheroides</i>	6	14,68	1,17	3	4,83	0,68	2,04
<i>A. ahngeri</i>	-	?	?	1	6,63	0,82	?
<i>A. amoena</i>	8	20,01	1,30	-	5,50 ^{a&b}	0,74	2,64
<i>A. anasuja</i>	9	10,71	1,03	5	2,60	0,41	3,12
<i>A. anomalopalpis</i>	-	?	?	1	4,27	0,63	?
<i>A. argentata</i>	6	13,69	1,14	3	3,72	0,57	2,68
<i>A. aurantia</i>	3	17,57	1,24	1	5,31	0,72	2,31
<i>A. aurocincta</i>	4	11,19	1,05	1	3,78	0,58	1,96
<i>A. australis</i>	4	24,07	1,38	3	6,28	0,80	2,83
<i>A. blanda</i>	2	12,11	1,08	-	3,50 ^c	0,54	2,46
<i>A. boesenbergi</i>	5	14,04	1,15	-	5,50 ^b	0,74	1,55
<i>A. bruennichi</i>	4	17,43	1,24	4	7,25	0,86	1,40
<i>A. catenulata</i>	6	14,43	1,16	1	4,50	0,65	2,21
<i>A. dang (Laos)</i>	2	9,78	0,99	1	3,50	0,54	1,79
<i>A. dang (Singapore)</i>	6	7,37	0,87	5	3,40	0,53	1,17
<i>A. dietrichae</i>	1	9,67	0,99	-	?	?	?
<i>A. flavipalpis</i>	8	16,18	1,21	5	3,59	0,56	3,50
<i>A. jinghongensis</i>	5	11,69	1,07	3	4,26	0,63	1,74
<i>A. katherina</i>	4	11,61	1,06	-	3,60 ^d	0,56	2,23
<i>A. keyserlingi</i>	2	10,35	1,01	10	4,60	0,66	1,25
<i>A. levii</i>	5	16,31	1,21	-	3,20 ^e	0,51	4,10
<i>A. lobata</i>	10	17,86	1,25	7	5,46	0,74	2,27
<i>A. luzona</i>	3	16,27	1,21	1	5,40	0,73	2,01
<i>A. macrochoera</i>	1	8,20	0,91	-	?	?	?
<i>A. mascordi</i>	2	10,30	1,01	-	?	?	?
<i>A. minuta</i>	8	10,85	1,04	2	4,50	0,65	1,41
<i>A. modesta</i>	2	15,95	1,20	-	?	?	?
<i>A. ocula</i>	9	19,55	1,29	10	5,34	0,73	2,66
<i>A. ocyaloides</i>	1	7,26	0,86	1	2,93	0,47	1,48
<i>A. picta</i>	14	15,99	1,20	4	5,03	0,70	2,18
<i>A. protensa</i>	6	13,53	1,13	1	4,70	0,67	1,88
<i>A. pulchella</i>	14	13,15	1,12	4	3,61	0,56	2,65
<i>A. radon</i>	3	15,88	1,20	-	6,00 ^d	0,78	1,65
<i>A. ranomafanensis</i>	3	8,33	0,92	-	1,47 ^e	0,17	4,67

»continued«

»continuation of Annex A. Average size (BL) of each sex and size dimorphism index (SDI) used in study 1«

Species	Female			Male			SDI
	N	BL	log(BL)	N	BL	log(BL)	
<i>A. reinwardti</i>	1	14,52	1,16	1	4,56	0,66	2,18
<i>A. sector</i>	8	19,07	1,28	12	6,22	0,79	2,07
<i>A. sp4</i>	5	9,20	0,96	4	4,34	0,64	1,12
<i>A. submaronica</i>	2	14,35	1,16	1	3,17	0,50	3,53
<i>A. trifaciata</i>	8	17,67	1,25	7	5,58	0,75	2,17
<i>A. versicolor</i>	5	11,75	1,07	-	3,45 ^a	0,54	2,41
<i>C. citricola</i>	12	7,17	0,86	-	2,75 ^a	0,44	1,61
<i>C. exanthematica</i>	4	10,44	1,02	4	3,51	0,55	1,97
<i>C. moluccensis</i>	8	14,17	1,15	4	3,02	0,48	3,70
<i>C. spB</i>	10	11,98	1,08	-	?	?	?
<i>C. unicolor</i>	5	18,53	1,27	-	4,00 ^b	0,60	3,63
<i>G. heptagon</i>	-	5,05 ^c	0,70	-	3,35 ^c	0,53	0,51
<i>G. spl</i>	10	6,58	0,82	-	?	?	?
<i>G. spinipes</i>	13	5,48	0,74	3	3,31	0,52	0,65
<i>G. theridioides</i>	3	6,68	0,83	1	4,25	0,63	0,57
<i>N. spl</i>	1	5,30	0,72	-	1,85 ^a	0,27	1,87

a (Yin et al. 1997); b (Tanikawa 2009); c (Levi 2004); d (Levi 1983); e (Bjørn 1997)

Annex B

The species list used in study 2 with the size and shape measurements. All size measurements are in millimeters. Abbreviations: BL = total body length; TPL = first leg tibia-patella length; CW= carapace width; adL = abdominal length.

Species	FBL	FTPL	FCW	FadL	MBL	MTPL	MCW	MadL	SSD BL	SSD TPL	SSD CW	Fshape	Mshape	SShD
<i>A. aemula</i>	16,89	9,42	5,61	11,80	3,86	2,70	2,16	2,28	4,38	3,49	2,60	Oval	Oval	Monomorphism
<i>A. aetherea</i>	10,22	7,56	3,97	6,64	4,90	3,73	2,60	2,66	2,08	2,02	1,53	Oval	Oval	Monomorphism
<i>A. aetheroides</i>	14,68	10,76	5,75	9,59	4,83	3,63	2,41	2,70	3,04	2,96	2,38	Pentagonal	Oval	Dimorphism
<i>A. ahngeri</i>					6,63	4,62	2,58	3,98				Lobed	Cylindrical	Dimorphism
<i>A. amoena</i>	20,01	10,85	6,94	13,16	5,50				3,64			Oval		
<i>A. anasuja</i>	10,72	6,67	3,89	6,82	2,73	2,03	1,45	1,70	3,93	3,29	2,69	Pentagonal	Oval	Dimorphism
<i>A. anomalopalpis</i>					4,27	2,90	2,09	2,30					Oval	
<i>A. argentata</i>	11,86	7,32	3,74	9,41	3,35	2,43	1,57	2,03	3,54	3,02	2,38	Lobed	Cylindrical	Dimorphism
<i>A. aurantia</i>	16,70	9,40	4,89	11,58	5,53	4,54	1,95	3,30	3,02	2,07	2,51	Cylindrical	Cylindrical	Monomorphism
<i>A. aurocincta</i>	11,19	5,51	4,40	7,46	3,78	2,53	1,98	2,27	2,96	2,17	2,22	Oval	Oval	Monomorphism
<i>A. australis</i>	24,07	12,50	6,80	17,11	6,28	3,91	2,17	3,82	3,83	3,20	3,13	Lobed	Cylindrical	Dimorphism
<i>A. blanda</i>	12,11		3,93	8,30	3,50				3,46			Lobed		
<i>A. boesenbergi</i>	14,04	9,20	5,13	9,93	5,50				2,55			Oval		
<i>A. bruennichi</i>	17,43	9,39	5,14	12,02	7,25	2,60	5,97	4,12	2,40	3,61	0,86	Cylindrical	Cylindrical	Monomorphism
<i>A. catenulata</i>	14,43	8,07	4,45	9,56	4,50	2,50	1,74	2,26	3,21	3,23	2,56	Cylindrical	Cylindrical	Monomorphism
<i>A. dang</i>	7,97	5,44	2,89	5,12	3,41	2,57	1,68	1,94	2,34	2,11	1,72	Cylindrical	Cylindrical	Monomorphism
<i>A. dietrichae</i>	9,67	8,50	4,51	6,41								Oval		
<i>A. flavipalpis</i>	15,71	8,68	5,42	10,80	3,59	2,37	1,78	2,03	4,37	3,66	3,04	Lobed	Oval	Dimorphism
<i>A. jinghongensis</i>	11,69	9,53	4,89	9,84	4,26	3,31	2,12	2,32	2,74	2,88	2,30	Pentagonal	Oval	Dimorphism
<i>A. katherina</i>	11,61	7,25	3,80	8,05	3,60	2,80	2,00		3,23	2,59	1,90	Oval		
<i>A. keyserlingi</i>	10,35	7,20	3,88	6,90	4,60	3,51	2,47	2,55	2,25	2,05	1,57	Oval	Oval	Monomorphism
<i>A. levii</i>	16,31	9,69	5,60	10,75	3,20	2,19	1,70		5,10	4,42	3,29	Lobed		
<i>A. lobata</i>	18,01	8,95	5,51	12,98	5,41	3,57	2,04	3,21	3,33	2,51	2,70	Lobed	Cylindrical	Dimorphism
<i>A. luzona</i>	16,27	8,55	5,62	10,66	4,44	3,57	2,41	2,44	3,66	2,40	2,33	Pentagonal	Oval	Dimorphism
<i>A. macrochoera</i>	8,20	5,68	2,82	5,13								Oval		
<i>A. mascordi</i>	10,30	6,27	3,67	7,04	3,30	2,73	1,80	1,87	3,12	2,30	2,04	Oval	Oval	Monomorphism
<i>A. minuta</i>	10,85	7,72	4,07	6,53	4,50	3,51	2,16	2,62	2,41	2,20	1,88	Oval	Oval	Monomorphism
<i>A. modesta</i>	13,14	8,19	4,59	8,48								Pentagonal		
<i>A. ocula</i>	19,55	12,92	6,71	17,75	5,34	4,47	2,44	3,31	3,66	2,89	2,75	Cylindrical	Cylindrical	Monomorphism
<i>A. ocyaloides</i>	7,26	3,15	2,09	5,18	2,93			1,18	1,73	2,48	1,77	Oval	Oval	Monomorphism
<i>A. picta</i>	15,99	10,03	5,37	9,47	5,03	3,78	2,49		3,18	2,66	2,16	Oval		
<i>A. protensa</i>	13,53	6,62	2,59	9,26	4,70	3,30	1,48	3,04	2,88	2,00	1,74	Cylindrical	Cylindrical	Monomorphism
<i>A. pulchella</i>	12,29	8,26	4,46	8,28	3,57	2,65	1,75	1,90	3,44	3,12	2,54	Pentagonal	Oval	Dimorphism
<i>A. radon</i>	15,88	10,52	5,38	10,34	3,72	3,28	2,01	2,23	4,27	3,21	2,68	Cylindrical	Cylindrical	Monomorphism
<i>A. ranomafanensis</i>	8,33	4,38	2,45	5,72	1,47	0,88	0,68		5,67	4,97	3,60	Cylindrical		
<i>A. reinwardti</i>	14,52	11,98	5,96	8,40	4,56	3,42	2,08	2,57	3,18	3,50	2,87	Pentagonal	Cylindrical	Dimorphism
<i>A. sector</i>	19,00	10,10	6,21	12,64	6,22	4,35	2,49	3,52	3,06	2,32	2,49	Lobed	Cylindrical	Dimorphism
<i>A. sp4</i>	8,72	5,63	3,44	5,61	4,34	3,35	2,18	2,58	2,01	1,68	1,58	Oval	Oval	Monomorphism

»continued«

»continuation of Annex B. The species list used in study 2 with the size and shape measurements.«

Species	FBL	FTPL	FCW	FadL	MBL	MTPL	MCW	MadL	SSD BL	SSD TPL	SSD CW	Fshape	Mshape	SShD
<i>A. submaronica</i>	14,02	8,31	4,28	9,15	3,17	2,38	1,60	1,75	4,42	3,50	2,68	Lobed	Oval	Dimorphism
<i>A. trifaciata</i>	16,48	7,56	5,12	12,28	5,06	3,95	2,01	3,02	3,26	1,92	2,55	Cylindrical	Cylindrical	Monomorphism
<i>A. versicolor</i>	11,75	7,49	3,85	8,18	3,45				3,41			Pentagonal		
<i>G. heptagon</i>	4,53	2,43	1,72	3,36	3,21	2,46	1,59	1,61	1,41	0,99	1,08	Pentagonal	Oval	Dimorphism
<i>G. spl</i>	6,58	3,43	2,70	4,55								Oval		Dimorphism
<i>G. spinipes</i>	5,48	3,23	2,15	3,59	3,31	2,59	1,80	1,82	1,65	1,25	1,19	Oval	Oval	Monomorphism
<i>G. theridioides</i>	6,54	3,32	2,23	4,53	4,25	3,32	2,04	2,47	1,54	1,00	1,09	Oval	Oval	Monomorphism
<i>N. spl</i>	5,30	2,70	2,23	4,23	1,85				2,87			Oval		

Annex C

The genera used in study 2 with SShD, SSD and reference.

Genus	SShD	SSD	Reference
<i>Acanthepeira</i>	Monomorphism	1,38	(Levi, 1976).
<i>Acusilas</i>	Monomorphism	3,30	(Schmidt and Scharff, 2008)
<i>Araneus</i>	Monomorphism	1,57	(Ono, 2009)
<i>Argiope</i>	Dimorphism	3,52	This study
<i>Caerostris</i>	Dimorphism	4,67	(Grasshoff, 1984)
<i>Cyclosa</i>	Monomorphism	1,64	(Ono, 2009)
<i>Cyrtophora</i>	Monomorphism	3,68	(Ono, 2009)
<i>Deliochus</i>	Monomorphism	2,29	From: http://www.findaspider.org.au/find/spiders/186.htm
<i>Gasteracantha</i>	Dimorphism	2,60	(Ono, 2009)
<i>Gnolus</i>	Monomorphism	1,10	(Platnick and Shadab, 1993)
<i>Larinioides</i>	Monomorphism	1,43	(Ono, 2009)
<i>Leviellus</i>	Monomorphism	1,29	(Levi, 1974)
<i>Mangora</i>	Monomorphism	1,53	(Levi, 2007)
<i>Mastophora</i>	Monomorphism	6,86	(Levi, 2003)
<i>Mecynogea</i>	Monomorphism	1,32	(Levi, 1997)
<i>Metepeira</i>	Monomorphism	1,51	(Piel, 2001)
<i>Micrathena</i>	Dimorphism	1,91	(Levi, 1985)
<i>Neoscona</i>	Monomorphism	1,41	(Ono, 2009)
<i>Phonognatha</i>	Monomorphism	1,81	From: http://australianmuseum.net.au/Leaf-curling-Spider
<i>Zygiella</i>	Monomorphism	1,40	(Levi, 1974)
<i>Agyneta</i>	Monomorphism	1,10	(Duperre, 2013)
<i>Australolinyphia</i>	Monomorphism	1,17	(Millidge, 1984)
<i>Bathyphantes</i>	Monomorphism	1,06	(Song et al., 1999)
<i>Bolyphantes</i>	Monomorphism	1,15	(Ono, 2009)
<i>Diplostyla</i>	Monomorphism	1,08	(Heimer et al., 1991)
<i>Drapetisca</i>	Monomorphism	1,00	(Tu and Li, 2006)
<i>Dubiaranea</i>	Monomorphism	1,10	(Millidge, 1991)
<i>Erigone</i>	Monomorphism	1,07	(Ono, 2009)
<i>Floronia</i>	Monomorphism	1,00	(Ono, 2009)
<i>Frontinella</i>	Monomorphism	1,10	(Song et al., 1999)
<i>Gonatium</i>	Monomorphism	1,13	(Ono, 2009)
<i>Gongylidiellum</i>	Monomorphism	1,00	(Ono, 2009)
<i>Helophora</i>	Monomorphism	1,10	(Song et al., 1999)
<i>Labulla</i>	Monomorphism	0,98	(Hormiga and Scharff, 2005)
<i>Laetesia</i>	Monomorphism	1,01	(Millidge, 1988)
<i>Lepthyphantes</i>	Monomorphism	1,07	(Ono, 2009)
<i>Linyphia</i>	Monomorphism	1,06	(Ono, 2009)
<i>Meioneta</i>	Monomorphism	1,02	(Ono, 2009)
<i>Microlinyphia</i>	Monomorphism	1,17	(Ono, 2009)
<i>Nanoa</i>	Monomorphism	1,09	(Hormiga et al., 2005)
<i>Neriene</i>	Monomorphism	1,02	(Ono, 2009)
<i>Notholepthyphantes</i>	Monomorphism	1,10	(Millidge, 1988)
<i>Novafroneta</i>	Monomorphism	1,50	(Blest, 2004)
<i>Oedothorax</i>	Monomorphism	1,08	(Song et al., 1999)
<i>Orsonwelles</i>	Monomorphism	1,17	(Hormiga, 2002)
<i>Ostearius</i>	Monomorphism	1,00	(Ono, 2009)
<i>Pimoa</i>	Monomorphism	1,35	(Xu and Li, 2007)
<i>Pocobletus</i>	Monomorphism	1,00	(Chickering, 1969)

»continued«

Genus	SShD	SSD	Reference
<i>Tenuiphantes</i>	Monomorphism	1,00	(Tu et al., 2006)
<i>Weintrauboa</i>	Monomorphism	1,40	(Ono, 2009)
<i>Alaranea</i>	Monomorphism	1,00	(Griswold, 1997)
<i>Nephila_1</i>	Monomorphism	3,84	(Ono, 2009)
<i>Clitaetra</i>	Monomorphism	1,93	(Kuntner, 2006)
<i>Herennia</i>	Dimorphism	3,73	(Kuntner, 2005)
<i>Nephila</i>	Monomorphism	7,03	(Ono, 2009)
<i>Nephilengys</i>	Monomorphism	3,20	(Kuntner, 2007)
<i>Nephilingis</i>	Monomorphism	5,64	(Kuntner et al., 2013)
<i>Acrobleps</i>	Monomorphism	1,16	(Lopardo and Hormiga, 2008)
<i>Allende</i>	Monomorphism	1,06	(Álvarez-Padilla, 2007)
<i>Anapisona</i>	Monomorphism	1,27	(Platnick and Shadab, 1979)
<i>Azilia</i>	Monomorphism	1,38	(Álvarez-Padilla and Hormiga, 2011)
<i>Chrysometa</i>	Monomorphism	1,24	(Levi, 1986)
<i>Cyrtognatha</i>	Monomorphism	1,35	(Dimitrov and Hormiga, 2009)
<i>Dolichognatha</i>	Monomorphism	1,10	(Smith, 2008)
			From:
<i>Eidmanella</i>	Monomorphism	1,00	http://www.bio.brandeis.edu/fieldbio/Spiders_Savransky_Suhd_Brondstatter/Pages/Nesticidae_Eidmannella_pallida.html
<i>Epeirotypus</i>	Monomorphism	1,37	(Miller et al., 2009)
<i>Gertschanapis</i>	Monomorphism	1,00	(Platnick and Forster, 1990)
<i>Leucauge</i>	Monomorphism	1,55	(Ono, 2009)
<i>Maymena</i>	Monomorphism	1,14	(Miller et al., 2009)
<i>Meta</i>	Monomorphism	1,25	(Ono, 2009)
<i>Metabus</i>	Monomorphism	1,10	(Álvarez-Padilla, 2007)
<i>Metellina</i>	Monomorphism	1,05	(Deltshev, 2011)
<i>Mimetus</i>	Monomorphism	1,46	(Song et al., 1999)
<i>Mollemeta</i>	Monomorphism	1,46	(Álvarez-Padilla and Hormiga, 2011)
<i>Mysmena</i>	Monomorphism	1,32	(Lin and Li, 2013)
<i>Nanometa</i>	Monomorphism	1,33	From:
			http://www.arachne.org.au/01_cms/details.asp?ID=2566
<i>Nesticus</i>	Monomorphism	1,09	(Ono, 2009)
<i>Opadometa</i>	Monomorphism	2,30	(Ono, 2009)
<i>Pachygnatha</i>	Monomorphism	1,11	(Ono, 2009)
<i>Patu</i>	Monomorphism	1,19	(Lin and Li, 2009)
<i>Symphytognatha</i>	Monomorphism	1,10	(Forster and Platnick, 1977)
<i>Synotaxus</i>	Monomorphism	1,29	(Santos and Rheims, 2005)
<i>Tetragnatha</i>	Monomorphism	1,26	(Ono, 2009)
<i>Theridiosoma</i>	Monomorphism	1,29	(Ono, 2009)
<i>Trogloneta</i>	Monomorphism	1,24	(Lin and Li, 2013)
<i>Tylorida</i>	Monomorphism	1,31	(Zhu et al., 2003)
<i>Achaeearanea</i>	Monomorphism	1,70	(Buckup et al., 2012)
<i>Anelosimus</i>	Monomorphism	1,70	(Ono, 2009)
<i>Argyrodes</i>	Monomorphism	1,00	(Ono, 2009)

»continued«

»continuation of Annex C. The genera used in study 2 with SShD, SSD and reference.«

Genus	SShD	SSD	Reference
<i>Ariamnes</i>	Monomorphism	1,80	(Ono, 2009)
<i>Cerocida</i>	Monomorphism	0,90	(Levi, 1963c)
<i>Chrosiothes</i>	Monomorphism	2,00	(Ono, 2009)
<i>Chryso</i>	Monomorphism	1,60	(Ono, 2009)
<i>Coleosoma</i>	Monomorphism	1,01	(Ono, 2009)
<i>Dipoena</i>	Monomorphism	1,00	(Levi, 1963b)
<i>Echinotheridion</i>	Monomorphism	2,10	(Knoflach and Van Harten, 2006)
<i>Enoplognatha</i>	Monomorphism	1,30	(Ono, 2009)
<i>Episinus</i>	Monomorphism	1,10	(Ono, 2009)
<i>Euryopis</i>	Monomorphism	1,25	(Ono, 2009)
<i>Helvibis</i>	Monomorphism	1,30	(Levi, 1964b)
<i>Keijia</i>	Monomorphism	1,30	(Ono, 2009)
<i>Latrodectus</i>	Monomorphism	2,50	(Ono, 2009)
<i>Neottiura</i>	Monomorphism	1,00	(Ono, 2009)
<i>Nesticodes</i>	Monomorphism	1,50	(Ono, 2009)
<i>Pholcomma</i>	Monomorphism	1,00	(Levi, 1964a)
<i>Phoroncidia</i>	Monomorphism	1,50	(Ono, 2009)
<i>Rhomphaea</i>	Monomorphism	1,10	(Ono, 2009)
<i>Robertus</i>	Monomorphism	1,10	(Ono, 2009)
<i>Spintharus</i>	Monomorphism	1,60	(Levi, 1963a)
<i>Steatoda</i>	Monomorphism	1,20	(Ono, 2009)
<i>Styposis</i>	Monomorphism	1,10	(Ono, 2009)
<i>Takayus</i>	Monomorphism	1,30	(Ono, 2009)
<i>Theridion</i>	Monomorphism	1,20	(Ono, 2009)
<i>Thwaitesia</i>	Monomorphism	1,60	(Song et al., 1999)
<i>Thymoites</i>	Monomorphism	1,10	(Levi, 1964c)
<i>Tidarren</i>	Monomorphism	4,76	(Knoflach and Van Harten, 2006)
<i>Polys</i>	Dimorphism	5,57	(Smith, 2006)
<i>Arachnura</i>	Dimorphism	11,00	(Ono, 2009)

Annex D

The average values for the somatic (mm) and genital characters (mm³). The data derived from the literature are marked with superscripted data source. Sexual size dimorphism (SSD) is the female size divided by male size, and the sexual genital size dimorphism (SGD) is the female genital size divided by male genital size.

Abbreviations: BL = total body length; TPL = first leg tibia-patella length; CW= carapace width; CL = carapace length; SV = spermathecal volume; EV =epigynal volume; PV = pedipalp volume.

Species	Female						Male					SSD				SGD	
	Somatic characters				Genital characters		Somatic characters				Genital characters	BL	TPL	CW	CL	SV/PV	EV/PV
	BL	TPL	CW	CL	SV	EV	BL	TPL	CW	CL	PV						
<i>A. aemula</i>	16,66	9,34	5,54	6,64	0,031	0,267	3,86	2,70	2,16	2,28	0,186	4,32	3,46	2,57	2,91	0,17	1,44
<i>A. aetherea</i>	10,22	7,56	3,97	4,68	0,020	0,504	4,90	3,73	2,60	2,77	0,354	2,08	2,02	1,53	1,69	0,06	1,42
<i>A. aetheroides</i>	14,68	10,76	5,75	6,61	0,024	0,712	4,83	3,63	2,41	2,61	0,251	3,04	2,96	2,38	2,53	0,10	2,84
<i>A. ahngeri</i>	13,00 ^a	7,00 ^a	3,40 ^a	4,90 ^a			6,63	4,62	2,58	3,10	0,417	1,96	1,52	1,32	1,58		
<i>A. amoena</i>	20,01	10,85	6,94	8,10	0,070	0,889	5,50 ^b	4,70 ^b	2,90 ^b	3,00 ^b		3,64	2,31	2,39	2,70		
<i>A. anasuja</i>	11,02	6,84	3,99	4,66	0,011	0,133	2,73	2,03	1,45	1,57	0,089	4,04	3,38	2,76	2,97	0,12	1,49
<i>A. anomalopalpis</i>							4,27	2,90	2,09	2,34	0,099						
<i>A. appensa</i>	16,97	8,40	5,87	6,52	0,044	1,987	5,48	4,69	3,20	3,23	0,620	3,10	1,79	1,84	2,02	0,07	3,20
<i>A. argCU</i>	8,11	6,12	2,85	3,44			2,66	1,86	1,25	1,46	0,050	3,05	3,29	2,28	2,36		
<i>A. argentata</i>	11,88	7,33	3,74	4,91	0,014	0,247	3,35	2,43	1,57	1,87	0,109	3,55	3,02	2,38	2,63	0,13	2,27
<i>A. aurantia</i>	16,70	9,40	4,89	6,54	0,013	1,371	5,53	4,54	1,95	2,72	0,304	3,02	2,07	2,51	2,41	0,04	4,51
<i>A. aurocincta</i>	11,93	5,93	4,67	5,09	0,010	0,225	3,78	2,53	1,98	2,22	0,146	3,15	2,34	2,36	2,29	0,07	1,54
<i>A. australis</i>	21,48	11,80	6,28	7,58	0,039	1,307	6,04	4,18	2,17	2,74	0,385	3,56	2,83	2,89	2,76	0,10	3,39
<i>A. blanda</i>	12,11	6,40 ^c	3,93	4,75	0,007	0,118	3,40 ^c	2,10 ^c	1,40 ^c	1,70 ^c		3,56	3,05	2,8	2,79		
<i>A. boesenbergi</i>	14,04	9,20	5,13	6,04	0,049	0,803	5,80 ^d	3,41 ^d	2,63 ^d	2,87 ^d		2,42	2,70	1,95	2,10		
<i>A. bruennichi</i>	16,68	7,83	5,14	6,05	0,019	1,211	5,50	4,10	1,80	2,40		3,03	1,91	2,86	2,52		
<i>A. buehleri</i>	16,01	10,78	5,70	6,49	0,018	0,474											
<i>A. caesarea</i>	18,43	13,68	7,23	8,62	0,041	0,944											
<i>A. catenulata</i>	14,63	8,64	4,55	5,56	0,029	0,520	4,69	2,86	1,98	2,25	0,162	3,12	3,02	2,29	2,47	0,18	3,21
<i>A. chloreis</i>	6,67	3,79	2,42	2,66	0,006	0,034	2,57	1,64	1,17	1,35	0,030	2,59	2,31	2,07	1,98	0,20	1,13
<i>A. dang</i>	7,97	5,44	2,89	3,37	0,009	0,434	3,41	2,57	1,68	1,82	0,095	2,34	2,11	1,72	1,85	0,09	4,57
<i>A. dietrichae</i>	13,50 ^e	10,20 ^e	4,50 ^e	5,30 ^e													
<i>A. flavipalpis</i>	15,71	8,68	5,42	6,34	0,129	0,116	3,59	2,37	1,78	2,01	0,104	4,37	3,66	3,04	3,15	1,24	1,12
<i>A. florida</i>	16,00 ^c	8,70 ^c	8,00 ^c	10,00 ^c			4,50 ^c	4,20 ^c	2,10 ^c	2,20 ^c		3,56	2,07	3,81	4,55		
<i>A. hinderlichi</i>	19,45	11,95	6,80	7,85	0,084	1,355											
<i>A. jinghongensis</i>	11,45	9,44	4,77	5,62	0,033	0,871	4,09	3,22	2,08	2,28	0,248	2,80	2,93	2,29	2,46	0,13	3,51
<i>A. katherina</i>	11,61	7,25	3,80	4,70	0,020	0,234	3,60 ^c	2,80 ^c	2,00 ^c	2,20 ^c		3,23	2,59	1,90	2,14		
<i>A. keyserlingi</i>	10,35	7,20	3,88	4,69	0,014	0,225	4,60	3,51	2,47	2,64	0,214	2,25	2,05	1,57	1,78	0,07	1,05
<i>A. levii</i>	16,31	9,69	5,60	6,41	0,026	0,271	3,60 ^f	2,19 ^f	1,70 ^f	1,80 ^f		4,53	4,42	3,29	3,56		
<i>A. lobata</i>	17,32	8,68	5,21	6,52	0,025	0,931	5,41	3,57	2,04	2,51	0,350	3,20	2,43	2,56	2,6	0,07	2,66

»continued«

»continuation of Annex D. The average values for the somatic (mm) and genital characters (mm³).«

Species	Female						Male					SSD				SGD	
	Somatic characters			Genital characters			Somatic characters				Genital characters	BL	TPL	CW	CL	SV/PV	EV/PV
	BL	TPL	CW	CL	SV	EV	BL	TPL	CW	CL	PV						
<i>A. luzona</i>	16,27	8,55	5,62	6,55	0,031	0,898	4,44	3,57	2,41	3,96	0,294	3,66	2,40	2,33	1,65	0,11	3,05
<i>A. macrochoera</i>	8,20	5,68	2,82	3,38	0,010	0,136											
<i>A. mascordi</i>	10,30	6,27	3,67	3,86	0,014	0,168	3,30	2,73	1,80	1,94	0,149	3,12	2,30	2,04	1,99	0,09	1,13
<i>A. minuta</i>	10,85	7,72	4,07	4,92	0,029	0,365	4,50	3,51	2,16	2,38	0,201	2,41	2,20	1,88	2,07	0,14	1,82
<i>A. modesta</i>	14,11	8,58	4,84	5,50	0,036	1,244	4,76	3,46	2,25	2,36	0,234	2,97	2,48	2,15	2,34	0,15	5,32
<i>A. ocyaloides</i>	7,26	3,15	2,09	2,40	0,001	0,014	2,93		1,18	1,39	0,015	2,48		1,77	1,72	0,07	0,93
<i>A. perforata</i>	7,23	4,37	2,52	2,84	0,012	0,066	5,30 ^b					1,36					
<i>A. picta</i>	16,09	10,14	5,31	6,57	0,031	0,840	5,16	3,89	2,62	2,80	0,476	3,12	2,61	2,03	2,35	0,07	1,76
<i>A. protensa</i>	13,53	6,62	2,59	3,91	0,004	0,425	4,70	3,30	1,48	2,00	0,135	2,88	2,00	1,74	1,95	0,03	3,15
<i>A. pulchella</i>	12,60	8,20	4,58	5,32	0,020	0,349	3,58	2,69	1,77	1,83	0,128	3,52	3,05	2,58	2,91	0,16	2,73
<i>A. pulchelloides</i>	17,66	11,18	6,47	6,83	0,018	0,272	3,47	2,41	1,62	1,75	0,103	5,09	4,65	3,99	3,91	0,17	2,64
<i>A. radon</i>	15,88	10,52	5,38	6,58	0,054	1,174	3,72	3,28	2,01	2,24	0,167	4,27	3,21	2,68	2,94	0,32	7,03
<i>A. ranomafanensis</i>	8,33	4,38	2,45	2,95	0,006	0,041	1,47 ^f					5,67		3,58	3,38		
<i>A. reinwardti</i>	14,49	11,12	5,36	6,06	0,014	0,455	4,50	3,38	2,12	2,39	0,249	3,22	3,29	2,53	2,53	0,06	1,83
<i>A. sector</i>	19,00	10,10	6,21	7,58	0,036	1,368	6,22	4,35	2,49	3,11	0,609	3,06	2,32	2,49	2,43	0,06	2,25
<i>A. submaronica</i>	14,02	8,31	4,28	5,30	0,008	0,092	3,17	2,38	1,60	1,73	0,068	4,42	3,50	2,68	3,06	0,12	1,35
<i>A. trifaciata</i>	16,48	7,56	5,12	5,91	0,016	0,332	5,06	3,95	2,01	2,43	0,160	3,26	1,92	2,55	2,43	0,10	2,08
<i>A. versicolor</i>	12,11	7,62	3,94	4,62	0,015	0,301											
<i>A. sp4</i>	8,51	5,43	3,34	3,76	0,006	0,143	4,34	3,35	2,18	2,26	0,133	1,96	1,62	1,53	1,66	0,05	1,08
<i>A. sp8</i>	12,12	9,00	4,96	5,42	0,039	0,577											
<i>G. heptagon</i>	4,53	2,43	1,72	2,09	0,001	0,032	3,21	2,46	1,59	1,84	0,046	1,41	0,99	1,08	1,14	0,02	0,70
<i>G. spinipes</i>	5,48	3,23	2,15	2,63	0,002	0,029	3,31	2,59	1,80	2,10	0,070	1,65	1,25	1,19	1,25	0,03	0,41
<i>G. theridioides</i>	6,54	3,32	2,23	2,69	0,001	0,035	4,25	3,32	2,04	2,27	0,111	1,54	1,00	1,09	1,18	0,01	0,32
<i>G. sp1</i>	6,61	3,42	2,69	3,10	0,002	0,008											
<i>G. sp7</i>	6,68	2,98	2,58	3,16	0,006	0,041											
<i>N. nocticolor</i>	9,39	5,35	3,20	3,89	0,003	0,067											
<i>N. spl</i>	5,30	2,70	2,23	2,28	0,002	0,039											

a (Tiunov and Esyunin, 2015); b (Schenkel, 1963); c (Levi, 1968); d (Namkung et al., 1994); e (Levi, 1983); f (Bjørn, 1997); g (Yin et al., 2012).