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# Nephilid spider eunuch phenomenon induced by female or rival male aggressiveness

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**Abstract.** Plugging of female genitals via male sexual mutilation is a common sexual repertoire in some nephilid spiders (*Herennia*, *Nephila*, *Nephilengys*), but the behavioral pathways leading to emasculation are poorly understood. Recent work suggests that copulating *Herennia* males damage their reproductive organs during copulation and then voluntarily, and stereotypically, remove their pedipalps to become eunuchs. Presumably, such emasculation increases agility allowing the male to better fend off rival males. However, through our observation of male antagonism in *Nephilengys borbonica* (Vinson 1863) in La Reunion (Indian Ocean), we discovered that genital severance involving the entire male palp is induced by a rival eunuch. Additionally, laboratory matings of the same species from Mayotte provide the first observations of female sexual cannibalism in this species, one such forceful copulation termination leading to emasculation of the entire palp. These novel behaviors suggest that mate plugging and the eunuch phenomenon are more plastic repertoires than hitherto thought, and thus our observations add to possible pathways leading to them. Based on our examination of 791 samples of *Nephilengys* spp. from museum collections and of a freshly collected representative sample of *N. borbonica*, we conclude that i) palpal severance is common (50% of males from the wild were eunuchs lacking both palps), but ii) the females (or perhaps subsequent males) must possess a mechanism for removing severed palps from the epigyna (none had a whole palpal bulb), leaving behind only partial, embolic plugs, and iii) the disparity between male palpal damage (50%) and visible mating plugs in females (21%) merits further research as the relative numbers of severed males and plugged females can offer insight into which sex may have the upper hand in an evolutionary arms race.

**Keywords:** Sexual conflict, emasculation, plugging, behavioral plasticity, Nephilidae, *Nephilengys borbonica*

Mate plugging via sexual mutilation and eunuchs, males with severed sperm- transferring organs (palps), are common sexual repertoires and outcomes in males of the nephilid spiders *Herennia* and *Nephilengys* (Robinson & Robinson 1978; Robinson & Lubin 1979; Kuntner 2005, 2007). The sparse behavioral data suggest that copulating males typically first produce a mating plug consisting of the distal two palpal sclerites stuck in the female reproductive apparatus (epigynum), thereby preventing additional access to the used female copulatory opening by rival males. Then the males voluntarily remove their copulatory organ and continue in a sterile, and more agile state, to mate-guard the female from competing suitors (Robinson & Robinson 1980). Such a stereotyped pathway leading to the eunuch phenomenon was partially supported by our recent work, which i) establishes the phylogenetic pattern of these behaviors as homologous in nephilids (Kuntner et al. 2008, 2009a); all nephilids with eunuchs also produce mating plugs; ii) provides the evidence that severed organs in *Herennia* indeed function as mating plugs (Kuntner et al. 2009b); and iii) confirms that sexual mutilation and palpal severance (or eunuch behavior) in *Herennia* is voluntary as previously reported for *Nephilengys* (Robinson & Robinson 1980). Plugging in *Herennia*, however, is more likely when the female shows aggression towards the male during copulation (Kuntner et al. 2009b).

However, our new observations reveal behavioral plasticity that complicates the apparently clear pattern of voluntary, post-copulatory self-emasculation. We here report the first observations of plugging that involve immediate, forced whole bulb loss in a nephilid. One such case was induced by male-male antagonistic behavior. We observed eunuch male mate-guarding and male-male antagonism in *Nephilengys borbonica* (Vinson 1863) (Nephilidae) in La Reunion (Indian Ocean) revealing that palpal severance may be forcefully induced by the male (eunuch) rival, and that such plugging may involve the entire male palp. Furthermore, laboratory mating observations of *N. borbonica* from Mayotte (Indian Ocean) revealed that female sexual aggressiveness, which may result in sexual cannibalism, may also induce male whole palp severance. Although based only on a few observations, we report on them because novel behaviors were noted that elucidate the ethological repertoires leading to plugging and the eunuch phenomenon, and because behavioral observations on *Nephilengys* sexual behavior are so scarce. To investigate the prevalence of palpal severance and the apparent absence of whole palpal plugs in preserved museum material, we here also report examinations of 791 samples of *Nephilengys* spp. from museum collections and of a freshly collected representative sample of *N. borbonica* from islands in the Indian Ocean.

## METHODS

**Field observations.**—We monitored male-male and male-female interactions in *Nephilengys borbonica* on the Indian Ocean island of La Reunion (France), in a forest at Colorado-La Montagne (20°54′23.5″S; 55°25′29.4″E; 680 m elev.) on 12 April 2008. Our daytime, non-manipulative observations utilized digital photography (Canon EOS 20D with a 50 mm macro lens and macro flash) and voice recording.

**Laboratory observations.**—We collected juvenile and adult *Nephilengys borbonica* in their natural environment on the Indian Ocean island of Mayotte (France) at Plage Tahiti (12°51′49.0″S; 45°06′39.4″E; 1 m elev.) on 8 April 2008 and

transported them live in foam stopper vials to the Ljubljana laboratory on 10 April 2008. We kept larger spiders in 60 × 60 × 10 cm perspex frames and smaller spiders in smaller plastic containers, at a constant 27° C temperature, 70% humidity, and 12:12 h photoperiod. We misted the webs daily and fed the adult and subadult spiders houseflies and crickets twice a week, and the younger spiders fruit flies or small crickets daily. We used final molting to adulthood as evidence for virginity, but also mated individuals of unknown mating histories (i.e., those collected as adults). Following the experimental mating protocols of Kuntner et al. (2009b), we placed in contact each experimental pair for up to four hours. Before experiments took place, we moved and opened the frames housing the females and kept them undisturbed for the first 60 min, followed by the transfer of a male onto the female web using a fine paintbrush. We used video (Sony HDV 1080i) and voice recording to document behaviors.

**Morphological examination.**—All adult spiders from mating experiments were examined for genital damage under a Leica MZ16 stereomicroscope using KOH maceration, methyl salicilate treatment, and spermathecal clipping (see Kuntner et al. 2009b). Spermathecal clipping reveals male parts (emboli) hidden within the female genitals (spermathecae and ducts) and thus provides evidence of male plugging. Microscope imaging was done using a Leica DFC420C digital camera. Two of the three spiders (eunuch male and female) from La Reunion and all those that mated from Mayotte are available as vouchers and will be deposited in the collections of the Smithsonian Institution's National Museum of Natural History.

In order to assess the prevalence of eunuchs and external plugs in nature, we examined a representative sample of newly collected *N. borbonica* from Madagascar and neighboring Indian Ocean Islands, including La Reunion. External examination of these samples with a Leica MZ16 stereomicroscope would have easily revealed whole palpal plugs in females and palpal loss in males.

## RESULTS

**Field observations.**—A web contained the host female and two males, of which the larger one was a full eunuch (individual that had severed both palps) and the smaller one was intact with both palps (Fig. 1A). Our observation started when the female was residing in her retreat above the hub, the full eunuch (hereafter "eunuch") resting 10 cm above her and the palped male (hereafter "male") 10 cm below her (Fig. 1A). Showing no apparent courtship behavior, the male approached the female, entered her retreat, climbed on her venter, and attempted copulation. At the same time, the eunuch aggressively pursued the wooing male into the retreat, forcing him immediately to jump off the female and out of the retreat into a free fall (Fig. 1B), stopped by his signal line that was attached to the female web. The eunuch climed on the female, and while remaining on her, she moved out of the retreat (yet showed no aggressiveness to either male), and remained around her web hub. The male returned on his signal line, re-approached the female, climbed on her venter, and resumed copulatory attempts despite continued eunuch aggression. One copulation attempt was apparently successful resulting in left palp insertion (Fig. 1C-arrow). However, eunuch antagonism (not female aggressiveness) forced the male to flee (Fig. 1D), this time inducing the copulating male to leave behind his entire left palpal bulb, stuck in the female epigynal opening (Fig. 1D-inset). The severed palp broke in the typical position between the tibia and the tarsus (Fig. 1E-inset). The male, now half-eunuch, returned facing the female's front (she remained unaggressive) and climbed back onto her venter in a new copulation attempt, this time with his remaining right palp (Fig. 1E, F). Continued eunuch attacks (Fig. 1F) again forced the male to flee, but only to yet again approach the female, which had returned to her retreat (Fig. 1G). Walking by the prosoma and chelicerae of the unaggressive female, the male moved towards the eunuch, now protecting the female epigynum on her venter (Fig. 1H). The eunuch forced the male to withdraw again, this time onto the female dorsum (Fig. 1I), and the eunuch remained by the female epigynum (plugged by the male severed bulb, Fig. 1I-arrow). Apparently irritated by the two males on her abdomen, the female moved abruptly out of her retreat and the eunuch attacked the male by aggressively biting with his chelicerae (Fig. 1J shows them still widely open). The male jumped again, and the eunuch resumed his mate-guarding, keeping close to the female abdomen in her retreat (Fig. 1K).

**Laboratory observations.**—The first two male-female encounters on 15 May 2008 did not result in copulation. Male 1 (virgin) attempted to mate repeatedly using both palps with female 1 (unknown history), but failed. As predicted from this episode, subsequent examination revealed an embolic plug in each female opening. Male 2 (unknown history) attempted to copulate with female 2 (previously mated as witnessed by a clutch of offspring), but his approach resulted in female's aggression immediately upon contact and in the first recorded case of sexual cannibalism, where she consumed her suitor (video available at www.nephilidae.com). Again, subsequent examination revealed an embolic plug in each epigynal opening.

On 28 May 2008 at 10:07 h, male 1 (still virgin after unsuccessful copulation attempts) and female 3 (virgin, one week after final molt) were placed together. The male, being placed about 15 cm diagonally below the female, started walking away from her for several seconds, then turned around and headed towards the female at the top of the web. He stopped 10 cm before the female and cleaned his legs in the order 1-right, 1-left, 2-right, 2-left, 4-left, 4-right, then continued towards the female and paused for 68 s about 5 cm away from her. He continued towards the female shaking his whole body twice and pausing again with his first legs 0.5 cm away from the female's first legs. The contact (video available at www.nephilidae.com) occurred after 10 min of idleness. The male walked onto the female's venter, tapped and probed briefly with his palps on the epigynum, and inserted his right palp into the right genital opening (ipsilateral insertion), followed by the male's lateral body twist (abdomen to the left). After only a brief copulation (about 4 s, at 10:20 h), the female aggressively terminated it by manipulating the male with her first and second legs, grabbing him in her chelicerae, and holding on and consuming him. During seizure, the male's right palp broke off and remained stuck in the female's genital opening (Fig. 2A). Her devouring of the male continued (video available at www.nephilidae.com) until

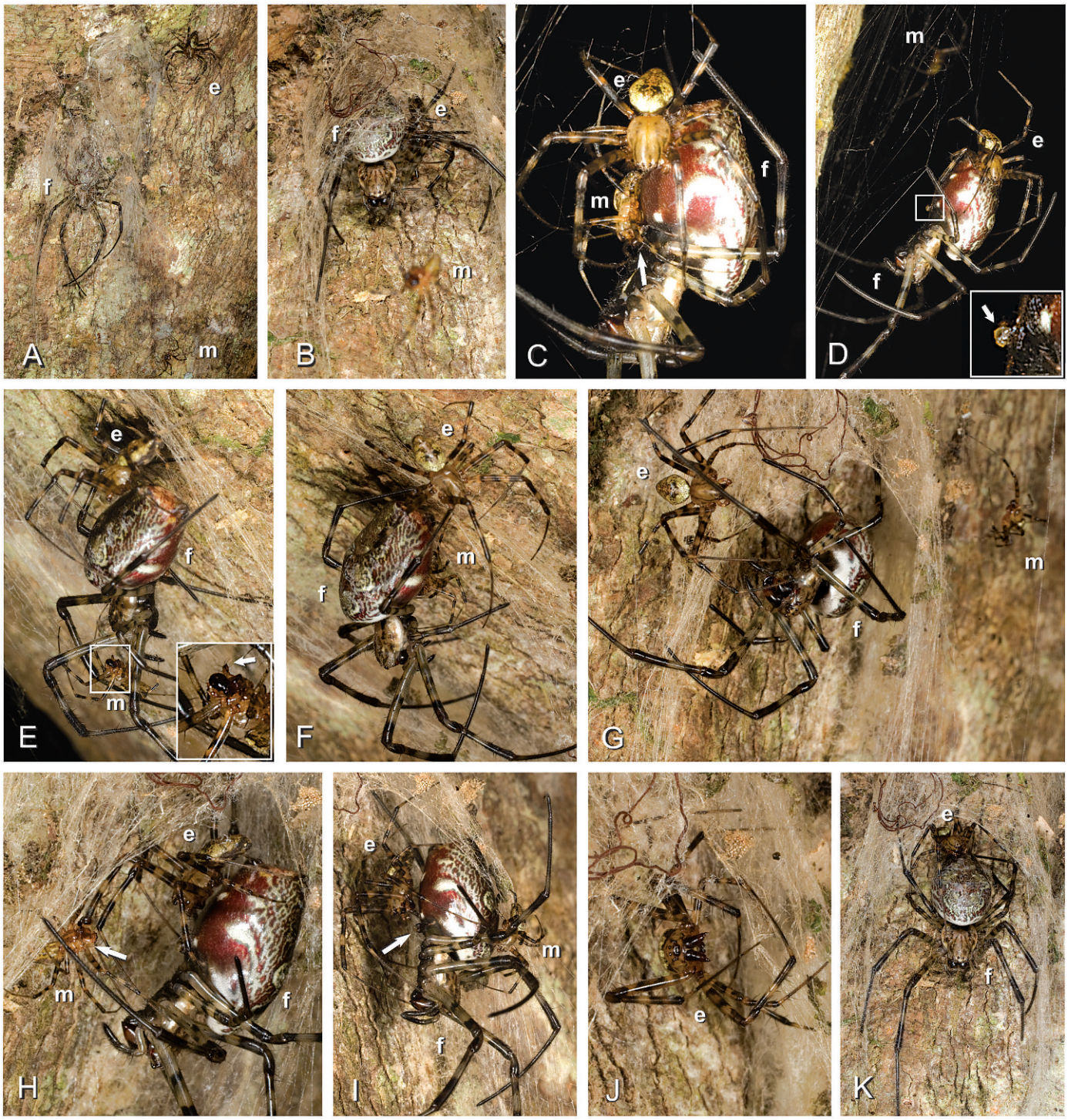


Figure 1.—Male-female and (eunuch) male-male interactions of *Nephilengys borbonica* from La Reunion in chronological order (see text). The male (m) with both palps approached the female (f) (A), but the palpless eunuch (e) repeatedly chased him off her (B–I) and, ultimately, successfully off her web (J–K). A male-male fight during brief copulation (C) resulted in a forceful severance of the male copulating palpal bulb, that remained stuck in the female genital opening (D-inset, arrow, I-arrow) and rendered the sexually active male a half-eunuch (E-inset, arrow, H-arrow).

we interrupted it to secure the male voucher needed for morphological examination. The male lost his right palp at the tibia-tarsus joint (Fig 2B-arrow), as is common in self-castrated eunuch nephilids (Kuntner 2005, 2007).

**Morphological examination.**—We have previously examined 791 samples containing preserved *Nephilengys* specimens (Table 1, specimen data examined by Kuntner 2007 available at www.nephilidae.com). In *Nephilengys*, all four species are

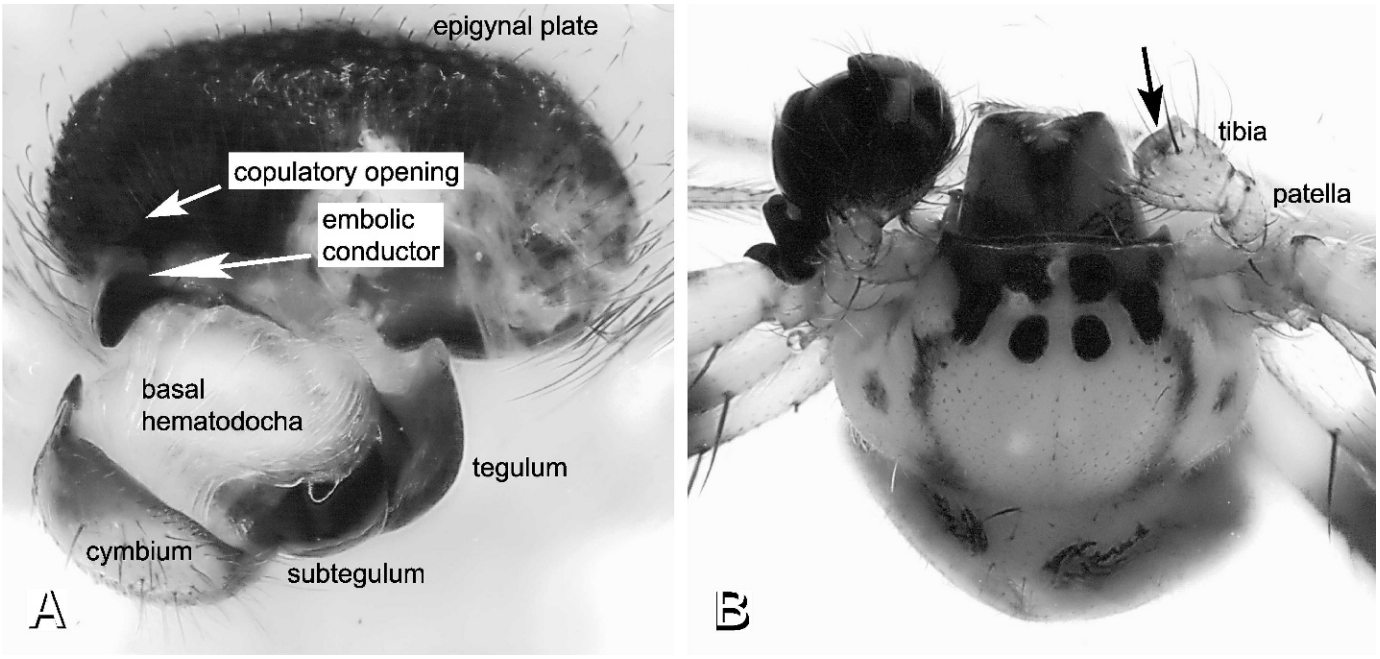


Figure 2.—Outcome of the laboratory mating experiment with *Nephilengys borbonica* from Mayotte. Female forcefully terminated copulation resulting in whole bulb plug in epigynal opening (A) and male becoming eunuch (B) just before being cannibalized by the female. Arrow in (B) indicates the point of palpal breakage.

uniform in gross morphology and behavior, including common eunuchs (Kuntner 2007). However, none of these samples contained females plugged with whole palps.

Table 2 lists the *N. borbonica* specimens examined externally within the representative sample from the 2008 collected material ($n = 33$ females, 30 males; specimens from mating trials and samples containing only juveniles are omitted). Again, eunuchs were common (50% of males were both palp eunuchs), but while 21% of females had mating plugs in the form of externally visible embolic parts in their copulatory ducts, none of the females had whole palps in their epigyna.

## DISCUSSION

The nephilid eunuch phenomenon is not homologous to the voluntary emasculation by theridiid spiders *Echinotheridion* and *Tidarren* (Agnarsson 2006; Kuntner et al. 2008), where the subadult males remove one of their palps before seeking mates. However, increased male agility may explain both phenomena. In these tiny theridiid males, also known for whole palpal plugs and fierce male-male competition (Miller 2007), two large palps reduce the mobility of the male and subsequent emasculation thus facilitates mate searching (Ramos et al. 2004). As sexual cannibalism characterizes these theridiids, males do not have the opportunity to defend mated females. Nephilid males, on the other hand, always start their sexually active post-molting period with a fully functional pair of palps, and become eunuchs after copulation. Hence, unlike their theridiid counterparts, their emasculation serves for post-copulatory agility. In the theridiids, emasculation is highly stereotypical (Knoflach & Harten 2000, 2001). In contrast, our findings here suggest that emasculation does not always follow the same behavioral pattern in nephilids, as previously thought.

Eunuchs are known for post-copulatory mate-guarding and aggressiveness towards rival males (Robinson & Robinson 1978). Our own work on *Herennia* (Kuntner et al. 2009b) has recently confirmed that the eunuch phenomenon typically entails initial sclerite damaging during copulation, followed by voluntary post-copulation palpal removal, corroborating only two previous observations on *Nephilengys* (Robinson & Robinson 1980). However, even if the males of *Herennia* removed their palps themselves after initial mutilation, mutilation itself (resulting in plugging) was more likely when the female was aggressive towards the male during copulation

Table 1.—Numbers of samples containing *Nephilengys* spp. examined morphologically by Kuntner (2007; specimen and depository data available at www.nephilidae.com).

| Species | Samples containing female(s) | Samples containing immature(s) | Samples containing male(s) | Total |
|---|---|---|---|---|
| *N. borbonica* | 86 | 10 | 49 | 145 |
| *N. cruentata* | 360 | 49 | 46 | 455 |
| *N. malabarensis* | 99 | 8 | 45 | 152 |
| *N. papuana* | 24 | 5 | 10 | 39 |
| Total | 569 | 72 | 150 | 791 |

Table 2.—Representative sample of female (f) and male (m) *N. borbonica* collected in nature and examined externally for palpal severance and plugs. Left/right scores are for embolic plug counts in females per opening (0 = no plug; 1 = one plug) and damaged palp in male (u = undamaged; p = palpal severance). More detailed specimen data available from the authors. Note absence of simple embolic severance in males and no half-eunuchs (see Discussion).

| Sex | Left | Right | Number of specimens |
|---|---|---|---|
| f | 0 | 0 | 26 |
| f | 1 | 0 | 3 |
| f | 0 | 1 | 2 |
| f | 1 | 1 | 2 |
| m | p | p | 15 |
| m | u | u | 15 |

(Kuntner et al. 2009b). This suggests the possibility that aggressiveness could play a role in inducing palpal severance at various weak points. Although data are sparse, our mating observations suggest that sexual aggression/cannibalism may be common in *N. borbonica* (two out of three mating sequences observed) and, in one case, induced the male to release and leave behind a whole palpal plug in the female opening. Furthermore, the newly observed behavioral pattern of rival-male-induced palpal severance suggests that intraspecific aggression from the female or rival males may induce whole palpal plugging.

Most available observations on nephilid sexual mutilation rely on very few data points (*Nephila* being an exception, see Schneider et al. 2001, 2005; Fromhage & Schneider 2006) and the sexual behavior of *N. borbonica* has not been studied in detail (but see Kuntner 2007). However sparse the data, whole bulb plugging has not been observed before. Certain behaviors such as plugging and bulb loss can be deduced via morphological examination (Kuntner et al. 2008, 2009b). We have examined numerous samples of male and female individuals of *N. borbonica* from its entire range and of *Nephilengys* spp. in total (Tables 1 and 2). Presumably, these examinations would have easily detected any evidence of whole bulb-plugs, yet did not. Similarly, whole bulb-plugs are unknown in *Herennia* or any other nephilid (Kuntner 2005, 2006). The data at hand, if interpreted uncritically, would thus suggest that mating plugs in *Nephilengys* are usually formed by the terminal two sclerites as in most other nephilids (Kuntner 2005, 2007; Kuntner et al. 2008). However, in light of our study, we propose an alternative explanation: that whole palps may be frequently severed and left in the female, but that they subsequently break off at the standard point (the remaining morphological evidence of females would then fail to detect such involuntary palp loss). Because a palpal bulb consists of the entire palpal tarsus sexually modified in adult males to contain a number of interconnected sclerites and membranes (Fig. 2A), it is thus a relatively large and cumbersome device for the female to keep on her. Additionally, the sexual conflict theory (Arnqvist & Rowe 2005) would predict that it is in the female's interest to resist such a male monopolization mechanism (Kuntner et al. 2009a). We suggest that such plugged females do remove whole palpal plugs (perhaps using their legs or rubbing against the substrate), and that such removal would then result in the common morphological outcome: palpless (eunuch) males, and females plugged only by the male's two terminal sclerites. In fact, the second breaking point between the two sclerites and the remainder of the male palp may be another twist in the male-female conflict story: the male's response to female palp-plug removal. As our preliminary data show, even the two-sclerite plugs seem effective in preventing further matings.

Alternatively, subsequent males, in addition to the female, may be able to remove previous whole palp plugs. Embolic plug removal by the next male is known to occur sporadically in *Nephila fenestrata* (J. Schneider pers. comm.), which is phylogenetically only a few nodes away from *Nephilengys* (Kuntner et al. 2008), although in that species plugs are simpler and generally functional (Fromhage & Schneider 2006). A similar mechanism in *N. borbonica* would then explain the second breaking point in the palpal bulb as another result of sperm competition, in addition to the plugs themselves, rather than a result of sexual conflict between the male and the female. We find this mechanism possible, but unlikely, because it would imply some percentage of females that find no mate subsequent to being plugged, and thus retain the whole palp plug. In our case, this percentage was zero.

Examination of over 800 preserved *Nephilengys* specimens shows high frequencies of female plugging (21%) and male palpal severance (50%). Given that a considerable portion of samples must represent unmated specimens, palpal severance in mated males is likely universal. Our ongoing work (Kuntner, unpublished data) suggests that external genital examination in nephilids underestimates the number of embolic mating plugs (broken emboli may be lodged deep in spermathecae), and thus the real number of plugged females is certainly higher than 21%. Further work is thus necessary to determine if the percentage of plugged females equals the percentage of severed males. Such work should lead to interesting conclusions. If differences in frequencies are confirmed, this would suggest that females are successful in removing mating plugs so that even whole palpal sacrifice by the male may not guarantee his mating success and/or female monopolization. If, however, the numbers of severed males and plugged females are approximately equal, this would imply that male plugs are successful and that males may be winning an evolutionary arms race.

It is worth noting, that the 15 *N. borbonica* eunuchs collected in nature were all full eunuchs, meaning they lacked both palps. Kuntner (2005, 2007) reported also half-eunuchs (males with only one functional palp) in *Nephilengys* and *Herennia*, and we have documented half-eunuchs of *H. multipuncta* in nature (Kuntner et al. 2009b: fig. 2b–c). It remains to be seen whether half-eunuchs are rarer in *N. borbonica* than in *Herennia* and other *Nephilengys* species.

In sum, the newly discovered behavior where sexual mutilation is induced by male or female aggressive behavior and where the mating plug consists of the whole palpal bulb, adds to the documented plasticity in the pathways leading to the nephilid eunuch phenomenon. We conclude that palpal severance in *N. borbonica* is common (50% of males are eunuchs) and that the females are likely able to remove severed palps from their epigyna, resulting in embolic mating plugs.

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