

# The eunuch phenomenon: adaptive evolution of genital emasculation in sexually dimorphic spiders

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## ABSTRACT

Under natural and sexual selection traits often evolve that secure paternity or maternity through self-sacrifice to predators, rivals, offspring, or partners. Emasculation—males removing their genitals—is an unusual example of such behaviours. Known only in insects and spiders, the phenomenon's adaptiveness is difficult to explain, yet its repeated origins and association with sexual size dimorphism (SSD) and sexual cannibalism suggest an adaptive significance. In spiders, emasculation of paired male sperm-transferring organs—secondary genitals—(hereafter, palps), results in 'eunuchs'. This behaviour has been hypothesized to be adaptive because (i) males plug female genitals with their severed palps (plugging hypothesis), (ii) males remove their palps to become better fighters in male–male contests (better-fighter hypothesis), perhaps reaching higher agility due to reduced total body mass (gloves-off hypothesis), and (iii) males achieve prolonged sperm transfer through severed genitals (remote-copulation hypothesis). Prior research has provided evidence in support of these hypotheses in some orb-weaving spiders but these explanations are far from general. Seeking broad macroevolutionary patterns of spider emasculation, we review the known occurrences, weigh the evidence in support of the hypotheses in each known case, and redefine more precisely the particular cases of emasculation depending on its timing in relation to maturation and mating: 'pre-maturation', 'mating', and 'post-mating'. We use a genus-level spider phylogeny to explore emasculation evolution and to investigate potential evolutionary linkage between emasculation, SSD, lesser genital damage (embolic breakage), and sexual cannibalism (females consuming their mates). We find a complex pattern of spider emasculation evolution, all cases confined to Araneoidea: emasculation evolved at least five and up to 11 times, was lost at least four times, and became further modified at least once. We also find emasculation, as well as lesser genital damage and sexual cannibalism, to be significantly associated with SSD. These behavioural and morphological traits thus likely co-evolve in spiders. Emasculation can be seen as an extreme form of genital mutilation, or even a terminal investment strategy linked to the evolution of monogyny. However, as different emasculation cases in araneoid spiders are neither homologous nor biologically identical, and may or may not serve as paternity protection, the direct link to monogyny is not clear cut. Understanding better the phylogenetic patterns of emasculation and its constituent morphologies and behaviours, a clearer picture of the intricate interplay of natural and sexual selection may arise. With the here improved evolutionary resolution of spider eunuch behaviour, we can more specifically tie the evidence from adaptive hypotheses to independent cases, and propose promising avenues for further research of spider eunuchs, and of the evolution of monogyny.

**Key words:** genital mutilation, terminal investment, monogyny, sexual selection, sperm competition, antagonistic co-evolution, sexual size dimorphism, SSD, female gigantism, mate choice, plugging, sexual cannibalism, sexual conflict.

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## I. INTRODUCTION

## (1) General introduction

Under both natural and sexual selection traits are expected to evolve that increase the likelihood of successful transfer of genes to the next generation. Traits securing paternity or maternity often appear to be self-sacrificial, such as selfless parental care defending offspring against predators (Svageľ, Trivellini & Quintana, 2012), the often risky male defence of females against competing males (Milner, Jennions & Backwell, 2010; Kralj-Fišer *et al.*, 2011), and in some cases pure self-sacrifice such as a mother offering herself as food for her offspring (matrphagy) (Schneider, Salomon & Lubin, 2003), or a male offering himself as food for his mate (male complicity to sexual cannibalism) (Andrade, 1996). Emasculation, where males break off their genitals to become eunuchs (Lee *et al.*, 2012) may be seen as an extreme case of organic sacrifice. From the male standpoint, rendering oneself incapable of future mating incurs obvious and immediate costs that must be offset by some positive trade-off. Seemingly maladaptive and ranking amongst

nature's most bizarre evolutionary outcomes, emasculation could nevertheless be explained functionally and adaptively, e.g. if it increased male paternity. However, being known only in very few groups of dipterans (Downes, 1978) and spiders (reviewed here) the phenomenon has received too limited attention, despite its repeated origins suggesting evolutionary significance through common selection pressures. For example, both in ceratopogonine dipterans and orb-web spiders emasculation seems to be associated with female-biased sexual size dimorphism (SSD) and sexual cannibalism (Downes, 1978; Knoflach & van Harten, 2001; Knoflach, 2002; Ramos, Irschick & Christenson, 2004; Agnarsson, 2006; Miller, 2007; Kuntner, Agnarsson & Gregorič, 2009; Kuntner, Coddington & Schneider, 2009; Kralj-Fišer *et al.*, 2011, 2012; Kralj-Fišer & Kuntner, 2012; Lee *et al.*, 2012; Li *et al.*, 2012). The lack of literature reports in other animal clades does not necessarily mean that the phenomenon is not more widespread than currently understood. Furthermore, we do not consider emasculation in plants, which is a consequence of plant–animal interactions (e.g. Hargreaves, Harder & Johnson, 2009), nor in humans, where genital mutilation and castration are 'cultural', not evolutionary

inventions (e.g. Wilson, 2008; Eng, Zhang & Zhu, 2010).

Spiders represent excellent models to study emasculation in an evolutionary context for several reasons. First, our understanding of emasculation in spiders has seen recent phylogenetic, observational and experimental advances (Knoflach & van Harten, 2001; Agnarsson, 2006; Kralj-Fišer *et al.*, 2011; Kralj-Fišer & Kuntner, 2012; Lee *et al.*, 2012; Li *et al.*, 2012). Second, because they possess paired genitalia (Fig. 1A, B), spiders make interesting models for sexual selection research (Eberhard, 2004); specifically in the emasculation context, losing one organ does not mean zero chances to remate. This increases the possible number of scenarios through which males become full or partial (half-) eunuchs, and brings into play strategies that are related to monogyny, and to sperm competition (Miller, 2007; Kuntner *et al.*, 2009b). Third, spider eunuchs are known mostly in groups that are highly sexually dimorphic, and those are the ones where sexual cannibalism is also common (Andrade, 2003; Elgar & Schneider, 2004; Foellmer & Fairbairn, 2004; Kralj-Fišer *et al.*, 2012; Li *et al.*, 2012), and where sperm competition among small males is most severe (Andrade & Kasumovic, 2005; Kuntner *et al.*, 2009c,b). Sexual cannibalism is particularly interesting as a potential evolutionary correlate of emasculation (Li *et al.*, 2012). To simplify, two scenarios can account for sexual cannibalism, one is female excessive aggression [either through aggressive spillover or mate-check (Andrade, 1998; Kralj-Fišer *et al.*, 2012)], and the other is male sacrifice (Andrade, 1996; Schwartz, Wagner & Hebets, 2013). Both have been proposed as important strategies in spiders, and eunuchs have been shown to lose their palpal tarsi including the sperm-transferring organ (the palpal bulb) and the cymbium (Fig. 1A–D), in response to both female and male aggression (Kuntner *et al.*, 2009a). Fourth, at least in some taxa males leave parts of their broken genitals (emboli) lodged deep in the female copulatory tract prior to full emasculation; these embolic leftovers can function as mating plugs (Kuntner, 2005; Kralj-Fišer *et al.*, 2011). In fact, emasculation may be interpreted as an extreme form of genital mutilation (Miller, 2007), below defined as ‘lesser genital damage’. Thus, an intricate interplay of male–female and male–male interactions takes place in spider sexual encounters (Schneider & Andrade, 2011), and eunuchs may play a prominent role in elucidating evolutionary mechanisms pertaining to sexual selection, and more specifically to monogyny in spiders (Fromhage, Elgar & Schneider, 2005; Fromhage, McNamara & Houston, 2008).

## (2) Conventions

Different spider groups perform anatomically different versions of emasculation (reviewed below), but the common theme to all is a predetermined breaking point,

usually between the palpal tibia and tarsus (Fig. 1A–D) (Knoflach, 2002; Kuntner, Coddington & Hormiga, 2008). Thus, the phenomenon in spiders has so far been treated as analogous, if not homologous, with common hypotheses proposed to explain its evolutionary significance (Kuntner, 2005, 2007; Kuntner *et al.*, 2008). By convention, we refer to ‘emasculation’ in spiders when males remove/lose at least the entire sperm-transferring organ — palpal bulb — if not the whole palpal tarsus (Fig. 1A–D) (Knoflach & van Harten, 2001; Knoflach, 2002; Kuntner, 2005, 2007; Kuntner *et al.*, 2009a; Kralj-Fišer *et al.*, 2011; Kralj-Fišer & Kuntner, 2012; Lee *et al.*, 2012; Li *et al.*, 2012). Eunuchs that arise from this action are termed ‘full eunuchs’ if they have lost both palpal tarsi (Fig. 1A), and ‘half eunuchs’ if they have dropped only one but retain the other (Fig. 1B) (Kralj-Fišer *et al.*, 2011; Kralj-Fišer & Kuntner, 2012; Lee *et al.*, 2012; Li *et al.*, 2012). Emasculation in this review thus refers to effective inability to mate in those males without one or both palpal tarsi (Knoflach & van Harten, 2001; Knoflach, 2002; Lee *et al.*, 2012), not to mere breakage/mutilation of the distal palpal sclerite, the embolus (Fromhage & Schneider, 2006; Snow, Abdel-Mesih & Andrade, 2006; Nessler, Uhl & Schneider, 2007; Uhl, Nessler & Schneider, 2007). The latter, termed here ‘lesser genital damage’ (Fig. 1E, F), is a much more widespread and common phenomenon in spiders (Kuntner, 2005; Miller, 2007; Uhl, Nessler & Schneider, 2010; Izquierdo & Rubio, 2011; Schneider & Andrade, 2011) and likely primarily a male strategy to plug females. Other types of sacrifice are known in male spiders such as spontaneous death or behavioural offering by the male to the female. Being linked to emasculation only in a single family, we do not consider these forms of sacrifice as synonymous with emasculation. However, we discuss these biologies in the broader context of monogyny (Section V).

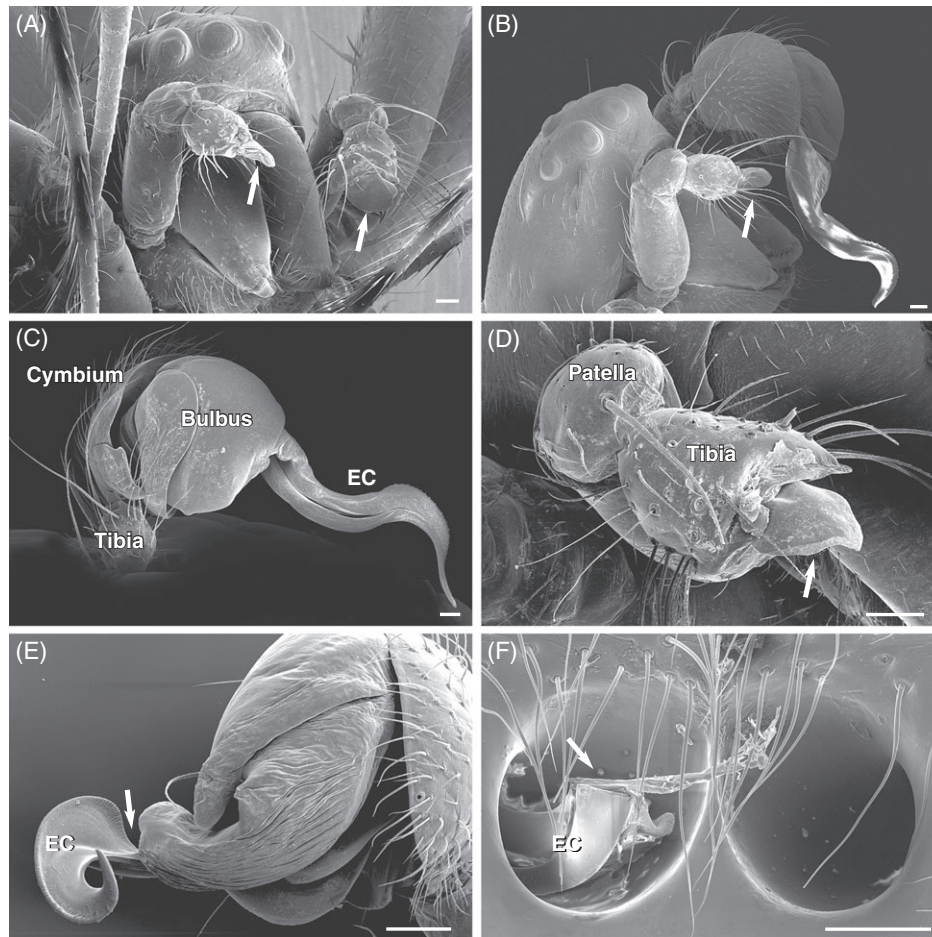
## (3) Emasculation types redefined

Based on the timing of palp severance, this review defines more precisely the types of spider emasculation. We define ‘pre-maturation emasculation’ as palp severance before the final (maturation) moult (Fig. 2) as exemplified by *Tidarren* spp. We define ‘mating emasculation’ as the direct case of palp severance during copulation as exemplified by *Nephilengys malabarensis*. We define ‘post-mating emasculation’ as indirect, voluntary severance of the palp subsequent to copulation as exemplified by *Herennia multipuncta*. For detailed accounts of these behaviours, see Section III.

## (4) Aims

In order to discern among different types, and adaptiveness, of emasculation, we review and redefine the phenomenon more precisely to reflect important differences in various taxa. Several hypotheses have been





**Fig. 1.** The anatomy of nephilid spider emasculation (A–D, *Nephilengys malabarensis*) and lesser genital damage (E, F, *Herennia multipuncta*). (A) A ‘full eunuch’ having severed tarsi of both sperm-transferring organs (palps). (B) A ‘half eunuch’ having severed tarsus of a single palp. (C) Intact palp, its tarsus consisting of the cymbium, bulb and the embolic conductor (EC). (D) Detail of emasculation breakage point between the palpal tibia and tarsus. (E) Detail of intact palp before lesser genital damage (embolic breakage). (F) Result of lesser genital damage—a broken EC plugging a female copulatory opening. Arrows indicate breakage points. Scale bars = 100 µm.

proposed to explain spider eunuch behaviour as adaptive. In Section II, we critically review the evidence in support of and against the (i) plugging hypothesis, (ii) better-fighter hypothesis, (iii) remote-copulation hypothesis, and (iv) other hypotheses. The null hypothesis, finally, is that emasculation is not necessarily adaptive in all cases. We then use up-to-date phylogenies to trace the evolution of spider emasculation and to establish the number of its origins and losses, and use comparative phylogenetic methods to test whether emasculation is evolutionarily associated with SSD, with lesser genital damage, and with sexual cannibalism. If these traits correlate, this would imply possible co-evolution among various biological phenomena, which could all plausibly evolve predictably with extreme female-biased SSD, a male-biased sex ratio, and strategies related to the evolution of monogyny in spiders. Finally, we propose promising avenues for further research focusing on individual evolutionary cases

of emasculation, and more generally on the evolution of monogyny. As with other complex evolutionary phenomena, there may be no single explanation of the causes and underlying forces responsible for the evolution of spider emasculation.

## II. EVIDENCE FOR ADAPTIVE HYPOTHESES

Below and in Table 1 we introduce, and provide a brief review of research that experimentally tested adaptive hypotheses explaining the eunuch phenomenon, and comment on support for each of them.

### (1) Plugging hypothesis

The plugging hypothesis (Kuntner, 2005) sees emasculation as an extension of lesser genital damage



**Fig. 2.** The behaviour leading to, and the anatomy of theridiid spider emasculation (A, B, D–F, *Echinotheridion gibberosum*; C, G, *Tidarren argo*). After moulting, subadult male (A) drops one undeveloped palp (B, arrow) and matures with a single palp (C; arrow points to prior palp severance between trochanter and femur). Adult male (D, E, top) mates with a single female (bottom) and typically has the functional palp severed by the female (arrow). The typical result of emasculation is the bulb and cymbium firmly lodged in the female epigynum (F), and the palp severed between the tibia and tarsus (G). Scale bars = 200  $\mu$ m. All images courtesy of Barbara Thaler-Knoflach.

where terminal ends of palps (emboli/embolic conductors; Fig. 1E, F) function as genital plugs (Robinson, 1982; Uhl *et al.*, 2010), and the mutilated palp is thus permanently disfigured, and cannot be used for further mating (Kuntner *et al.*, 2009c). Therefore, males may remove it entirely using predetermined weak points. This hypothesis was based on legacy literature observations of *Nephilengys papuana* (as *N. malabarensis*) males voluntarily severing their palps, post-mating, after

having irreversibly disfigured them during copulation (Robinson & Robinson, 1980). We refer to this scenario as post-mating emasculation. However, newer literature reported that palp severance may be direct, i.e. during mating, leaving no time for disfiguring only a part of the palp, but rather leaving the female with the entire palpal bulb stuck in her copulatory openings (Kuntner *et al.*, 2009a). This does not mean, however, that the male palp cannot function as copulation barrier, as even



Table 1. Research that established emasculation in various spider taxa and proposed or experimentally tested adaptive hypotheses

Clade	Species	Full or half eunuchs	Timing of emasculation	Plugging	Better fighter	Remote copulation	Sources
'zygiellids'	<i>Deliochus</i> spp.	Full	Unknown	Likely	Unknown	Unknown	Kuntner <i>et al.</i> (2008)
'zygiellids'	<i>Leviellus thorelli</i>	Half	Post-mating	No	Unknown	n/a	Kralj-Fišer <i>et al.</i> (2013)
'zygiellids'	<i>Phonognatha melanopyga</i>	Full	Unknown	Likely	Unknown	Unknown	Kuntner <i>et al.</i> (2008)
Araneidae	<i>Acusilas</i> spp.	Half	Unknown	Unknown	Unknown	Unknown	Kuntner <i>et al.</i> (2008)
Araneidae	<i>Argiope</i> spp.	Half	Unknown	Likely	Unknown	Unknown	This paper
Araneidae	<i>Cerostris darwini</i>	Half	Mating	Likely	Unknown	Unknown	Kuntner & Agnarsson (2010)
Nephilidae	<i>Herennia</i> spp.	Full	Post-mating	Yes	Yes	n/a	Kuntner (2005), Kuntner <i>et al.</i> (2009c) and M. Kuntner, U. Pristovšek & S. Kralj-Fišer
Nephilidae	<i>Nephilengys malabarensis</i>	Full	Mating	Yes	Yes	Yes	Kuntner (2007), Kralj-Fišer <i>et al.</i> (2011), Li <i>et al.</i> (2012) and Lee <i>et al.</i> (2012)
Nephilidae	<i>Nephilengys</i> spp.	Full	Mating	Yes	No	Likely	Kuntner (2007), Kuntner <i>et al.</i> (2009), Kuntner & Agnarsson (2011) and Kralj-Fišer & Kuntner (2012)
Theridiidae	<i>Echinotheridion</i> spp.	Full	Pre-maturation and mating	Yes	Unknown	Likely	Knoflach (2002) and Knoflach & Van Harten (2006)
Theridiidae	<i>Tidarren</i> spp.	Full	Pre-maturation and mating	Yes	Unknown	Likely	Knoflach & Benjamin (2003) and Knoflach & Van Harten (2000, 2001, 2006)

in those females that removed the palp, terminal sclerites may still remain lodged inside the female tract, perhaps functioning as paternity protection plugs (Kuntner *et al.*, 2009a; Kralj-Fišer *et al.*, 2011).

There is good evidence in support of the plugging hypothesis in those cases where males become eunuchs during mating and post-mating. Early (non-experimental) evidence came from morphological examinations of female *Herennia* spp.: the plugs consisting of the massive male embolic division lodged in female genital opening (and extending well to the outside, thus highly conspicuous, see Fig. 1F) were always single per copulatory opening (Kuntner, 2005), which suggested the inability of the subsequent male to lodge another plug, a strategy typical of some *Nephila* species, for example. Experimental evidence for the plugging hypothesis in species known for eunuchs is relatively recent. By staging mating and remating trials in *Nephilengys malabarensis*, Kralj-Fišer *et al.* (2011) showed that plugs consisting of the entire or a part of the male palp lodged in female genitals were effective in 75% of cases, meaning a solid prevention of subsequent male reuse of the same opening. Trials with *Nephilengys livida* produced similar results, although the plugging effectiveness was slightly lower at 64% (Kralj-Fišer & Kuntner, 2012), a difference that the authors attributed to significantly different genital anatomy between *Nephilengys* (as *malabarensis*) and *Nephilengys* (as *livida*). In *Herennia multipuncta*, known for eunuchs, the extensive male plugs were fully successful in preventing female remating, while in *Nephila pilipes*, a species with no eunuchs, the plugs were ineffective (Kuntner *et al.*, 2009c). This available evidence in nephilids suggests that eunuchs who plug, also more successfully enforce female monandry, while in *Nephila* spp. the females are highly polyandrous. In theridiids, however, the ability of females to remove the male palp from her genitalia allows for her remating (Knoflach & van Harten, 2001), and thus the effectiveness of plugging by eunuchs varies across spiders.

The behaviours resulting in male eunuchs seem to be directly linked with sperm competition mechanisms that result in higher mating success of those males that undertake plugging female genital openings with male genital parts. In nephilids, the plugging hypothesis receives solid support, but plugging effectiveness varies among genera, which is an expected outcome under the antagonistic co-evolution scenario (Kuntner *et al.*, 2009b), where females may be expected to evolve counter-adaptations to effective male plugs.

## (2) Better-fighter hypothesis

Several authors (Robinson & Robinson, 1980; Kuntner, 2005) noted that *Herennia* spp. eunuchs persist around the female post-emasculation and hypothesized that they continue mate guarding through male–male fights. Along these lines, the 'better-fighter hypothesis'

(Kralj-Fišer *et al.*, 2011) posits that palpless eunuchs will possess superior contest abilities compared with intact males. Note that this hypothesis does not necessarily explain the proximate cause of such hypothesized differences in fighting ability. In sexually highly dimorphic spider groups, paired palps represent a relatively large portion of the male's overall mass and may thus represent an evolutionary conflict (Ramos *et al.*, 2004). Therefore, those males that drop one or both palps may be able to achieve higher agility (Ramos *et al.*, 2004; Kuntner *et al.*, 2009a), and the edge in male–male contests. Thus, a proximate explanation for the better fighting abilities is the ‘gloves-off’ hypothesis (Kuntner *et al.*, 2009c) predicting enhanced physical stamina in emasculated eunuchs (Ramos *et al.*, 2004; Kralj-Fišer *et al.*, 2011; Lee *et al.*, 2012).

The better-fighter hypothesis was first tested in *Nephilengys malabarensis*. Kralj-Fišer *et al.* (2011) performed male–male contests in the presence of a female, where one of the males was (half) eunuch and the other was intact, virgin. The results were overwhelmingly in support of the hypothesis as the *Nephilengys malabarensis* eunuchs were more aggressive, initiated more fights, and were superior fighters. However, contrary to predictions, repeating these tests in *Nephilengys* (as in *Nephilengys livida*) failed to recover the same pattern, and this second study rather concluded that *Nephilengys* eunuchs are less aggressive and do not show superior fighting abilities (Kralj-Fišer & Kuntner, 2012). As much as the better fighter hypothesis is intuitively satisfying – already mated and effectively sterile males have little to lose but much to gain from initiating rivalry – (Kralj-Fišer *et al.*, 2011; Kralj-Fišer & Kuntner, 2012), it does not seem to be general.

As to what proximate mechanisms may be responsible for eunuchs being better fighters, if at all, the gloves-off hypothesis was recently tested in *N. malabarensis* (Lee *et al.*, 2012). The logical prediction was that due to decreased overall mass after emasculation, palpless males would be more agile and show higher levels of physical endurance compared with intact ones. This prediction received strong support: half-eunuchs and full eunuchs were 4 and 9% lighter than the intact males, respectively, and their corresponding endurance (time till exhaustion) increased by 32 and 80%, respectively (Lee *et al.*, 2012). Similarly, in *Tidarren sisypoides*, a single palp that is voluntarily amputated by the male represents a 10% mass loss, and his maximum speed correspondingly increased by 44%, his total endurance by 63%, and the total travelled length before exhaustion by a dramatic 300% (Ramos *et al.*, 2004). The gloves-off, or agility hypothesis, thus seems to be strongly supported.

### (3) Remote-copulation hypothesis

This intriguing hypothesis, which predicts that the severed palp may continue to transfer sperm, was proposed for the theridiids *Tidarren cuneolatum* and

*Echinotheridion gibberosum* (Knoflach & van Harten, 2001; Knoflach, 2002). Understanding better the process of direct, during-mating emasculation in nephilids shed new light on the eunuch paradox (Kuntner *et al.*, 2009a), and thus the remote-copulation hypothesis was tested, and corroborated, in *Nephilengys malabarensis* (Kralj-Fišer *et al.*, 2011; Li *et al.*, 2012). Through continuous sperm transfer, males should gain a higher share of paternity after darting away from an aggressive female, or even after having fallen victim to sexual cannibalism, in some cases through self-sacrifice.

As predicted (Kralj-Fišer *et al.*, 2011), the test study found strong evidence for remote copulation *via* a severed male palp (Li *et al.*, 2012). These authors varied the time elapsed from palp severance during mating of virgin spiders, and its experimental removal, and measured the quantity of sperm remaining in both the male and female genital tract (Li *et al.*, 2012). Males were only able to transfer 28–33% of sperm before palp breakage. However, as predicted, the severed palp lodged in the female epigynum continued to transfer sperm, and the amount of transferred sperm was positively correlated with the time of continuous palp attachment to the female genitals (Li *et al.*, 2012). Furthermore, perhaps unexpectedly, the amount and speed of continuous sperm transfer were higher in the cases where palp severance was initiated by the female compared with those cases where it was initiated by the male (Li *et al.*, 2012). Although these levels of variation in remote copulation are difficult to interpret, they may represent antagonistic responses to sexual conflict. Female control of mating may be a mate-check (Kralj-Fišer *et al.*, 2012), and her early aggressive termination of mating may indicate to the male that a cannibalism attempt is imminent, and that the sperm need to be rapidly transferred. If true, the mechanism behind such male control of the speed of remote copulation is not understood. Regardless, the evidence for the remote-copulation hypothesis in *Nephilengys malabarensis* is strong, while we lack any tests in other spiders known for eunuchs. Of course, the hypothesis only logically applies to those taxa that engage in mating, not post-mating, emasculation.

### (4) Other hypotheses

Since at least in *Herennia* spp. (and in part in *Nephilengys* spp.) emasculation follows partial palpal mutilation and is thus a post-mating, voluntary act, Kuntner (2005) speculated that palp severance may be advantageous for the male in order to stop haemolymph leakage from the previously mutilated organ. This hypothesis has not been experimentally tested.

Finally, a null hypothesis would state that emasculation serves no function at all (Kuntner, 2005). Since there is at least partial support for hypotheses 1–3 (Table 1), the null hypothesis is generally rejected. However, it would be interesting to examine more closely adaptive differences in emasculation-related traits between

phylogenetically differently placed taxa that exhibit evolutionary origins *versus* maintenance of such behaviour.

### III. REVIEW OF KNOWN SPIDER EMASCULATION CASES

Emasculation has been reported in the literature only for the nephilid genera *Herennia*, *Nephilengys* and *Nephilingis*, the theridiid genera *Echinotheridion* and *Tidarren*, and sporadic genera within the broadly defined Araneidae (*Acusilas*, *Caerostris*, *Deliochus*, *Leviellus*, *Phonognatha*). We here review these cases and report its likely prevalence also in certain species of *Argiope*, also in Araneidae. While all other cases may represent optional strategies, judging from the lack of reports and from examined museum specimens, emasculation in certain nephilids and theridiids seems to be common if not obligate. We have no doubt that emasculation will also be discovered in other genera or families. However, for several taxa we can claim that emasculation does not take place as their mating biology has been subject to intense morphological and behavioural studies, e.g. most species of *Nephila* s.l. (Schneider *et al.*, 2000; Schneider, Thomas & Elgar, 2001; Schneider & Elgar, 2002, 2005; Schneider, Fromhage & Uhl, 2005; Fromhage & Schneider, 2005*a,b*, 2006; Fromhage, Jacobs & Schneider, 2007; Kuntner *et al.*, 2009*b,c*, 2012; Schneider & Michalik, 2011; Zhang, Kuntner & Li, 2011) and many species of diverse theridiid genera (Knoflach, 2004; Agnarsson, 2004*a*).

#### (1) Nephilidae

Robinson & Robinson (1980, pp. 42–43) observed male *Nephilengys papuana* (as *N. malabarensis*) deliberately severing their bulbs after having mutilated them during mating: ‘... (The mated) “Male now has one pedipalp in which the embolus is all twisted and points permanently forwards, bulbus (?) extruded ... (12 minutes later) male bites off one pedipalp, the used-up one, wraps it ... in jaws ... and hangs it from the web.” After another such presumed copulation, we saw the male repeatedly groom at the disfigured pedipalp with the tarsi of legs I and II as though trying to push the end off. This male was subsequently captured, and overnight, in a holding vial, he removed this part.’

At the time, the significance of eunuchs was not understood, although the authors noted that eunuchs remain around the female further to guard her from rivals. Until recently, the nephilid process of palp severance was believed to follow the above scenario, thus be voluntary, males removing disfigured palps after mating (Kuntner, 2005, 2007; Kuntner *et al.*, 2008).

Today, the nephilid eunuch phenomenon is much better recorded and understood. Our own examinations of hundreds of specimens have established the absence

of eunuchs in all *Nephila* s.l. and in all *Clitaetra* species whose males are known (Kuntner, 2006; Kuntner *et al.*, 2008, 2009*b*), and the commonness of eunuch males in both *Nephilengys* species, in all *Nephilingis* species, and in those *Herennia* species whose males are described (Kuntner, 2005, 2007; Kuntner *et al.*, 2008, 2009*b*). Thus, as discussed below, it is likely that emasculation in nephilids is uniform within the three genera.

#### (a) *Herennia*

A taxonomic revision of *Herennia* (Kuntner, 2005) described several new species for which males are mostly unknown, but for those species with known males it confirmed the presence of eunuchs. Subsequently, Kuntner *et al.* (2009*c*) established that palp removal in *H. multipuncta* takes place post-mating, confirming Robinson & Robinson’s (1980) report for *Nephilengys papuana*. Through finding that genital plugs prevent female remating, these authors also found support for the plugging hypothesis in *Herennia* spp. (Kuntner *et al.*, 2009*c*). Work is currently underway testing to what extent predictions from the other adaptive hypotheses apply to *Herennia* spp. eunuchs (M. Kuntner, U. Prisotšek & S. Kralj-Fišer).

#### (b) *Nephilengys*

Until recently, *Nephilengys* was believed to be a globally distributed genus of highly sexually dimorphic nephilid spiders with a characteristic semi-arboreal web with retreat, less elongate female abdomen compared with *Nephila*, and with male eunuch behaviour (Kuntner, 2007; Kuntner *et al.*, 2008; Kuntner & Agnarsson, 2011). However, new phylogenetic evidence (Kuntner *et al.*, 2013) is fairly conclusive about the name actually applying to species falling into two parallel clades. Within true *Nephilengys*, only two species are known, the S and SE Asian *N. malabarensis* and the Australasian *N. papuana*. In both, eunuch males are common (Kuntner, 2007).

*N. malabarensis* eunuch behaviour is the best understood among all spiders. More detailed investigations could only corroborate Robinson & Robinson’s (1980) descriptions of post-mating palp severance in the minority of cases, but rather suggest that direct severance during mating is by far more common; 87.5% of mating cases resulted in direct emasculation while mating, and only 12.5% resulted in partial palpal damage and post-mating severance (Kralj-Fišer *et al.*, 2011; Li *et al.*, 2012). Those males that severed palps directly during mating did so either voluntarily or in response to female aggression (Li *et al.*, 2012), which often ends with the male being cannibalized (Kralj-Fišer *et al.*, 2011). Typically, the whole palp bulb remains lodged in the female tract, which both plugs her genital opening (the one used), and enables the sperm to be continuously transferred in the absence of the male (Li *et al.*, 2012). The surviving half- and full eunuchs remain around the



female and aggressively guard her by chasing away rival males in fierce attack displays (Kralj-Fišer *et al.*, 2011). These eunuchs are not only more aggressive, but were also shown to be more agile and have higher endurance compared with intact males (Lee *et al.*, 2012), which may give them the edge in male–male competition. It thus seems that all hypotheses for eunuch adaptiveness are supported in *N. malabarensis*.

### (c) *Nephilingis*

The genus *Nephilingis* Kuntner, 2013 was created for African and W Indian Ocean species formerly placed in *Nephilengys* in order to reflect genealogy (Kuntner *et al.*, 2013). As in *Nephilengys*, all *Nephilingis* species are known for eunuch behaviour (Kuntner, 2007). The prevalence of eunuch males in nature is about 50% (Kuntner *et al.*, 2009a), but because this estimation includes also unmated males, the real eunuch prevalence among mated males must be substantially higher. It was in *N. borbonica* that direct emasculation during mating was first observed (Kuntner *et al.*, 2009a), which set the phenomenon apart biologically from the post-mating emasculation in *Herennia* spp. Kuntner *et al.* (2009a) reported that direct palpal loss may co-occur with male or female aggressiveness towards the copulating male. Since there were no museum records of females with whole palp plugs, the authors hypothesized that plugged females (or subsequent males) must be able to remove cumbersome whole-palp plugs subsequent to mating, perhaps causing another breakage of the embolus within her tract, or that the plug simply falls off with time.

While these preliminary findings triggered intensive research into several species, a study of the sexual biology of *Nephilingis livida* (then *Nephilengys livida*) from Madagascar (Kralj-Fišer & Kuntner, 2012) found it to differ substantially from its ‘congener’ *N. malabarensis* (Kralj-Fišer *et al.*, 2011). For example, counter to predictions, eunuch *N. livida* were not found to be more aggressive in fights with intact males. This difference in comparison with *N. malabarensis* was explained by lower population densities and relaxed male–male competition in *N. livida*, and their more complex genital anatomy increasing genital plug effectiveness (Kralj-Fišer & Kuntner, 2012). The differences are less suprising considering the subsequent classification of these species into two distinct and unrelated genera (Kuntner *et al.*, 2013) whose eunuch behaviour apparently evolved in parallel (see below). We predict that support for some but not all adaptive hypotheses will be typical of all *Nephilingis* species.

Kuntner *et al.* (2009a) also noted that all emasculated male *N. borbonica* were full eunuchs, which may imply that these males mate with a single female using, and sacrificing, both palps. If true, this would be another difference in sexual biology between *Nephilingis* and the better understood *Nephilengys* and *Herennia*, where both

full- and half eunuchs coexist. This would imply a strictly monogynous system in *Nephilingis* spp.

### (2) Theridiidae

Emasculation is known in several representatives of two highly sexually dimorphic genera, *Tidarren* and *Echinotheridion* (Knoflach & van Harten, 2001, 2006; Knoflach, 2002; Agnarsson, 2006). Uniquely in these genera, a first emasculation resulting in one-palped males occurs prior to maturity (Fig. 2A, B). Subadult males affix one palp in their web and then rotate around the palp until it twists off (Branch, 1942; Knoflach & van Harten, 2000, 2001; Knoflach, 2002; Knoflach & Benjamin, 2003). Also uniquely, the males typically display ‘auto-cannibalism’ by consuming their own detached palp. The males then moult to adulthood retaining only one palp (Fig. 2C), which they charge, find and mate with a female (Fig. 2D), and typically die during copulation, in some cases instantaneously upon genital contact (Knoflach, 2002). The female is cataleptic as copulation starts, but comes around in a few minutes and the dead male is then typically cannibalized by the female (Fig. 2E), although females of at least one *Tidarren* species appear not to cannibalize males (Knoflach & Benjamin, 2003). Before, or during cannibalism, the female twists the dead male body thus detaching the second palp, which remains attached to the female genitalia (Fig. 2E), and probably continues sperm transfer through ‘remote copulation’. The detached bulb and cymbium form a temporary mating plug (Fig. 2F), but females are able to remove it, and typically do so within hours of terminating copulation, and may subsequently remate with other males (Knoflach & van Harten, 2001). The anatomy of *Tidarren* spp. and *Echinotheridion* spp. eunuchs resembles that of nephilids (but not *Caerostris darwini*) in eunuch males typically retaining the tibia but not the cymbium (Fig. 2G). However, the precise breaking point in theridiid eunuchs may sometimes be more proximal on the palp and thus varies more than in nephilids (Knoflach, 2002). This mating behaviour is not unlike male sacrifice in widow spiders *Latrodectus hasselti* (Andrade, 1996), except that widow males retain both functional palps.

Ramos *et al.* (2004) proposed an intriguing hypothesis, that developmental male severance in *Tidarren sisyphoides* reduces male mass significantly enough for them to become more agile (see also gloves-off hypothesis). Agnarsson (2006) reviewed the emasculation phenomenon in *Tidarren* and *Echinotheridion*, and found weak phylogenetic evidence refuting the hypothesis that these genera are each other’s closest relatives. Hence, it appears that emasculation is convergent in the two genera. We revisit this problem below.

Michalik *et al.* (2010) showed that in *Tidarren argo*, the extreme monogynous strategy is accompanied by a developmental cessation of sperm production termed

permanent sperm depletion, and together with emasculation, these traits may represent an evolutionary ‘dead end’ (Michalik & Rittschof, 2011). Some additional facts may be worth noting here. First, *Tidarren*, also known for remarkable spermatozoan morphology (T. argo; Michalik *et al.*, 2005), probably represents a case of evolutionary male dwarfism in addition to female gigantism (Hormiga, Scharff & Coddington, 2000), which may further hint at strong male adaptations in this lineage. Second, the evolutionary association of emasculation and permanent sperm depletion seems to gain further support from a study on the theridiid genus *Latrodectus* where neither trait is present despite strong female-biased SSD and male terminal investment strategies (Modanu, Michalik & Andrade, 2013).

An unknown theridiid from Singapore has been observed with both intact and eunuch males (S. Zhang, personal communication), which would imply the presence of a different type of emasculation than known in the theridiids discussed above.

### (3) Araneidae

Although never tested experimentally, eunuchs have been reported in at least some species of the araneid genera *Acusilas*, *Argiope*, *Caerostris*, *Deliochus*, *Leviellus*, and *Phonognatha*. All except *Caerostris* perform palp severance at the precisely homologous weak point between the palpal tibia and tarsus, as nephilids do (Kuntner *et al.*, 2008; Kuntner & Agnarsson, 2010). According to the available museum material, several species of the Australian genus *Deliochus* sever their palps, and thus the behaviour may be uniform within the genus, which exhibits moderate SSD (Kuntner *et al.*, 2008). Its sister genus, *Phonognatha* with Australasian species ranging from almost monomorphic to highly dimorphic, only exhibits eunuchs in the highly dimorphic *P. melanopyga* and not in others such as the common, and less dimorphic, *P. graeffei* (Kuntner *et al.*, 2008). *Acusilas* is largely unstudied, but eunuchs have been recorded in some specimens (Kuntner *et al.*, 2008).

Kralj-Fišer *et al.* (2013a) also established eunuchs in 2 out of 17 mated *Leviellus thorelli* males. Subsequent to mating and separation from females, they found half-eunuchs along with their severed palps, implying post-mating palpal removal resembling that in *Herenia* spp.

Although *Argiope* males are famous for breaking distal parts of their palps (emboli) during copulation (Nessler *et al.*, 2007; Uhl *et al.*, 2007; Ghione & Costa, 2011), we are not aware of literature records of eunuchs. However, we have examined half-eunuch males in collections of at least the following species: *A. lobata*, *A. sector*, *A. aetherea*, *A. flavipalpis*, and *A. pulchella* (R.-C. Cheng & M. Kuntner, personal communication). Since *Argiope* palps are huge and complex relative to other araneids, we find it likely that eunuch behaviour will be further documented in many more species.

That *Caerostris darwini* males sometimes break their palp at an unusual weak point between the palpal cymbium and the bulb (Kuntner & Agnarsson, 2010), unlike all other known eunuchs, may suggest that in *C. darwini* emasculation is not homologous to the case in other araneids.

### (4) Other cases

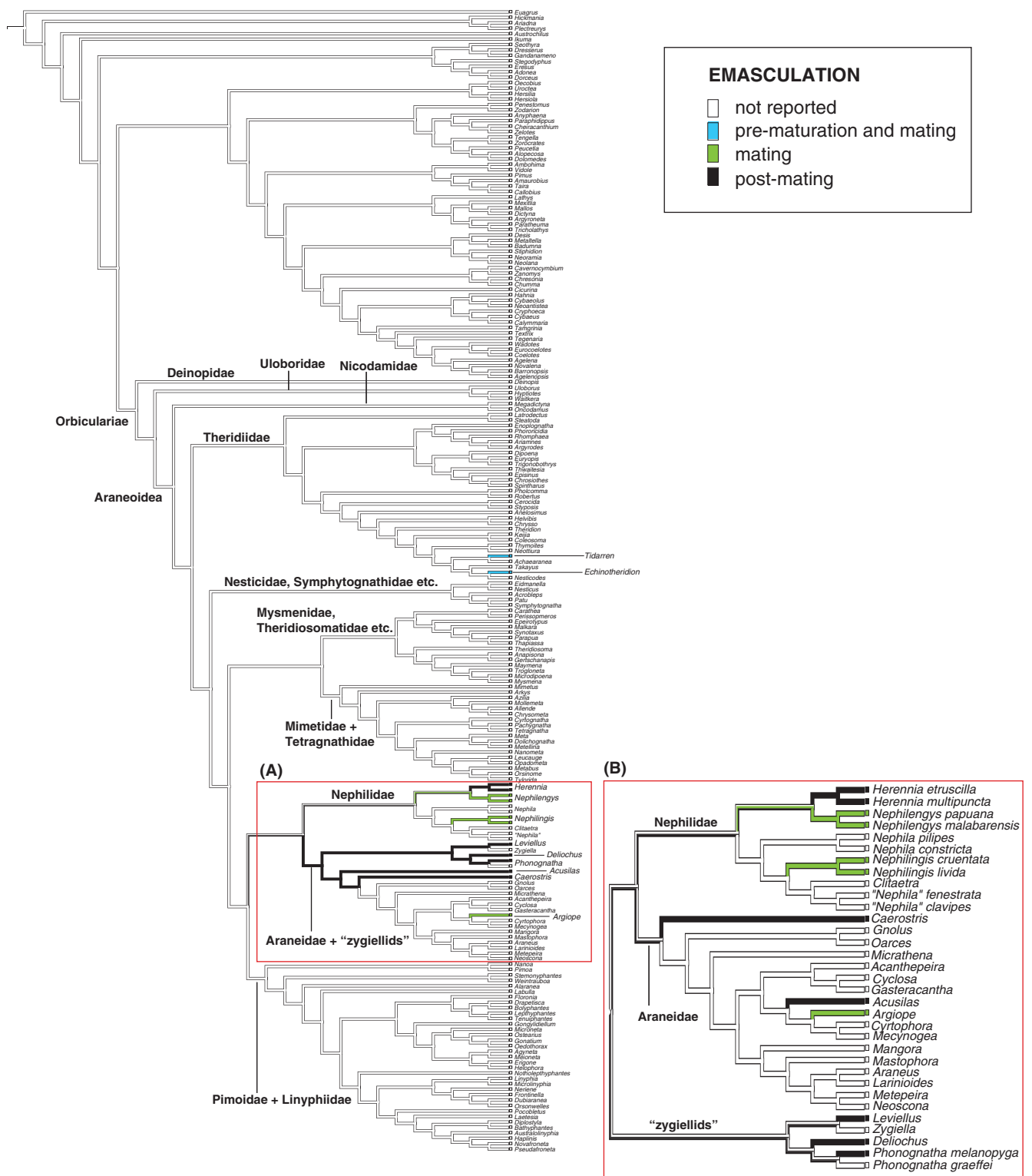
Vetter (2011) reported rare cases of fatal moulting in adulthood of *Loxosceles laeta* males whereby they lost their palps. This seems to be an entirely different, perhaps anomalous, case of developmental autotomy, which we do not explore further.

## IV. EVOLUTION OF EMASCULATION

### (1) Taxon selection and phylogenetic hypotheses

To gain an evolutionary perspective, we used as baseline topology the most up-to-date phylogenetic hypothesis of spider relationships at the genus level emphasizing orbicularian spiders (Dimitrov *et al.*, 2012; Fig. 3). We used their maximum-likelihood tree on selected 272 taxa called ‘dataset1 v1 ml’ (supporting information from Dimitrov *et al.*, 2012). In order to retain a strictly genus-level tree, we first excluded from the full tree all generic repeats, and also those higher level terminals that did not place a generic name with certainty (e.g. Metinae). Thus, we retained 198 generic terminals directly reflecting a pruned phylogenetic hypothesis of Dimitrov *et al.* (2012). Of these, ‘*Nephila*’ was represented by *Nephila clavipes* and *Nephilengys* was represented by *Nephilengys malabarensis*. To this genus-level tree we added additional nephilids to reflect the newest nephilid genealogy (Kuntner *et al.*, 2013), thus *Nephila* s. str. was represented by *N. pilipes* + *N. constricta*, *Cliataetra* was represented by *C. irenae*, and the new genus *Nephilingis* (Kuntner *et al.*, 2013) was represented by *N. cruentata* and *N. livida*. We also added *Caerostris* and *Acusilas*, both known for eunuchs, to the base of araneids minus ‘zygiellids’ to reflect their most current phylogenetic placement (Kuntner *et al.*, 2013). Finally, we added *Leviellus* as sister to *Zygiella* (Gregorič, Kostanjšek & Kuntner, 2010). We used this topology as one of the two alternatives (Fig. 3A).

The relative position of Nephilidae, Araneidae and ‘zygiellids’ is far from resolved and differs somewhat among all analyses performed in the above phylogenetic studies. An alternative phylogeny focusing on Araneidae (T. A. Blackledge & I. Agnarsson) differs in several important clade rearrangements, relevant to emasculation evolution. Thus, a second, alternative topology (Fig. 3B), rearranged the relative positions of Nephilidae, Araneidae and ‘zygiellids’, and the placement of *Acusilas*. Both alternative phylogenies had 210 terminal taxa.



**Fig. 3.** The evolution of emasculation in spiders. Type of emasculation defined according to the precise timing in relation to maturation and mating (legend). Ancestral states are reconstructed with the parsimony criterion, double branch colour implies ambiguity. A genus-level phylogeny, based on latest phylogenetic literature (see text for details) has two alternative topologies (A, B), which affects the reconstruction of emasculation. Major orbicularian clades relevant for emasculation evolution are labelled.



## (2) Phylogenetic scoring

Based on our redefinition of spider emasculation types, we score them as a multistate character for the taxa represented in the phylogeny. Where full eunuchs are known, but the timing of palp severance is not, we conservatively score it as post-mating (Kuntner *et al.*, 2009a). Where no direct or indirect evidence indicate emasculation, we score emasculation as absent. The assumptions of this approach are that (i) the known cases are already reported in the literature, and (ii) lacking direct biological observations, museum specimens should hint at emasculation and we know from our own examination of thousands of specimens that the vast majority of male spiders retain two functional palps throughout their adult lives. However, we do acknowledge vast differences in the understanding of mating biology of certain species and higher taxa. The mating biology of nephilids is much better known than, for example, most araneids, and we have high confidence that if prior work on the sexual biology of, say, *Nephila pilipes* has failed to document emasculation (Kuntner *et al.*, 2009c, 2012; Zhang *et al.*, 2011), the phenomenon is not going to be discovered in this taxon in the future, but the same may not hold for many taxa whose mating biology has not been subject to any studies and/or are rare in collections.

This scoring approach does not take into account levels of plasticity in the eunuch behaviour. As noted above, *Nephilengys* and *Nephilingis* may lose their palps directly or indirectly (Robinson & Robinson, 1980; Kuntner *et al.*, 2009a; Kralj-Fišer *et al.*, 2011). Thus, mating and post-mating emasculation could be viewed as alternative strategies in certain taxa. Nevertheless, for an insight of macro-evolutionary patterns, we score what we interpret as the predominant strategy in each taxon.

## (3) Evolutionary analyses

We used Mesquite (Maddison & Maddison, 2012) for data manipulation and to trace character evolution. For ancestral-state reconstruction, we used the parsimony criterion as implemented in Mesquite, as any model-based method would ‘evolve’ traits all over the tree for biologically less-meaningful interpretations. The resulting reconstructions (Fig. 3) represent two alternative macro-evolutionary patterns of spider emasculation.

Literature suggests two primary biological phenomena to which emasculation may plausibly be related: extreme SSD, and sexual cannibalism (Kuntner *et al.*, 2009b; Kralj-Fišer *et al.*, 2011; Li *et al.*, 2012). Furthermore, emasculation is known in those spiders that regularly damage palps at the distal, embolic end (Kuntner, 2005; Miller, 2007; Uhl *et al.*, 2010); it is then logical to expect the correlation of emasculation and lesser genital damage. To explore possible evolutionary correlates

of these traits, we scored them as independent variables for orbicularian spiders (Orbiculariae) on the two alternative topologies pruned for non-orbicularians. SSD in spiders is routinely defined as female average size being over twice male size (Kuntner & Coddington, 2009), and thus we scored for each taxon mean female/mean male body length ratios. These were obtained from the literature, mostly from the works of Hormiga *et al.* (2000); Kuntner & Coddington (2009); Miller (2007); for all other sources and data, see online Appendix S1. We then rescored these SSD indices into discrete binary characters with the alternative states as (i) none to low (female/mean male body length ratio less or equal to 2.0), *versus* (ii) moderate to extreme (ratio > 2.01), see online Appendix S2. Although the evolution of size is best reconstructed as a continuous variable, and for each sex separately (Hormiga *et al.*, 2000; Kuntner & Coddington, 2009), we here explored all independent traits, SSD included, as categorical (Miller, 2007; Kuntner *et al.*, 2008) to conform to the requirements of the phylogenetic comparative tests. We thus rescored the multistate emasculation character in a binary fashion (present/absent). Similarly, we scored sexual cannibalism as a simple presence/absence character, however, lacking a more recent taxonomic review of the phenomenon than Elgar’s (1992), the unknowns were left as ‘?’ (see online Appendix S2) in all taxa not investigated for cannibalism in recent literature (e.g. Andrade, 1996, 1998, 2003; Knoflach & van Harten, 2000, 2001; Schneider *et al.*, 2001, 2005; Knoflach, 2002, 2004; Schneider & Elgar, 2002; Elgar *et al.*, 2003; Knoflach & Benjamin, 2003; Elgar & Schneider, 2004; Uhl *et al.*, 2007; Wilder & Rypstra, 2008; Wilder, Rypstra & Elgar, 2009; Kuntner *et al.*, 2009c; Kralj-Fišer *et al.*, 2011, 2012, 2013a; Kralj-Fišer & Kuntner, 2012). In addition to the pairwise correlational tests between emasculation as the dependent variable and SSD and cannibalism as independent variables, respectively, we also tested potential correlation between emasculation and lesser genital damage defined as presence *versus* absence of male embolic breakage (Fig. 1E, F; see online Appendix S2) gained from the literature (Kuntner *et al.*, 2008, 2009b,c; Uhl *et al.*, 2010). Although total emasculation may be seen as an extension of lesser genital damage, and thus these phenomena may be taxonomically dependent, we nevertheless tested this assumption. We caution here that potential correlation of several traits with SSD would imply that they all evolve in concert with SSD, and that any further correlations between them may be simply a result of such morphological–behavioural co-evolution. However, phylogenetic correlation does not imply causation; our multiple pairwise tests should thus be interpreted cautiously, as they cannot discern among potential processes. Furthermore, it is plausible that emasculation could arise as a consequence of morphology (e.g. SSD), or mating biology (e.g. cannibalism).

We used three comparative methods that test for character correlation on phylogenies, the pairwise comparison (Maddison, 2000) implemented in Mesquite, the concentrated changes test (Maddison, 1990) implemented in MacClade (<http://macclade.org/>), and the stochastic mutational map analysis (Bollback, 2006) implemented in SIMMAP version 1.5 (<http://www.simmap.com/>). The first two methods rely on parsimony reconstructions of binary character states on a given tree. The pairwise comparison method examines whether the presence of a particular state in one binary character may predict the presence of a particular state in the other in taxon pairs that are phylogenetically separate (not necessarily each other's sister taxa but unique, meaning that no two taxa share a path on the tree connecting them) (Maddison, 2000). We used default settings in Mesquite which maximize taxon pairs with no constraints as to the states that each pair shares; this approach examined 105 taxon pairs. The concentrated changes test examines whether the states in one binary character are significantly distributed along the parts of the tree with a state in the other character (Maddison, 1990) and has been used for similar evolutionary analyses (Miller, 2007). The mutational map analysis, on the other hand, uses a stochastic simulation of character evolution including state distribution and the timing of phylogenetic changes on any character, binary or multistate (Bollback, 2006). Since our trees were derived from several sources, we do not have good estimates of branch lengths and thus set all branch lengths as equal. Unlike in the two parsimony-based methods where the usual significance is  $P < 0.05$ , in mutational map analysis significant associations between specific states that occur more frequently than expected if the characters were evolving independently are indicated with  $P > 0.95$ . Analyses were run for 100 replicates.

#### (4) Macro-evolutionary patterns

Emasculation in spiders, according to the available data, is confined to Araneioidea where it evolved at least five times and up to 11 times, was lost at least four times, and became further modified at least once (Fig. 3). The reconstruction of the ancestral states at the node subtending the araneoid clades Nephilidae + Araneidae + 'zygiellidae' is ambiguous. An accelerated reconstruction (ACCTRAN, see Agnarsson & Miller, 2008) would evolve post-mating emasculation only once, in the common ancestor to Nephilidae + Araneidae. Such a strategy would thus be homologous in those araneids and nephilids that exhibit post-maturity emasculation, and furthermore would suggest that it evolved into a modified behaviour, which takes place during mating, in *Nephilengys* and independently in *Nephilingis*. Alternatively, a delayed reconstruction (DELTRAN, see Agnarsson & Miller, 2008) would imply that post-mating emasculation evolved independently in the common araneid ancestor

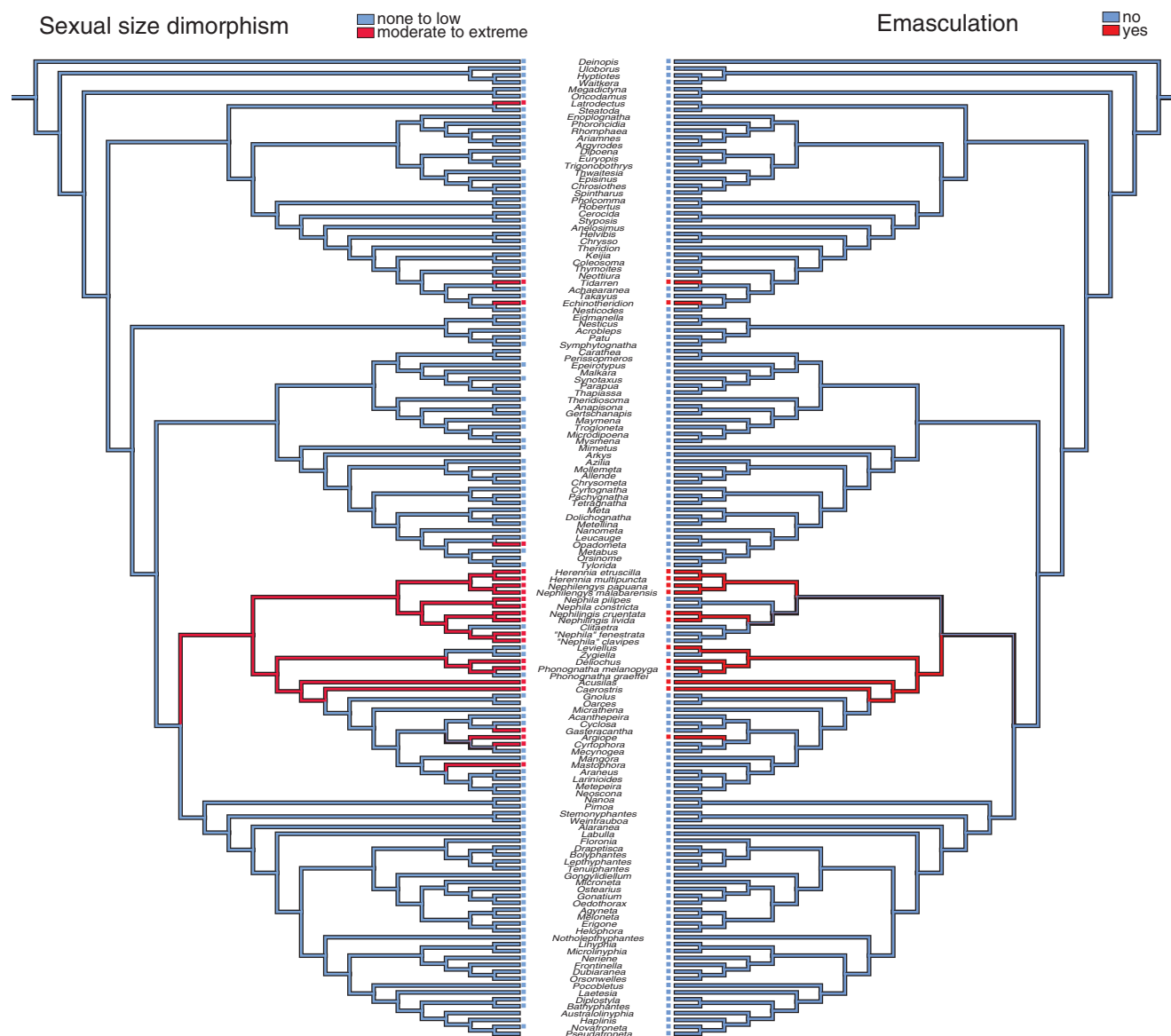
and in *Herennia* (or perhaps in the ancestor of *Herennia* and *Nephilengys* with subsequent modification, as above). Regardless of this ambiguity, it is clear that the three cases of emasculation during mating evolved independently in *Nephilengys*, in *Nephilingis*, and in *Argiope*. Similarly, the current reconstruction weakly favours two independent origins of pre-maturation emasculation in *Tidarren* and in *Echinotheridion*.

Further data are needed to resolve these ambiguities better, and no doubt, the picture will change as phylogenetic resolution further improves and more behavioural data accumulate. However, what is already clear is that the evolution of spider emasculation is more complicated than has been appreciated in prior phylogenetic literature (Kuntner *et al.*, 2008). Independently acquired cases of emasculation may result from similar selection pressures related to SSD, however, each case may be best explained by more than one of the hypotheses outlined above; we thus cannot conclude that any single hypothesis emerges as strongest.

#### (5) Evolutionary correlates of SSD and emasculation

A simple co-reconstruction of SSD *versus* emasculation already implies a strong phylogenetic association (Fig. 4). Indeed, all evolutionary analyses recovered a significant positive correlation between the presence of emasculation as a dependent, and levels of SSD as an independent variable (pairwise:  $P = 0.031$ ; concentrated changes:  $P < 0.001$ ; SIMMAP:  $P = 1.0$ ; note again that the latter significance is taken as  $P > 0.95$ ). Lesser genital damage also correlated positively with SSD (pairwise:  $P = 0.031$ ; SIMMAP:  $P = 1.0$ ), as did sexual cannibalism in some (SIMMAP:  $P = 1.0$ ), but not all analyses (pairwise:  $P = 1.0$ ). These results suggest that both types of genital mutilation (lesser genital damage and full emasculation), as well as sexual cannibalism, all co-evolve with SSD, although our analyses cannot unequivocally interpret emasculation to be a consequence of SSD. It is thus surprising that emasculation did not also significantly correlate with sexual cannibalism (pairwise:  $P = 1.0$ ) or alternatively, their correlation was weakly significant (SIMMAP:  $P = 0.97$ ). Our dataset likely did not provide enough power as the occurrence of cannibalism in most taxa remains unknown. Hence, it is too preliminary to conclude that emasculation should not at least partially be driven by female cannibalism or other sexually conflicted behaviours (Li *et al.*, 2012). As expected, emasculation was significantly correlated with lesser genital damage (pairwise:  $P = 0.031$ ), since it is a taxonomic subset of those males that practice embolus breakage that also break their palps entirely to become eunuchs.

Previous studies have detected a phylogenetic correlation between sexual cannibalism and SSD in spiders both with species treated as data points and with comparative analyses correcting for common ancestry



**Fig. 4.** Co-evolution of sexual size dimorphism (SSD) and emasculation. In orbicularian spiders, these traits—simplified as binary categorical variables—are phylogenetically correlated (pairwise comparison:  $P = 0.031$ ; concentrated changes test:  $P < 0.001$ ) and together with lesser genital damage (embolus breakage) and sexual cannibalism likely form a morphological–behavioural syndrome (i.e. predictable co-evolutionary scenario).

(Wilder & Rypstra, 2008). Males are thus more vulnerable to cannibalism in those clades where females are much larger, yet SSD persists through evolution where males retain ancestral sizes rather than increasing in size along with females (Hormiga *et al.*, 2000; Kuntner & Coddington, 2009; Wilder *et al.*, 2009). Our results suggest that several male adaptations co-evolve with SSD, and may in fact maintain it. For example, lesser genital damage that results in plugging of the female genital tract and increases paternity (Nessler *et al.*, 2007; Uhl *et al.*, 2010) may be seen as male monopolization of females (Schneider *et al.*, 2001; Kuntner *et al.*, 2009b,c), in other words a male adaptation to sexual conflict when

gender sizes differ dramatically. Females may respond to male monopolization with increased cannibalism levels and males follow the arms race by total emasculation, which, as we review here, is adaptive. This scenario seems to be a likely explanation of SSD and emasculation evolution in nephilids (Elgar & Schneider, 2004; Fromhage & Schneider, 2006; Kuntner *et al.*, 2009b; Kralj-Fišer *et al.*, 2011; Lee *et al.*, 2012; Li *et al.*, 2012). On the other hand, an evolutionary link between male genital mutilation and male sacrifice to females (also sexual cannibalism) (Miller, 2007) may be seen in the light of male complicity to cannibalism and thus less of sexual conflict, although Miller's (2007) study did



not distinguish precisely between different types of genital mutilation and sexual cannibalism. This explanation seems more likely to reflect the evolution of SSD and total emasculation in theridiid spiders.

Further studies are needed in order to test co-evolutionary dependence of these traits and whether or not they indeed form, with SSD, a morphological–behavioural co-evolutionary syndrome (meant as a predictable case of co-evolving traits). Nevertheless, it is becoming clear that independent emasculation cases have different causes.

## V. EMASCULATION AND MONOGYNY IN SPIDERS

Recent years have seen lively new research on the evolution of monogyny in spiders (e.g. Fromhage *et al.*, 2005, 2008). Theory predicts that monogyny should evolve where operational sex ratios are male-biased and benefits to males of paternal protection outweigh those of searching for additional females (Fromhage *et al.*, 2005). Precisely such conditions are present in highly sexually size dimorphic orb web spiders, where asynchronous development (small males undergo fewer moults to adulthood compared with large females) results in skewed functional sex ratios (Higgins *et al.*, 2011). Increased male–male agonistic interactions in such systems facilitate adaptations lowering sperm competition, for example mate guarding, genital mutilation and plugging of female genitals (Fromhage *et al.*, 2005). Increasingly, authors have argued that these adaptations to monopolize females represent male persistence mechanisms through sexual conflict and that females evolve predictable sexually antagonistic resistance mechanisms, such as sexual cannibalism (Kuntner *et al.*, 2009b) or other types of sexual aggression (Kralj-Fišer, Schneider & Kuntner, 2013). In order to protect their paternity, small males engage in terminal investment strategies, which in spiders involve spontaneous death *in copula*, as in *Argiope aurantia* (Foellmer & Fairbairn, 2003) and *Dolomedes tenebrosus* (Schwartz *et al.*, 2013), or self-sacrifice in *Latrodectus hasselti* (Andrade, 1996).

Emasculation during mating, where palps form genital plugs, may arguably also be interpreted in the context of terminal investment. However, several facts complicate the comparison. First, self-castrated (nephilid) eunuchs do not die (theridiids do), but rather persist as aggressive fighters and hence emasculation is not strictly ‘terminal’. Secondly, we showed that the known cases of spider emasculation differ in phylogenetic origin, in timing in relation to maturation and mating, and in the extent of palpal loss (one or both). Consequently, one of the main messages of our review is that emasculation cases across spiders evolved multiple times independently and thus are not homologous, nor are convergent instances biologically identical. Because of paired male genitals, half-eunuch nephilid males

may mate again and be bigynous, whereas half-eunuch (virgin) *Tidarren* spp. and *Echinotheridion* spp. males are fully monogynous. The obligate pre-maturation palp severance by subadult male theridiids may have more to do with natural than sexual selection, e.g. by facilitating moulting where two large palps would complicate maturation. While mating and post-mating emasculation may both be related to paternity protection, they too are biologically distinct. Emasculation during mating may serve mostly a plugging function, while post-mating palp severance primarily increases eunuch endurance. Since post-mating emasculation is not known to be obligate, those eunuchs that sever their palp after mating may not fit well into the monogynous evolution picture. Indeed, theoretical modelling predicts that mono- and bigyny can both alternate in a system as stable strategies (Fromhage *et al.*, 2008), and empirical work supports such conditional monogyny in spiders (Welke, Zimmer & Schneider, 2012). Nevertheless, in order to understand spider sexual mechanisms, different types and phylogenetic cases of emasculation should become one of the central traits under investigation when discussing the evolution of spider monogyny and terminal investment.

## VI. OUTLOOK

Although the pattern detected in our analyses will no doubt be modified with phylogenetic updates and new studies of mating biology, we gained a better understanding of the evolution of spider emasculation. Clearly, cases of emasculation differ from one another, and can now be linked better to the predictions arising from the adaptive hypotheses. For example, all three main hypotheses are supported in the case of emasculation during mating as shown in *Nephilengys malabarensis*, and perhaps in the cases of pre-maturation emasculation in theridiids, but clearly not in those taxa known only for post-mating emasculation such as *Herennia*. It would be worthwhile to explore specific differences in the biology of *Nephilengys* spp. and *Herennia* spp. eunuchs and test whether the cases are evolutionarily linked or are convergent, something that can be addressed only ambiguously using phylogenetic tests. Similarly, more specific research could untangle further important functional differences in the two independent cases of previously congeneric *Nephilengys* and *Nephilengys* species.

The evolution of extreme SSD in spiders seems to trigger a unique combination of morphologies and behaviours that evolve in concert and may be responsible for sexual biology that is often interpreted as sexually conflicted (Kuntner *et al.*, 2009b; Li *et al.*, 2012). We showed that lesser genital damage, total emasculation and sexual cannibalism all co-evolve with female-biased SSD. Modelling studies could put these

macro-evolutionary patterns to test, and link eunuchs to other sexually selected traits such as skewed sex ratios, plugging and mate guarding, and to the evolution of monogyny (Fromhage *et al.*, 2005, 2008). However, as pointed out by Hormiga *et al.* (2000) single explanations, or models, are unlikely generally to explain complex biological phenomena, but instead, such traits should first be understood historically. Furthermore, integrative approaches that combine comparative research with evidence from functional and experimental work (Weber & Agrawal, 2012) should advance our understanding of seemingly bizarre evolutionary outcomes, such as emasculation. By studying this and other similar evolutionary phenomena, and their constituent morphologies and behaviours across other animal clades, we may obtain a clearer picture of the intricate interplay of natural and sexual selection in animals.

## VII. CONCLUSIONS

(1) We took a fresh and critical look at spider emasculation resulting in male eunuchs, redefined the types of emasculation according to its timing and mechanism, reviewed the evidence in support of adaptive hypotheses proposed so far, and used updated phylogenies to test broad macro-evolutionary patterns and potential biological correlates of spider SSD and emasculation.

(2) We conclude that three types of spider emasculation exist, pre-maturation, mating, and post-mating, with varying outcomes and behaviours.

(3) Although levels of plasticity in emasculation exist in some but not all taxa, one emasculation type is always predominant.

(4) All emasculation cases in spiders are confined to Araneioidea (Table 1: clades Theridiidae, Nephilidae, Araneidae and 'zygiellidae').

(5) Within these clades, there are replicated origins of each emasculation type, several subsequent losses, and transitions from one type to the other.

(6) The evolution of emasculation is clearly correlated with extreme SSD, as are lesser genital damage and sexual cannibalism, and these probably represent a morphological and behavioural syndrome (by which we mean a predictable form of co-evolution) that arises, we hypothesize, as a consequence of extreme SSD.

(7) Emasculation may be interpreted as a terminal investment strategy related to the evolution of spider monogyny, but because different emasculation cases in araneoid spiders are not biologically identical, this link is not clear-cut.

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## X. SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

**Appendix S1.** Size data for the reconstruction of sexual size dimorphism (SSD) additional to those of the three main literature sources, Hormiga, Scharff, & Coddington (2000); Kuntner & Coddington (2009), and Miller (2007). F = female, M = male. ‘Total’ refers to total body length.

**Appendix S2.** Character matrix with categorical data for emasculation, SSD, lesser genital damage and sexual cannibalism scored for 210 taxa.

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