# Independent origins of female penis and its coevolution with male vagina in cave insects (Psocodea: Prionoglarididae)

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Abstract

The cave dwelling psocid tribe Sensitibillini (*Afrotrogla*, *Neotrogla* and *Sensitibilla*) is of special morphological and evolutionary interest because of its possession of reversed copulatory organs:, i.e., females of *Afrotrogla* and *Neotrogla* have a penis-like organ. The female penis structure is highly variable among taxa, as is the case of the male penis in animals with normal copulatory organs. Here, we present the first molecular phylogeny of Sensitibillini and analyse the evolutionary pattern of their genitalia. *Afrotrogla* and *Neotrogla* did not form a monophyletic clade, and their female penis structures are significantly different, suggesting two independent origins of the female penis within Sensitibillini. In *Neotrogla*, the species that has a simple female penis is embedded among species that have an elaborate penis, and detailed structures of the female penis elaborations are in exact agreement among species, suggesting a secondary simplification of the female penis. A correlated evolutionary pattern between male and female genitalia was also detected. This coevolution of genitalia may suggest that sexual conflict or cryptic "male" choice drove the diversity of the female penis, as is the case of male penile diversity in animals with conventional genitalia.

Keywords: genital evolution, reversed direction of sexual selection, cryptic mate choice, sexual conflict, sexually antagonistic coevolution

## 1. Introduction

Debate is ongoing regarding the evolutionary forces underlying extraordinarily rapid diversification of genital traits in animals [1]. Male reproductive success increases with the number of mates, whereas female fitness does not generally increase with multiple matings because it is limited by the number of ova. Thus, sexual conflict over mating can cause sexually antagonistic coevolution (SAC) in genital traits between the sexes [2]. Mounting evidence shows that males develop persistence traits in their genitalia, such as claspers and spines, that enable them to coercively mate with females, while females develop traits for resistance or tolerance, such as anti-clasping projections and pouches for accommodating spines, as a counter-adaptation for mitigating the male-imposed costs [3–5]. Alternatively, female genital pouches could function to allow the female to favor the males that have mechanically compatible genitals (cryptic female choice [1, 6]).

Sperm transferring structures have evolved multiple times in the animal kingdom but almost always in males. Females of the cave insect genus *Neotrogla* (Psocodea: Prinoglarididae: Sensitibillini: figure 1*a*) possess a penis-like organ, termed a gynosome (figure 1*b–f*). This organ is used to anchor male vagina-like genitalia in a species-specific manner for a long time (40–70 hours in *N. curvata*), during which voluminous and probably nutritious semen is passed to the female [7]. Because they inhabit dry and oligotrophic caves, severe competition for seminal gifts has likely reversed their propensity for multiple mating (reversed direction of sexual selection). Among the three known genera of Sensitibillini, a well-developed female penis is known also in *Afrotrogla* (figure 1*h*), whereas *Sensitibilla* does not have such a structure (figure 1*g*) [8–10]. Thus, the members of Sensitibillini provide an exceptionally rare opportunity to study the evolutionary origins of this novel penis-like organ and the generality of coevolutionary patterns between male and female genital traits.

In this study, we provide the first molecular phylogeny of Sensitibillini based on six gene markers selected from both nuclear and mitochondrial genomes. Based on the estimated tree, we discuss the coevolutionary pattern of female and male genitals.

## 2. Material and methods

The cave psocids with reversed genitalia are classified under the family Prionoglarididae (Insecta: Psocodea: Trogiomorpha). In this study, all generic or higher taxa of the family were sampled except for the genus *Speleopsocus*, which is known only from a single specimen. *Neotrogla* shows the most elaborate condition of the female penis, from which all four named species plus an undescribed species were sampled. Outgroups were selected from all suborders of "Psocoptera" (non-parasitic Psocodea) (electronic supplementary material, table S1). Nuclear 18S rRNA and Histone 3 and mitochondial 12S rRNA, 16S rRNA, COI and CytB genes were used. Trees were estimated by the maximum likelihood and Bayesian methods. Ancestral state reconstruction was conducted under the parsimony and likelihood models. For the likelihood ancestral state estimation, the branch length of the ML tree was re-estimated under the molecular clock model. A detailed account of the methods is given in the electronic supplementary material.

## 3. Results and discussion

In the present tree, *Sensitibilla* and *Afrotrogla* formed a clade, and *Neotrogla* was placed to its sister group (figure 2; electronic supplementary material, figures S1–S2). *Sensitibilla* and *Afrotrogla* are distributed in southern Africa [8–10], whereas *Neotrogla* is distributed in South America [11–13]. Therefore, the result is reasonable geographically. In contrast, a well-developed and protruding female penis-like organ is known from *Afrotrogla* and *Neotrogla*, whereas females of *Sensitibilla* only have a small sclerotized shaft near the opening of the spermathecal duct that lacks a protruding portion [9,10] (figure 1*c–h*). This implies that the well-developed female penis either evolved independently in *Afrotrogla* and *Neotrogla* or reduced secondarily in *Sensitibilla*. Correlated with the evolution of the female penis, an absence of the male paramere (grasping organ) was also detected [9,11]. The result of the parsimonious reconstruction was ambiguous, but by likelihood criterion, “absence of female penis (88.7%)” and “presence of male paramere (84.6%)” were estimated to be more likely as the ancestral states of Sensitibillini (electronic supplementary material, table S2), supporting the independent origins of the female penis and independent coevolutionary losses of the male parameres (figure 2). The

female penises of *Afrotrogla* and *Neotrogla* are considerably different morphologically: That of *Neotrogla* is fully sclerotized apically and possesses an inflatable balloon-like structure *basally* (red and green in figure 1*d-f*), and the latter acts as an anchor during copulation [7]. In contrast, the female penis of *Afrotrogla* is mostly membranous (and probably inflatable) *apically*, and no *basal* inflatable structure is observed [9]. In addition, the penis of *Afrotrogla* bears a pair of lateral sclerites that are not homologous to any female penis structure of *Neotrogla* (figure 1*c–f*, *h*). These morphological differences reinforce the independent origin hypothesis of their female penis.

Evolution of a female penis is an extraordinary rare event, even among the animals with reversed direction of sexual selection [14]. Nevertheless, our results show that it has arisen twice in this small group of insects. In addition to competition for male-driven seminal gifts [7], some factors unique to the biology of Sensitibillini, such as extremely oligotrophic cave environments and the evolution of a specialized sperm storage organ for simultaneously holding two seminal gifts [11,12], probably drove the evolution of the female penis. In addition, evolution of a small penis-like structure, as observed in *Sensitibilla* (figure 1*e*), likely had functioned as a preadaptation. The detailed genital morphology and function of *Afrotrogla* and *Sensitibilla* are still unknown, and they deserve further studies.

As in cases of animals with conventional direction of sexual selection, genital coevolution is not straightforward within *Neotrogla*. In most species, the basal membrane of the female penis has one dorsal and two lateral lobes bearing sclerotized spines (red and green in figure 1). Females of *N. curvata* possess an additional set of spines on the ventral side (purple in figure 1*d*). In contrast, the basal membrane of *N. truncata* is covered with tiny denticles and has no lobes or spines (figure 1*c*, *i*). Male genitalia also show corresponding patterns: males of the species with spiny female penises have vaginal pouches for receiving the spines (figure 1*l*), whereas males of *N. truncata* do not have such structures (figure 1*j*). No wound scar has been detected in the male pouches [7]. The present tree placed *N. truncata* within the species with anchoring female spines and male pouches, implying that these structures might have evolved independently or may have evolved in their common ancestor and reversed to a simple condition in *N. truncata*. The result of the parsimonious reconstruction was ambiguous, but by likelihood

criterion, female penile spines and male vaginal pouches were estimated to be the plesiomorphies of *Neotrogla* (92.5% for both: electronic supplementary material, table S2), suggesting coevolutionary secondary reduction of the anchoring spines and pouches in *N. truncata* (figure 2).

Similar de-escalation has been reported for animals with conventional direction of sexual selection. In a *Drosophila* species endemic to island mountains, male genital spines and the corresponding female genital pouches are reduced [15]. In a diving beetle species endemic to a single small pond in Japan, female-grasping male legs are slightly reduced compared with close congeners, in parallel with the reduction of anti-grasping setae on the female elytra [16]. Though exact causes are unclear for these cases, theories predict that both concerted escalation and de-escalation of coercive and resistant traits occur depending on the balance between the benefits of coercion and its costs for the counter sex [17–19]. Among *Neotrogla* spp., *N. truncata* inhabits notably dry caves where only limited potential prey and predator species are found (electronic supplementary material, table S3). Lower densities of conspecific competitors (females) and/or predators in extreme environments might reduce the risk of copulating pairs being disturbed and thus can relax selection pressure for secure mate holding. Alternatively, male *Neotrogla* might control the transfer of sperm and/or nutrients based on the stimuli provided by the elaborated female penises (i.e., cryptic "male" choice), in case they reflect female quality [20]. With possibly many undiscovered species/populations adapted to each isolated cave environment, accumulations of basic biological data of *Neotrogla* are highly desired to discriminate between these hypotheses.

**Ethics**. Not applicable.

**Data accessibility**. Raw sequence data are available from GenBank (electronic supplementary material, table S1), and Nexus formatted aligned DNA matrix and Mesquite file for morphological evolution data are available from FigShare [21].

**Author contribution**. KY and YK designed the study. IY and KY conducted PCR and sequencing. KY conducted phylogenetic analyses and ancestral state estimations. KY, CL and YK analyzed morphology. RLF, KY and YK conducted field work. RLF obtained behavioral and environmental data. KY and YK wrote the first draft and all authors contributed for the final

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159
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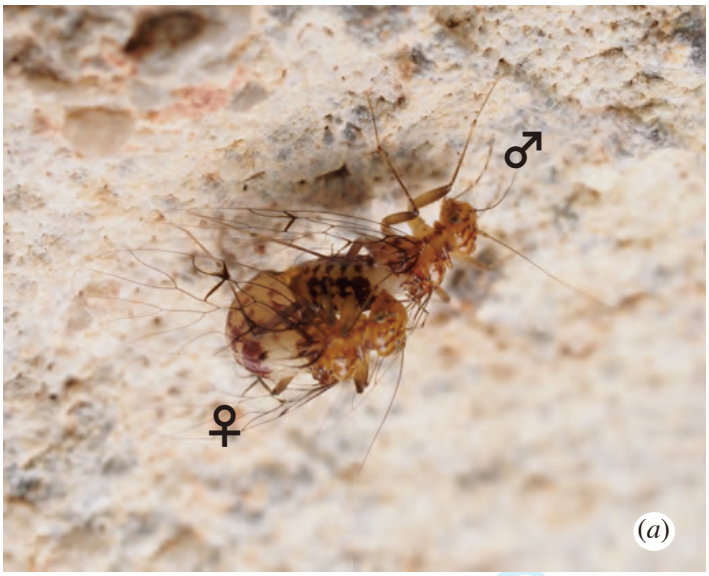
218 **Figure legends**

219

220 **Figure 1.** (*a*) *Neotrogla* sp. in copula. (*b*) Terminal abdomen of *N. curvata* in copula. Female
221 structures are highlighted by red. (*a–l*) Morphology of the female (*c–i*, *k*) and male (*j*, *l*) genitalia
222 in Sensitibillini, with the homology scheme indicated by colours. (*c*, *i–j*) *Neotrogla truncata.* (*d*)
223 *N. curvata.* (*e–f, k–l*) *N. aurora* (note that *N. brasiliensis* and *N.* sp. also have this type of
224 genitalia). (*g*) *Sensitibilla etosha.* (*h*) *Afrotrogla oryx.* Dotted regions of illustrations indicate
225 membranes and others are sclerite (*c-h*: approximately to scale). Arrowheads in (*c*)–(*h*) indicate
226 the opening of the spermathecal duct, and arrowheads in (*j*) and (*l*) indicate the presence (filled)
227 or absence (open) of male vaginal pouches. (*c*)–(*e*) lateral view. (*f*)–(*i*),(*k*) ventral view. (*j*),(*l*)
228 dorsal view.

229

230 **Figure 2.** Maximum likelihood tree (Troctomorpha and Psocomorpha omitted from the figure),
231 with character state changes estimated by the likelihood model. Numbers indicate bootstrap
232 support values/Bayesian posterior probabilities. See electronic supplementary material (figure
233 S1–S2) for details.


♂
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(a)


♂
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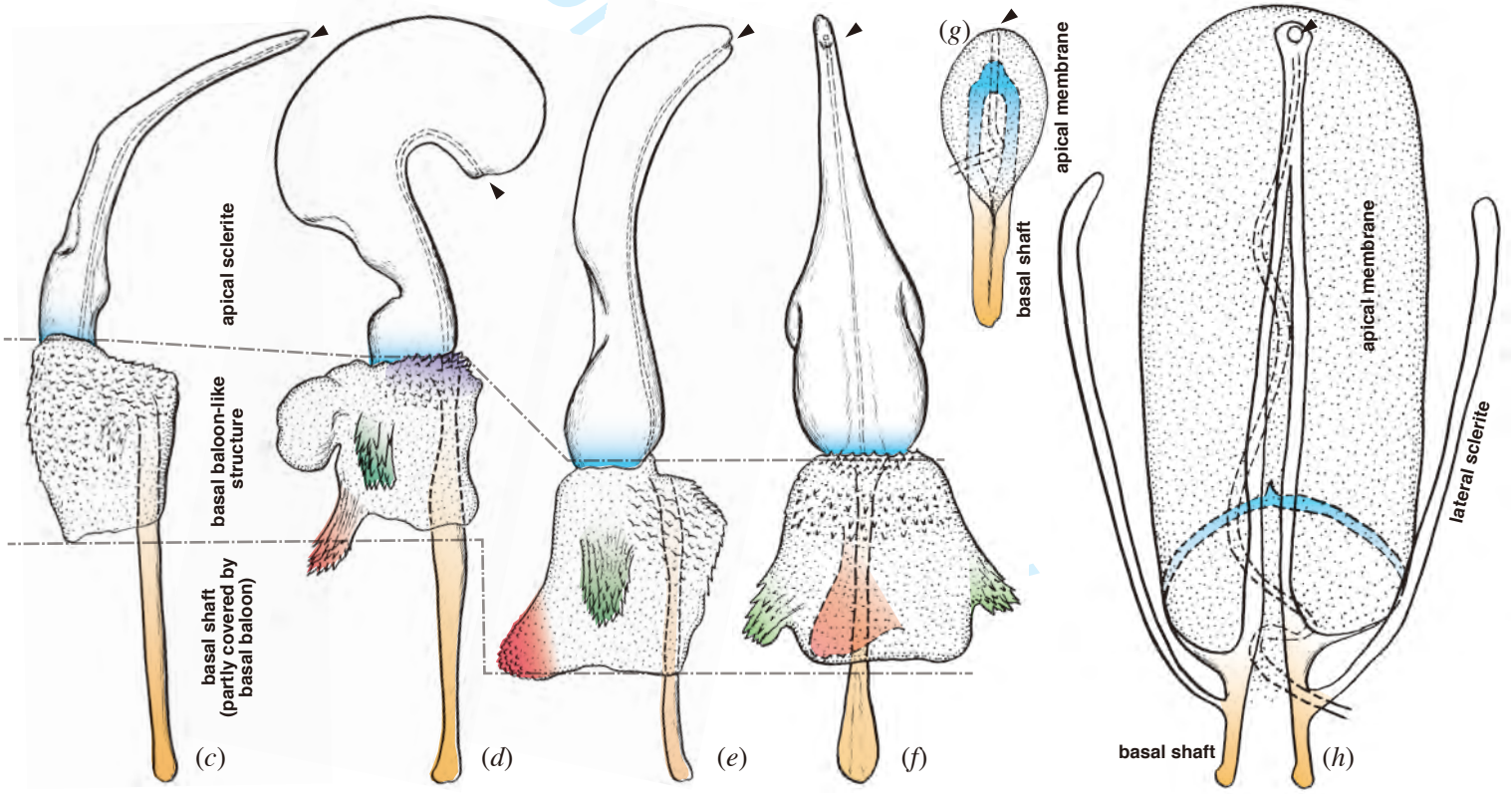
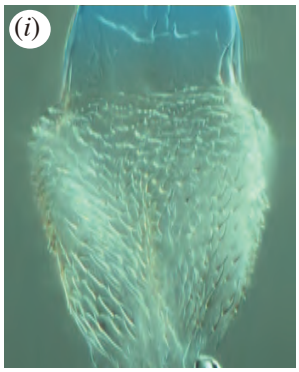
(b)


apical sclerite
basal baloon-like structure
basal shaft (partly covered by basal baloon)
(c)
(d)
(e)
(f)
(g)
apical membrane
basal shaft
apical membrane
lateral sclerite
basal shaft
(h)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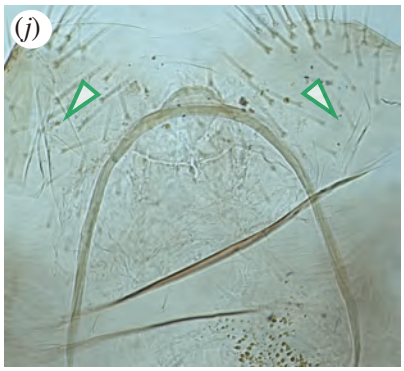
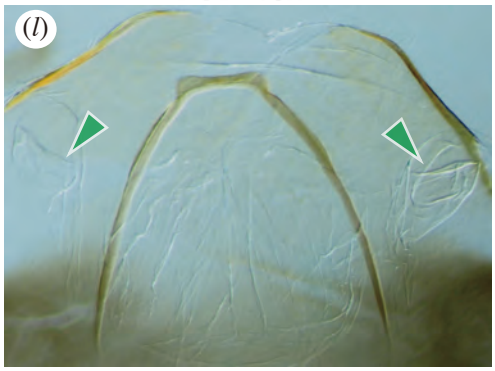

(j)


(k)


(l)